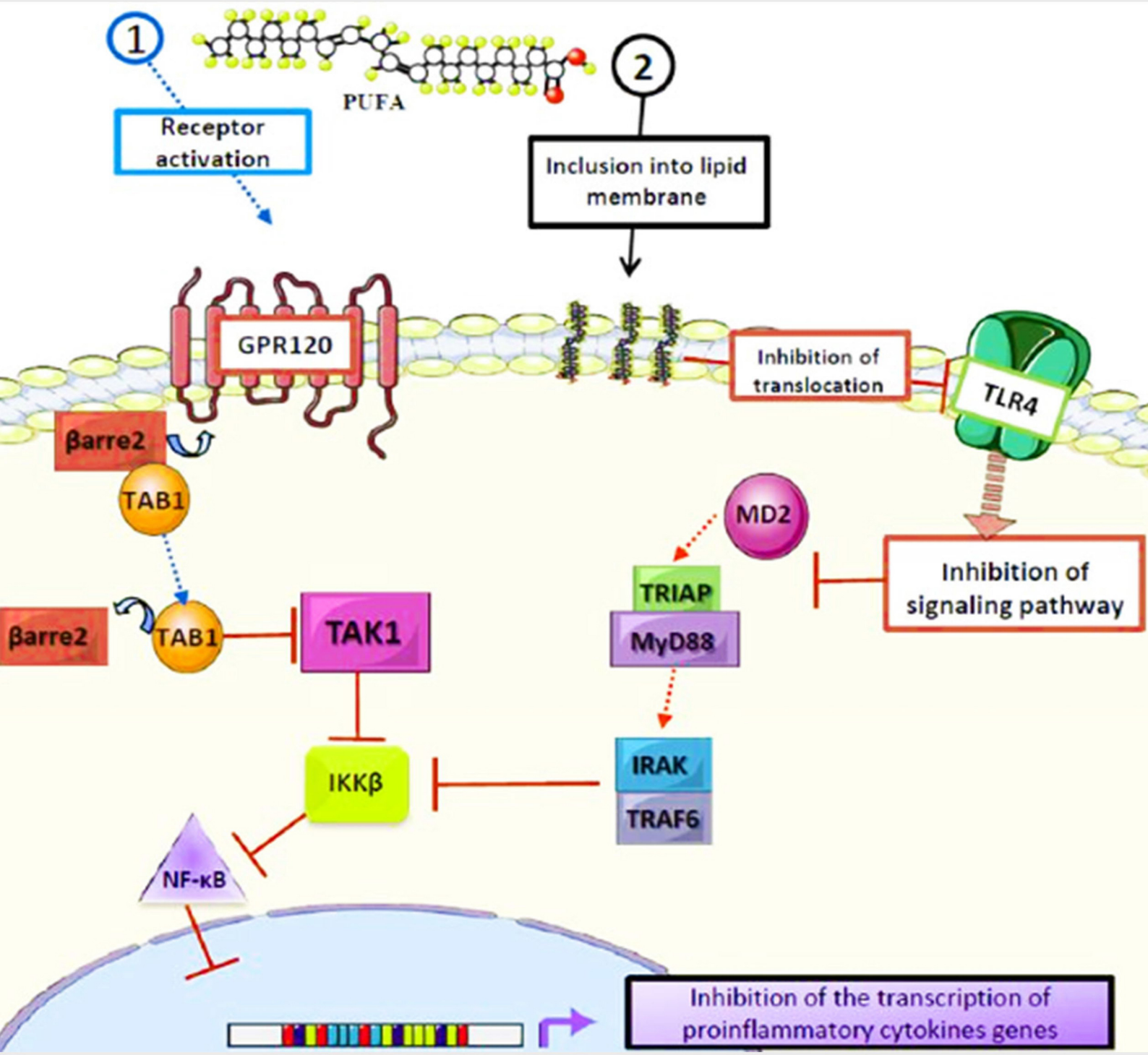
ISSN 2574-1209 (Online)
Volume 1 | 2017

VP Vessel Plus



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Publisher

OAE Publishing Inc.
245 E Main Street ste122, Alhambra, CA 91801, USA
Website: www.oaepublish.com

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Dumont. *Vessel Plus* 2017;1:1-2
DOI: 10.20517/2574-1209.2017.03

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Editorial **Open Access**

A foreword from the Editor-in-Chief

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How to cite this article: Dumont AS. A foreword from the Editor-in-Chief. Vessel Plus 2017;1:1-2.

Article history: Received: 22-01-2017 Accepted: 23-01-2017 Published: 31-03-2017

Dr. Dumont serves as the Charles B. Wilson Professor and Chair of the Department of Neurosurgery at Tulane University School of Medicine. He is a board-certified neurosurgeon who completed his residency program training at the University of Virginia as well as a fellowship in cerebrovascular, skull base and endovascular neurosurgery at the University of Virginia. He has active neurosurgical research interests and has published and presented extensively with more than 200 publications and more than 175 national and international presentations.

Welcome to the inaugural issue of *Vessel Plus*. This recently launched journal from OAE Publishing Inc., is an international, peer-reviewed, open access journal dedicated to publishing articles focused on vascular physiology, biology and disease.

I would like to thank and extend my gratitude to my co-editors, editorial board members, reviewers, members of OAE Publishing Inc., especially Sylvia Wang, as well as the contributing authors for creating this first issue.

Vessel Plus publishes articles related to all diseases caused by pathological change of blood vessels, including stroke, hypertension, aneurysm and coronary artery and peripheral vascular diseases and thrombosis. This journal focuses on the latest clinical and basic research on the prevention, treatment and prognosis of disease of the blood vessels including those of the arterial and venous circulation as well as the microcirculation.

In this issue, readers will find a diverse group of manuscripts. Sobenin *et al.*[1] have provided new insight into the nature of low density lipoprotein modifications in diabetic compared to non-diabetic patients. Schiavone *et al.*[2] have compared the mechanical performance of metallic compared to bioresorbable polymeric stents during the process of crimping and deployment using finite element modelling. Additionally, Dong *et al.*[3] have reviewed the potential therapeutic role of a mimetic peptide of the ubiquitin-interacting motif of epsin, through regulation of VEGFR2 signaling, in brain tumors.

This is indeed an exciting time for *Vessel Plus*. Our journal will allow rapid publication of manuscripts after rigorous peer review, free of charge to the authors with open access, facilitating widespread dissemination and impact on the field. Future special issues may include a thematic focus on original research as

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well as reviews and editorials by luminaries in the field. We look forward to helping advance the field of vascular research by providing a premier medium for publication.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. Sobenin IA, Galitsyna EV, Grechko AV, Orekhov AN. Small dense and desialylated low density lipoprotein in diabetic patients. *Vessel Plus* 2017 Jan 19. [Online First]
2. Schiavone A, Qiu TY, Zhao LG. Crimping and deployment of metallic and polymeric stents -- finite element modelling. *Vessel Plus* 2017 Jan 19. [Online First]
3. Dong Y, Wu H, Dong J, Song K, Rahman HA, Towner R, Chen H. Mimetic peptide of ubiquitin-interacting motif of epsin as a cancer therapeutic-perspective in brain tumor therapy through regulating VEGFR2 signaling. *Vessel Plus* 2016 Dec 27. [Online First]

Dong *et al. Vessel Plus* 2017;1:3-11
DOI: 10.20517/2574-1209.2016.01

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Review **Open Access**

Mimetic peptide of ubiquitin-interacting motif of epsin as a cancer therapeutic-perspective in brain tumor therapy through regulating VEGFR2 signaling

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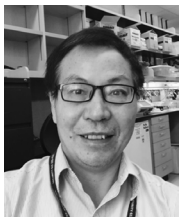
How to cite this article: Dong Y, Wu H, Dong J, Song K, Rahman HA, Towner R, Chen H. Mimetic peptide of ubiquitin-interacting motif of epsin as a cancer therapeutic-perspective in brain tumor therapy through regulating VEGFR2 signaling. Vessel Plus 2017;1:3-11.



Dr. Yunzhou Dong is a senior investigator at the Vascular Biology Program of Boston Children's Hospital and Harvard Medical School. He received his PhD in Genetics and Molecular Biology in 2000. Dr. Dong had worked at the Cardiovascular Biology Program in the Oklahoma Medical Research Foundation (OMRF) and the Section of Endocrinology and Diabetes in the University of Oklahoma Health Sciences Center. In his earlier career, Dr. Dong worked at the College of Veterinary Medicine, University of Tennessee at Knoxville. Dr. Dong has substantial publications in prestigious journals about the mechanistic study and therapeutic development in cardiovascular diseases, metabolic diseases and cancer field.

Article history:
Received: 31-08-2016
Accepted: 01-11-2016
Published: 31-03-2017

Key words:
UPI peptide,
epsin,
cancer therapy,
glioma,
tumor angiogenesis

ABSTRACT

Epsins, endocytic adaptor proteins required for internalization of ubiquitylated receptors, are generally upregulated in human cancers. It has been characterized that mice deficient of epsins in the endothelium inhibit tumor growth by dysregulating vascular endothelial growth factor receptor-2 (VEGFR2) signaling and non-productive tumor angiogenesis. Binding of the epsin ubiquitin (Ub)-interacting motif (UIM) with ubiquitylated VEGFR2 is a critical mechanism for epsin-dependent VEGFR2 endocytosis and degradation, indicative of epsin UIM as a potential therapeutic target. A Computer Assisted Drug Design approach was utilized to create the UIM mimetic peptides for the functional competition of epsin binding sites in ubiquitylated VEGFR2 *in vivo*. Specifically targeting VEGFR2 in the tumor vasculature, the chemically synthesized chimeric UIM peptide, UPI, causes non-functional tumor angiogenesis, retards tumor growth, and increases survival rates in several tumor models. The authors showed that UPI binds ubiquitylated VEGFR2 to form a supercomplex in an Ub-dependent fashion. Collectively, the UPI targeting strategy offers a potentially novel treatment for cancer patients who are resistant to current anti-angiogenic therapies. In this review, the authors outline the main points of this research specifically as a potential application for glioma tumor therapy.

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INTRODUCTION

Angiogenesis is essential for embryogenesis and postnatal tissue repair. Deadly cancers, however, can emerge from tumor angiogenesis, a physiological process involving the production of functional vessels for cancer cell embedment, colonization, growth, and metastasis. In 1971, Folkman[1] hypothesized that inhibition of tumor angiogenesis is a potentially powerful tool for cancer therapy.[2-6] With the identification of more and more new molecules modulating angiogenesis,[6-8] targeting tumor angiogenesis has become increasingly likely, and the concept of inhibiting tumor vessel growth has led to the discovery of vascular endothelial growth factor (VEGF) and the anti-VEGF antibody, Bevacizumab (Avastin). Bevacizumab is an angiogenic inhibitor approved by the U.S. Food and Drug Administration (FDA) for certain metastatic cancers such as colorectal cancer and lung cancers.[9] However, this approach centered around the major pathways including vascular endothelial growth factor receptor (VEGFR) or Notch signaling via direct or indirect modulations.[5,10-13] Because the therapeutic efficacy of Bevacizumab is mild in clinical applications where patients could develop resistance to the drug during the course of the treatment, it was imperative to develop alternative compounds to modulate tumor angiogenesis and complement the efficacy of Bevacizumab for those who are resistant to anti-angiogenic therapies.

EPSIN UBIQUITIN-INTERACTING MOTIF AS A THERAPEUTIC TARGET FOR CANCER

Epsins are adaptor proteins in endocytosis

Epsins were originally isolated as adaptor proteins in the clatherin-mediated endocytosis of ubiquitylated cell surface receptors.[14,15] Using molecular, cellular, genetic, and mutant mouse models, we have identified that epsins modulate embryogenesis,[16] angiogenesis vasculature,[17] lymph angiogenesis,[18] tumor angiogenesis,[19,20] and cancer progression.[21] Mechanistic studies have demonstrated that epsins target the Notch[16] or ubiquitylated receptor VEGFR2,[17,19,20,22] VEGFR3 or Wnt signaling pathway,[21] and modulate angiogenesis or epithelial cell proliferation. In tumor angiogenesis, epsins bind to the ubiquitylated VEGFR2 via the ubiquitin (Ub)-interacting motif (UIM) to facilitate endocytosis and inactivate VEGFR2 signaling [Figure 1].[19,20]

Epsins regulate tumor growth and tumor angiogenesis by targeting VEGFR2 signaling: role of UIM in epsins

We previously reported that the UIM-dependent binding of epsins with VEGFR2 is required for VEGFR2

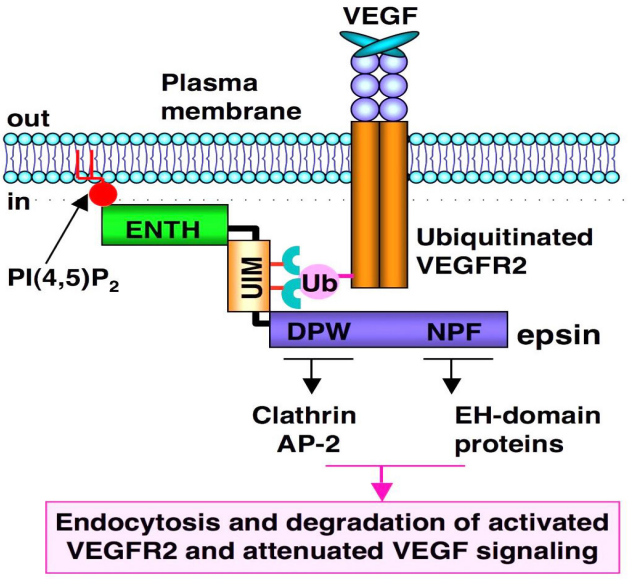

Figure 1: Epsins are adaptor proteins in endocytosis. VEGFR2 is activated by ligand-VEGF binding. Activated VEGFR2 is ubiquitylated, followed by epsin binding via UIM motif. ENTH domain in epsin "hooks" into the plasma membrane (PM) via PiP2. Clathrin and other endocytotic proteins such as AP2 bind to the DPW domain (Asp-Pro-Trp), while the EH-domain (Eps15 homology [EH] domain) proteins bind to the NPF domain (Asn-Pro-Phe) so that a pit is formed on the PM. Receptor-containing vesicles are endocytosed to cytosolic degradation machinery to deactivate cell signaling

internalization, degradation, and signaling attenuation in tumor angiogenesis.[20] Strikingly, the UIM sequence is highly conserved in both human and mouse epsins 1 and 2, indicative of a central element in epsin function and potential clinical applications.[20] In endothelial-specific loss of epsin mouse models (EC-iDKO), tumor growth is significantly inhibited in Lewis lung carcinoma (LLC), melanoma (B-16), glioma, and Tramp (Transgenic Adenocarcinoma of the Mouse Prostate) mouse models.[20] The loss of epsins drastically increased not only the number of vessels but also the diameters of tumor vessels.[20] Functional perfusion analysis suggests that loss of epsins leads to tumor vessel hyper leakage and dysfunction [Figure 2].[20] Our data also suggest that loss of epsins modulates VEGFR2 endocytosis by upregulating its expression. The heightened VEGFR2 is anchored on the plasma membrane (PM) in EC-iDKO mice, leading to augmented VEGFR2 signaling and tumor angiogenesis.[20] Because these vessels are not functional, the tumors are actually much smaller.[20] A domain mapping experiment suggests that the UIM in epsins is critical for regulating the epsin-VEGFR2 interaction, and that loss of UIM in epsin 1 blocks the interaction between epsin and VEGFR2.[20]

Adoption of Computer Assisted Drug Design to mimic "*in vivo*" knockout phenotype in tumor models: specificity, enrichment, and stability

To increase therapeutic efficacy, the design of the

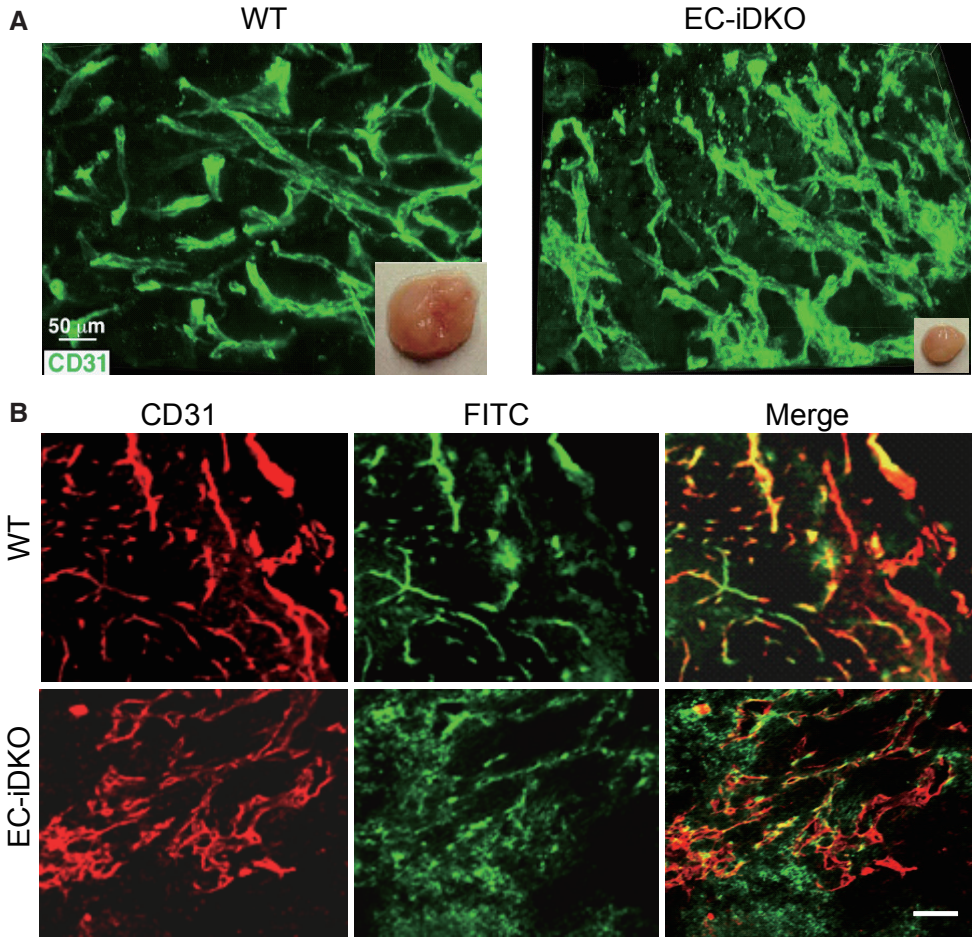

Figure 2: Loss of epsins in endothelial cells inhibits tumor growth by producing upregulated, but non-functional tumor angiogenesis. (A) LLC tumor is much smaller in EC-iDKO mice, accompanied by upregulated, but dilated vessels in tumors; (B) Vessel function assay: FITC-Dextran perfusion suggests that tumor vessels in EC-iDKO mice are hyper leaky. Scale bars = 50 μm for A and B. Figure 2 is adapted with permission from ref. 20. Copyright 2012 ASCI

peptide is crucial. The top gear, peptide targeting specificity, peptide working mechanism *in vivo*, and peptide stability in circulation are important factors that need to be carefully considered.

Specific targeting

We hypothesize that if a synthetic UIM-containing peptide can be targeted to tumor vessels, it could competitively bind to the ubiquitylated VEGFR2 receptor and therefore block the epsin-VEGFR2 interaction, which could photocopy the knockout of epsins in tumor endothelial cells (TECs). Molecular modeling revealed that the UIM peptide forms a helical structure known as yeast Vps27-UIM.[19] To ensure exclusive delivery of the UIM peptide to tumor vasculature, a tumor EC-homing peptide, iRGD, was conjugated to the N-terminus of the UIM peptide.[23] iRGD binds to αvβ3 or αvβ5 integrin, then to neuropilin, and thus can be specifically internalized into TECs.[24,25]

Peptide working mechanism in vivo

To increase the local concentration of peptides near the PM, an inner PM-anchoring peptide from the Lyn kinase H4 domain[26-28] bound to lipid rafts through palmitoylation and myristoylation sites was inserted between iRGD and UIM. The resulting peptide is referred to as UPI [Figure 3].[19] To explore the specificity in the molecular interaction, we undertook docking studies and used a de novo structural prediction method to generate the atomic model for the interaction between UIM/UPI and the VEGFR2 kinase domain (KD) [Figure 4].[19] Our model predicts that the unique residues Q9, A13, and K16, present only in epsin UIM but not in UIMs from a number of other endocytic proteins, play a critical role in the specific interaction with residues R1027 and R1080 in VEGFR2 [Figure 4]. Furthermore, molecular modeling revealed that interactions between the UIM helix and Ub in both UIM-Ub and UPI-Ub models are similar to the binding of yeast Vps27 UIM-Ub complex by nuclear magnetic resonance (NMR) spectroscopy [Figure 5A].[29] Remarkably, the interaction surfaces of UIM-Ub or UPI-Ub and UIM-VEGFR2 or UPI-VEGFR2 are clearly complementary in terms of charges from

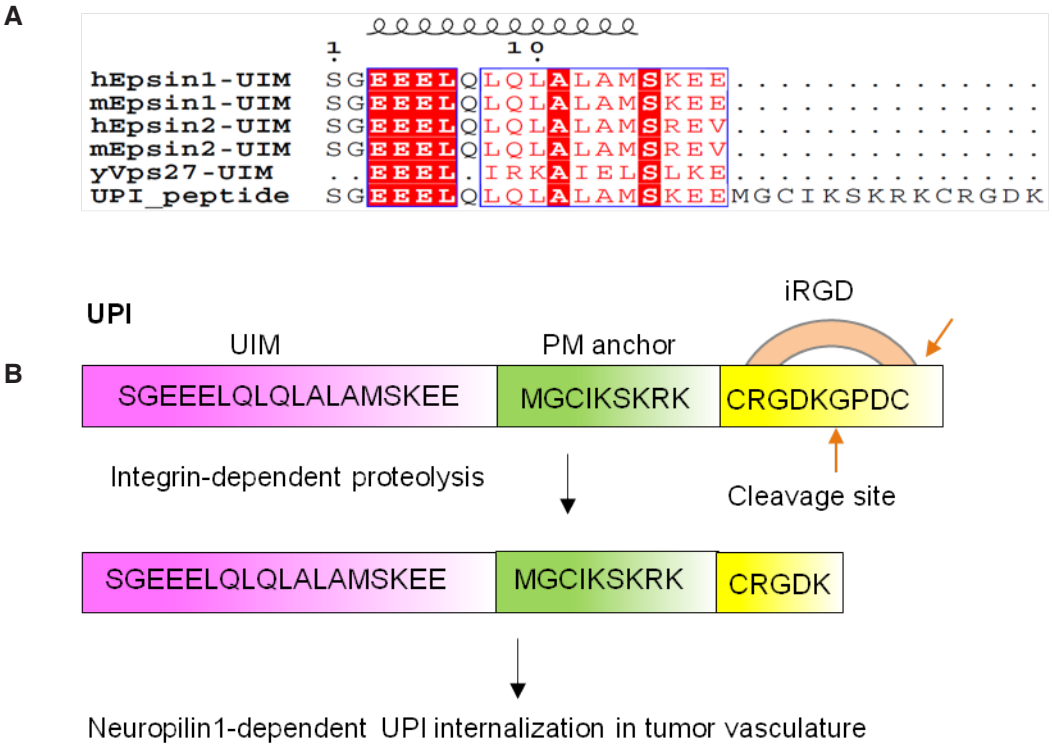

Figure 3: Design of the UPI peptide. (A) Alignment of human epsin UIM, mouse epsin UIM, and yeast Vps27 UIM with UPI chimeric peptide; (B) iRGD binds integrin and goes through proteolysis. The last 4 amino acids (GPDC) will be removed by circulating proteases.[23] UPI peptide contains an epsin UIM, a PM targeting sequence from the Lyn kinase H4 domain, and a tumor homing sequence (iRGD). Figure 3 is adapted with permission from ref. 19. Copyright 2015 ASCI

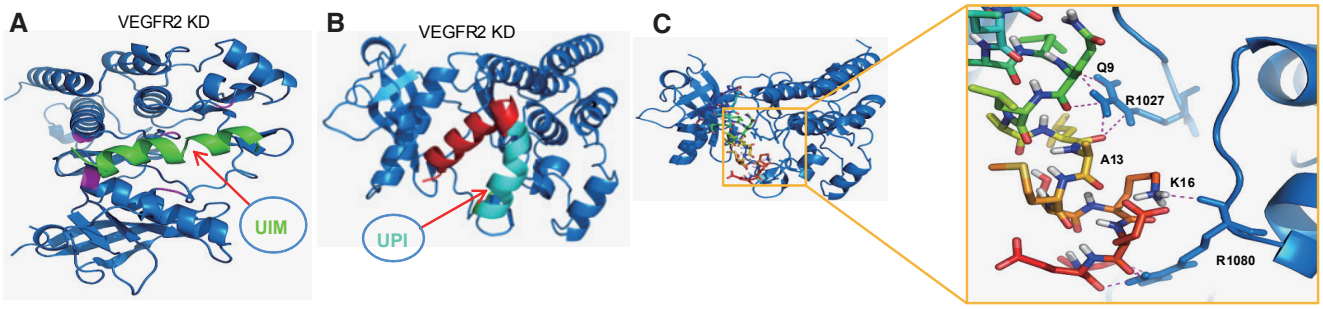

Figure 4: Molecular modeling to study the interaction between UIM or UPI with VEGFR2 kinase domain (VEGFR2-KD). The 3D models of UIM and UPI were predicted using the PEP-FOLD program with 200 computational simulations. The best score models of UIM and UPI were docked into VEGFR2-KD respectively using the ClusPro2.0 program. (A) Ribbon representation of the interaction between UIM and VEGFR2-KD, which are colored green and blue, respectively. The interaction residues His891, His816, Arg1022, Arg1027, and Arg1080 on the hairpin-shaped binding cleft of VEGFR2-KD are denoted in pink;[17,19] (B) Ribbon representation of the association between UPI peptide and VEGFR2-KD. In the same manner as UIM:VEGFR2-KD, UPI binds into the same binding pocket of VEGFR2-KD. VEGFR2-KD is denoted in blue. In UPI peptide, UIM is denoted in red, and the inner plasma membrane anchoring peptide and a tumor homing peptide (iRDG) are denoted in cyan; (C) Cartoon representation of the model of UIM-VEGFR2 complex. VEGFR2 is denoted in blue and shown as a ribbon; UIM is denoted in multicolor and shown as a stick (left). On the right: A close-up view of interaction residues between UIM and VEGFR2 is shown in the right panel. The key residues Q9, A13, and K16 of UIM form hydrogen bonds with R1027 and R1080 of VEGFR2.[19]

the electrostatic point of view [Figure 5]. By binding to the Ub moiety conjugated to VEGFR2, UPI, and Ub, VEGFR2 forms a supercomplex [Figure 6]. The UPI peptide can specifically hone in to tumor vasculature and enrich itself in the inner part of the TEC's PM as expected, which increases the therapeutic efficacy and minimizes the dosage of the peptide when used in animal administration.[19]

Optimization of peptide stability in vivo

In order to avoid the degradation of our peptide from peptidases in circulation, we used the D-isomer of amino acids to synthesize the UIM sequence, while the iRGD sequence was synthesized using the L-isomer of amino acids for efficient binding to integrin. During synthesis, the iRGD is circled by the disulfide bond of two cysteines for *in vivo*

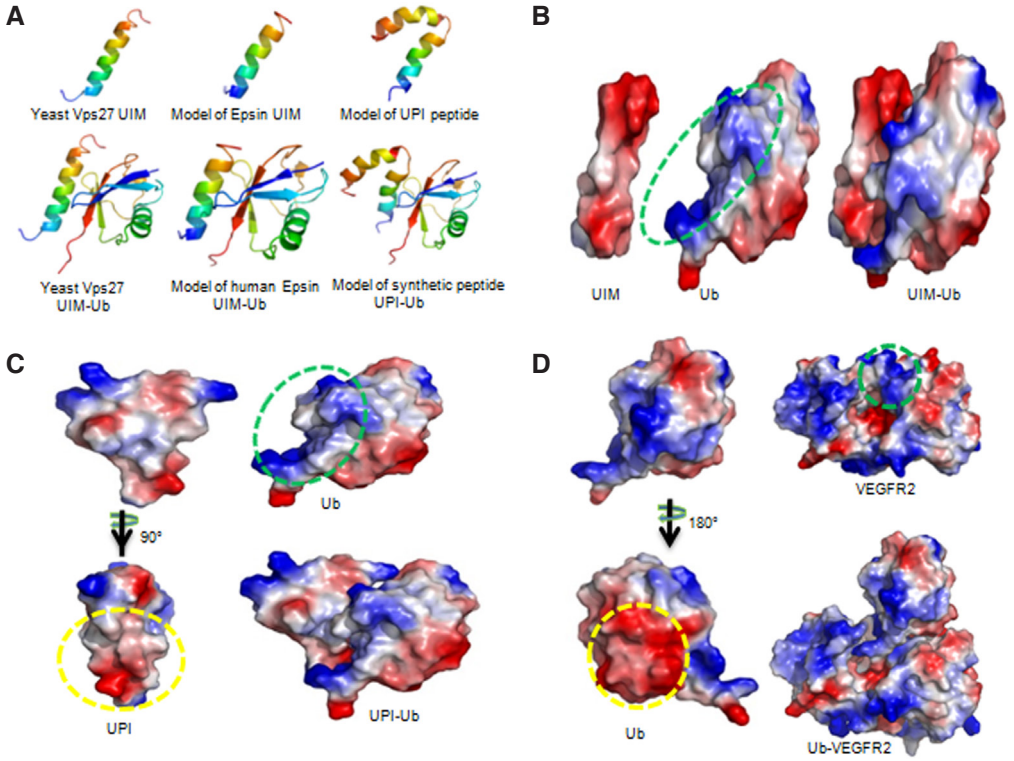

Figure 5: Models of UIM-Ub, UPI-Ub, and Ub-VEGFR2 complexes. (A) Ribbon diagrams show yeast Vps27 UIM interaction with Ub (left), and models of human epsin UIM-Ub complex (middle) and UPI-Ub complex (right). The NMR structure of yeast Vps27 and X-ray structure of Ub were taken from the Protein Data Bank, entries 1Q0W and 3JVZ, respectively. The top scoring models of epsin UIM and UPI (top panel) were selected and docked into Ub. The models with high scores and good topologies are shown in the bottom panel. Yeast Vps27 UIM, epsin UIM, and UIM of UPI peptide interact with Ub in a highly similar manner (bottom panel). Structures are multi-colored, with the N and C termini denoted in blue and red, respectively; (B-D) Electrostatic surface representations of UIM-Ub, UPI-Ub, and Ub-VEGFR2 complex models. Red and blue represent negative and positive potential, respectively. The proposed binding surfaces with negatively charged amino acids are indicated by yellow circles. The green circles highlight the proposed interaction surfaces of positively charged amino acids. The figures were prepared using PyMol (Schrödinger, Inc, Cambridge, MA). Note: Figure 5A is adapted with permission from ref. 19. Copyright 2015 ASCI

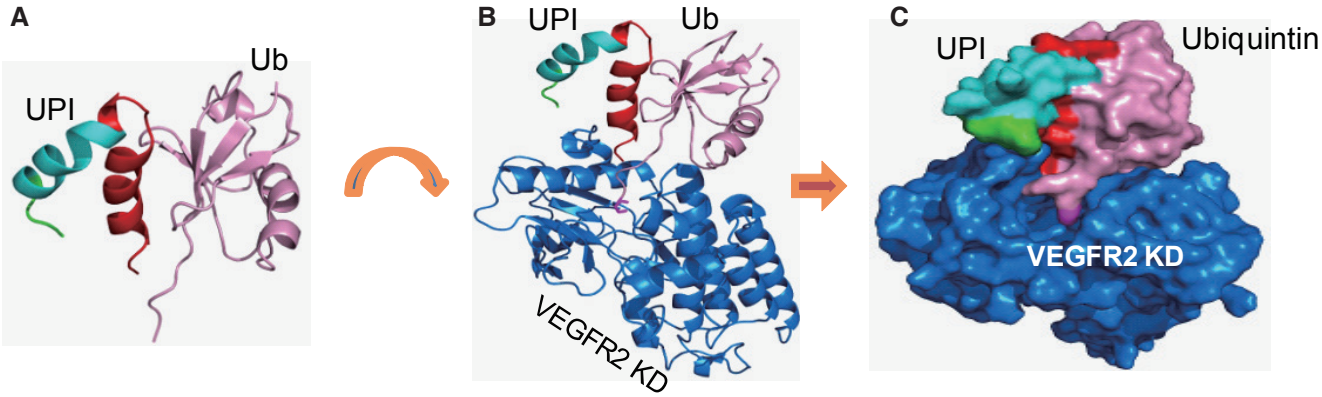

Figure 6: UPI-Ub-VEGFR2 KD forms a supercomplex. (A) UPI hybrid peptide (red: UIM, cyan: plasma membrane-anchoring peptide, green: tumor homing peptide) interacts with Ub (pink). (B) Supercomplex of UPI-Ub-VEGFR2. The model of UPI-Ub was docked onto VEGFR2 kinase domain (marine, PDB entry 3U6J). The UIM domain of UPI tightly binds to Ub, and the C-terminal tail of Ub (8-amino acid stretches, Gly76 side chain, magenta) inserts into the binding pocket of VEGFR2. (C) Surface representation of UPI-Ub-VEGFR2 KD supercomplex

stabilization.[30-32] Molecular modeling suggests that L-UIM and D-UIM show symmetric binding features to the same pocket of VEGFR2 KD [Figure 7], implying that the UIM peptide in D-isomer does not change the UIM peptide docking sites in VEGFR2 KD. Collectively, the UPI peptide is a multistep-targeting peptide to tumor vascularization and inner PM enrichment. The introduced D-isomer of amino acids in UIM and the circled iRGD can increase the UPI peptide stability *in vivo*. This design strategy could empower the function of the UPI peptide *in vivo* for better therapeutic efficacy.

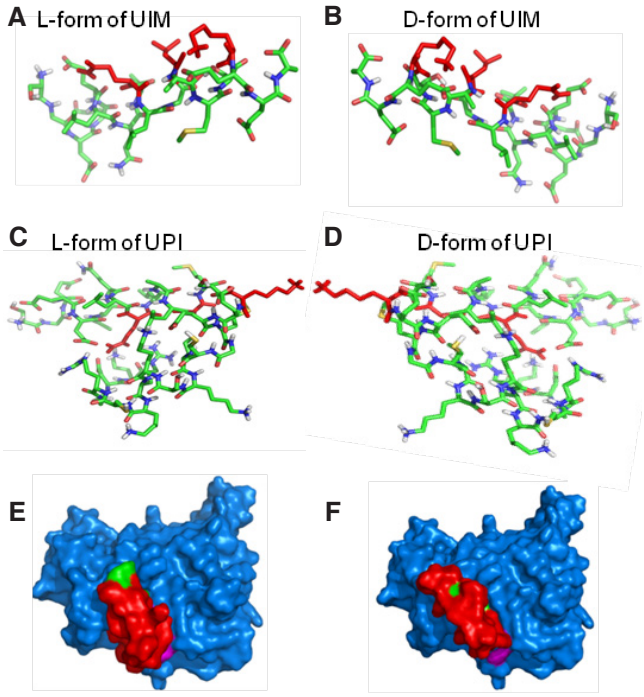

Figure 7: Molecular modeling to compare the interaction between D-amino acids and L-amino acids of UIM or UPI with VEGFR2 kinase domain. The 3D models of L- and D-amino acids UIM and L- and D-amino acids UPI were predicted by the PEP-FOLD program (L-form and D-form). Stick representations of the L-form of UIM (A) and D-form of UIM (B) form mirror-images of the actual structures. In the same manner, the L-form of UPI (C) and D-form of UPI (D) form mirror-images of the actual structures. (E) Surface representation of the L-form of UPI (UIM, red; anchoring peptide and iRGD, green) interacting with VEGFR2-KD (blue). (F) In the same manner as the L-form of UPI interacts with VEGFR2-KD, surface representation shows the D-form of UPI (UIM, red; anchoring peptide and iRGD, green) binds to the same binding pocket of VEGFR2 (blue)

Therapeutic efficacy of UPI peptide in animal cancer models: role of UPI in tumor angiogenesis and metastasis

UPI peptide administration can drastically inhibit tumor growth and metastasis in animal models of LLC, B16-F10, glioma brain tumor, and Tramp prostate.[19] In GL261 brain tumor models, the UPI peptide can obtain a similar therapeutic efficacy and survival rate to anti-VEGF antibodies.[19] More importantly, in the human U87 glioma tumor model (an immune deficient mouse model), we demonstrated that UPI peptide treatment can significantly retard tumor growth and increase the survival rate, accompanied by dysregulated VEGFR2 signaling and tumor angiogenesis.[19] Mechanistically, the UPI peptide treatment generates hyper leakage vessels via upregulated VEGFR2 signaling [Figure 8] and impairs metastasis in the prostate and B-16 melanoma animal models likely due to dysfunctional tumor angiogenesis.[19]

UPI peptide targeting specificity

We tested the UPI peptide specificity *in vitro* and *in vivo*. In cultured human umbilical vein endothelial cells (HUVECs), the UPI peptide treatment had no effect on other angiogenic growth factor signaling such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), and transforming growth factor beta (TGFβ) pathways.[19] We previously obtained similar results in UPI-treated mouse tumors.[19] This targeting specificity may be interpreted from the length of the UIM. It has been suggested that the core components of UIMs contain 18 amino acid (aa) residues including a core Ø-XX-Ala-XXX-Ser-XX-Ac (Ø: a large hydrophobic residue; Ac: an acidic residue), which likely form a short helix embedded into different protein folds.[33] Ahead of this core sequence, a short 10-aa peptide may create specificity to its binding partners. Secondly, the 3D structure of epsin-UIM may fit the binding to VEGFR2 pockets more suitably than other angiogenic receptor molecules. Third, tumor cells in general secrete more VEGF than other angiogenic substances so that VEGFR2 may have more chances to be activated and ubiquitylated. Therefore, the epsin-UPI peptide may predominantly modulate VEGFR2 signaling rather than other angiogenic receptors and signaling. This conclusion has been further confirmed by *in vitro* and *in vivo* experiments, as well as by the specific designed peptide binding assay using isolated tumor ECs.[19] Interestingly, the Hrs-UIM or Eps15-UIM peptide does not show promising therapeutic efficacy in animal tumor models,[19] further suggesting the specificity of the epsin-UIM peptide to VEGFR2 KD binding.

BIOSAFETY OF UPI PEPTIDE ADMINISTRATION

We have measured the main metabolic parameters after UPI peptide administration for 3 months (10-50 mg/kg, twice a week by i.v. injection) in mice, and our results showed that UPI peptide injection had minimal side effects (data not shown). Glucose and lipid metabolism remained normal, likely owing to a relatively lower dosage used in the homing strategy. Histology and immunofluorescent staining revealed that the UPI peptide targeted to other tissues was neglected. However, whether the UPI peptide causes drug resistance in the long term warrants further investigation.

PERSPECTIVE

Serving as a potential candidate for cancer therapeutics, the UPI peptide requires more in-depth research in commonly associated cancer models such as prostate and glioma tumors [Figure 9]. Our data have shown that the UPI peptide can efficiently attenuate prostate cancer progression in Tramp mouse models and glioma mouse models [Figure 9A and B].[19] Mechanistically,

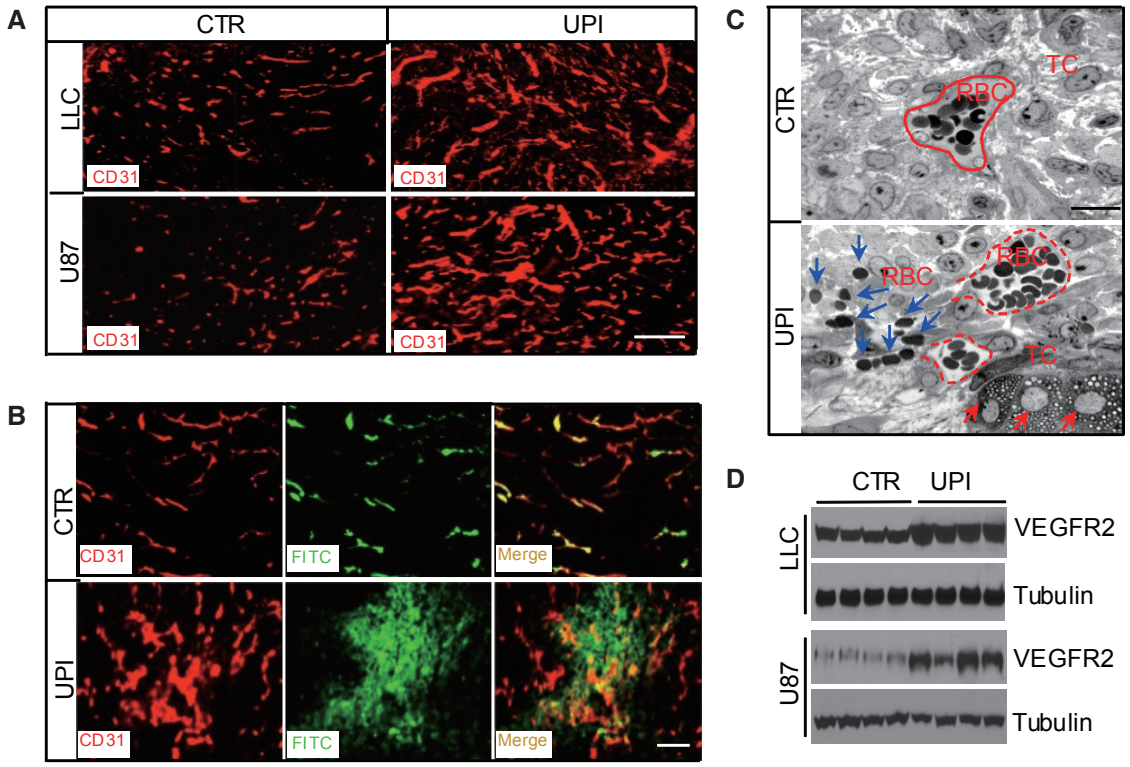

Figure 8: UPI peptide treatment produces upregulated but leaky vessels in tumors. (A) CD31 immunofluorescent staining for LLC or U87 tumors, showing the upregulated vessels in UPI-treated tumors. Scale bar: 100 μm; (B) Control or UPI peptide-treated s.c. U87 tumor-bearing mice were perfused with FITC-dextran for 10 min, following which the mice were killed. Tumors were fixed and processed for CD31 staining. Note that vessels in UPI-treated tumors were hyper leaky. Scale bar: 100 μm. (C) Transmission electron microscopy analysis of semi-thin sections from control and UPI peptide-treated s.c. implanted U87 tumors. Dotted red lines indicate tumor vessels; blue arrows depict red blood cell leakage from tumor vessels; and red arrows indicate dying tumor cells. Scale bar: 50 μm. (D) VEGFR2 expression in control or UPI peptide-treated s.c. LLC and U87 tumors. Figure 8 is adapted with permission from ref. 19. Copyright 2015 ASCI

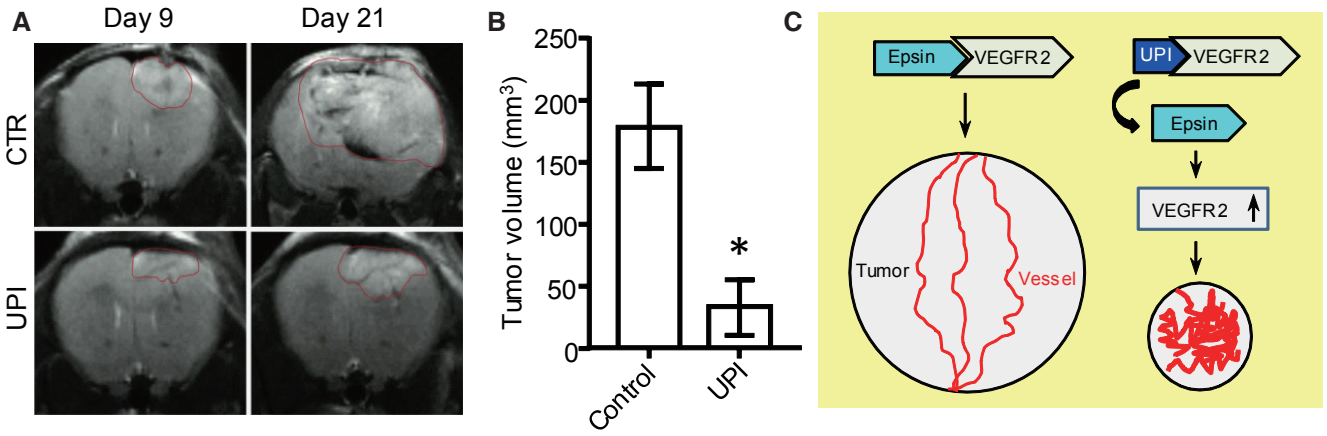

Figure 9: UPI peptide treatment significantly retards tumor growth in glioma tumor models. GL261 glioma cells (2×10^4) were implanted to the right forebrain of C57BL/6 mice. At day 9, UPI peptide was administered by intravenous injection at 20 mg/kg dosage every alternate day. Gliomas were monitored via magnetic resonance imaging (MRI). (A) Representative MRI images. (B) Statistical analysis of tumor volume of terminal mice treated by control or UPI peptide; $n = 5$ in each group, Student *t*-test, $^*P < 0.001$ *vs.* control. (C) Sketch of the UPI peptide therapeutic mechanism. UPI administration inhibits Epsin-VEGFR2 interaction *in vivo*, promotes non-functional tumor angiogenesis, and retards tumor growth

UPI peptide inhibits Epsin-VEGFR2 interaction *in vivo* and produces non-functional tumor angiogenesis [Figure 9C]. Specific targeting and therapeutic efficacy can be further improved by modifying the peptides, where clinical trials would then require further follow-up assessments. Because epsins are ubiquitously expressed proteins, it is reasonable to assume that epsins may modulate a wide array of cellular processes including cell development, differentiation, proliferation, migration, and genetics. Targeting epsins in different disease models, as well as the emergence of new technologies such as nanoparticles,[34] liposomes,[35,36]

and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9) genome editing[37-39] may also provide new directions to develop novel therapeutic agents.

CONCLUSION

The UPI peptide is a promising compound to treat cancers. The UPI peptide can efficiently inhibit tumor growth and metastasis and specifically targets VEGFR2 signaling to create upregulated, nonfunctional tumor vessels. It is expected that the peptide may be applicable to treat cancer patients as a first or second line compound; or as an alternative replacement to the anti-VEGF antibody in patients who are resistant to anti-angiogenic therapies.

Acknowledgments

We thank Dr. Pierre Tuffery in the Institut National de la Santé et de la Recherche Médicale (INSERM) for his help in performing the molecular modeling of D- and L-amino acids for UIM or UPI peptide docking. Scott Wong helped us to edit the manuscript.

Financial support and sponsorship

This work was supported in part by National Institutes of Health (NIH) grants R01HL-093242, R01 HL118676, R01HL-130845, P20 RR018758; an AHA Established Investigator Award; a National Scientific Development Grant from the American Heart Association (0835544N); and a grant from the Department of Defense W81XWH-11-1-00226 to author HC; by OCAST grants AR11-043, HR14-056; and American Heart Association AHA-SDG grant 12SDG8760002 to author YD. Author JD is a recipient of the Fleming Scholar Award from the Oklahoma Medical Research Foundation.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;285:1182-6.
2. Cao Y, Langer R. A review of Judah Folkman's remarkable achievements in biomedicine. *Proc Natl Acad Sci U S A* 2008;105:13203-5.
3. Gaur P, Bose D, Samuel S, Ellis LM. Targeting tumor angiogenesis. *Semin Oncol* 2009;36:S12-9.
4. Blázquez C, Casanova ML, Planas A, Gómez Del Pulgar T, Villanueva C, Fernández-Aceñero MJ, Aragonés J, Huffman JW, Jorcano JL, Guzmán M. Inhibition of tumor angiogenesis by cannabinoids. *FASEB J* 2003;17:529-31.
5. Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. *Nature* 2005;438:967-74.
6. Carmeliet P. Angiogenesis in health and disease. *Nat Med* 2003;9:653-60.
7. Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nat Rev Mol Cell Biol* 2007;8:464-78.
8. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature* 2011;473:298-307.
9. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. *Nat Clin Pract Oncol* 2006;3:24-40.
10. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249-57.
11. Noguera-Troise I, Daly C, Papadopoulos NJ, Coetzee S, Boland P, Gale NW, Lin HC, Yancopoulos GD, Thurston G. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Nature* 2006;444:1032-7.
12. Ridgway J, Zhang G, Wu Y, Stawicki S, Liang WC, Chanthery Y, Kowalski J, Watts RJ, Callahan C, Kasman I, Singh M, Chien M, Tan C, Hongo JA, de Sauvage F, Plowman G, Yan M. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature* 2006;444:1083-7.
13. Niethammer AG, Xiang R, Becker JC, Wodrich H, Pertl U, Karsten G, Eliceiri BP, Reisfeld RA. A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat Med* 2002;8:1369-75.
14. Chen H, Fre S, Slepnev VI, Capua MR, Takei K, Butler MH, Di Fiore PP, De Camilli P. Epsin is an EH-domain-binding protein implicated in clathrin-mediated endocytosis. *Nature* 1998;394:793-7.
15. Tessneer KL, Cai X, Pasula S, Dong Y, Liu X, Chang B, McManus J, Hahn S, Yu L, Chen H. Epsin family of endocytic adaptor proteins as oncogenic regulators of cancer progression. *J Can Res Updates* 2013;2:144-50.
16. Chen H, Ko G, Zatti A, Di Giacomo G, Liu L, Raiteri E, Perucco E, Collesi C, Min W, Zeiss C, De Camilli P, Cremona O. Embryonic arrest at midgestation and disruption of Notch signaling produced by the absence of both epsin 1 and epsin 2 in mice. *Proc Natl Acad Sci U S A* 2009;106:13838-43.
17. Rahman HN, Wu H, Dong Y, Pasula S, Wen A, Sun Y, Brophy ML, Tessneer KL, Cai X, McManus J, Chang B, Kwak S, Rahman NS, Xu W, Fernandes C, McDaniel JM, Xia L, Smith L, Srinivasan RS, Chen H. Selective targeting of a novel epsin-VEGFR2 interaction promotes VEGF-mediated angiogenesis. *Circ Res* 2016;118:957-69.
18. Liu X, Pasula S, Song H, Tessneer KL, Dong Y, Hahn S, Yago T, Brophy ML, Chang B, Cai X, Wu H, McManus J, Ichise H, Georgescu C, Wren JD, Griffin C, Xia L, Srinivasan RS, Chen H. Temporal and spatial regulation of epsin abundance and VEGFR3 signaling are required for lymphatic valve formation and function. *Sci Signal* 2014;7:ra97.
19. Dong Y, Wu H, Rahman HN, Liu Y, Pasula S, Tessneer KL, Cai X, Liu X, Chang B, McManus J, Hahn S, Dong J, Brophy ML, Yu L, Song K, Silasi-Mansat R, Saunders D, Njoku C, Song H, Mehta-D'Souza P, Towner R, Lupu F, McEver RP, Xia L, Boerboom D, Srinivasan RS, Chen H. Motif mimetic of epsin perturbs tumor growth and metastasis. *J Clin Invest* 2015;125:4349-64.
20. Pasula S, Cai X, Dong Y, Messa M, McManus J, Chang B, Liu X, Zhu H, Mansat RS, Yoon SJ, Hahn S, Keeling J, Saunders D, Ko G, Knight J, Newton G, Luscinskas F, Sun X, Towner R, Lupu F,

Xia L, Cremona O, De Camilli P, Min W, Chen H. Endothelial epsin deficiency decreases tumor growth by enhancing VEGF signaling. *J Clin Invest* 2012;122:4424-38.
21. Chang B, Tessneer KL, McManus J, Liu X, Hahn S, Pasula S, Wu H, Song H, Chen Y, Cai X, Dong Y, Brophy ML, Rahman R, Ma JX, Xia L, Chen H. Epsin is required for Dishevelled stability and Wnt signalling activation in colon cancer development. *Nat Commun* 2015;6:6380.
22. Tessneer KL, Pasula S, Cai X, Dong Y, McManus J, Liu X, Yu L, Hahn S, Chang B, Chen Y, Griffin C, Xia L, Adams RH, Chen H. Genetic reduction of vascular endothelial growth factor receptor 2 rescues aberrant angiogenesis caused by epsin deficiency. *Arterioscler Thromb Vasc Biol* 2014;34:331-7.
23. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, Hanahan D, Mattrey RF, Ruoslahti E. Tissue-penetrating delivery of compounds and nanoparticles into tumors. *Cancer Cell* 2009;16:510-20.
24. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Greenwald DR, Ruoslahti E. Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs. *Science* 2010;328:1031-5.
25. Weis SM, Cheresh DA. αV integrins in angiogenesis and cancer. *Cold Spring Harb Perspect Med* 2011;1:a006478.
26. Kovárová M, Tolar P, Arudchandran R, Dráberová L, Rivera J, Dráber P. Structure-function analysis of Lyn kinase association with lipid rafts and initiation of early signaling events after Fcepsilon receptor I aggregation. *Mol Cell Biol* 2001;21:8318-28.
27. Chen H, De Camilli P. The association of epsin with ubiquitinated cargo along the endocytic pathway is negatively regulated by its interaction with clathrin. *Proc Natl Acad Sci U S A* 2005;102:2766-71.
28. Kurosaki T. Regulation of B-cell signal transduction by adaptor proteins. *Nat Rev Immunol* 2002;2:354-63.
29. Solution structure of Vps27 UIM-ubiquitin complex important for endosomal sorting and receptor downregulation. *EMBO J* 2003;22:4597-606.
30. Bogdanowich-Knipp SJ, Jois DS, Siahaan TJ. The effect of conformation on the solution stability of linear vs. cyclic RGD peptides. *J Pept Res* 1999;53:523-9.
31. Liu S. Radiolabeled cyclic RGD peptides as integrin alpha(v)beta(3)-targeted radiotracers: maximizing binding affinity via bivalency. *Bioconjug Chem* 2009; 20:2199-213.
32. Roxin Á, Zheng G. Flexible or fixed: a comparative review of linear and cyclic cancer-targeting peptides. *Future Med Chem* 2012;4:1601-18.
33. Hofmann K, Falquet L. A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal protein degradation systems. *Trends Biochem Sci* 2001;26:347-50.
34. Dahlman JE, Barnes C, Khan OF, Thiriot A, Jhunjunwala S, Shaw TE, Xing Y, Sager HB, Sahay G, Speciner L, Bader A, Bogorad RL, Yin H, Racie T, Dong Y, Jiang S, Seedorf D, Dave A, Singh Sandhu K, Webber MJ, Novobrantseva T, Ruda VM, Lytton-Jean AK, Levins CG, Kalish B, Mudge DK, Perez M, Abezgauz L, Dutta P, Smith L, Charisse K, Kieran MW, Fitzgerald K, Nahrendorf M, Danino D, Tuder RM, von Andrian UH, Akinc A, Panigrahy D, Schroeder A, Koteliansky V, Langer R, Anderson DG. In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. *Nat Nanotechnol* 2014;9:648-55.
35. Guo P, Yang J, Jia D, Moses MA, Auguste DT. ICAM-1-targeted, Lcn2 siRNA-encapsulating liposomes are potent anti-angiogenic agents for triple negative breast cancer. *Theranostics* 2016;6:1-13.
36. Guo P, You JO, Yang J, Jia D, Moses MA, Auguste DT. Inhibiting metastatic breast cancer cell migration via the synergy of targeted, pH-triggered siRNA delivery and chemokine axis blockade. *Mol Pharm* 2014;11:755-65.
37. Cong L, Zhang F. Genome engineering using CRISPR-Cas9 system. *Methods Mol Biol* 2015;1239:197-217.
38. Heckl D, Charpentier E. Toward whole-transcriptome editing with CRISPR-Cas9. *Mol Cell* 2015;58:560-2.
39. Sternberg SH, Doudna JA. Expanding the biologist's toolkit with CRISPR-Cas9. *Mol Cell* 2015;58:568-74.

Schiavone *et al. Vessel Plus* 2017;1:12-21
DOI: 10.20517/2574-1209.2016.03

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Original Article **Open Access**

Crimping and deployment of metallic and polymeric stents -- finite element modelling

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How to cite this article: Schiavone A, Qiu TY, Zhao LG. Crimping and deployment of metallic and polymeric stents -- finite element modelling. Vessel Plus 2017;1:12-21.

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Article history:
Received: 19-10-2016
Accepted: 03-01-2017
Published: 31-03-2017

Key words:
Polymeric stents,
metallic stents,
finite element,
crimping,
deployment

ABSTRACT

Aim: This paper aims to compare the mechanical performance of metallic (Xience) and bioresorbable polymeric (Elixir) stents during the process of crimping and deployment. **Methods:** Finite element software ABAQUS was used to create the geometrical models and meshes for the balloon, stent and diseased artery. To simulate the crimping of stents, 12 rigid plates were generated around the stent and subjected to radially enforced displacement. The deployment of both stents was simulated by applying internal pressure to the balloon, where hard contacts were defined between balloon, stent and diseased artery. **Results:** Elixir stent exhibited a lower expansion rate than Xience stent during deployment. The stent diameter achieved after balloon deflation was found smaller for Elixir stent due to higher recoiling. Lower level of stresses was found in the plaque and artery when expanded by Elixir stent. Reduced expansion, increased dogboning and decreased vessel stresses were obtained when considering the crimping-generated residual stresses in the simulations. **Conclusion:** There is a challenge for polymeric stents to match the mechanical performance of metallic stents. However, polymeric stents impose lower stresses to the artery system due to less property mismatch between polymers and arterial tissues, which could be clinically beneficial.

INTRODUCTION

Over the past three decades, significant improvements have been made in stent designs and materials, especially the development of drug-eluting stents (DESs, approved by Food and Drug Administration (FDA) of USA in 2002). The vast majority of DESs used so far have non-degradable polymer coatings,

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incorporated with anti-proliferative drugs, to overcome the notorious in-stent restenosis (ISR) that represented an undesired drawback of bare metal stents.[1] Although ISR rates can be substantially reduced by DESs (lowered by 74% in high-risk patients), the life-long presence of metallic alloy stents, under loads and corrosive conditions, could increase the risk of strut fracture and failure with time. This may lead to possible complications such as delayed healing, lethal migration and unstable angina.[2,3] Bioresorbable stents provide the advance of overcoming these possible long-term complications of metallic stents. Biodegradable polymers are the most promising materials investigated to date.[4-6] Fully expandable polymeric biodegradable stents initially support the vessel wall as scaffolds with sufficient radial strength to prevent mechanic recoil after implantation. In about 6 months, arterial remodelling enters a stable phase, and no substantial scaffolding is required. Consequently, it is desirable for stents to dissolve after 6 months in the body, leaving behind an intact vessel with no pro-inflammatory substances or obstacles for future treatments.[7] During the process, the polymers gradually soften, which allows for a smooth disappearance of high stresses, imposed by the permanent stents, in the recovering artery. It should be noted that recent reports have suggested an independent relationship between bioresorbable stents and stent thrombosis.[8] Instead, the higher incidence rates of stent thrombosis were associated with the 2.5 mm platforms.[9]

One of the major concerns for polymeric BRSs is their mechanical performance, especially their interaction with blood vessels during and after deployment. Agrawal *et al*.[10] carried out the earliest work in assessing the *in vitro* performance of the Duke biodegradable stents made of Poly-L lactide (PLLA) fibres. Results showed that a successful biodegradable stent could be achieved by carefully balancing the mechanical properties of PLLA fibres and geometrical design of stents. Nuutinen *et al*.[11] conducted *in vitro* tests of a woven fibre polymeric braided stent subjected to radial compression. The performance of such stents was not as good as metal ones, and the collapse pressure was found lower, even for thicker PLLA fibres. Bünger *et al*.[12] compared the *in vivo* performance of stents made of biodegradable PLLA and stainless steel, by monitoring the stents after the implantation in carotid arteries of living pigs. Both stents achieved initial technical success. However, PLLA was reported to be significantly softer than metallic alloys, which affected the radial stiffness of the implanted stents and needed to be further investigated. Over the last two decades, great effort has been made for the development of bioresorbable stents, and there have been many devices available in preclinical and clinical evaluations. Elixir DESolve (Elixir Medical Corporation, USA) stent, made of PLLA, is one of the major biodegradable stents currently available on the market. The polymer stents showed sufficient radial strength within 6 months of implantation, with the occurrence of bioresorption between one and two years.[13]

Finite element (FE) analyses have been largely used in modelling of stent expansion and deformation during the deployment process.[14-16] However, majority FE analyses were performed on metallic stents. For example, Imani *et al*.[17] modelled the effects of design on vessel wall stresses for Palmaz-Schatz, Xience V and NIR stents. The comparative study confirmed that Palmaz-Schatz stent generated 15.6% and 7.6% higher stresses in the arterial wall than Xience V stent and NIR stent, respectively. The results suggested a direct correlation between vessel wall stresses and in-stent restenosis rate, as Palmaz-Schatz stent gave the highest restenosis rate in clinical trials. Recent FE simulations on metallic stents by Schiavone *et al*.[18] also confirmed that stent design is a major factor that controls the expansion of the device and also the stresses generated in the artery. In addition, shape optimization of metallic stents, in terms of improved fatigue resistance and radial flexibility, can also be achieved by FE modelling.[19] On the contrary, there is very limited work dedicated to modelling the deformation of bioresorbable polymer stents, particularly during the process of deployment in diseased arteries.

In this paper, FE modelling was carried out to simulate the deployment of Elixir polymer stent in a diseased artery, with head-to-head comparison against Xience metallic stent. Particularly, the simulations considered the crimping step, which is necessary to fix the as-produced stent to the catheter. The mechanical performance of the two stents has been compared directly, in terms of radial expansion and stresses in the stent-vessel system. In addition, results were also compared with those obtained without considering the residual stresses generated from the crimping process.

METHODS

Models for stents, balloon and artery

Geometries for Xience and Elixir stents were built using the NX design software. Both stents were created in expanded shape, with a diameter of 3 mm and a length of 10 mm. The geometries of both stents were based on dimensions found in open resource. The strut thickness is 80 μm and 150 μm for Xience stent and Elixir stent, respectively. The FE mesh contains eight-

node incompatible brick elements, with full integration (C3D8I). The incompatible mode is chosen for the purpose of modelling large bending deformation during stent crimping and subsequent expansion. There are 4-layer elements through the width and the thickness of all struts. The geometrical designs and FE meshes for both stents are given in Figure 1.

The balloon used to inflate the stents had a tri-folded geometry which was produced using the NX software. The diameter of the fully folded part is 1.25 mm and the total length of the balloon is 14 mm. To create the pattern, the tri-folded cross section was sketched first, and subsequently extruded for a length of 12 mm. Towards the ends, the balloon smoothly transits into a circle, 0.75 mm in diameter, over a length of 1 mm. This was done by using the sweeping tools in NX. The balloon was totally constrained at both ends as they are fixed to a catheter. The diameter of the expanded balloon was set to be 3 mm, matching the targeted stent or vessel diameter after deployment. Four-node shell elements, with reduced integration (S4R), were adopted to mesh the tri-folded balloon.

The diseased artery has a total length of 40 mm and a lumen diameter of 3 mm for the heathy part. The middle portion of the artery is covered by 10 mm plaque. The stenosis, defined as the ratio of plaque thickness to healthy lumen radius, was chosen to be 50%. The artery comprises three individual tissue layers, and the wall thickness is 0.27 mm, 0.35 mm and 0.38 mm for the intima, media and adventitia layers, respectively. Eight-node brick elements with reduced integration (C3D8R) were used to mesh the artery and the plaque. Four layers of elements were assigned through the wall of each tissue layer and eight layers of elements were assigned through the plaque thickness.

Geometry and mesh of the balloon-artery assembly are shown in Figure 2. Mesh-sensitivity studies confirmed the convergence of numerical results, with regards to stent expansion, stent recoiling and stresses in the stent-artery system, for the mesh adopted in this work.

Material model

Both stents were modelled as elastic-plastic, with non-linear strain hardening [Figure 3]. The tensile stress-strain curves for Co-Cr L605 and PLLA were taken from literature.[20,21] It can be noted that PLLA is much weaker than Co-Cr L605. Table 1 gives the essential properties for both materials as obtained from the tensile curves. Strain hardening was realised in ABAQUS by stating the yield stress as a function of the plastic strain, as also obtained from the tensile curves. The plaque was assumed to be hypocellular, and its behaviour was described by the Ogden hyperelastic model. The hyperelastic model parameters were provided in Zahedmanesh and Lally.[22] The tri-folded balloon was treated as a linear elastic material. The material density, Young's modulus and Poisson's ratio were taken as 1.1×10^6 kg/mm^3, 900 MPa and 0.3, respectively.[23]

It is well recognised that the arterial layers possess distinct anisotropic behaviour as they are reinforced by two families of collagen fibres. Here, the established Holzapfel-Gasser-Ogden (HGO) anisotropic hyperelastic model[24] was employed to describe the anisotropic behaviour of individual coronary arterial layer. In this model, the hyperelastic strain energy potential W is given by:[24]

$$W = C_{10}(I_1 - 3) + (k_1/2k_2)[\exp(k_2\langle E_f\rangle^2) - 1] + (1/D)[(J^2-1)/2 - \ln J]$$

$$E_f = \kappa(I_1 - 3) + (1 - 3\kappa)(I_4 - 1)$$

where C_{10}, D, k_1, k_2 and κ are model parameters, I_1 and J are the first and third stretch invariants, and I_4 is the invariant of Cauchy-Green deformation tensor. The Macauley bracket is indicated by the operator <>, whilst γ represents the angle between the mean directions of the two families of fibres whose deformation is defined by E_f. The model parameters [Table 2] were calibrated against the experimental data.[25] Both longitudinal and circumferential stress-stretch responses, computed by using the HGO model, agreed with the experimental data very well[25] for all three vessel layers [Figure 4].

The HGO model used in this work is for incompressible hyperelastic materials. To consider compressible deformation, the HGO-C model was suggested, with the anisotropic part expressed by isochoric invariants

Table 1: Properties for the Co-Cr L605 and Poly-L lactide stent materials[20,21]

Material	ρ (kg/mm^3)	E (GPa)	ν	σy (MPa)
Co-Cr L605	9.1E-6	243	0.30	476
Poly-L lactide	1.4E-6	2.2	0.30	60

Table 2: Parameter values of the anisotropic Holzapfel-Gasser-Ogden model for the arterial layers

Layer	ρ (kg/mm^3)	C_{10}	D	k_1	k_2	κ	γ
Intima	1.066E-6	0.03	8.95E-7	4.0	1200.0	0.303	60°
Media	1.066E-6	0.005	5.31E-6	0.57	80.0	0.313	20°
Adventitia	1.066E-6	8.32E-3	4.67E-6	1.0	1000.0	0.303	67°

(insensitive to volumetric deformation). However, Nolan *et al*.[26] found that the HGO-C model was unable to simulate compressible anisotropic behavior correctly, because it used isochoric anisotropic invariants which is insensitive to volumetric deformation. Consequently, they formulated a modified anisotropic (MA) model by using the full anisotropic invariants which accounted for a volumetric anisotropic contribution. The MA model correctly predicted the material's anisotropic response to hydrostatic tensile loading, pure shear and uniaxial deformations. They also found the HGO-C model significantly underpredicted arterial compliance, which might affect the simulation results of stent deployment in diseased arteries. To fully clarify this effect, a considerable amount of new work is required, especially the efforts required for coding a user-defined material subroutine for the MA model (interface with the FE package Abaqus). This is beyond the scope of this paper, and will be investigated in our future studies.

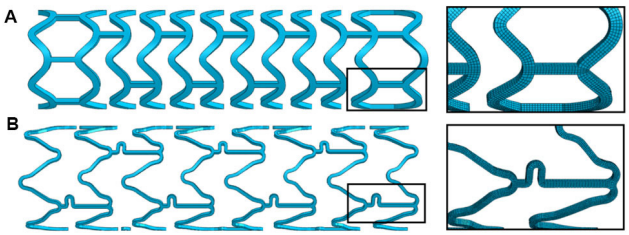

Figure 1: Geometry and mesh for (A) Elixir and (B) Xience stents

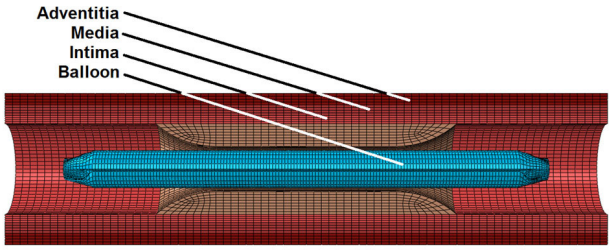

Figure 2: Geometry and mesh of the artery with stenosis and angioplasty balloon

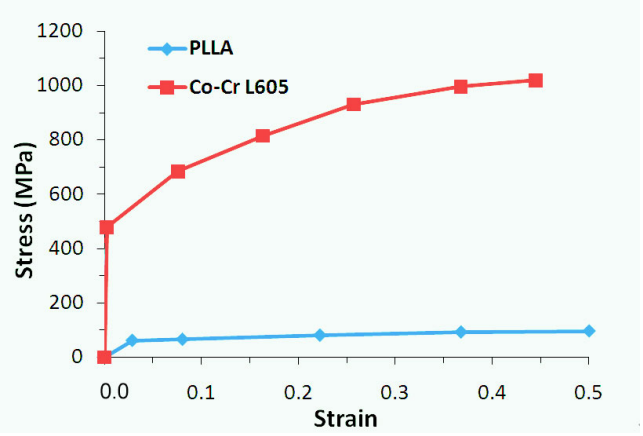

Figure 3: Stress-strain curves for the Co-Cr L605 alloy and Poly-L lactide[20,21]

Nevertheless, the HGO model used in this work was calibrated properly against the longitudinal and circumferential tensile data of arteries.[25] It was proved to be a reliable anisotropic formulation for modeling deformation of the arterial layers with collagen fibre reinforcement.

Crimping simulation

To model stent crimping, twelve rigid plates were generated around the stent as shown in Figure 5. The rigid plates were modelled as shell surfaces. Uniform radial displacement, linearly increasing to 1 mm within a step time of 0.1 s, was applied to the plates to enforce the crimping of stent. Spring back of the stent after crimping, due to the recovery of elastic deformation, resulted in a final stent diameter of 1.5 mm, which is able to fit in the diseased vessel.

Abaqus explicit was adopted for crimping simulations (0.1 s step time). No constraint was applied to the stent. Hard contact was assigned between the outer surface of the stent and the rigid plates. The friction coefficient was assumed to be 0.8. Following crimping, an additional step was used to simulate the spring back of the stent within 0.1 s. In this step, the contacts between the rigid plates and the stent were deleted, allowing for the stent to recover the elastic deformation freely.

Expansion simulation

The expansion procedure was simulated using two steps, namely inflation and deflation. During the inflation step (0.1 s), a pressure linearly increasing to 1.2 MPa was applied inside the balloon. While in the deflation step (0.1 s), the balloon pressure was brought linearly down to zero. All analyses were carried out by considering the residual stresses generated from crimping. Again, simulations were performed using the explicit solver in Abaqus. Change of stent outer diameter was tracked for the middle ring and the two end rings of both stents. The data outputs were used to quantify stent expansion as well as the recoiling and dogboning effects.[27]

RESULTS

Stent crimping

During crimping, both stents underwent severe bending deformation, as illustrated in Figure 6 for Elixir stent. The two stents were squashed to a diameter of only 1.25 mm at the fully crimped state. After crimping, stresses were highly localised at the U-bend regions for both devices. The von Mises stress in the Xience and the Elixir stents had a maximum magnitude of 750 MPa [Figure 7A right] and 96 MPa [Figure 7A left], respectively. After spring back, the maximum von

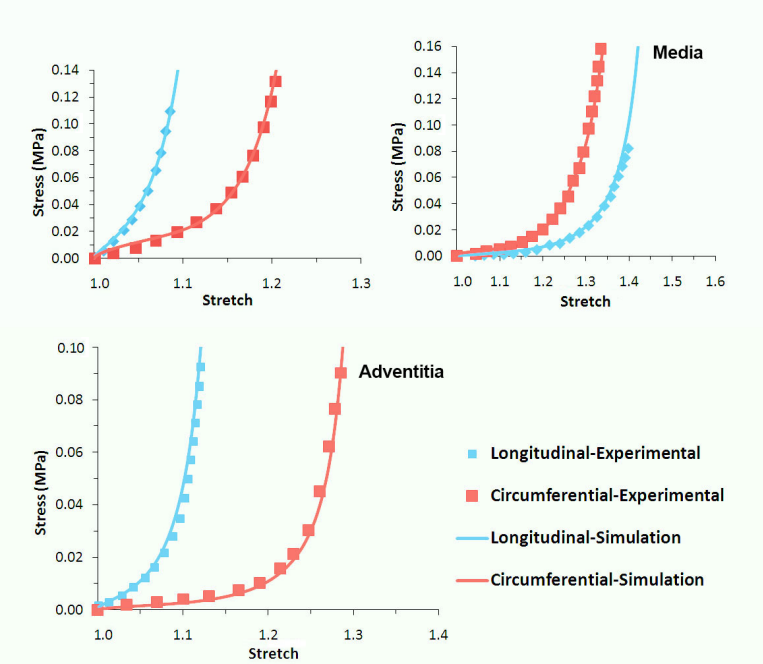

Figure 4: Stress-stretch curves for the three artery layers: Holzapfel-Gasser-Ogden model *vs.* experimental data[25]

Mises stress in the Xience and Elixir stents relaxed to 706 MPa and 50 MPa, respectively [Figure 7B]. These are also the residual stresses generated in the stent after crimping. And the diameter of both stents settled at about 1.5 mm.

Stent expansion

Pressure-diameter plots in Figure 8 showed that Elixir stent experienced a lower rate of expansion than Xience stent. Both stents developed a saturation state in expansion with the increasing pressure, but Elixir stent reached the saturation earlier. At the maximum pressure, the stent outer diameter was computed as 2.66 mm and 2.61 mm for the Xience and Elixir stents, respectively. The recoiling effect was 11% for the Xience stent [Figure 8B], which is significantly lower than that for the Elixir stent (20%; Figure 8B). Consequently, a larger final diameter was achieved for the Xience stent (2.40 mm) when compared to the Elixir stent (2.10 mm only). Consistently, the Xience stent also showed considerably less dogboning effect (24%) than the Elixir stent (45%).

High levels of stresses were developed in both stents following their deployment in the artery [Figure 9]. Similar to the crimping process, the maximum von Mises stresses were found in the U-bend districts, with a value of 935 MPa for the Xience and 95 MPa for the Elixir stent. In the artery, the peak stresses were generally found on the plaque, especially towards the ends of the stenosis. This was caused by the dogboning effect of the implanted stent. There is a direct connection between stress concentration and localized rupture for plaque as evidenced in numerous studies.[28,29] In our case, plaque rupture is likely to happen towards the ends of plaque region, a condition that can lead to heart attack (coronary artery) or stroke (carotid artery). In addition, there could be

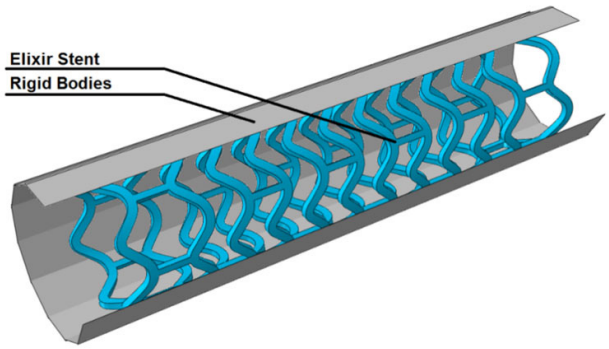

Figure 5: Illustration of assembly used in crimping simulation for Elixir stent

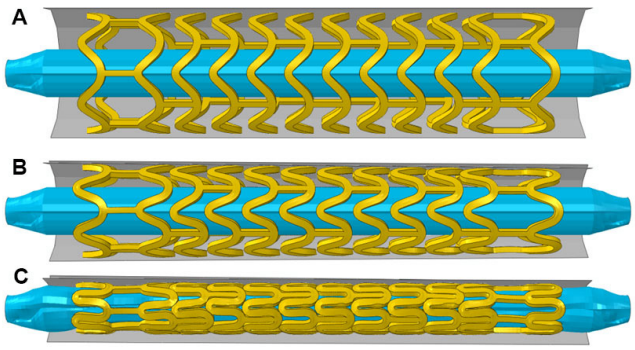

Figure 6: Deformation of Elixir stent and balloon (A) before, (B) during and (C) at the end of the crimping process

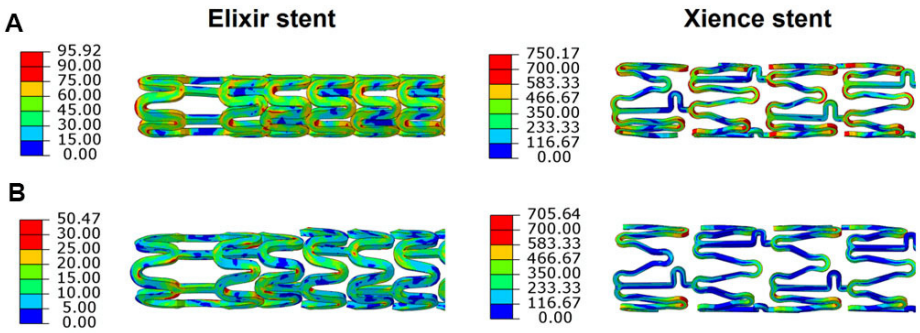

Figure 7: Von Mises stress (MPa) contour plot for the Elixir (left) and Xience (right) stents in (A) fully crimped state and (B) spring-back state

local damage to plaque/artery where the high stresses are located. This condition also leads to the growth of new tissue, e.g. proliferation of smooth muscle cells, around the stent, causing in-stent restenosis.

The maximum principal stress on the plaque had a peak value of 1.43 MPa and 0.54 MPa for the Xience and Elixir stents, respectively [Figure 10]. The considerably lower stress level for Elixir stent is consistent with the reduced property mismatch between the polymer and the artery as well as less arterial expansion achieved by the polymer stent. It should be noted that stress triaxiality was not explored for the stents and diseased artery. Stress triaxiality is defined as the ratio of hydrostatic stress to the equivalent (or von Mises) stress, and it is a key parameter used in ductile fracture analysis. In this study, we mainly focus on the stress state of stent-artery system during crimping and expansion, instead of fracture or failure. Consequently, stress triaxiality is not presented. But we aim to look into this in future studies when considering the failure and fracture of stent, plaque and arteries in the simulations.

Effect of residual stresses caused by crimping

As shown in Figure 7, crimping introduced severe residual stresses into the stent, which can affect the subsequent expansion of the device. To understand such effect, simulations of stent deployment were also carried out for both stents without including the residual stresses generated from crimping.

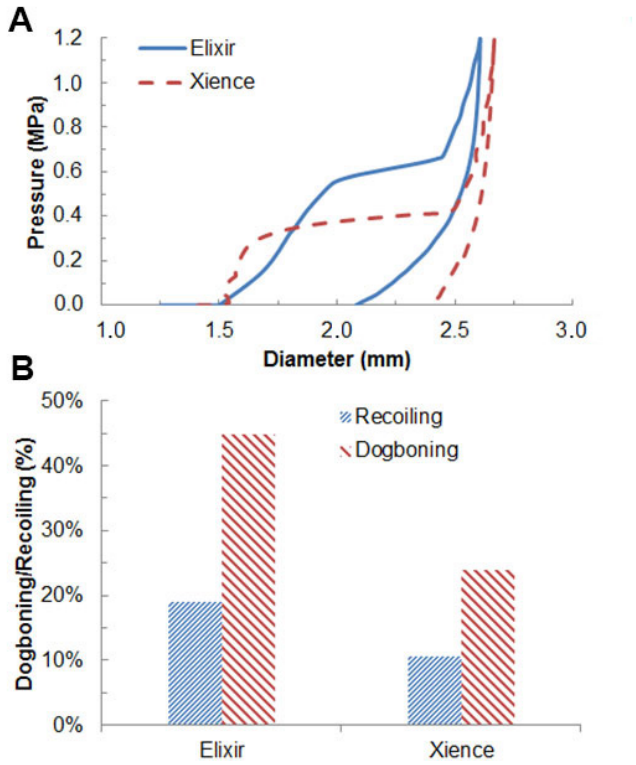

Figure 8: (A) Diameter change against pressure; (B) recoiling and dogboning effects during deployment of Elixir and Xience stents

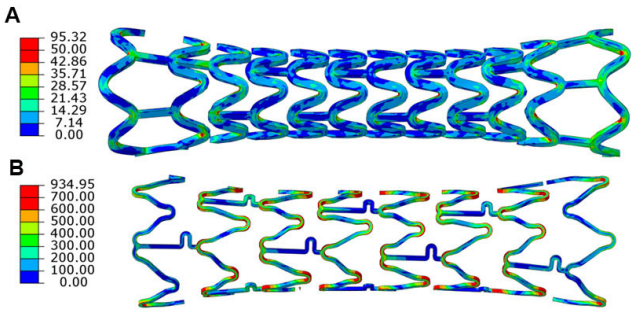

Figure 9: Contour plot of the von Mises stress (MPa) on (A) Elixir and (B) Xience stents after deployment

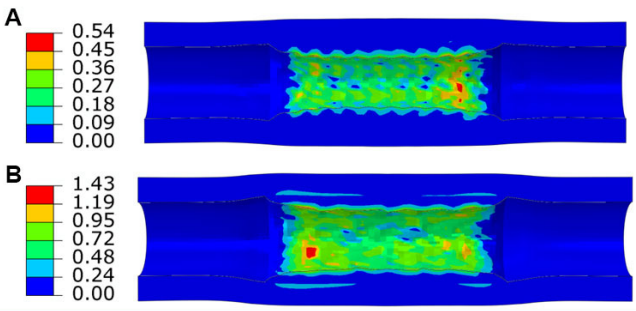

Figure 10: Contour plot of maximum principal stress (MPa) on the stenotic plaque after stent deployment: (A) Elixir and (B) Xience stents

For Elixir stent, it is confirmed that crimping-caused residual stresses do affect stent expansion [Figure 11]. When stent expansion was simulated without considering residual stress, the stent expanded faster and reached saturation earlier, as shown by the solid blue line in Figure 11A. Overall, residual stresses tend to compromise stent expansion both at peak pressure and after balloon deflation. At peak pressure, stent outer diameter was found to be 2.61 mm and 2.75 mm for simulations with and without considering residual stresses, respectively, and settled as 2.10 mm and 2.19 mm, respectively, after balloon deflation. Although the recoiling had similar magnitude for both cases (~20%; with and without residual stresses), less dogboning was found when residual stresses were excluded in simulations (i.e. 41%; versus 45% for simulations with residual stresses). As shown in Figure 12, residual stresses did not affect the pattern of stress distribution in the stent and artery. On the stent, stresses are still highly localised at the U-bend regions, regardless of the residual stress state. Also, the maximum stress on the stent changed only by ~0.4% (95.69 to 95.32 MPa) when residual stresses were considered in the simulations. In the artery [Figure 12B], stresses were again concentrated towards the ends of the plaque as a result of stent dogboning effect. The peak value of the maximum principal stress changed by ~15% (0.64 to 0.54 MPa) when the residual stresses were included.

For Xience stent, residual stresses affected the early stage of expansion of the stent as shown in Figure 13. The achieved final diameter (~2.40 mm) and the recoiling effect (~11%) were similar for the two cases (with and without residual stresses). Dogboning increased when residual stresses were excluded, with a value of 36% (compared to 24% for the case considering residual stresses). As shown in Figure 14, the stresses developed in the stent and the diseased artery were similar, in terms of distribution, for the two cases (with and without residual stresses). In terms of magnitude, the maximum stress in the stent changed only by ~0.7% (928.9 to 935.0 MPa) when residual stresses were included in the simulations. For the diseased artery, the peak value of the maximum principal stress changed by ~5% (1.50 MPa to 1.43 MPa) when the residual stresses were considered.

DISCUSSION

Clinically, in-stent restenosis (ISR), i.e. re-narrowing of stented artery, is one of the major drawbacks associated with stent implantation.[30] ISR is a direct consequence of the formation of neointima, largely caused by proliferating smooth muscle cells and accumulated extracellular matrix.[30] According to recent investigations, arterial wall biomechanics plays a key role in ISR. The stenting-caused alteration of biomechanical environment controls the inflammatory and remodelling processes of vessel walls. As reported by Timmins *et al.*,[31] stent designs that induced higher

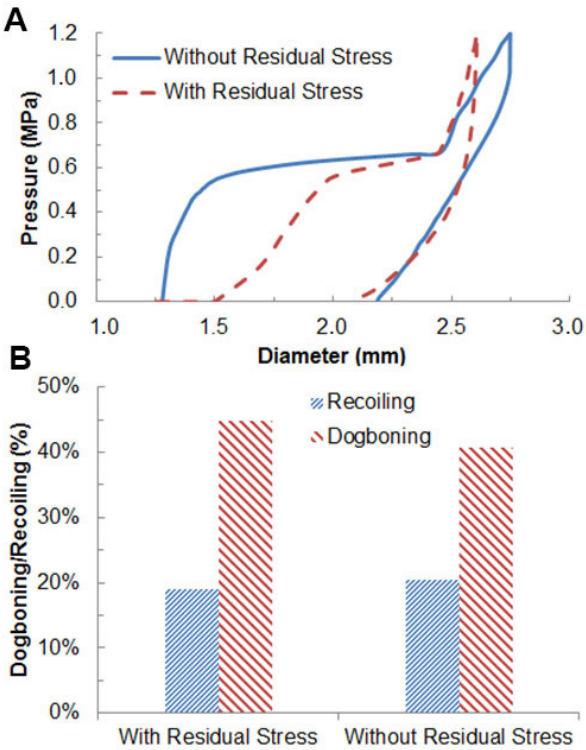

Figure 11: (A) Diameter change against pressure; and (B) recoiling and dogboning effects obtained from simulations with and without considering crimping-caused post-crimping stresses on Elixir stent

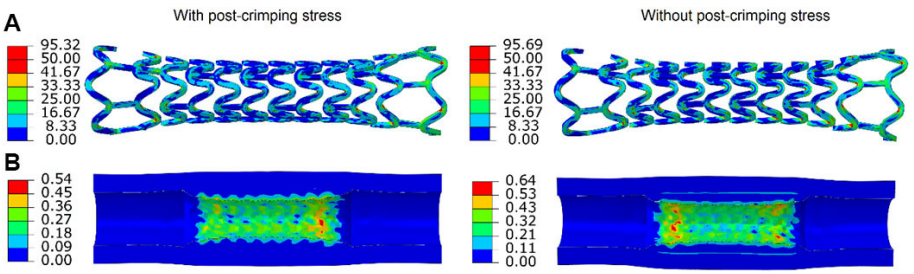

Figure 12: (A) von Mises stress (MPa) on the Elixir stent; and (B) maximum principal stress (MPa) on the artery-plaque system for simulations with (left) and without (right) considering residual stresses

levels of non-physiologic stresses provoked a more aggressive pathobiological response of the vessel walls, leading to a higher degree of neointimal formation. *In-vitro* studies have also proved that mechanical stresses regulated the proliferation and migration of vascular cells,[32] and the synthesis and reorganization of extracellular matrix.[33] Stent-induced vessel stresses are closely linked with the level of artery injury, also promoting the development of restenosis. From this study, it is clear that the stresses in the artery appear to be largely affected by the stent materials and designs. PLLA has lower modulus, yield strength and strain hardening, which soothed the stent-artery interaction and led to stress reduction in vessel layers as shown in Figure 10. This is clinically beneficial. Bioresorbable polymeric stents are also more compliant than metallic stents, diminishing associated vascular responses over the scaffolded vessel segments.[34]

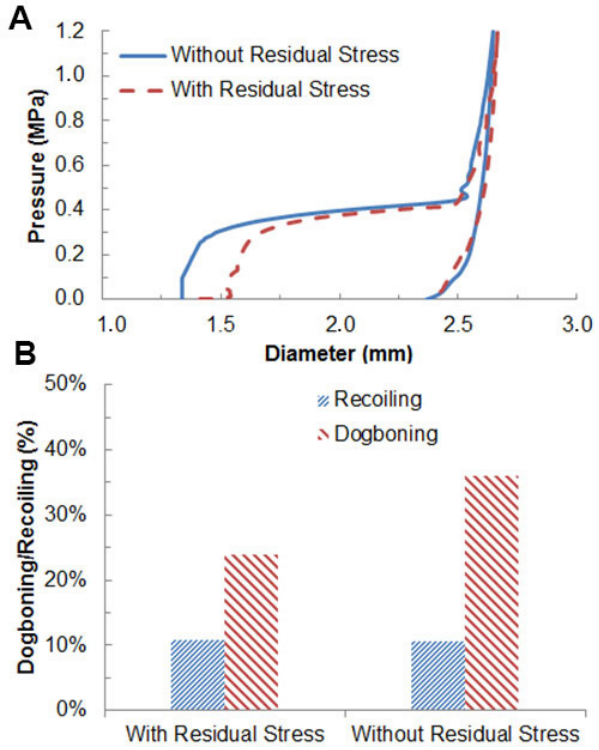

Figure 13: (A) Diameter change against pressure; and (B) recoiling and dogboning effects obtained from simulations with and without considering crimping-caused residual stresses on Xience stent

Residual stresses in stents were studied by Möller *et al.*[35] using X-ray diffraction method. Their work confirmed that even for as-produced stents, a significant amount of microstresses can be developed in the stent during crimping (this is also the case found in our work). Subsequent stent expansion caused an increase of stress due to tension. According to their study, the level of stresses introduced by crimping and expansion can considerably affect the fatigue life of the stent. Based on our results, crimping and expansion processes were found to induce comparable levels of stresses in the stent struts. Also, residual stresses developed during crimping affected the expansion behaviour, though only slightly, of stent in the deployment step. It is also thought that residual stresses in the stent contributed to the flexibility of the device, thus imposing less stresses on the plaque during further deformation as confirmed by our simulation results, although only marginally.

The targeted vessel diameter (i.e. 3 mm) could not be achieved by stent expansion only. This is the case for both polymer and metallic stents. Firstly, it was due to the saturated expansion of the artery layers, developed at a later stage of vessel stretching. This happened when the stiffness of vessel layers, especially the intima layer, increased steeply upon large stretch [Figure 4]. Secondly, vessel layers were assumed to deform purely elastically which imposed a large recovery force on the expanded stent after balloon deflation. Generally, the expansion of polymer stent was slower than that of metallic stent. Higher recoiling was also observed for polymeric stent due to the weaker mechanical properties. This indicates that there is a challenge to use polymer stents to achieve desired lumen diameter, especially for patients with stiffer artery and heavily calcified plaques. However, it is recognised that polymer stent induced significantly lower stresses in the artery than metallic stents, which could reduce the occurrence of arterial injuries and in-stent restenosis.

In clinical practice, most stents are post-dilated with

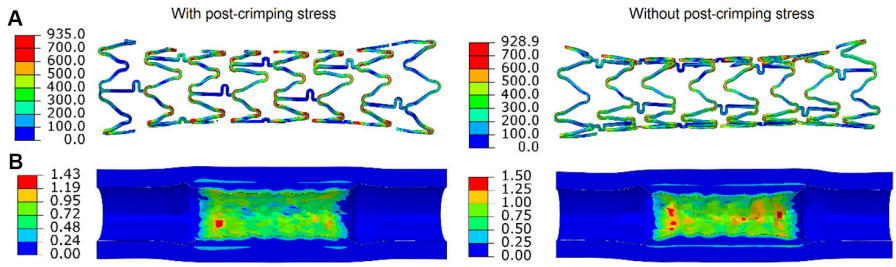

Figure 14: (A) von Mises stress on the Xience stent; and (B) maximum principal stress on the artery-plaque system for simulation with (left) and without (right) considering residual stresses

relatively non-compliant balloons, i.e. stiffer and larger than the stent delivery system balloon, to ensure good apposition and to ensure that the intended diameter is achieved. However, multiple cycles of inflation were not simulated here which is a limitation of this study. The simulation in this work was a single deployment of stent or direct stenting of a vascular lesion. In clinical practice, pre-dilatation of diseased artery can also occur. Direct-stenting is common, but not with bioresorbable polymer stents. For bioresorbable polymer stents (or any new technology), the users generally acknowledge the importance of careful lesion preparation by pre-dilatation as well as post-dilatation. The pre-dilatation balloon inflation step modifies the plaque and results in less recoil after deployment of a stent or scaffold. After a stent is deployed, an additional post-dilatation generally occurs with a second larger balloon. Sometimes this is to achieve a better deployment through a tapered vessel, or to "crack" a stubborn plaque. Sometimes, this is required when two stents are deployed with an overlap. This type of balloon pre-dilatation and post-dilatation is essential for polymer stents to be effective in difficult lesions. In fact, larger degrees of recoil and dogboning predicted by the simulations indicate that adequate pre-dilatation and post-dilatation are potentially critical for polymeric stents to achieve optimal clinical results. Simulation of pre-dilation or post-dilation requires proper inelastic or damage models to describe unrecoverable deformation for the plaque and artery wall, which is currently beyond the scope of the paper. In this study, the artery and plaque were assumed to behave purely elastically (hyperelastic model), and the efforts of pre-dilation or post-dilation will be nullified as soon as the dilation pressure is removed. Modelling of inelastic deformation and damage will be attempted in our future work.

In addition, the stresses in the vessel layers at peak inflating pressure are generally beyond the ultimate tensile strength of the tissue layer.[25] This is also the case for the plaques. Therefore, tissue damage will need to be modelled in the FE simulations at high pressure levels. Tissue damage is associated with unrecoverable deformation in the artery, which could reduce the stent recoiling considerably upon balloon deflation. Consequently, stent is expected to expand further if tissue damage is considered due to irrecoverable mechanical deformation of the artery-plaque system, thus resulting in a larger lumen diameter. Damage modeling of arterial layers is a limitation of this study and will be studied in our future work. However, it does not affect the general conclusion of this paper, as we aim to make like-for-like comparison of the scaffolding capability and mechanical performance between bioresorbable polymeric and metallic stents.

On the other hand, the plaque can be classified as hypocellular, cellular or calcified, depending on the composition. It is more difficult to treat calcified plaque by stenting due to its strong resistance to stretch when compared to hypocellular or cellular plaques.[18] So far, the effect of plaque composition on stent expansion was only evaluated for isotropic tissue model, and further work is required to study such effects by considering vessel anisotropy. Finally, polymers generally possess anisotropy and viscoplasticity effects which could also affect the simulation results and will need to be addressed in future studies.

In conclusion, crimping and deployment of polymeric and metallic stents have been simulated using finite element method to give a direct comparison of their mechanical performances. Results demonstrated that polymer stent has a lower rate of expansion than metallic stent. The overall expansion, reached at peak inflating pressure and after balloon deflation, was lower for polymer stent due to weaker material properties. This is also associated with the higher recoiling effect for polymer stent. Thus, it is a challenge to use polymer stent to treat patients with heavily calcified plaques or stiffer vessels, without pre-dilation or post-dilation. Crimping generated severe residual stresses in the stent, which tend to affect stent expansion and increase dogboning for Elixir polymer stent. However, they did not alter the stress distribution during the deployment process, and only imposed small changes to the stress magnitude.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Patient consent

There is no patient involved.

Ethics approval

This article does not contain any studies with human participants or animals.

REFERENCES

1. Hoffmann R, Mintz GS. Coronary in-stent restenosis - predictors, treatment and prevention. *Eur Heart J* 2000;21:1739-49.
2. Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, Kutys R, Skorija K, Gold HK, Virmani R. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. *J Am Coll Cardiol* 2006;48:193-202.
3. Yang TH, Kim DI, Park SG, Seo JS, Cho HJ, Seol SH, Kim SM, Kim

DK, Kim DS. Clinical characteristics of stent fracture after sirolimus-eluting stent implantation. *Int J Cardiol* 2009;131:212-6.
4. Flege C, Vogt F, Höges S, Jauer L, Borinski M, Schulte VA, Hoffmann R, Poprawe R, Meiners W, Jobmann M, Wissenbach K, Blindt R. Development and characterization of a coronary polylactic acid stent prototype generated by selective laser melting. *J Mater Sci Mater Med* 2013;24:241-55.
5. Ormiston JA, Serruys PW. Bioabsorbable coronary stents. *Circ Cardiovasc Interv* 2009;2:255-60.
6. Onuma Y, Serruys PW. Bioresorbable scaffold: the advent of a new era in percutaneous coronary and peripheral revascularization? *Circulation* 2011;123:779-97.
7. Waksman R. Biodegradable stents: they do their job and disappear. *J Invasive Cardiol* 2006;18:70-4.
8. Serruys PW, Chevalier B, Dudek D, Cequier A, D Carrié D, Iniguez A, Dominici M, van der Schaaf RJ, Haude M, Wasungu L, Veldhof S, Peng L, Staehr P, Grundeken MJ, Ishibashi Y, Garcia-Garcia HM, Onuma Y. A bioresorbable everolimus-eluting scaffold versus a metallic everolimus-eluting stent for ischaemic heart disease caused by de-novo native coronary artery lesions (ABSORB II): an interim 1-year analysis of clinical and procedural secondary outcomes from a randomised controlled trial. *Lancent* 2015; 385:43-54.
9. Gao R, Abizaid A, Banning A, Bartorelli AL, DŽavík V, Ellis S, Jeong MH, Legrand V, Spaulding C, Urban P. One-year outcome of small-vessel disease treated with sirolimus-eluting stents: a subgroup analysis of the e-SELECT registry. *J Interv Cardiol* 2013,26:163-72.
10. Agrawal CM, Haas KF, Leopold DA, Clark HG. Evaluation of poly (L-lactic acid) as a material for intravascular polymeric stents. *Biomaterials* 1992;13:176-82.
11. Nuutinen JP, Clerc C, Reinikainen R, Törmälä P. Mechanical properties and *in vitro* degradation of bioabsorbable self-expanding braided stents. *J Biomater Sci Polym Ed* 2003;14:255-66.
12. Bünger CM, Grabow N, Sternberg K, Kröger C, Ketner L, Schmitz KP, Kreutzer HJ, Ince H, Nienaber CA, Klar E, Schareck W. Sirolimus-eluting biodegradable poly-L-lactide stent for peripheral vascular application: a preliminary study in porcine carotid arteries. *J Surg Res* 2007;139:77-82.
13. Verheye S, Ormiston JA, Stewart J, Webster M, Sanidas E, Costa R, Costa JR Jr, Chamie D, Abizaid AS, Pinto I, Morrison L, Toyloy S, Bhat V, Yan J, Abizaid A. A next-generation bioresorbable coronary scaffold system: from bench to first clinical evaluation: 6-and 12-month clinical and multimodality imaging results. *JACC Cardiovasc Interv* 2014;7:89-99.
14. Chua SND, MacDonald BJ, Hashmi MSJ. Finite element simulation of stent and balloon interaction. *J Mater Process Technol* 2003;143-144:591-7.
15. Lally C, Dolan F, Prendergast PJ. Cardiovascular stent design and vessel stresses: a finite element analysis. *J Biomech* 2005;38:1574-81.
16. Gijsen FJ, Migliavacca F, Schievano S, Socci L, Petrini L, Thury A, Wentzel JJ, van der Steen AF, Serruys PW, Dubini G. Simulation of stent deployment in a realistic human coronary artery. *Biomed Eng Online* 2008;7:23.
17. Imani SM, Goudarzi AM, Ghasemi SE, Kalani A, Mahdinejad J. Analysis of the stent expansion in a stenosed artery using finite element method: application to stent versus stent study. *Proc Inst Mech Eng H* 2014;228:996-1004.
18. Schiavone A, Zhao LG, Abdel-Wahab AA. Effects of material, coating, design and plaque composition on stent deployment inside a stenotic artery -- finite element simulation. *Mater Sci Eng C Mater Biol Appl* 2014;42:479-88.
19. Clune R, Kelliher D, Robinson JC, Campbell JS. NURBS modeling and structural shape optimization of cardiovascular stents. *Struct Multidiscipl Optim* 2014;50:159-68.
20. Pauck RG, Reddy BD. Computational analysis of the radial mechanical performance of PLLA coronary artery stents. *Med Eng Phys* 2015;37:7-12.
21. Medtronic Technical Bulletin (2003) Advantages of cobalt alloy for coronary stents. Available from:http://wwwp.medtronic.com/newsroom/content/1110132739468.pdf [Last Accessed on 11-01-2017].
22. Zahedmanesh H, Lally C. Determination of the influence of stent strut thickness using the finite element method: implications for vascular injury and in-stent restenosis. *Med Biol Eng Comput* 2009;47:385-93.
23. Gervaso F, Capelli C, Petrini L, Lattanzio S, Di Virgilio L, Migliavacca F. On the effects of different strategies in modelling balloon-expandable stenting by means of finite element method. *J Biomech* 2008;41:1206-12.
24. Holzapfel GA, Gasser TC, Ogden RW. A new constitutive framework for arterial wall mechanics and a comparative study of material models. *J Elast Phys Sci Solids* 2000;61:1-48.
25. Holzapfel GA, Sommer G, Gasser CT, Regitnig P. Determination of layer-specific mechanical properties of human coronary arteries with nonatherosclerotic intimal thickening and related constitutive modeling. *Am J Physiol Heart Circ Physiol* 2005;289:H2048-58.
26. Nolan DR, Gower AL, Destrade M, Ogden RW, McGarry JP. A robust anisotropic hyperelastic formulation for the modelling of soft tissue. *J Mech Behav Biomed Mater* 2014;39:48-60.
27. Migliavacca F, Petrini L, Colombo M, Auricchio F, Pietrabissa R. Mechanical behavior of coronary stents investigated through the finite element method. *J Biomech* 2002;35:803-11.
28. Cheng GC, Loree HM, Kamm RD, Fishbein MC, Lee RT. Distribution of circumferential stress in ruptured and stable atherosclerotic lesions. A structural analysis with histopathological correlation. *Circulation* 1993;87:1179-87.
29. Li ZY, Howarth S, Trivedi RA, U-King-Im JM, Graves MJ, Brown A, Wang L, Gillard JH. Stress analysis of carotid plaque rupture based on *in vivo* high resolution MRI. *J Biomech* 2006;39:2611-22.
30. Lowe HC, Oesterle SN, Khachigian LM. Coronary in-stent restenosis: current status and future strategies. *J Am Coll Cardiol* 2002;39:183-93.
31. Timmins LH, Miller MW, Clubb FJ Jr, Moore JE Jr. Increased artery wall stress post-stenting leads to greater intimal thickening. *Lab Invest* 2011;91:955-67.
32. Haga JH, Li YS, Chien S. Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells. *J Biomech* 2007;40:947-60.
33. Chung IM, Gold HK, Schwartz SM, Ikari Y, Reidy MA, Wight TN. Enhanced extracellular matrix accumulation in restenosis of coronary arteries after stent deployment. *J Am Coll Cardiol* 2002;40:2072-81.
34. Sharkawi T, Cornhill F, Lafont A, Sabaria P, Vert M. Intravascular bioresorbable polymeric stents: a potential alternative to current drug eluting metal stents. *J Pharm Sci* 2007;96:2829-37.
35. Möller D, Reimers W, Pyzalla A, Fischer A. Residual stresses in coronary artery stents. *J Biomed Mater Res* 2001;58:69-74.

Bilovol. *Vessel Plus* 2017;1:22-8
DOI: 10.20517/2574-1209.2016.11

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Predictors of hormonal and metabolic disorders of arterial hypertension and type 2 diabetes mellitus comorbidity

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How to cite this article: Bilovol O. Predictors of hormonal and metabolic disorders of arterial hypertension and type 2 diabetes mellitus comorbidity. Vessel Plus 2017;1:22-8.

Article history:
Received: 26-10-2016
Accepted: 02-03-2017
Published: 31-03-2017

Key words:
Hypertension,
type 2 diabetes mellitus,
adipokines,
interleukins

ABSTRACT

Aim: These experiments studied adipokine and interleukin imbalances during the development and progression of metabolic disorders in patients with arterial hypertension (AH) and type 2 diabetes mellitus (T2DM). **Methods:** Ninety-five patients with stage II second degree AH (53 males and 42 females, mean age 54.7 ± 5.4 years) were observed. The cohort was separated into: group 1 (n = 48) patients with AH and group 2 (n = 47) patients with both AH and T2DM. Serum protein levels of omentin, adiponectin, tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), resistin, interleukin (IL)-1β, IL-4, and IL-6 were measured. Lipid and carbohydrate metabolism rates were assessed. **Results:** Peak homeostatic model assessment for insulin resistance (HOMA-IR) index values, as well as circulating insulin and CRP levels, were observed in group 2. Reduced adiponectin levels negatively correlated with HOMA-IR indices (r = -0.52, P < 0.05), triglycerides (r = -0.52, P < 0.05), glucose levels (r = -0.44, P < 0.05), body mass indices (r = -0.44, P < 0.05), and HbA1c levels (r = -0.57, P < 0.01). Group 2 patients demonstrated low omentin levels and high resistin and TNF-α levels. Negative correlations between IL-6 and both omentin and adiponectin (r = -0.46, P < 0.01; r = -0.42, P < 0.01, respectively) were observed. **Conclusion:** A novel pathogenic link was demonstrated between metabolic disorders, adipokines, and pro-inflammatory IL-6 levels as negative regulators of comorbid AH and T2DM.

INTRODUCTION

Arterial hypertension (AH) is an important public health challenge in both economically developing and developed countries.[1] The reported prevalence of AH has varied globally; the lowest prevalences was found in rural India (3.4% in men and 6.8% in women) and the highest was observed in Poland (68.9% in men and 72.5% in women).[2] Sur *et al.*[3] in 2010 reported that more than 3 million people in Romania are hypertensive. In 2009, one-fifth of the adult Danish population was hypertensive.[4] Hypertension affects nearly 75 million adults in the USA and is a major risk factor for myocardial infarction, ischemic stroke, chronic kidney disease, and vascular disease. According to an analysis of American data from the Nationwide Emergency Department

Sample, there was a 25% increase in the number of people visiting emergency rooms for hypertension-related procedures between 2006 and 2011.[5] A 25-year epidemiological study has been devoted to situational dynamics related to AH in the Ukraine. The report enrolled urban residents aged 18-64 years and indicated that AH prevalence grew from 30.6% to 35.3% ($P > 0.05$) over the observation period, probably due to increased average systolic arterial pressure levels.[6] The study also revealed a preponderance of risk factors among patients with AH. Only 5.6% of AH patients exhibited zero risk factors. Strikingly, 25.4% of AH patients had one risk factor, 33.2% of patients had 2 risk factors, and 3 or more risk factors were reported in 35.8% of patients. Unfortunately, the epidemiologic environment contributing to AH remains unfavorable. The Ukrainian data suggest that the AH high-risk profile is common.[6] Thus, it is unlikely that a noticeable decline in the death rate of this population will occur in the near future. A significant number of individuals with AH are unaware of their disease. Among those with diagnosed hypertension, treatments are frequently inadequate and incidence of deaths due to hypertension complications is high.

Isolated AH is currently rare. More commonly, physicians encounter clinical situations in which cases of AH are associated with endocrinopathies. The turn of the 21st century was marked by significant prevalence of type 2 diabetes mellitus (T2DM). Increases in incidence worldwide led to discussions about the global diabetes epidemic. The World Health Organization reported in 2016 that the number of people with diabetes mellitus rose from 108 million in 1980 to 422 million in 2014. Among adults over 18 years of age, diabetes mellitus rates rose from 4.7% in 1980 to 8.5% in 2014.[7] Given that diabetes mellitus disables patients at very early ages and leads to high mortality rates, the fight against diabetes has been elevated to a top priority for national health care systems across the world.

According to epidemiological studies, in patients with a combination of hypertension and T2DM the risk of fatal coronary heart disease increases 3 to 5 times, stroke increases 3 to 4 times, complete loss of vision increases 10 to 20 times, uremia increases 20 to 25 times, and gangrene of lower extremities becomes 20 times more likely.[8]

One explanation for the frequent observation of comorbid type 2 diabetes in hypertensive patients is that the prevalence of overweight and obesity in the population has increased.[9] Prospective studies have shown a strong relationship between obesity and type 2 diabetes. Almost 90% of patients with T2DM are obese. Similarly, the risk of hypertension is 50% higher in obese patients compared to those in normal weight ranges.

In the last few years, inflammatory mechanisms have been shown to regulate initiation, maintenance, and development of cardiovascular disease. Associations between cardiovascular diseases and obesity are mediated, in part, by secretory regulation of adipose tissue. Adipose tissue secretes bioactive peptides (adipokines), which have local and distal influences on organ systems through autocrine, paracrine, and endocrine functions. Increased production of adipokines in obese patients impacts multiple bodily functions, many of which are linked with cardiovascular diseases, such as: insulin sensitivity, immunity, angiogenesis, hemostasis, lipid metabolism, and blood pressure. Adipocytes, like T-lymphocytes and macrophages, produce cytokines and are involved in complement activation-stimulating inflammatory signaling cascades.[3,8] Cytokine imbalance is a well-established mechanism contributing increased risk of vascular complications. Therefore, elucidating the interaction between adipokines, interleukins, and metabolic disorders in patients with AH and T2DM is relevant.

The present study was undertaken to investigate the effects of adipokine and interleukin imbalances on the development and progression of metabolic disorders in patients managing hypertension with comorbid type 2 diabetes.

METHODS

Patients

Ninety-five patients with stage II second degree arterial hypertension (53 males and 42 females) were assessed. The mean age of the cohort was 54.7 ± 5.4 years. The patients were sub-divided into 2 groups. Group 1 (n = 48) consisted of hypertensive patients without T2DM. Group 2 (n = 47) was a comorbid population with both AH and T2DM. The control group consisted of 20 age- and gender-matched healthy subjects. AH diagnoses were conducted according to guidelines for the management of arterial hypertension from the European Society of Cardiology (ESC) and the European Society of Hypertension (2013), as well as Ukrainian Cardiology Association recommendations (2013).[10]

Criteria supporting diagnosis of abdominal obesity were established based on recommendations by the World Health Organization (1997). Anthropometric measurements were used to calculate body mass

index (BMI). The severity of obesity was determined following guidance from the International Diabetes Federation (2015). Diagnoses of type 2 diabetes were made following general recommendations of the European Association for the Study of Diabetes (2013) and the ESC (2013).[11] Inclusion criteria: fasting blood glucose ≤ 8.5 mmol/L, postprandial hyperglycemia ≤ 11 mmol/L, and HbA1c level ≤ 9%. Exclusion criteria: pregnancy or lactation; self-reporting with insulin-dependent diabetes, previous acute myocardial infarction or ischemic stroke; cardiovascular damage ≥ II by New York Heart Association criteria; liver disease indicated by transaminase values more than 3 times greater than normal; chronic kidney disease with serum creatinine > 2 mg/dL; ongoing dialysis; human immunodeficiency virus positive disease; history of chronic alcohol abuse in the last 2 years; or history of cancer, stroke, or organ transplantation.

Enzyme-Linked ImmunoSorbent Assay

Serum concentrations of omentin (BioVendor, Czech Republic), adiponectin, tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP) - DRG Elisa, USA and resistin (MBL International Corporation, USA) were determined with ELISA. Serum concentrations of interleukin (IL)-1, IL-4, and IL-6 were also determined by Enzyme-Linked ImmunoSorbent Assay (ELISA, Protein Contour, St. Petersburg). All assays were tested per the manufacturers' guidelines.

Lipid metabolism assays

Total cholesterol (TC) in plasma, high-density lipoprotein (HDL), and triglyceride (TG) levels were determined in all patients by enzymatic methodologies on a Humalyser autoanalyzer (Human Company, Germany). Low-density lipoprotein (LDL) cholesterol content was calculated using the Friedewald W. T. formula: LDL cholesterol (mmol/L) = TC - HDL cholesterol - TG/2.22. Whole blood HbA1c levels were determined by Reagent Test-Systems (Ukraine). Fasting plasma glucose levels was determined using glucose oxidase methodology. Fasting serum insulin levels were determined by ELISA. Glucose tolerance was measured using oral glucose tolerance tests. Homeostatic model assessment for insulin resistance (HOMA-IR) levels were calculated based on laboratory insulin and fasting glucose levels. HOMA-IR = fasting insulin × fasting glucose (mmol/L)/22.5. HOMA-IR levels > 2.77 were diagnosed as insulin resisted.

Statistical analysis

Statistical data analyses were performed using the general-purpose data processing software package in Statistica 8.0. Analyses of statistical significance were performed using Student's *t*-tests and non-parametric statistical methods.

RESULTS

Analyses of trophologic parameters revealed specific patterns in both groups. Patients with isolated AH (group 1) exhibited BMI measurements ranging from 18.5 to 24.9 kg/m^2 (4 patients). The majority of patients with isolated AH and comorbid AH with T2DM (group 2; 65.1% and 54.6%, respectively) had BMI measurements ranging from 30 to 34.9 kg/m^2. Third-degree obesity (BMI > 40.0 kg/m^2) was observed in 2 patients with isolated hypertension and in 6 patients with comorbid AH and T2DM.

Serum lipid spectra were altered with significantly higher frequency in patients with concomitant AH and T2DM rather than AH alone (62.6% and 44.2%, respectively; $P < 0.05$). Serum TG levels [Table 1] were 1.4 fold ($P = 0.0002$) higher in group 2 patients (AH plus T2DM) than in group 1 patients (AH alone), and 1.5 fold higher than in the controls ($P = 0.0020$). HDL-C levels in group 2 patients were significantly reduced compared to controls (53.2% and 21.0%, respectively; $P = 0.0020$). Group 2 patients with BMI measurements of 30 to 34.9 kg/m^2 had significantly lower levels of HDL-C compared with control patients ($P < 0.05$). Progression of lipid disorders in patients with comorbid AH and T2DM directly depended on BMI. Maximum TC and TG levels were observed in patients with BMI measurements of 35 to 40 kg/m^2 ($P = 0.242$, $P = 0.062$, respectively), while HDL cholesterol serum concentrations were the lowest in these patients ($P = 0.042$).

Next, insulin resistance (IR) indices were analyzed in the cohort. Patients in group 2 exhibited significantly higher HOMA-IR index values, as well as insulin and C-peptide measurements, when compared to group 1 and the controls ($P < 0.0001$, $P = 0.0003$, $P = 0.0004$, respectively) [Table 2]. These data indicated that hyperinsulinemic IR progression was associated with T2DM incidence. HOMA-IR indices in patients with AH alone were 2-fold higher than in controls ($P = 0.00001$). In patients with both AH and T2DM, HOMA-IR indices were 2.2-fold higher than in controls ($P = 0.00001$).

Regression analyses of these data identified statistically significant relationships between HOMA-IR levels and: glucose levels ($r = 0.42$, $P = 0.06$), C-peptide levels ($r = 0.64$, $P = 0.0001$), BMI ($r = 0.54$, $P = 0.0054$) and cholesterol levels ($r = 0.64$, $P = 0.056$). These results supported the hypothesis that the development and progression of IR correlated with hyperinsulinemia and dyslipidemia. These data also suggest that IR was associated with inflammation and the development of

Table 1: Characteristic of patient's lipid metabolism (mean ± SD)

Index	1. Control group (n = 20)	2. AH (n = 48)	3. AH + T2DM (n = 47)	P value
Total cholesterol, mmol/L	4.90 ± 0.64	5.80 ± 1.30	6.10 ± 1.70	P_{1-2} = 0.0470 P_{1-3} = 0.0320 P_{2-3} = 0.7200
Cholesterol of high-density lipoprotein, mmol/L	1.20 ± 0.06	1.00 ± 0.05	0.700 ± 0.045	P_{1-2} = 0.5400 P_{1-3} = 0.0020 P_{2-3} = 0.0070
Triglycerides, mmol/L	1.80 ± 0.07	1.90 ± 0.09	2.70 ± 0.16	P_{1-2} = 0.7300 P_{1-3} = 0.0020 P_{2-3} = 0.0002
Cholesterol of low-density lipoprotein, mmol/L	3.20 ± 0.54	3.68 ± 0.60	4.04 ± 0.97	P_{1-2} = 0.2300 P_{1-3} = 0.0330 P_{2-3} = 0.0530

AH: arterial hypertension; T2DM: type 2 diabetes mellitus

Table 2: Characteristic of insulin resistance indexes in observed patients (mean ± SD)

Index	1. Control group (n = 20)	2. AH (n = 48)	3. AH + T2DM (n = 47)	P value
HOMA-IR	1.64 ± 0.56	4.47 ± 0.60	5.44 ± 0.72	P_{1-2} = 0.00001 P_{1-3} = 0.00001 P_{2-3} = 0.1500
Insulin, μU/mL	5.58 ± 1.30	11.10 ± 2.70	13.70 ± 2.60	P_{1-2} = 0.0003 P_{1-3} = 0.0002 P_{2-3} = 0.0470
C-reactive protein, ng/mL	0.490 ± 0.025	0.960 ± 0.053	1.300 ± 0.075	P_{1-2} = 0.0004 P_{1-3} = 0.0001 P_{2-3} = 0.0620

HOMA-IR: homeostatic model assessment for insulin resistance; AH: arterial hypertension; T2DM: type 2 diabetes mellitus

vascular wall atherosclerotic lesions in patients facing both AH and T2DM.[12,13]

Next, glucose tolerance was tested. Impaired glucose tolerance (IGT) was observed in 9.6% of patients with hypertension only. In contrast, 96.5% of patients with both AH and T2DM were glucose intolerant. Also, HbA1c was significantly increased in group 2 patients compared to controls (P < 0.05). These data affirmed that excess body weight had negative impacts carbohydrate metabolism [Table 3]. Furthermore, fasting serum glucose (FG) levels were significantly elevated in group 1 patients (6.2%) compared to controls (P = 0.034). This may result from abdominal obesity given that: (1) excess body weight is causally associated with IR development; and (2) we observed the highest FG levels in patients with both AH and T2DM.

Serum adiponectin levels were evaluated. Adiponectin levels were reduced in patients with both isolated AH and comorbid AH/T2DM when compared to controls [Table 4]. Hypoadiponectinemia was most apparent in group 2 patients (P < 0.05). Adiponectin levels negatively correlated with HOMA-IR indices (r = -0.52, P < 0.05), TG levels (r = -0.52, P < 0.05), glucose levels (r = -0.44, P < 0.05), BMI (r = -0.44, P < 0.05) and HbA1c (r = -0.57, P < 0.01). These data supported that adiponectin regulated carbohydrate and lipid metabolisms and was deregulated in cases of IR. Currently, IR is considered a major risk factor contributing to etiology of T2DM, hypertension, dyslipidemia, atherosclerotic vascular disease, and, potentially, coronary heart disease and stroke.[13-15] IR can also predict development of T2DM in individuals who are normoglycemic. Therefore, it is important to identify IR in the pre-diabetic or early disease stages when therapeutic interventions are most likely to succeed.

Further analyses identified a correlation between adiponectin levels and BMI. In patients with AH and T2DM, those with a BMI ranging from 25.0 to 29.9 kg/m^2 had an average adiponectin level of 12.2 ± 3.6 ng/mL. When group 2 patients had BMIs ranging from 35.0 to 39.5 kg/m^2, average adiponectin level dropped to 7.4 ± 2.2 ng/mL (P < 0.05). These results suggest that adiponectin levels could be used to identify the development of vascular atherosclerotic lesions in patients with comorbid AH and T2DM [Table 4].

Next, omentin serum levels were evaluated. Patients with both AH and T2DM had 1.5-fold lower serum omentin than control patients (P = 0.044), as well as significantly lower omentin than AH patients (P = 0.052). There were negative correlative relationships between omentin levels and: systolic blood pressure levels (r = -0.61, P < 0.05), diastolic blood pressure levels (r = -0.68, P < 0.001), BMI (r = -0.36, P < 0.05), TG levels (r = -0.44, P < 0.001), CRP (r = -0.38, P < 0.001), and TNF-α (r = -0.44, P < 0.001). Also, omentin levels positively correlated with HDL-C (r =

Table 3: Characteristic of carbohydrate metabolism indexes in observed patients (mean ± SD)

Index	1. Control group (n = 20)	2. AH (n = 48)	3. AH + T2DM (n = 47)	P value
Fasing glucose, mmol/L	4.27 ± 0.94	6.02 ± 1.05	7.76 ± 1.92	P_{1-2} = 0.0340 P_{1-3} = 0.0003 P_{2-3} = 0.0740
HbA1c (%)	4.50 ± 0.85	6.30 ± 1.23	8.70 ± 1.52	P_{1-2} = 0.0966 P_{1-3} = 0.0002 P_{2-3} = 0.0054
Oral glucose tolerance test, mmol/L	5.16 ± 1.06	10.42 ± 1.89	13.90 ± 2.26	P_{1-2} = 0.0001 P_{1-3} = 0.0000 P_{2-3} = 0.0100

AH: arterial hypertension; T2DM: type 2 diabetes mellitus

Table 4: Levels of adipose tissue hormones and inflammatory markers in the studied patients (mean ± SD)

Index	1. Control group (n = 20)	2. AH (n = 48)	3. AH + T2DM (n = 47)	P value
Omentin, ng/mL	397.60 ± 5.30	319.52 ± 11.92	264.52 ± 3.76	P_{1-2} = 0.063 P_{1-3} = 0.044 P_{2-3} = 0.052
Adiponectin, ng/mL	13.60 ± 2.10	11.40 ± 2.70	7.30 ± 1.83	P_{1-2} = 0.097 P_{1-3} = 0.0002 P_{2-3} = 0.0056
Resistin, ng/mL	10.20 ± 2.58	10.30 ± 2.80	23.40 ± 3.80	P_{1-2} = 0.0971 P_{1-3} = 0.043 P_{2-3} = 0.049
TNF-α, pg/mL	5.24 ± 1.03	12.24 ± 1.60	27.36 ± 1.74	P_{1-2} = 0.008 P_{1-3} = 0.0001 P_{2-3} = 0.0038
IL-1β, pg/mL	36.8 ± 5.6	86.2 ± 6.3	93.1 ± 9.5	P_{1-2} = 0.008 P_{1-3} = 0.001 P_{2-3} = 0.064
IL-6, pg/mL	19.1 ± 1.2	33.5 ± 3.7	36.4 ± 4.3	P_{1-2} = 0.006 P_{1-3} = 0.002 P_{2-3} = 0.072
IL-4, pg/mL	42.9 ± 2.4	69.1 ± 3.4	79.4 ± 2.1	P_{1-2} = 0.0031 P_{1-3} = 0.002 P_{2-3} = 0.041

AH: arterial hypertension; T2DM: type 2 diabetes mellitus; TNF-α: tumor necrosing factor-alfa; IL: interleukin

0.46, P < 0.001) and adiponectin (r = 0.44, P < 0.05). Additionally, negative correlations were observed between omentin and glucose levels (r = -0.34, P < 0.05), as well as HOMA-IR indices (r = -0.46, P < 0.001). These data suggested that omentin levels were involved in progression of metabolic disorders as well as atherosclerosis development in patients with comorbid hypertension and T2DM. Data gathered here support the hypothesis that abnormal omentin production may contribute to the pathogenesis of obesity-related complications, including T2DM and cardiovascular diseases such as AH.[16-18]

The resistin cytokine is attracting considerable research interest, given that it may link obesity and diabetes. Resistin regulates inflammatory cascade activities, endothelial cell activations and proliferation of vascular smooth muscle cells. The cytokine is of interest as a potential link between metabolic and cardiovascular diseases. Resistin plasma levels were measured here.

Results revealed expression dynamics similar to those observed with insulin and glucose levels. Thus, patients with AH and T2DM experienced significantly higher resistin levels than patients with AH alone or than control patients (P = 0.049, P = 0.043, respectively). However, resistin levels between AH patients and controls were not significantly different (P > 0.05). In AH/T2DM comorbid patients, circulating blood resistin increased significantly, which correlated with fasting insulin, fasting glucose and lipid (TC and TG) concentrations. These data suggested that increased adiposity contributed to the etiology of changes in resistin levels. Previous data have provided ambiguous results when characterizing the role of resistin secretion in the pathogenesis of insulin resistance and T2DM.[18-20] The presence of high serum resistin in these comorbid AH and T2DM patients suggested that it was an additional regulator of IR.

Next, the groups were tested for TNF-α serum levels. Both group 1 and group 2 patients experienced significant increases in serum TNF-α levels when compared with controls (P < 0.05). At their highest, TNF-α levels were 4.1-fold increased in AH/T2DM patients (P = 0.0001) [Table 4]. Increased TNF-α levels

correlated with increased concentrations of TG and glucose (r = 0.415, P = 0.001; r = 0,042, P = 0.014, respectively).

Finally, both AH and AH/T2DM patients experienced significant increases in IL-1β levels when compared to controls. The most pronounced differences were observed in patients with both AH and T2DM (P = 0.008). These results have been associated with presumptive stimulation of acute-phase protein syntheses occurring as a result of AH and T2DM. Additionally, IL-4 activities were increased and positive correlations were observed between IL-4 and IL-1β (r = 0.42, P < 0.01) and IL-6 (r = 0.44, P < 0.01). These data suggested that compensatory signaling activities were occurring, given that IL-4 has been regulated to promote stabilization of inflammation. The regularity in which comorbid AH and T2DM experienced alterations in metabolic signaling factors emphasized the systematic and patterned etiologies of metabolic disorders. Negative correlations between IL-6 and both omentin and adiponectin (r = -0.46, P < 0.01; r = -0.42, P < 0.01, respectively) were observed. These findings were consistent with previous studies indicating that IL-6 regulated adipokine production.[18,21,22]

DISCUSSION

Presence of comorbid hypertension and type 2 diabetes is often associated with underlying metabolic disorders and central obesity. The combined sequelae lead to increased incidence of disease complications, and ultimately decreased patient life span. In this study, features of hormonal and metabolic disorders in patients with arterial hypertension and type 2 diabetes were investigated. The most significant signals identified were regulators of lipid and carbohydrate metabolisms, insulin resistance, and systemic inflammation development.

The data presented here indicated that all known manifestations of metabolic abnormalities dramatically increase with BMI in the cohort. The results suggested that in hypertensive patients with diabetes mellitus adipose tissue signaling was dysfunctional. Additionally, carbohydrate metabolism signaling alterations demonstrated that metabolic and hormonal signals were disrupted when hypertensive patients also had comorbid T2DM. The data suggested close pathogenetic links between onset of metabolic disorders and expressions of adipokines (omentin, adiponectin, and resistin). Pro-inflammatory IL-6 secretion was also altered, which was a negative regulator of comorbid arterial hypertension and T2DM.

Mounting evidence supports that adipokines are essential to etiology of cardiometabolic disorders. Despite these findings, research directions continue to focus on obesity as the essential therapeutic target for reducing the risk of metabolic syndrome, T2DM, and associated cardiovascular complications. Given the widespread incidence of comorbid hypertension in diabetic patients, identifying new markers responsible for increased blood pressure during insulin resistance is a paramount research concern.

Authors' contributions

O. Bilovol contributed solely to this paper.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

All involved patients gave their consent forms.

Ethics approval

The study protocol was approved by the Local Ethics Committee of the Kharkov National Medical University and was performed in accordance to the Declaration of Helsinki.

REFERENCES

1. Pereira M, Lunet N, Azevedo A, Barros H. Differences in prevalence, awareness, treatment and control of hypertension between developing and developed countries. *J Hypertens* 2009;27:963-75.
2. Kearney PM, Whelton M, Reynolds K, Whelton PK, He J. Worldwide prevalence of hypertension: a systematic review. *J Hypertens* 2004;22:11-9.
3. Sur G, Sur M, Kudor-Szabadi L, Sur L, Sporis D, Sur D. Arterial hypertension - prevalence of risk factors and morbide associations that increase cardiovascular risk. *Maedica (Buchar)* 2010;5:34-40.
4. Kronborg CN, Hallas J, Jacobsen IA. Prevalence, awareness, and control of arterial hypertension in Denmark. *J Am Soc Hypertens* 2009;3:19-24.
5. Harrison L. Hypertension ER Visits Surge 25% in Five Years. Medscape 2014. Available from: http://www.medscape.com/viewcollection/33205. [Last accessed on 21-03-2017].
6. Gorbas I, Smyrnova I. Epidemiology of arterial hypertension in Ukraine: results of the 25-year observation: PP.28.124. *J Hypertens* 2010;28:e485-6.
7. World Health Organization. Global report on diabetes. Geneva, 2016. Available from: http://www.who.int/diabetes/global-report/en/. [Last Accessed on 08-03-2017].
8. Ametov AS, Demidova TIu, Galieva OR. Angiotensin converting enzyme inhibitors in the prevention and treatment of vascular complications of diabetes. *Kardiologiia* 2005;45:109-12. (in Russian)
9. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 2001;409:307-12.
10. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M,

Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F, Redon J, Dominiczak A, Narkiewicz K, Nilsson PM, Burnier M, Viigimaa M, Ambrosioni E, Caufield M, Coca A, Olsen MH, Schmieder RE, Tsioufis C, van de Borne P, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S, Clement DL, Coca A, Gillebert TC, Tendera M, Rosei EA, Ambrosioni E, Anker SD, Bauersachs J, Hitij JB, Caulfield M, De Buyzere M, De Geest S, Derumeaux GA, Erdine S, Farsang C, Funck-Brentano C, Gerc V, Germano G, Gielen S, Haller H, Hoes AW, Jordan J, Kahan T, Komajda M, Lovic D, Mahrholdt H, Olsen MH, Ostergren J, Parati G, Perk J, Polonia J, Popescu BA, Reiner Z, Rydén L, Sirenko Y, Stanton A, Struijker-Boudier H, Tsioufis C, van de Borne P, Vlachopoulos C, Volpe M, Wood DA. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013;34:2159-219.

11. Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N, Deaton C, Escaned J, Hammes HP, Huikuri H, Marre M, Marx N, Mellbin L, Ostergren J, Patrono C, Seferovic P, Uva MS, Taskinen MR, Tendera M, Tuomilehto J, Valensi P, Zamorano JL; ESC Committee for Practice Guidelines (CPG), Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S; Document Reviewers, De Backer G, Sirnes PA, Ezquerra EA, Avogaro A, Badimon L, Baranova E, Baumgartner H, Betteridge J, Ceriello A, Fagard R, Funck-Brentano C, Gulba DC, Hasdai D, Hoes AW, Kjekshus JK, Knuuti J, Kolh P, Lev E, Mueller C, Neyses L, Nilsson PM, Perk J, Ponikowski P, Reiner Z, Sattar N, Schächinger V, Scheen A, Schirmer H, Strömberg A, Sudzhaeva S, Tamargo JL, Viigimaa M, Vlachopoulos C, Xuereb RG. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur Heart J* 2013;34:3035-87.
12. Piya MK, McTernan PG, Kumar S. Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. *J Endocrinol* 2013;216:T1-15.
13. Gutch M, Kumar S, Razi SM, Gupta KK, Gupta A. Assessment of insulin sensitivity/resistance. *Indian J Endocrinol Metab* 2015;19:160-4.
14. Trzeciak-Ryczek A, Tokarz-Deptula B, Deptula W. Adipocytokines affecting the immune system - selected data. *Centr Eur J Immunol* 2011;36:92-4.
15. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011;11:85-97.
16. Abd-Elbaky AE, Abo-ElMatty DM, Mesbah NM, Ibrahim SM. Omentin and apelin concentrations in relation to obesity, diabetes mellitus type two, and cardiovascular diseases in Egyptian population. *Int J Diabetes Dev Ctries* 2016;36:52-8.
17. Zhou JY, Chan L, Zhou SW. Omentin: linking metabolic syndrome and cardiovascular disease. *Curr Vasc Pharmacol* 2014;12:136-43.
18. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. *Front Endocrinol (Lausanne)* 2013;4:71.
19. Benomar Y, Gertler A, De Lacy P, Crépin D, Ould Hamouda H, Riffault L, Taouis M. Central resistin overexposure induces insulin resistance through Toll-like receptor 4. *Diabetes* 2013;62:102-14.
20. Ronti T, Lupattelli G, Mannarino E. The endocrine function of adipose tissue: an update. *Clin Endocrinol (Oxf)* 2006;64:355-65.
21. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol* 2010;316:129-39.
22. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. *Arch Med Sci* 2013;9:191-200.

Sobenin *et al. Vessel Plus* 2017;1:29-37
DOI: 10.20517/2574-1209.2016.12

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Small dense and desialylated low density lipoprotein in diabetic patients

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How to cite this article: Sobenin IA, Galitsyna EV, Grechko AV, Orekhov AN. Small dense and desialylated low density lipoprotein in diabetic patients. Vessel Plus 2017;1:29-37.

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Article history:
Received: 26-10-2016
Accepted: 29-12-2016
Published: 31-03-2017

Key words:
Diabetes mellitus,
atherosclerosis,
low density lipoprotein,
desialylated LDL,
small dense LDL,
electronegative LDL

ABSTRACT

Aim: This study was undertaken to investigate the physicochemical properties of modified low density lipoprotein (LDL) in diabetes. **Methods:** LDL from 10 type 1 and 10 type 2 diabetic patients, as well as LDL from 10 non-diabetic subjects, was subdivided into bound and non-bound fractions by affinity chromatography on *Ricinus communis* agglutinin-agarose, and further characterized by sialic acid content, hydrated density, electrophoretic mobility, and the ability to induce cholesterol deposition in cultured cells. **Results:** The non-bound LDL fraction was similar to native LDL from healthy subjects, with respect to its physicochemical properties, and did not produce intracellular cholesterol accumulation. The bound LDL fraction was characterized by several alterations differentiating it from non-bound LDL, namely, significantly lowered sialic acid content (by 35-50%, compared with non-bound LDL), increased electrophoretic mobility (by 40-50%), increased hydrated density (difference in modae, 5.6-5.9 mg/mL), and smaller particle size (difference in modae, 3.8-4.9 nm). Bound LDL possessed the ability to induce a 2.1- to 2.7-fold increase in intracellular cholesterol content. **Conclusion:** The results showed the presence of a dense, small, more electronegative, desialylated LDL subfraction in the blood of diabetic patients, which is *in vivo* modified atherogenic LDL.

Quick Response Code:

INTRODUCTION

Accelerated coronary and peripheral vascular atherosclerosis is the most common long-term complication of diabetes mellitus.[1-3] The mortality rate of coronary heart disease (CHD) is up to four times higher in diabetic than nondiabetic individuals. Therefore, CHD is the leading cause of death in diabetic patients.[4-6] Many factors contribute to the increased rate of atherosclerosis progression in diabetes, including alterations in plasma lipid profile, platelet function, clotting factors, metabolism of arterial wall cells, and elevated blood pressure. The precise mechanisms of premature atherogenesis in diabetic patients, however, remain unclear.

At the cellular level, the deposition of intracellular cholesterol in the arterial wall and subsequent foam-cell formation is a typical feature of early atherosclerotic lesions.[7] Low-density lipoprotein (LDL) has been associated with sourcing of accumulating lipids.[6,8,9] However, LDL isolated from healthy individuals failed to produce notable cholesterol accumulation in cultured arterial smooth muscle cells or macrophages.[10,11] It was hence accepted that LDL is required to undergo structural alterations or chemical modifications to become atherogenic.[12] However, modified LDL particles are still not considered as clinical biomarkers or therapeutic targets because of insufficient evidence. Therefore, additional studies, both basic and clinical, are necessary to fill the existing gap in the knowledge.

Previously, we have shown that LDL from diabetic patients, unlike LDL from healthy individuals, is able to induce significant lipid deposition in cultured cells derived from uninvolved (non-atherosclerotic) human aortic intima.[13] LDL from diabetic patients was subdivided into two fractions by lectin chromatography on Ricinus communis agarose, wherein bound (desialylated) LDL showed substantial dissimilarity with non-bound (native) LDL with respect to chemical composition and atherogenic properties, i.e. it was just a fraction of *in vivo* modified LDL presumably responsible for lipid accumulation in cultured cells.[13,14]

In the past few years, it was demonstrated that even in healthy individuals, LDL is heterogeneous in size and hydrated density, and the presence of small dense LDL (sdLDL) in blood is associated with a higher risk of clinical manifestations of atherosclerosis.[15-17] We have shown that multiple-modified atherogenic LDL occurring in blood of atherosclerotic patients is characterized also by increased hydrated density, and therefore may be easily regarded as sdLDL.[18]

This study was undertaken to obtain more information on the desialylated LDL isolated from the blood of diabetic patients and healthy individuals, to examine their distribution by hydrated density and the relationship between the LDL density and atherogenicity.

METHODS

Patients

This study was conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983. It was approved by the local ethics committee of the Institute for Atherosclerosis Research, Skolkovo Innovation Center, Moscow, Russia. All participants gave their written informed consent prior to inclusion in the study. The study group comprised of 10 type 1 diabetic patients, 10 type 2 diabetic patients, and 10 healthy control subjects, free from coronary artery disease [Table 1]. The diagnosis of diabetes mellitus was verified according to the 1997 criteria by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria.[19] Type 1 diabetic patients were on insulin therapy, and type 2 diabetic patients were treated with oral hypoglycemic agents, namely sulfonylurea derivatives.

LDL isolation, lectin chromatography, and density fractionation

Venous blood (15 mL) was drawn after overnight fasting in plastic tubes containing 0.1% EDTA. Plasma was separated by centrifugation, and ε-aminocaproic acid (1 mmol/L) was added. LDL (density, 1.025-1.063 g/mL) was isolated by sequential preparative ultracentrifugation according to Lindgren[20] in a Beckman L8-55 ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) using Type 50Ti fixed angle rotor operated at 40,000 *g* at 10 ºC, and sterilized by filtration (pore size, 0.45 µm). The LDL preparations were dialyzed against 2,000 volumes of phosphate buffered saline (PBS) at pH 7.4, overnight at 4 ºC. LDL was subfractionated into two fractions [non-bound (sialylated) LDL and bound

Table 1: Demographic findings and subject characteristics of study groups

Characteristic	Healthy subjects	Type 1 diabetic patients	Type 2 diabetic patients
Gender, M/F	4:6	4:6	5:5
Age, years	32.3 (2.1)	46.0 (17.7)	56.3 (3.5)*
Diabetes, years	-	24.7 (17.0)	16.0 (6.1)
Glycemia, mmol/L	4.6 (0.3)	9.1 (2.1)*	11.3 (1.8)*
TG, mmol/L	1.6 (0.2)	1.7 (0.6)	2.2 (0.4)
Cho, mmol/L	4.7 (0.2)	5.2 (1.0)	5.5 (0.8)
HDL-Cho, mmol/L	1.3 (0.1)	1.4 (0.2)	1.3 (0.3)

*Significant difference from healthy subjects, $P < 0.05$. TG: plasma triglycerides; Cho: plasma cholesterol; HDL-Cho: plasma high-density lipoprotein-cholesterol

(desialylated) LDL] using affinity chromatography on Ricinus communis agglutinin (RCA_{120})-agarose (Sigma Chemical Co., St. Louis, MO) as described elsewhere.[21] Briefly, approximately 2 mg of LDL (by protein) was applied on the column containing 2 mL of the affinity gel equilibrated with PBS. Non-bound (sialylated) LDL was washed from the gel with 30 volumes of PBS containing 0.5 mol/L NaCl, and bound (desialylated) LDL was eluted with 5 volumes of PBS containing 50 mmol/L galactose (Sigma Chemical Co., St. Louis, MO). LDL fractions were adjusted with solid NaBr to a density of 1.065 g/mL and concentrated by ultracentrifugation. For cell culture experiments, aliquots of LDL preparations were dialyzed against PBS and sterilized by filtration (pore size, 0.45 μm). The larger portions of LDL fractions were subdivided in a density gradient as described elsewhere.[22,23] Briefly, total, non-bound, and bound LDL were dialyzed against 2000 volumes of NaCl/NaBr solution with a density of 1.050 g/mL overnight at 4 ºC. The gradients were formed into 14 × 95-mm tubes by successively layering 2.6 mL of NaCl/KBr solutions having densities of 1.1289, 1.0637, 1.0500 (containing the LDL sample), 1.0398, and 1.0282 g/mL. The gradients were centrifuged for 42 h in a Beckman L-80 ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) using Type SW40Ti swinging bucket rotor operated at 36,000 *g* at 10 ºC. After centrifugation, the gradients were fractionated by upward displacement with KBr solution (density, 1.300 g/mL) at a speed of 0.5 mL/min (Isco Density Gradient Fractionator, Model 640, Isco, Lincoln, NE). The absorbance of the effluent was monitored with an Isco UA-5 absorbance detector (Isco, Lincoln, NE) at 280 nm, and 0.4 mL fractions were collected. Equal salt gradients without LDL were run and fractionated simultaneously, and the densities of these fractions were measured to reconstruct the density profile of the gradient (DMA 45 Calculating Digital Density Meter, Anton Paar, Graz, Austria).

Cell culture studies

Subendothelial cells were isolated from uninvolved human aortic intima by dispersion with 0.15% collagenase.[7] Subendothelial cells represented a mix of typical smooth muscle cells (about 30-40%), atypical (pericyte-like or stellate) smooth-muscle cells (about 40-45%), resident macrophages (3-8%), and very small number of cells that migrated from circulation (monocytes and lymphocytes). Because atherosclerosis develops only in the subendothelial intima, this type of primary culture is adequate for reproducing *in vitro* the major traits of atherogenesis at the cellular level (e.g. lipid deposition, proliferation, fibrosis, and inflammation), although this model has certain limitations with the respect to inter-experiment standardization. These limitations were avoided when the whole set of experiments was performed in the primary culture obtained from one autopsy sample. The results were reproduced twice in primary cultures obtained from two other autopsy samples and, hence, considered reliable. The autopsy material was taken from men aged 52-62 years who had died suddenly in an accident. The cells were suspended in Medium 199 containing standard additives: 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL Fungizone, and 10% fetal calf serum (FCS). Cells were seeded onto 24-well tissue culture plates at a density of 3×10^4 cells/cm^2 of growth area, and cultured at 37 ºC in a humidified CO_2-incubator (95% air and 5% CO_2). The medium was changed every other day. On the 7th day in culture, the total, non-bound, and bound LDL fractions (50 μg/mL) were added in quadruplicate in Medium 199 containing 10% human lipoprotein-deficient serum (LDS). Control cells were incubated in Medium 199 containing 10% LDS without LDL addition. After 24 h of incubation, cells were rinsed thoroughly with PBS, and cellular lipids were extracted thrice with n-hexane/isopropanol (3:2, v/v) according to Hara and Radin's protocol.[24] Total cholesterol in the extracts was determined enzymatically.[25] Cellular protein was measured according to Lowry *et al*.[26]

Electrophoresis

The aliquots of LDL samples (20 μg LDL by protein) were run on agarose gel films (Ciba Corning Diagnostics GmbH, Giessen, Germany) according to Lipid Research Clinics Manual of Laboratory Operations.[27] The same LDL sample from healthy donors was used as the reference sample throughout all experiments, and its mobility was assumed as 1.00. Nondenaturing polyacrylamide gradient gel electrophoresis was run on continuous 2-16% gradient gels as described elsewhere.[28] After gel staining with Coomassie Brilliant Blue R-250, LDL-migration distance was measured by scanning densitometry at 560 nm. LDL particle size was calculated according to the calibration curve reconstructed from migration distances of standards with known diameters using Exponential Curve Fit utility in the Sigmaplot 12.0 program package.

Other analyses

LDL sialic acid content was measured according to Warren's modified method.[29] LDL protein was measured according to Lowry *et al*.[26]

Statistical methods

Results are reported as mean and standard deviation (SD). Significance of differences was evaluated using ANOVA and Mann-Whitney tests (IBM SPSS 20.0 statistical program package), and P values < 0.05 were considered statistically significant.

RESULTS

Sialic acid content of LDL fractions

Sialic acid content of total LDL preparations accounted for 40.0 (SD 3.0) nmol/mg LDL protein in non-diabetic individuals, 32.9 (SD 2.8) nmol/mg in type 1, and 26.4 (SD 3.9) nmol/mg in type 2 diabetic patients. Sialic acid content in diabetic patients' LDL was significantly lower than non-diabetic individuals (P = 0.027). Upon LDL sub-fractionation into non-bound and bound fractions by affinity chromatography on RCA_{120}-agarose, non-bound LDL in all studied groups were characterized by high levels of sialic acid, quite comparable to normal values [41.5 (SD 1.7), 38.7 (SD 1.4), and 35.1 (SD 1.8) nmol/mg] LDL protein for non-diabetic individuals, type 1, and type 2 diabetic patients, respectively; (P > 0.1). However, bound LDL had significantly lower sialic acid levels than total LDL (P = 0.029) and non-bound LDL (P = 0.009) in all studied groups; the levels accounted for 32.3 (SD 2.5), 24.8 (SD 4.0), and 17.9 (SD 2.3) nmol/mg LDL protein for non-diabetic individuals, type 1, and type 2 diabetic patients, respectively. Thus, the obtained LDL fractions (non-bound and bound) differed significantly in sialic acid content and might be readily regarded as sialylated and desialylated LDL.

Density of sialylated and desialylated LDL

The density distributions of sialylated and desialylated LDL are shown in Figure 1. In non-diabetic individuals, the density peak of desialylated LDL was slightly shifted to the higher density region compared with that of sialylated LDL [1.0350 (SD 0.0003) *vs*. 1.0335 (SD 0.0003) g/mL, P = 0.013]. A more prominent shift was observed for desialylated LDL from type 1 diabetic patients and furthermore for desialylated LDL from type 2 diabetic patients [1.0395 (SD 0.0006) *vs*. 1.0338 (SD 0.0004), P < 0.001, and 1.0408 (SD 0.0004) *vs*. 1.0350 (SD 0.006) g/mL, P < 0.001, respectively]. Therefore, desialylated LDL was significantly denser than sialylated LDL, and this difference in LDL density was most evident in diabetic patients. It is also notable that even sialylated LDL in diabetic patients seemed to be slightly more dense than that in non-diabetic individuals [Figure 1], but this difference was not statistically significant.

Atherogenic potential of sialylated and desialylated LDL: correlation with LDL density

LDL atherogenicity was determined as the ability to induce cholesterol deposition in cells cultured from unaffected human aortic intima. As shown in Table 2, LDL from non-diabetic individuals did not affect cellular cholesterol content; neither did sialylated LDL, but desialylated LDL fraction increased intracellular cholesterol level moderately and significantly (P = 0.037). The same difference was observed for sialylated and desialylated LDL from type 1 and type 2 diabetic patients; however, in some cases, sialylated LDL seemed slightly atherogenic, and desialylated LDL caused extensive cholesterol accumulation, much more than that of desialylated LDL from non-diabetic individuals [Table 2]. The effect of total LDL from diabetic patients appeared to have intermediate value between that of sialylated and desialylated LDL [Table 2],

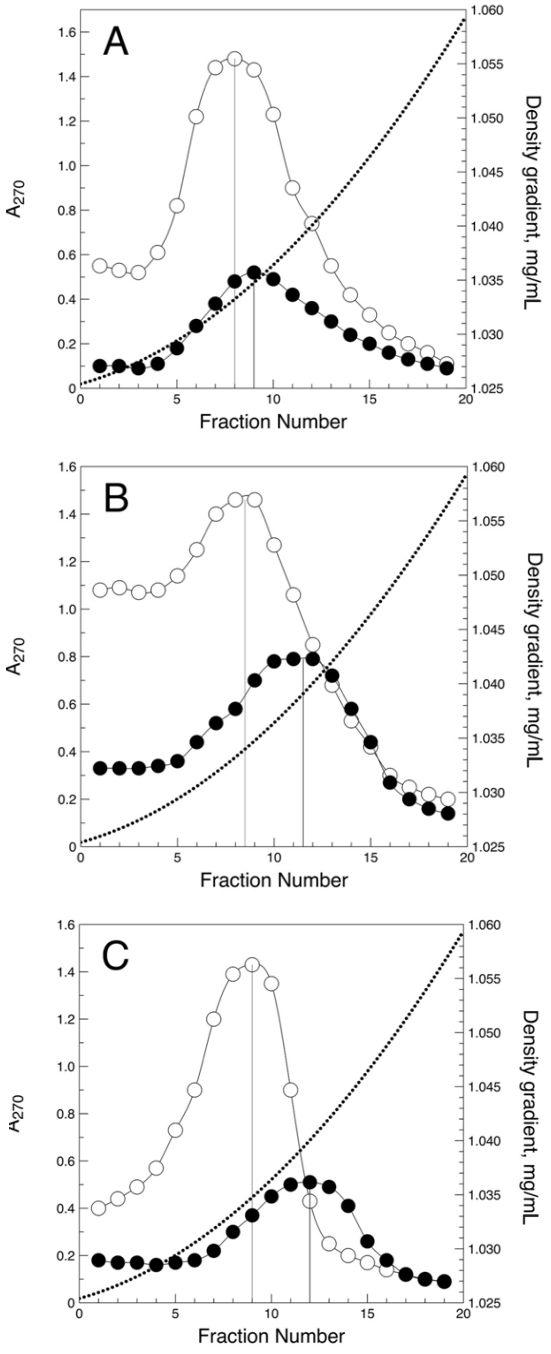

Figure 1: Density distribution of sialylated (hollow circles) and desialylated (filled circles) low-density lipoprotein in healthy subjects (A), type 1 (B), and type 2 diabetic patients (C). Y-axis denotes optical density at λ = 270 nm. Dotted line denotes the density gradient

and was supposedly dependent on the proportion of desialylated (atherogenic) LDL in total preparation and of the extent of its desialylation.

The effects of sialylated and desialylated LDL fractionated by density on intracellular cholesterol content was also studied. The results from the experiment performed using LDL subfractions obtained from type 1 and type 2 diabetic patients are shown in Figure 2. The LDL samples from all patients produced similar but quantitatively different effects. Sialylated LDL did not induce an increase in the intracellular cholesterol content after 24 h of incubation under the cell culture conditions. However, the densest sialylated LDL subfractions (d > 1.042 g/mL) produced a moderate but significant rise in intracellular cholesterol level. Subfractions of less dense desialylated LDL (1.025-1.035 g/mL) also produced no significant atherogenic effect, but LDL of greater density stimulated 1.3- to 2.3-fold cholesterol accumulation ($P < 0.05$). Denser desialylated LDL subfractions from type 2 diabetic patients were much more effective than that from type 1 diabetic patients, with respect to intracellular cholesterol accumulation.

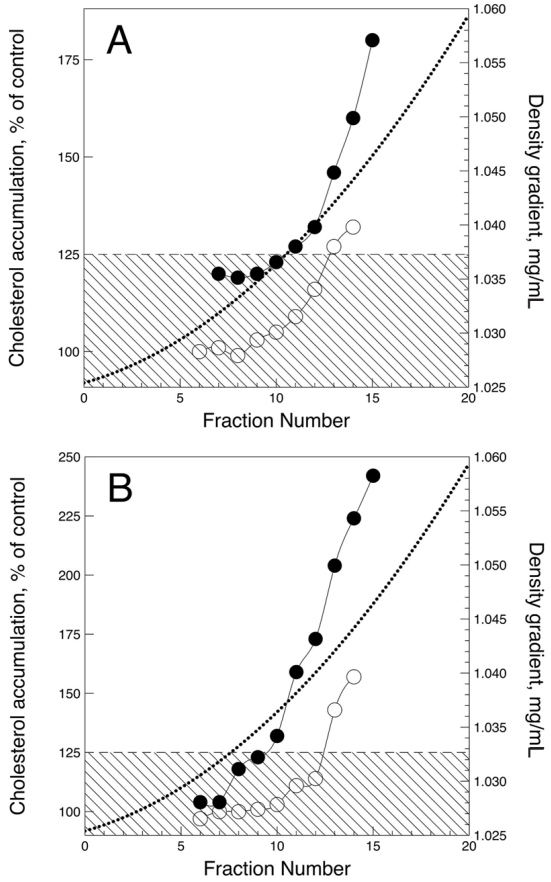

Figure 2: Atherogenic effects of sialylated (hollow circles) and desialylated (filled circles) LDL density subfractions from type 1 (A) and type 2 (B) diabetic patients. Atherogenic effect was assessed as the ability of LDL (50 μg/mL) to induce significant increase in the cholesterol content of subendothelial cells cultured from normal human aortic intima. Intracellular cholesterol content is expressed as the percentage of cholesterol level in control cells. The rectangular shaded areas denote the range of statistically insignificant changes in intracellular cholesterol level. LDL: low-density lipoprotein

Size of sialylated and desialylated LDL

The size of total, sialylated, and desialylated LDL particles was assessed according to their migration distance in polyacrylamide gradient gel electrophoresis performed under non-denaturing conditions. As shown in Table 3, in every studied group, desialylated LDL particles appeared significantly smaller than the those in the total LDL preparation and more so than those in the sialylated LDL fraction. However, there was no significant difference in LDL size between non-diabetic individuals, type 1, and type 2 diabetic patients, with respect to either total LDL, sialylated LDL, or desialylated LDL.

Electrophoretic mobility of sialylated and desialylated LDL

The surface net charge of sialylated and desialylated LDL was judged according to their electrophoretic mobility in agarose gel. As shown in Table 4, desialylated LDL fractions in both non-diabetic and diabetic patients had higher mobility than sialylated LDL fractions, and this difference was statistically significant among diabetic patients ($P = 0.041$). Sialylated LDL from diabetic patients also seemed to have higher mobility than sialylated LDL from normal controls, but this difference was not statistically significant [Table 4].

Table 2: The effect of total, sialylated and desialylated LDL on intracellular cholesterol level

Group	Cholesterol accumulation, % above control		
	Total LDL	Sialylated LDL	Desialylated LDL
Healthy subjects	5 (3)	3 (3)	26 (9)*†‡
Type 1 diabetic patients	47 (10)*	9 (2)†	110 (45)*†‡
Type 2 diabetic patients	100 (29)*	19 (10)†	169 (33)*†‡

*Significant intracellular cholesterol accumulation, $P < 0.05$ *vs.* control; †significant difference from total LDL, $P < 0.05$; ‡significant difference from sialylated LDL, $P < 0.05$. LDL: low-density lipoprotein

Table 3: The size of total, sialylated and desialylated LDL particles

Group	LDL particle size, nm		
	Total LDL	Sialylated LDL	Desialylated LDL
Healthy subjects	25.4 (1.2)	26.6 (1.4)	23.1 (0.9)*†
Type 1 diabetic patients	26.6 (1.9)	27.2 (1.0)	22.5 (0.6)*†
Type 2 diabetic patients	25.1 (0.7)	26.3 (1.2)	22.3 (0.5)*†

*Significant difference from total LDL, $P < 0.05$; †significant difference from sialylated LDL, $P < 0.05$. LDL: low-density lipoprotein

Table 4: Relative electrophoretic mobility of sialylated and desialylated LDL fractions

Group	Relative LDL mobility	
	Sialylated LDL	Desialylated LDL
Healthy subjects	1.00 (0.07)	1.27 (0.19)
Type 1 diabetic patients	1.05 (0.10)	1.55 (0.17)*
Type 2 diabetic patients	1.29 (0.21)	1.85 (0.29)*

*Significant difference from sialylated LDL, $P < 0.05$. LDL: low-density lipoprotein

DISCUSSION

We have shown previously that blood sera taken from diabetic patients, in contrast to sera from non-diabetic individuals, can induce cholesterol deposition in cultured cells derived from uninvolved human aortic intima, mainly because of LDL.[13] LDL particles carry a variety of modifications, which are usually inherently atherogenic, unlike native LDL.[10] The increase of a fraction of modified LDL can cause disruption of lipid metabolism and contribute to the development of atherosclerotic and metabolic diseases such as diabetes. According to Tsai *et al.*,[30] modified LDL levels were significantly higher in stroke survivors, thus attributing it as a risk factor for stroke outcome.

LDL particles in humans are heterogeneous in a polysaccharide moiety, and contain a single molecule of apolipoprotein B-100 per particle, and also 80-100 molecules of secondary proteins, approximately 1,500 molecules of esterified and non-esterified cholesterol, and a varying amount of other lipids.[31] It is possible to divide the LDL pool into two different sub-fractions [sialylated (sialic acid-rich) LDL and desialylated (sialic acid-poor) LDL]. Such separation is possible by using a column with RCA_{120} immobilized on CNBr-activated agarose.[12,21] Compared with sialylated LDL, desialylated LDL particles are smaller in size and contain more oxysterols, and less phospholipids and antioxidants.[12] In terms of the ability to induce intracellular deposition of lipids, the desialylated LDL fraction is atherogenic.[12,23,32] Several studies have reported an elevation of sialic acid serum levels in CHD patients, and also on its correlation with the severity of the coronary lesions.[33] Diabetic patients' LDL, by the first approach, appeared to have a decreased level of sialic acid, and further investigations have shown that this was due to an increased proportion of desialylated LDL fraction in patients' blood and a greater extent of its desialylation.[13,14] This LDL fraction was also characterized by increased non-enzymatic glycation and altered lipid composition, namely, decreased content of esterified cholesterol and elevated level of lyso-phospholipids.[13,14] Taken together, these findings indicate that this LDL fraction with multiple modifications has marked atherogenic potency. In the present study, desialylated LDL from diabetic patients was additionally examined by density, particle size, and electrophoretic mobility to obtain a more complete physicochemical characterization of this atherogenic LDL fraction.

The density distribution profile of desialylated LDL from diabetic patients differed from that of sialylated LDL, evidently owing to the presence of denser particles. While this difference in LDL density between sialylated and desialylated LDL was also observed in non-diabetic individuals, it was markedly distinct among diabetics. Desialylated LDL was also characterized by diminished particle size, approximately by 1.2-fold as compared with sialylated LDL.

It has been shown that sdLDL was characterized as highly atherogenic, and its level strongly correlated with cardiovascular disease risk.[15,16] The presence of sdLDL is claimed to be highly associated with atherosclerosis development, and several studies have attempted to identify the risk of ischemic atherosclerotic events according to LDL subclass pattern.[15,16,18] The higher prevalence of small dense LDL has been associated with cardiovascular events, acute ischemic stroke onset, and short-term mortality after acute ischemic stroke.[33-36] Our data demonstrate that in the blood of the same patient, there are at least two distinct forms of LDL differing by particle size and density. Obviously, the LDL pattern would strongly depend on what LDL fraction prevails in circulation.

The most prominent feature of desialylated LDL is its ability to induce intracellular cholesterol accumulation.[12,13,23,32] We have found previously that LDL atherogenicity in CAD patients and diabetic patients correlates negatively with sialic acid levels.[13] Indeed, *in vitro* studies have shown that desialylated LDL treated with neuraminidase, which removes the sialic acid residues, results in a significant increase in LDL potency to induce lipid accumulation in cultured monocyte-macrophages or intimal cells.[32] However, whether the loss of sialic acid in circulating LDL is the primary reason for their atherogenicity, remains unclear. The so-called desialylated LDL is characterized by several alterations to its chemical composition and physical properties, low sialic acid level being only one attribute of a wider scope of changes including the density, size, and electric charge of particles.[12,18,23]

Desialylated LDL showed higher mobility on agarose gel electrophoresis, thereby indicating that this LDL fraction has an elevated surface net charge, i.e. is a more electronegative LDL particle.[18] It is definitely known that numerous techniques of *in vitro* LDL modification (e.g.

acetylation, metal-dependent oxidation, methylation, malonic dialdehyde treatment, *etc*.) lead to the formation of anionic LDL thus rendering it atherogenic.[31] It is possible that LDL surface charge plays a significant role in the processes of lipoprotein-to-cell interaction, and charge alterations may substantially modify LDL cellular metabolism, thus resulting in lipid deposition. The elevated electrophoretic mobility of desialylated LDL may be readily explained considering the significantly reduced content of free amino groups of desialylated apoB.[30,31]

A smaller size and increased density of desialylated LDL particles seem to be typical attributes of modified atherogenic LDL.[18,31] In our study, LDL's ability to induce intracellular cholesterol accumulation increased with LDL density. Desialylated LDL of lower density was not atherogenic, and the densest sialylated LDL was capable of inducing a moderate increase in cellular cholesterol. However, this does not necessarily imply that the density or size of LDL particles are the primary determinants of their atherogenicity. As it was demonstrated previously, desialylated LDL is also characterized with decreased content of neutral sugars such as galactose and mannose and acetylated residues such as N-acetylglucosamine.[31] It may be speculated that some extensively modified LDL particles may be deprived of not only sialic acid, but also of galactose residues, that usually become terminal saccharides in biantennary polysaccharide chains in apoB after desialylation. Such particles cannot be isolated from the total LDL preparation by RCA_{120} affinity chromatography and would contaminate non-bound LDL fractions that are generally thought to be non-modified, sialic acid-rich LDL. Assuming that diminished particle size, increased density, and loss of sugar residues are the processes characteristic for LDL modification and work in parallel, the densest part of non-bound (so-called sialylated) LDL would contain some amount of exceptionally modified LDL that could raise the intracellular cholesterol levels. Additionally, desialylated or sialylated LDL from diabetic patients did not differ significantly with respect to particle size from corresponding LDL fractions from healthy subjects; the difference, however, was dramatic with regard to the atherogenic effect. Therefore, the size or density of LDL particles may be regarded as the marker of LDL atherogenic modification, but seem to be secondary events reflecting the alterations in chemical composition of LDL that have occurred before. However, it may be speculated that smaller LDL particle size may lead to conformational changes in those apoB domains responsible for interaction with cell receptors. However, in this case, one should provide mechanistic explanations of the effect of LDL particle size on its binding, uptake, and internalization by cells; such explanation was provided only for desialylated LDL.[37]

Interestingly, LDL desialylation is assumed to be a systemic process, which affects not only LDL as a single target. Recently Koska *et al*.[38] have reported that properly glycosylated apoC-III proteoform (i.e. apoprotein-bearing 2 sialic acid residues per every biantennary polysaccharide chain), in contrast to partially or totally desialylated proteoforms, is associated with beneficial lipid profile in prediabetic and type 2 diabetic patients. It is possible that apoC-III non-sialylated, monosialylated, and di-sialylated isoforms occur in circulation owing to desialylation (or another kind of deglycosylation), but not because of different pathways of posttranslational glycosylation in hepatocytes.

This study has certain limitations. First, it was of an experimental nature, and not aimed at estimating the clinical relevance of desialylated LDL with respect to diabetes and/or gender- and age-related differences, or association with metabolic control and therapy. Second, it was not possible to calculate the sample size prior to the study, because there were no available statistical data on variability of the studied parameters in healthy subjects and diabetic patients. Thus, only *post hoc* analysis was possible, which showed that the given sample size was sufficient to reach 96-100% statistical power at a 5% confidence level, with respect to observed inter-groups differences in LDL atherogenicity, electrophoretic mobility of desialylated LDL, and intra-group differences between sialylated and desialylated LDL particle sizes. Regardless, the results of our study conclude that there is a fraction of modified LDL along with native LDL in the blood of diabetic patients. This naturally occurring modified LDL is characterized by various alterations to its physicochemical properties. Our previous findings have shown that: (1) it is desialylated and non-enzymatically glycated lipoprotein; (2) it is characterized by lowered esterified cholesterol level and elevated content of lyso-phospholipids; and (3) it is atherogenic in terms of intracellular cholesterol accumulation. We believe our research presents new and significant abnormal peculiarities of this LDL: (1) it is a small, dense LDL; and (2) it is more electronegative than native LDL. Therefore, previously described atherogenic LDL fraction in diabetics can be regarded as multiple-modified LDL that may be assigned to have a significant role in early atherogenesis in diabetes mellitus. The origin and metabolic fate of this *in vivo* modified LDL remains to be studied.

Acknowledgments

We acknowledge the help of Dr. Olli Jaakkola and

Dr. Tiina Solakivi (University of Tampere, Tampere, Finland) in methodologic supervising of preliminary studies with LDL density fractionation.

Financial support and sponsorship

This study was supported in part by Russian Ministry of Education and Science, Project #RFMEFI61614X0010.

Conflicts of interest

There are no conflicts of interest.

Patient consent

All involved patients gave their consent forms.

Ethics approval

This study was approved by the local ethics committee of the Institute for Atherosclerosis Research, Skolkovo Innovation Center, Moscow, Russia.

REFERENCES

1. Ghosh P, Sahoo R, Vaidya A, Chorev M, Halperin JA. Role of complement and complement regulatory proteins in the complications of diabetes. *Endocr Rev* 2015;36:272-88.
2. Nathan DM, Bayless M, Cleary P, Genuth S, Gubitosi-Klug R, Lachin JM, Lorenzi G, Zinman B; DCCT/EDIC Research Group. Diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: advances and contributions. *Diabetes* 2013;62:3976-86.
3. Moore DJ, Gregory JM, Kumah-Crystal YA, Simmons JH. Mitigating micro-and macro-vascular complications of diabetes beginning in adolescence. *Vasc Health Risk Manag* 2009;5:1015-31.
4. Aronson D, Edelman ER. Coronary artery disease and diabetes mellitus. *Heart Fail Clin* 2016;12:117-33.
5. Kones R. Primary prevention of coronary heart disease: integration of new data, evolving views, revised goals, and role of rosuvastatin in management. A comprehensive survey. *Drug Des Devel Ther* 2011;5:325-80.
6. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015;161:161-72.
7. Shi H, Mao X, Zhong Y, Liu Y, Zhao X, Yu K, Zhu R, Wei Y, Zhu J, Sun H, Mao Y, Zeng Q. Lanatoside C promotes foam cell formation and atherosclerosis. *Sci Rep* 2016;6:20154.
8. Das A, Brown MS, Anderson DD, Goldstein JL, Radhakrishnan A. Three pools of plasma membrane cholesterol and their relation to cholesterol homeostasis. *Elife* 2014;3:02882.
9. Yang H, Mohamed AS, Zhou SH. Oxidized low density lipoprotein, stem cells, and atherosclerosis. *Lipids Health Dis* 2012;11:85.
10. Orekhov AN, Melnichenko AA, Sobenin IA. Approach to reduction of blood atherogenicity. *Oxid Med Cell Longev* 2014;2014:738679.
11. Bobryshev YV, Ivanova EA, Chistiakov DA, Nikiforov NG, Orekhov AN. Macrophages and their role in atherosclerosis: pathophysiology and transcriptome analysis. *Biomed Res Int* 2016;2016:9582430.
12. Zakiev ER, Sukhorukov VN, Melnichenko AA, Sobenin IA, Ivanova EA, Orekhov AN. Lipid composition of circulating multiple-modified low density lipoprotein. *Lipids Health Dis* 2016;15:134.
13. Sobenin, IA, Tertov VV, Koschinsky T, Bünting CE, Slavina ES, Dedov II, Orekhov AN. Modified low density lipoprotein from diabetic patients causes cholesterol accumulation in human intimal aortic cells. *Atherosclerosis* 1993;100:41-54.
14. Sobenin IA, Tertov VV, Orekhov AN. Characterization of chemical composition of native and modified low density lipoprotein occurring in the blood of diabetic patients. *Int Angiol* 1994;13:78-83.
15. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 1988;260:1917-21.
16. Musliner TA, Krauss RM. Lipoprotein subspecies and risk of coronary disease. *Clin Chem* 1988;34:B78-83.
17. Packard CJ. Small dense low-density lipoprotein and its role as an independent predictor of cardiovascular disease. *Curr Opin Lipidol* 2006;17:412-7.
18. Ivanova EA, Bobryshev YV, Orekhov AN. LDL electronegativity index: a potential novel index for predicting cardiovascular disease. *Vasc Health Risk Manag* 2015;11:525-32.
19. Puavilai G, Chanprasertyotin S, Sriphrapradaeng A. Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria. World Health Organization. *Diabetes Res Clin Pract* 1999;44:21-6.
20. Lindgren FT. Preparative ultracentrifugal laboratory procedures and suggestions for lipoproten analysis. In: Perkins EG, editor. Analysis of lipids and lipoproteins. New York: American Oil Chemical Society; 1975. p. 205-24.
21. Tertov VV, Sobenin IA, Tonevitsky AG, Orekhov AN, Smirnov VN. Isolation of atherogenic modified (desialylated) low density lipoprotein from blood of atherosclerotic patients: separation from native lipoprotein by affinity chromatography. *Biochem Biophys Res Commun* 1990;167:1122-7.
22. Jaakkola O, Solakivi T, Ylä-Herttuala S, Nikkari T. Receptor-mediated binding and degradation of subfractions of human plasma low-density lipoprotein by cultured fibroblasts. *Biochim Biophys Acta* 1989;1005:118-22.
23. Jaakkola O, Solakivi T, Tertov VV, Orekhov AN, Miettinen TA, Nikkari T. Characteristics of low-density lipoprotein subfractions from patients with coronary artery disease. *Coron Artery Dis* 1993;4:379-85.
24. Hara A, Radin NS. Lipid extraction of tissues with a low-toxicity solvent. *Anal Biochem* 1978;90:420-6.
25. Siedel J, Hägele EO, Ziegenhorn J, Wahlefeld AW. Reagent for enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem* 1983;29:1075-80.
26. Lowry OH, Rosebrough NJ, Farr AL, Randall BJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
27. Lipid Research Clinics Program. In: Manual of Laboratory Operations, Lipid and Lipoprotein Analysis, DHEW Publication No. (NIH) 75-628. 2nd ed. Washington, DC: U. S. Government Printing Office; 1974. p. 1-81.
28. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J Lipid Res* 1982;23:97-104.
29. Sobenin IA, Tertov VV, Orekhov AN. Optimization of the assay for sialic acid determination in low density lipoprotein. *J Lipid Res* 1998;39:2293-9.
30. Tsai NW, Lee LH, Huang CR, Chang WN, Chang YT, Su YJ, Chiang YF, Wang HC, Cheng BC, Lin WC, Kung CT, Su CM, Lin YJ, Lu CH. Statin therapy reduces oxidized low density lipoprotein level, a risk factor for stroke outcome. *Crit Care* 2014;18:R16.
31. Liu Y, Atkinson D. Enhancing the contrast of ApoB to locate the surface components in the 3D density map of human LDL. *J Mol Biol* 2011;405:274-83.

32. Aksenov DV, Medvedeva LA, Skalbe TA, Sobenin IA, Tertov VV, Gabbasov ZA, Popov EV, Orekhov AN. Deglycosylation of apo B-containing lipoproteins increase their ability to aggregate and to promote intracellular cholesterol accumulation in vitro. *Arch Physiol Biochem* 2008;114:349-56.
33. Nigam PK, Narain VS, Kumar A. Sialic acid in cardiovascular diseases. *Indian J Clin Biochem* 2006;21:54-61.
34. Zeljkovic A, Vekic J, Spasojevic-Kalimanovska V, Jelic-Ivanovic Z, Bogavac-Stanojevic N, Gulan B, Spasic S. LDL and HDL subclasses in acute ischemic stroke: prediction of risk and short-term mortality. *Atherosclerosis* 2010;210:548-54.
35. Oravec S, Krivosikova Z, Krivosik M, Gruber K, Gruber M, Dukát A, Gavorník P. Lipoprotein profile in patients who survive a stroke. *Neuro Endocrinol Lett* 2011;32:496-501.
36. Albers JJ, Slee A, Fleg JL, O'Brien KD, Marcovina SM. Relationship of baseline HDL subclasses, small dense LDL and LDL triglyceride to cardiovascular events in the AIM-HIGH clinical trial. *Atherosclerosis* 2016;251:454-9.
37. Orekhov AN, Tertov VV, Sobenin IA, Smirnov VN, Via DP, Guevara J Jr, Gotto AM Jr, Morrisett JD. Sialic acid content of human low density lipoproteins affects their interaction with cell receptors and intracellular lipid accumulation. *J Lipid Res* 1992;33:805-17.
38. Koska J, Yassine H, Trenchevska O, Sinari S, Schwenke DC, Yen FT, Billheimer D, Nelson RW, Nedelkov D, Reaven PD. Disialylated apolipoprotein C-III proteoform is associated with improved lipids in prediabetes and type 2 diabetes. *J Lipid Res* 2016;57:894-905.

Todua *et al. Vessel Plus* 2017;1:38-42
DOI: 10.20517/2574-1209.2016.06

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Short Communication **Open Access**

Homocysteine and D-dimer levels and multilayer computed tomography for diagnosing pulmonary artery thromboembolism

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How to cite this article: Todua F, Akhvlediani M, Vorobiova E, Tsivtsivadze G, Baramidze A. Homocysteine and D-dimer levels and multilayer computed tomography for diagnosing pulmonary artery thromboembolism. Vessel Plus 2017;1:38-42.

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Article history:
Received: 17-10-2016
Accepted: 29-12-2016
Published: 31-03-2017

Key words:
Homocysteine,
D-dimer,
pulmonary embolism

ABSTRACT

Aim: D-dimer reportedly plays a leading role in diagnosing pulmonary embolism. Additionally, homocysteine is an established risk factor for atherosclerosis, vascular disease, and thrombosis. Herein, the authors aimed to evaluate the diagnostic significance of D-dimer and homocysteine levels, together with multi-detector computed tomography (CT) in suspected pulmonary embolism. **Methods:** The authors examined patients suffering from conditions and complaints that are typical of pulmonary artery thromboembolism (PATE), such as chest pain, haemoptysis, dyspnoea, tachycardia, arterial hypotension, and signs of vein thrombosis in the inferior limbs. In these patients, PATE was found in different localizations with varying rates of severity. D-dimer levels were measured in patients with suspected PATE using enzyme-linked immunosorbent assays. Homocysteine levels were determined by an enzymatic method. All patients were examined to evaluate the presence of pulmonary embolism by multi-detector CT angiopulmonography. **Results:** Changes in homocysteine levels can be considered a separate independent factor for PATE diagnostics. The correlation between multi-detector CT angiopulmonography, elevated D-dimer levels, and concomitant hyperhomocysteinemia can be used not only for diagnostics but also for the assessment of the effectiveness of PATE treatment. **Conclusion:** Multi-detector CT angiopulmonography, D-dimer levels and related hyperhomocysteinemia can serve as significant laboratory markers in the diagnosis and treatment efficacy of PATE.

Quick Response Code:

INTRODUCTION

Pulmonary artery thromboembolism (PATE) is an acute occlusion of the pulmonary trunk, or its branches, with thrombi, formed in the veins of the greater circulation, or in the cavities of the right section of the heart. Modern medical literature uses the term pulmonary embolism. More than 500,000 cases of pulmonary embolism are diagnosed annually.[1-3]

Modern diagnostics of PATE have faced substantial difficulties, associated with polymorphism of developing clinical syndromes; suddenness and catastrophic speed of disease development, and the lack of highly informative methods of investigation (perfusive scintigraphy of the lungs, pulmoangiography) in some hospitals.[3-5]

The diagnostic problems that need to be solved when patients have suspected PATE are:
(1) to confirm the presence of an embolism;
(2) to determine the exact location of the thromboembolism in pulmonary vessels;
(3) to determine the rate of embolic damage of pulmonary vessel beds;
(4) to assess the haemodynamic state in the greater circulation and the lesser circulation;
(5) to ascertain the source of the embolism and to assess the probability of relapse.

Venous thromboembolism (VTE) is associated with a 5% to 27% annual risk of recurrence after the discontinuation of anticoagulant (AC) therapy, and indefinite AC treatment is recommended if the bleeding risk is low-to-moderate. However, in one-third of patients with unprovoked VTE, the risk of recurrence is so low (< 3% per year) that AC therapy > 3 months may not be necessary.[4,5]

Pulmoangiography is the method of choice in pulmonary embolism diagnostics. However, this examination is associated with discomfort, elevated cost, and the risk of serious complications, typical of invasive procedures. Ventilation-perfusion scintigraphy is widely used at the initial stage of revealing pulmonary embolism, but the validity of the method is limited due to numerous uncertain conclusions. Precise presentation of pulmonary architectonics has become possible since the invention of spiral, and then multilayer scanners, which can provide a higher accuracy of diagnostics.[3,5]

It should also be noted that numerous retrospective and prospective investigations have shown a close interdependence between homocysteine (a sulphur-containing amino acid that is an intermediate product of methionine and cysteine exchange) and cardiovascular diseases, such as venous and arterial thromboses, pulmonary embolism, stroke and myocardial infarction. Hyperhomocysteinemia can suppress a whole range of anti-coagulating mechanisms, which involve the mediation of the vascular endothelium in pathological processes, and as a result, even a moderate increase in homocysteine levels in the blood can cause not only arterial thrombosis and atherosclerosis, but also vein thrombosis.[6-8]

Evaluation of D-dimer levels has an active application in current clinical practice, and provides additional information for thrombosis diagnostics (D-dimers are molecules that circulate in the blood during the development of thrombi, which can be resolved under the action of a fibrinolytic system).[9,10]

METHODS

The aim of our investigation was to assess the importance of measuring D-dimer and homocysteine levels, along with the use of multilayer computer tomography (CT), in the diagnosis of patients with suspected PATE.

We examined 54 patients (31 males and 23 females) from 18 to 76 years of age, who were suffering from conditions and complaints that are typical of PATE, such as chest pain, haemoptysis, dyspnoea, tachycardia, arterial hypotension and signs of vein thrombosis in the inferior limbs. In 51 patients (94.4%), PATE was evident in different localizations at varying rates of severity.

A total of 27 healthy persons, with an average age of 52.3 ± 1.3 years old, formed a control group. The investigation was randomized, as we did not include patients with normal values of D-dimer, and there were no patients with the above mentioned complains (chest pain, haemoptysis, dyspnoea, tachycardia, arterial hypotension and signs of vein thrombosis in the inferior limbs). All investigations were conducted at the Research Institute of Clinical Medicine.

D-dimer levels were measured in patients with suspected PATE using enzyme-linked immunosorbent assays. Patients with D-dimer levels > 500 ng/L were subjected to ultrasonic scanning of proximal veins of inferior limbs with compression, and to multilayer CT. Homocysteine levels were measured by an enzymatic method using the biochemical analyser COBAS INTEGRA 400 PLUS (Roche Diagnostics).

Multilayer CT (MLCT) was carried out using the Siemens SOMATOM Sensation 16 Cardiac. During MLCT-pulmoangiography after native examination of

Table 1: Frequency (%) of DVT in extremities and PATE in patients at various risk rates

Risk rate	DVT/PATE risks (objective test data)			
	Crus vein thrombosis	Proximal phlebothrombosis	Clinical PATE	Mortal PATE
High	40-80	10-30	5-10	1-5
Average (moderate)	10-40	2-10	1-8	0.1-0.7
Low	< 10	< 1	< 1	< 0.01

PATE: pulmonary arteria thromboembolism; DVT: deep vein thrombosis

chest organs, we performed contrast enhancement of the pulmonary artery channel, by injecting 70-80 mL of a contrast substance using an automatic injector at 3 mL/s; the delay time was 9-11 s, which enabled the initiation of tomography when there was the highest concentration of the contrast substance (1,220 Hertz units) in the area of interest, namely the pulmonary artery trunk. During the CT procedure, the patient was instructed to hold his/her breath, or tried to breathe very shallowly. We assessed the condition of pulmonary arteries and their branches up to the sub-segmental level [Table 1].

Ultrasonic scanning was carried out in B-mode, examining common femoral and popliteal veins. The incomplete vascular embarrassment of these veins during compression was considered as the criterion for thrombosis.

All patients were examined with CT and ultrasonic scanning.

Anticoagulants were prescribed to the patients with thrombosis of proximal deep veins, (ultrasonic scanning and negative CT results). The main indication for analyses was the portion of patients with thrombosis of proximal deep veins and negative CT results. Risk of thromboembolism during the 3 months of attendance was the second indication, if ultrasonic scanning of inferior limbs was not carried out.

Modern methods of variation statistics (Windows 7, SPSS21 software), performed in Microsoft Excel, were used for statistical processing of obtained results. Sampled simple average (M), simple average of error (m), and average standard deviation (σ), were used. Student's criterion (T) was used to define reliability of the difference between simple average values. Comparison of the student's distribution was made. The value of P was < 0.5 in the groups which we studied. To determine the ratio between variables, we used Pearson's correlation coefficient, where x_i and y_i are values of compared variables, $\bar{x}$ and $\bar{y}$ are mean values of these variables, and r is the correlation coefficient.

Table 2: Homocysteine and D-dimer levels in patients with pulmonary embolism and control group

Laboratory parameters	Patients with pulmonary embolism (n = 54)	Control group (n = 27)
D-dimer (ng/mL)	950.0 ± 6.0	500.0 ± 3.0
Homocysteine (μmol/L)	26.2 ± 0.4	9.1 ± 0.3

RESULTS

Our studies showed that hyperhomocysteinemia of up to 26.2 ± 0.4 μmol/L was found in 50 out of 54 patients (92.6%). Homocysteine did not exceed admissible levels and was 8.64 ± 0.20 μmol/L in 4 patients [Table 2]. It should be noted that homocysteine levels in healthy males and females aged 30 years and above is between 4 and 14 μmol/L. A homocysteine concentration of > 15 μmol/L indicates a high risk of developing cardiovascular diseases.[6]

Correlation analysis showed a significant positive interdependence between laboratory test results of D-dimer and homocysteine levels with a correlation coefficient of 0.557.

The series of computer tomograms during MLCT angiography clearly show thrombi in the lumen of pulmonary trunk and its branches of lobar and segmental order.

Thrombi are seen in the lumen as defects of vessel filling, having clear, even outlines of various forms (oval, protruded, irregular form, V-form), dimensions of 2-16 mm and extensions of up to 35 mm. The thrombi have soft-tissue density (35-50 Hertz units), homogeneous structure and partially or completely occlude the damaged vessel.

Multilayer CT pulmoangiography revealed signs of PATE in 19 out of 21 patients (90.5%), who showed a high probability of disease, evaluated from clinical data. During ultrasound scanning, thrombosis of deep proximal veins was found in 9 out of 19 patients (47.4%). Only 1 out of 21 patients (0.5%) had thrombosis of proximal deep veins and negative CT results. PATE was not revealed by clinical results of CT or ultrasound scanning in 3 patients who had a high probability of PATE, and further angiography also gave negative results. A total of 23 out of 33 patients (69.6%) with a low-to-medium probability of PATE had D-dimer levels > 500 ng/L. CT revealed symptoms of PATE in 8 of these 33 patients (24%), and thrombosis of proximal deep veins was found in 3 of these 8 patients (37.5%) during ultrasound scanning. Only 2 patients (0.6%) with low-to-medium PATE probability had thrombosis of maximally deep veins and negative CT results

[Table 1]. MLCT and pulmoangiography revealed pulmonary infarction of various sizes and localization in 13 (24%) patients, while in 41 (76%) patients, these signs were absent. According to the statistics, PATE causes complications in the form of infarction in 10-30% of cases.

DISCUSSION

Pulmonary angiography is a gold standard of PATE diagnostics. However, this method is invasive, causes various complications and is not widely available. In most cases, PATE diagnosis is based on the combination of laboratory tests and radio-diagnostics, such as perfusive radionuclide scintigraphy and echocardiography (cardiac ultrasound), which reveal indirect symptoms of embolism (dilatation of the right ventricle of heart and pulmonary hypertension), observed in severe forms of PATE. CT allows the visualisation of pulmonary arteries and thrombi themselves, as well as the ability to assess structures of mediastinum and parenchyma, which is the main advantage of CT. According to some authors, two thirds of patients with suspected PATE were diagnosed as having aorta dissection, pneumonia, lung carcinoma, and pneumothorax. In addition, the presence of complicated pulmonary embolism (pulmonary infarction, pleural effusion, re-induced vascular picture) is possible.[5,8]

With the introduction of high resolution multilayer CT, all the problems associated with the lack of accuracy using spiral CT in PATE diagnostics have been overcome. Using of 14-16 and 64-layer CT decreases the number of unrevealed cases of deseases. Furthermore, visualization of proximal, lobar, and more distal artery branches (at segmental and sub-segmental levels) is provided, revealing peripheral emboli, with precise assessment of their extent being possible.[9]

Moreover, to reveal deep vein thromboses, it is possible to combine chest examination with vein CT angiography.

Using reconstructive images, left and right ventricles in axial and four-chamber projections can be assessed and measured. Right ventricle dilation is an unfavourable prognostic factor.[10]

We recommend CT examination to be performed at 3 months after diagnosis of PATE, as this period is sufficient for thrombus resolution. Perfusive radionuclide scintigraphy, recommended for a more accurate diagnosis is characterized with high radiation exposure, compared to MLCT, and is not useful for revealing an anatomic picture of the disease.[7,9]

On the basis of numerous results, obtained during the investigation of haemostasis and homocysteine, according to which homocysteine can cause a damaging effect on thrombocytes, the interdependence between the rate of D-dimer changes and observed hyperhomocysteinemia becomes evident. Moderate hyperhomocysteinemia, up to 19.0 μmol/L not complicated with thromboembolism was observed in patients with vein thrombosis in inferior limbs.[7,11] If we take considering that even moderate hyperhomocysteinemia can cause oxidative stress (homocysteine oxidation results in the formation of free radicals, which activate the formation of thrombi, in turn resulting in hypercoagulation), then the mechanism of the results observed in patients with thrombosis of proximal deep veins and in thromboembolism of pulmonary artery becomes evident.[11,12]

It can be concluded that multilayer CT pulmoangiography is a non-invasive highly informative method, providing a very low level of complications, and is thus indicated for acute PATE diagnostics, and for control of the disease dynamics after anticoagulation treatment. Moderate hyperhomocysteinemia, observed in cases of vein thrombosis of inferior limbs, uncomplicated by PATE, deserves further attention to determine the fate of these patients and the appropriate treatment to provide. The changes in homocysteine levels can be considered as a separate independent factor for PATE diagnostics. The interdependence between D-dimer and concomitant hyperhomocysteinemia can be used not only for diagnostics, but also for the assessment of the effectiveness of PATE treatment.

Authors' contributions

Concept: F. Todua
Design: M. Akhvlediani
Definition of intellectual content: M. Akhvlediani, E. Vorobiova
Literature search: A. Baramidze
Clinical studies: F. Todua, G. Tsivtsivadze
Data acquisition: G. Tsivtsivadze
Statistical analysis: A. Baramidze
Manuscript preparation, editing and review: M. Akhvlediani, E. Vorobiova

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

All the patients'consent is mentioned in their medical case history.

Ethics approval

The study has been approved by the responsible committee on human experimentation of research institute of clinical medicine (protocol 3 25.08.2016).

REFERENCES

1. Asfar S, Safar H. Homocysteine levels and peripheral arterial occlusive disease: a prospective cohort study and review of the literature. *J Cardiovasc Surg (Torino)* 2007;48:601-5.
2. Bělohlávek J, Dytrych V, Linhart A. Pulmonary embolism, part I: Epidemiology, risk factors and risk stratification, pathophysiology, clinical presentation, diagnosis and non-thrombotic pulmonary embolism. *Exp Clin Cardiol* 2013;18:129-38.
3. Kearon C, Akl EA, Comerota AJ, Prandoni P, Bounameaux H, Goldhaber SZ, Nelson ME, Wells PS, Gould MK, Dentali F, Crowther M, Kahn SR; American College of Chest Physicians. Antithrombotic therapy for VTE disease: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012;141:e419S-94S. Erratum in *Chest* 2012;142:1698-704.
4. Ling IT, Naqvi HA, Siew TK, Loh NK, Ryan GF. SPECT ventilation perfusion scanning with the addition of low-dose CT for the investigation of suspected pulmonary embolism. *Intern Med J* 2012;42:1257-61.
5. Patel S, Kazerooni EA, Cascade PN. Pulmonary embolism: optimization of small pulmonary artery visualization at multi-detector row CT. *Radiology* 2003;227:455-60.
6. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, Johnston C, Engbaek F, Schneede J, McPartlin C, Scott JM. Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem* 2004;50:3-32.
7. Rodger MA, Kahn SR, Wells PS, Anderson DA, Chagnon I, Le Gal G, Solymoss S, Crowther M, Perrier A, White R, Vickars L, Ramsay T, Betancourt MT, Kovacs MJ. Identifying unprovoked thromboembolism patients at low risk for recurrence who can discontinue anticoagulant therapy. *CMAJ* 2008;179:417-26.
8. Remy-Jardin M, Pistolesi M, Goodman LR, Gefter WB, Gottschalk A, Mayo JR, Sostman HD. Management of suspected acute pulmonary embolism in the era of CT angiography: a statement from the Fleischner Society. *Radiology* 2007;245:315-29.
9. Robinson K, Mayer E, Jacobsen DW. Homocysteine and coronary artery disease. *Cleve Clin J Med* 1994;61:438-50.
10. Troy B. Targeted D-dimer testing best for DVT diagnosis. *Ann Intern Med* 2013;158:93-100.
11. van Belle A, Büller HR, Huisman MV, Huisman PM, Kaasjager K, Kamphuisen PW, Kramer MH, Kruip MJ, Kwakkel-van Erp JM, Leebeek FW, Nijkeuter M, Prins MH, Sohne M, Tick LW; Christopher Study Investigators. Effectiveness of managing suspected pulmonary embolism using an algorithm combining clinical probability, D-dimer testing, and computed tomography. *JAMA* 2006;295:172-9.
12. Vedovati MC, Becattini C, Agnelli G, Kamphuisen PW, Masotti L, Pruszczyk P, Casazza F, Salvi A, Grifoni S, Carugati A, Konstantinides S, Schreuder M, Golebiowski M, Duranti M. Multidetector CT scan for acute pulmonary embolism: embolic burden and clinical outcome. *Chest* 2012;142:1417-24.

Vakhtangadze *et al. Vessel Plus* 2017;1:43-8
DOI: 10.20517/2574-1209.2016.05

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Editorial **Open Access**

Myocardial ischemia in women: problems and challenges

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How to cite this article: Vakhtangadze T, Gakhokidze N. Myocardial ischemia in women: problems and challenges. Vessel Plus 2017;1:43-8.

Article history: Received: 25-09-2016 Accepted: 26-12-2016 Published: 27-06-2017

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INTRODUCTION

Even in the 21st century, cardiovascular disease (CVD) remains an important public health issue because it is associated with high rates of mortality and morbidity.[1-5] In addition, the costs of treating and managing these patients are very high, as such patients have a constant need for medical treatment and their management often requires sophisticated and expensive tests.[6] The use of preventive measures, identification of high-risk groups,[7] early diagnosis, and treatment of women presenting with cardiovascular problems can all have a significant influence on reducing cardiovascular mortality, morbidity, and treatment costs, as has been shown in various countries.

For a long time, it was thought that CVD predominantly affects men. However, over the last two decades, it has been revealed women have higher rates of cardiovascuar morbidity and mortality. For example, 49% of European women die from CVD, compared with 41% of men.[3] At present, the basic statistical facts are as follows:[1,3,5,8]

• Worldwide, one-third of all deaths among women are due to CVD, with up to 8.6 million women dying from CVD each year. Furthermore, stroke kills more women than men (11% *vs*. 8.4%).

• More than two-thirds (approximately 42%) of women who have heart attacks die within 1 year, compared with 24% of men.

• Under the age of 50 years, women have double the risk of death after a heart attack than men.

• Heart attack and heart failure kill 6 times more women than breast cancer every year.

Quick Response Code:

By the end of the 1990s, cardiovascular morbidity in women started to drew particular attention, leading to studies and intensive work by the world's top cardiology societies. At the European Society of Cardiology 2005 conference, for example, very interesting data were presented about the situation in Europe.[9] It was revealed that:

- Women are underrepresented in cardiovascular research and trials.
- Women are less likely to analyze the influence of their risk factors on morbidity and mortality rates, and awareness about CVD among women is low.
- Women are less likely to undergo primary and secondary prevention.
- Women seek medical care less often than men.
- Fewer women than men undergo diagnostic tests, which results in a delay in diagnosis and a resulting delay in treatment.

Currently, the following categories of women's cardiovascular health are provided in modern guidelines:[10,11]

High risk (at least one high-risk state): clinical manifestations of coronary heart disease, cerebrovascular disease, peripheral arterial disease, abdominal aortic aneurysm, end-stage or chronic kidney disease, diabetes, coronary heart disease with a 10-year risk > 10%.

At risk (at least one risk factor from the following): tobacco consumption, arterial hypertension (systolic pressure > 120 mmHg, diastolic pressure > 80 mmHg, or treatment with antihypertensive drugs), total cholesterol > 200 mg/dL, high-density lipoprotein cholesterol < 50 mg/dL or treatment of dyslipidemia, obesity (especially central obesity), poor nutrition, low physical activity, family history of premature CVD occurring in a first-degree relative in men aged < 55 years or women aged < 65 years, the metabolic syndrome, evidence of subclinical atherosclerosis, poor exercise capacity on treadmill test and/or abnormal heart rate recovery, systemic autoimmune collagen vascular disease (e.g. lupus, rheumatoid arthritis), history of pre-eclampsia, gestational diabetes, or pregnancy-induced hypertension.

Ideal cardiovascular health: total cholesterol < 200 mg/dL, arterial blood pressure < 120/80 mmHg, fasting glucose < 100 mg/dL, non-smoker, healthy diet.

Raising awareness of gender-specific risk factors will have an impact on women's cardiovascular health. The purpose of this article is to evaluate the modern views with respect to the diagnostic tools used to determine ischemia in women.

PATHOPHYSIOLOGICAL FEATURES OF MYOCARDIAL ISCHEMIA IN WOMEN

In general, the pathophysiological features of myocardial ischemia differ in women and men. There is a significant amount of data implicating the influence of sex hormones on the presentation of chest pain and electrocardiograph (ECG) changes.

In the mid-1990s, the US National Heart, Lung, and Blood Institute sponsored the Women's Ischemia Syndrome Evaluation (WISE) study, one of the cornerstones in the evaluation of myocardial ischemia in women,[12] which assessed 936 women with chest pain. The aims of the study were to optimize the evaluation of symptoms and diagnostic tests; to explore the mechanisms of symptoms and of myocardial ischemia in the absence of epicardial coronary artery stenosis; and to evaluate the influence of reproductive hormones on symptoms and the results of diagnostic tests.[12-14]

The results of the study were published in scientific papers over a long period of time. Based on these results, four groups of women with chest pain have been described:

1. Women with severe obstructive coronary artery disease (CAD) and myocardial ischemia.
2. Women with obstructive CAD but without myocardial ischemia.
3. Women without obstructive CAD but with myocardial ischemia.
4. Women without obstructive CAD and without myocardial ischemia.

One of the conclusions of the WISE study: quality of life is determined more by chest pain than by the presence of myocardial ischemia.

Women falling into the above categories present daily at medical facilities with chest pain, dyspnea, or other symptoms, and the proper differentiation of any underlying conditions will determine the need for further investigations and the most appropriate treatment options. These underlying conditions are macrovascular diseases, obstructive coronary atherosclerosis, and microvascular disease.

Among the women included in the WISE study, 37% did not have angiographic evidence of obstructive CAD; rather, they presented with normal or nearly normal coronary arteries (< 20% stenosis). In 25% of women, non-obstructive CAD was found (at least one 20-50% stenosis). And in 38% of patients included in the study, severe obstructive CAD was revealed (> 50% stenosis). Therefore, 62% of women with angina included in the study did not have severe obstructive CAD.[12-14]

Similar results have been obtained in other studies. In 2015, Lee *et al*.[15] published the results of a prospective study that evaluated patients with non-obstructive CAD. Overall, 77% of patients in the cohort were women; 44% of all patients had endothelial dysfunction, 21% had microvascular impairment, and 5% had a reduced fractional flow reserve. In 23% of cases, it was not possible to determine the cause of coronary symptoms. This study confirmed the results of previous studies[16-18] indicating that while the symptoms of CAD are well understood, women tend to develop symptoms 10-15 years later than men, and have more risk factors by the time of symptom onset. The study by Lee *et al*.[15] did not evaluate the influence of hormonal factors on the clinical presentation of symptoms.

Thus, an incomplete understanding of the sex-specific physiology of myocardial ischemia and underdeveloped diagnostic and treatment options may lead to the inadequate management of a large proportion of the population and a large number of women without signs of obstructive CAD at coronary angiography presenting with symptom-related disability. All of this consumes a considerable amount of healthcare resources.[6]

A European study published in 2012 found that angina in patients with normal blood vessels or non-obstructive atherosclerosis was associated with an increased risk of the combined endpoint of cardiovascular death, hospitalization due to myocardial infarction, heart failure, or stroke of up to 52% in the case of patients with normal coronary arteries and 85% in those with non-obstructive coronary atherosclerosis. In addition, these patients had an increased risk of all-cause mortality of up to 29% and 52%, respectively, with no differences between men and women.[19] Such physiological patterns have also been reported in other studies evaluating invasive and non-invasive coronary flow reserve.[20,21] All of these findings demonstrate the importance of evaluating and managing women with non-obstructive CAD.

Morphological studies have shown that the development of myocardial infarction is based on plaque rupture, plaque ulceration, and plaque calcification.[22] Plaque erosion/ulceration is another pathophysiological mechanism of myocardial infarction. In this case, damage of the integrity of the plaque cap leads to the development of a thrombus, with emboli from the plaque travelling to areas distal to the plaque and eventually blocking the lumen of the vessel. In most cases, this mechanism underlies the development of myocardial infarcts in women, and this type of non-obstructive atherosclerosis of coronary arteries is found more commonly in women with myocardial infarction than in men.[14,17,20-22]

The difference between men and women also exists in stable coronary syndromes. As noted above, in the WISE study, only 38% of women with a stable coronary syndrome had severe obstructive CAD, and the rest (62%) showed evidence of non-obstructive CAD.[13,14,17,20]

CORONARY MICROVASCULAR DYSFUNCTION

Myocardial ischemia is usually caused by narrowing of epicardial coronary arteries. Over the past 30 years, however, many studies have revealed that impaired coronary microcirculation can also lead or contribute to the development of ischemia of myocardial cells.

Most of the articles published on myocardial ischemia have been designed to evaluate coronary obstruction and to determine strategies for the early detection of obstructive CAD. However, there is lack of research on detection of ischemia in patients with normal or non-obstructive coronary arteries, which mainly present in women. As previously mentioned, women are less likely than men to undergo diagnostic or preventive measures. Since the 1980s, the information about microvascular disease has expanded. In 2007, Camici and Crea[23] evaluated clinical settings in which myocardial ischemia occurs and proposed a classification of coronary microvascular dysfunction (CMVD) based on the underlying diseases in which it occurs (e.g. obstructive CAD, cardiomyopathy, and systemic diseases). Their classification is as follows:

- Class 1: CMVD in the absence of obstructive CAD and myocardial diseases.
- Class 2: CMVD in the presence of myocardial diseases.
- Class 3: CMVD in the presence of obstructive CAD.
- Class 4: CMVD caused by coronary recanalization (i.e. iatrogenic).

In an everyday setting, it is very difficult to distinguish the forms of CMVD because small coronary arteries cannot be visualized by angiography. During invasive investigations, complex, time-consuming, and costly methods are required to carefully assess the function of the coronary microcirculation. In patients suspected of having microvascular angina, accepted hallmarks of myocardial ischemia, such as stress-induced left ventricular contractile alterations,[23-26] are usually undetectable. A sparse distribution of myocardial ischemia in a patient presenting with CMVD is, on one hand, sufficient to produce ECG changes and myocardial perfusion defects on single-photon emission computed tomography (SPECT); but, on the other hand, might not result in detectable contractile abnormalities because of normal function of the surrounding myocardial tissue.[26,27]

EVALUATION AND DIAGNOSIS

The high incidence of cardiovascular death in women, particularly due to CAD, raises the need for the early evaluation of women at increased risk in order to determine the optimal therapeutic strategies. Coronary angiography remains the reference standard for diagnosing ischemic heart disease. However, it has very low possibilities to evaluating patients with microvascular angina. Because the majority of patients with microvascular dysfunction are women, it is very important to determine which tests are of value for their evaluation. Coronary angiography cannot provide information about the severity and extent of ischemia. Thus, in women, non-invasive tests that save money and reduce periprocedural risks, are of particular value.

The simplest formula - ischemia is mismatch between oxygen demand and delivery - indicates the importance of the direct visualization of ischemia in women, particularly because the rate of microvascular angina is higher in women than in men. Exercise stress testing remains the basis for the evaluation and risk-stratification of patients with suspected CAD. It is a valuable and informative tool in both men and women. However, the accuracy of interpreting the test depends not only on ST-segment changes, but also on the double product, heart-rate recovery time, and so on. To accurately interpret the results of the test, the pretest probability of the patient having ischemic heart disease and her hormonal state should be considered. It is well known that during the physical exercise test (treadmill or veloergometer), increased oxygen demand and energy consumption lead to ECG changes.[28-32]

It is also well known that an exercise stress test has relatively lower sensitivity and specificity for diagnosing ischemia. Meta-analyses have indicated that there are frequent false-positive and false-negative results, and that this test is more valuable in young patients compared with older ones.[29,30] This view has been echoed in other meta-analysis.[32,33] An analysis of ECG results acquired during exercise stress tests found sensitivity and specificity of 64% and 81%, respectively, in men, compared with 61% and 65%, respectively, in women -- quite a big difference in specificity between men and women. Analyses for other imaging modalities have found sensitivities and specificities, respectively, of: 77% and 81% for men compared with 78% and 86% for women for stress echocardiography; 88% and 74% for men compared with 82% and 81% for women for SPECT; and 86% and 82% for men compared with 78% and 74% for women for magnetic resonance imaging (MRI).[33] In all of these studies, the standard for diagnosis (and comparison) was coronary angiography and the presence of obstructive CAD.

But how about the detection of microvascular disease? Angiography cannot record it. Is there a standard for diagnosing microvascular disease? To answer these questions, the physiology of tests should be considered. The major advantage of the exercise ECG is that it is inexpensive, and therefore readily and widely available. However, quantification of the extent of microvascular dysfunction is not possible.

Direct visualization of the blood supply and hence ischemia is possible only by studying perfusion via the well-established tool SPECT and the emerging tool MRI.

The advantages of SPECT stress perfusion images include direct visualization of ischemia, high interobserver agreement, low operator dependence, a high technical success rate, high sensitivity, better accuracy when multiple resting left ventricular motion abnormalities are present, and the ability to detect ischemia in an infarct area. Higher specificity, and greater availability, versatility, and (arguably) convenience favor the use of stress echocardiography over SPECT.[33-35] However, the lower specificity of SPECT compared to stress-echocardiography may correspond to the presence of microvascular disease, which does not currently have clear diagnostic criteria. Data regarding the use of MRI in this context remain limited and insufficient. However, it is seems promising.

Quantitative rest/stress myocardial perfusion imaging [best documented using positron emission tomography (PET)] combined with clinical circumstances usually provides a definitive direct visualization of ischemia, and is therefore a highly informative tool in the diagnosis of patients and guiding management, including risk-factor management and revascularization for patients with physiologically severe epicardial stenosis by quantitative PET.[36]

Compared with negative tests, a positive result on computed tomography angiography (CTA) in women has been found to be more predictive of subsequent clinical events than a positive stress test (adjusted P = 0.028).[32] Among men, a positive CTA was slightly but not significantly less informative of risk detection than a positive stress test (adjusted P = 0.168).[28,37,38]

However, all of these results, and all of the sensitivity and specificity data, refer to the evaluation of patients with obstructive CAD. There are very few data on the value of these tests in diagnosing microvascular angina, and this represents a main limitation of current research.

Evaluating women with chest pain seems to be difficult, with various pathophysiologic mechanisms behind the condition, diverse clinical presentations, and limited

diagnostic standards. Hence there is no single test that will definitely diagnose ischemia due to microvascular disease in women.[39] Even after a normal exercise-stress test, further testing may be needed to gain important diagnostic information. Further research is aimed for optimizing the non-invasive identification and management of CMVD in such patients.

Authors' contributions

Study design: T. Vakhtangadze
Data collection: N. Gakhokidze
Manuscript writing: T. Vakhtangadze, N. Gakhokidze
Manuscript review: T. Vakhtangadze

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. Writing Group Members, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jiménez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart disease and stroke statistics-2016 update: a report from the American Heart Association. *Circulation* 2016;133:e38-360.
2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistic--2015 update: a report from the American Heart Association. *Circulation* 2015;131:e29-322.
3. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. *Eur Heart J* 2016;37:3232-45.
4. Center for Disease Control and Prevention. Women and heart disease fact sheet. Available from: http://www.cdc.gov/dhdsp/data_statistics/fact_sheets/fs_women_heart.htm. [Last Accessed on 2017 Jan 11].
5. American Heat Association; American Stroke Association. Heart disease and stroke statistics - at-a-glance. Available from: https://www.heart.org/idc/groups/ahamah-public/@wcm/@sop/@smd/documents/downloadable/ucm_470704.pdf. [Last Accessed on 2017 Jan 11].
6. Vieira RD, Hueb W, Hlatky M, Favarato D, Rezende PC, Garzillo CL, Lima EG, Soares PR, Hueb AC, Pereira AC, Ramires JA, Kalil Filho R. Cost-effectiveness analysis for surgical, angioplasty, or medical therapeutics for coronary artery disease: 5-year follow-up of medicine, angioplasty, or surgery study (MASS) II trial. *Circulation* 2012;126:S145-50.
7. Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, Finkelstein EA, Hong Y, Johnston SC, Khera A, Lloyd-Jones DM, Nelson SA, Nichol G, Orenstein D, Wilson PW, Woo YJ; American Heart Association Advocacy Coordinating Committee; Stroke Council; Council on Cardiovascular Radiology and Intervention; Council on Clinical Cardiology; Council on Epidemiology and Prevention; Council on Arteriosclerosis; Thrombosis and Vascular Biology; Council on Cardiopulmonary; Critical Care; Perioperative and Resuscitation; Council on Cardiovascular Nursing; Council on the Kidney in Cardiovascular Disease; Council on Cardiovascular Surgery and Anesthesia, and Interdisciplinary Council on Quality of Care and Outcomes Research. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 2011;123:933-44.
8. Women's Heart Foundation. Women and heart disease facts. Available from: http://www.womensheart.org/content/HeartDisease/heart_disease_facts.asp. [Last Accessed on 2017 Jan 17].
9. Stramba-Badiale M, Fox KM, Priori SG, Collins P, Daly C, Graham I, Jonsson B, Schenck-Gustafsson K, Tendera M. Cardiovascular diseases in women: a statement from the policy conference of the European Society of Cardiology. *Eur Heart J* 2006;27:994-1005.
10. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvänne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F; European Association for Cardiovascular Prevention & Rehabilitation (EACPR); ESC Committee for Practice Guidelines (CPG). European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The fifth joint task force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). *Eur Heart J* 2012;33:1635-701.
11. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Piña IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC Jr, Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V, Wenger NK. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American Heart Association. *Circulation* 2011;123:1243-62.
12. Merz CN, Kelsey SF, Pepine CJ, Reichek N, Reis SE, Rogers WJ, Sharaf BL, Sopko G. The Women's Ischemia Syndrome Evaluation (WISE) study: protocol design, methodology and feasibility report. *J Am Coll Cardiol* 1999;33:1453-61.
13. Gulati M, Shaw LJ, Bairey Merz CN. Myocardial ischemia in women: lessons from the NHLBI WISE study. *Clin Cardiol* 2012;35:141-8.
14. Gulati M, Cooper-DeHoff RM, McClure C, Johnson BD, Shaw LJ, Handberg EM, Zineh I, Kelsey SF, Arnsdorf MF, Black HR, Pepine CJ, Merz CN. Adverse cardiovascular outcomes in women with nonobstructive coronary artery disease: a report from the Women's Ischemia Syndrome Evaluation Study and the St James Women Take Heart Project. *Arch Intern Med* 2009;169:843-50.

15. Lee BK, Lim HS, Fearon WF, Yong AS, Yamada R, Tanaka S, Lee DP, Yeung AC, Tremmel JA. Invasive evaluation of patients with angina in the absence of obstructive coronary artery disease. *Circulation* 2015;131:1054-60.
16. Jespersen L, Hvelplund A, Abildstrøm SZ, Pedersen F, Galatius S, Madsen JK, Jørgensen E, Kelbæk H, Prescott E. Stable angina pectoris with no obstructive coronary artery disease is associated with increased risks of major adverse cardiovascular events. *Eur Heart J* 2012;33:734-44.
17. Pepine CJ, Ferdinand KC, Shaw LJ, Light-McGroary KA, Shah RU, Gulati M, Duvernoy C, Walsh MN, Bairey Merz CN; ACC CVD in Women Committee. Emergence of nonobstructive coronary artery disease: a woman's problem and need for change in definition on angiography. *J Am Coll Cardiol* 2015;66:1918-33.
18. Bairey Merz N, Bonow RO, Sopko G, Balaban RS, Cannon RO 3rd, Gordon D, Hand MM, Hayes SN, Lewis JF, Long T, Manolio TA, Maseri A, Nabel EG, Desvigne Nickens P, Pepine CJ, Redberg RF, Rossouw JE, Selker HP, Shaw LJ, Waters DD; National Heart, Lung and Blood Institute; American College of Cardiology Foundation. Women's Ischemic Syndrome Evaluation: current status and future research directions: report of the National Heart, Lung and Blood Institute workshop: October 2-4, 2002: executive summary. *Circulation* 2004;109:805-7.
19. Jespersen L, Hvelplund A, Abildstrøm SZ, Pedersen F, Galatius S, Madsen JK, Jørgensen E, Kelbæk H, Prescott E. Stable angina pectoris with no obstructive coronary artery disease is associated with increased risks of major adverse cardiovascular events. *Eur Heart J* 2012;33:734-44.
20. Pepine CJ, Anderson RD, Sharaf BL, Reis SE, Smith KM, Handberg EM, Johnson BD, Sopko G, Bairey Merz CN. Coronary microvascular reactivity to adenosine predicts adverse outcome in women evaluated for suspected ischemia results from the National Heart, Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) study. *J Am Coll Cardiol* 2010;55:2825-32.
21. Murthy VL, Naya M, Taqueti VR, Foster CR, Gaber M, Hainer J, Dorbala S, Blankstein R, Rimoldi O, Camici PG, Di Carli MF. Effects of sex on coronary microvascular dysfunction and cardiac outcomes. *Circulation* 2014;129:2518-27.
22. Mehta LS, Beckie TM, DeVon HA, Grines CL, Krumholz HM, Johnson MN, Lindley KJ, Vaccarino V, Wang TY, Watson KE, Wenger NK; American Heart Association Cardiovascular Disease in Women and Special Populations Committee of the Council on Clinical Cardiology, Council on Epidemiology and Prevention, Council on Cardiovascular and Stroke Nursing, and Council on Quality of Care and Outcomes Research. Acute myocardial infarction in women: a scientific statement from the American Heart Association. *Circulation* 2016;133:916-47.
23. Camici PG, Crea F. Coronary microvascular dysfunction. *N Engl J Med* 2007;356:830-40.
24. Nihoyannopoulos P, Kaski JC, Crake T, Maseri A. Absence of myocardial dysfunction during stress in patients with syndrome X. *J Am Coll Cardiol* 1991;18:1463-70.
25. Panza JA, Laurienzo JM, Curiel RV, Unger EF, Quyyumi AA, Dilsizian V, Cannon RO 3rd. Investigation of the mechanism of chest pain in patients with angiographically normal coronary arteries using transesophageal dobutamine stress echocardiography. *J Am Coll Cardiol* 1997;29:293-301.
26. Pargaonkar V, Khandelwal A, Kobayashi Y, Tanaka S, Mathur MB, Froelicher V, Yeung A, Tremmel J. The diagnostic value of stress echocardiography and electrocardiography in identifying occult coronary abnormalities in patients with angina and no obstructive coronary artery disease. *J Am Coll Cardiol* 2015;65:A1623.
27. Lanza GA, Crea F. Primary coronary microvascular dysfunction: clinical presentation, pathophysiology, and management. *Circulation* 2010;121:2317-25.
28. Visser F. Non invasive assessment of coronary microvascular dysfunction. *Heart Metab* 2008;40:15-19.
29. Banerjee A, Newman DR, Van den Bruel A, Heneghan C. Diagnostic accuracy of exercise stress testing for coronary artery disease: a systematic review and meta-analysis of prospective studies. *Int J Clin Pract* 2012;66:477-92.
30. Shah SF, Meo SA. Usefulness of standard treadmill stress testing in women. *J Pak Med Assoc* 2009;59:197-200.
31. Kohli P, Gulati M. Exercise stress testing in women: going back to the basics. *Circulation* 2010;122:2570-80.
32. Sanders GD, Patel MR, Chatterjee R, Ross AK, Bastian LA, Coeytaux RR, Heidenfelder BL, Musty MD, Dolor RJ. Noninvasive technologies for the diagnosis of coronary artery disease in women: future research needs. Future research needs paper no. 41. (Prepared by the Duke Evidence-based Practice Center under contract No. 290-2007-10066-I). AHRQ Publication No. 13-EHC072-EF. Rockville, MD: Agency for Healthcare Research and Quality. February 2013. Available from: www.effectivehealthcare.ahrq.gov/reports/final.cfm. [Last Accessed on 2017 Jan 17].
33. Kwok Y, Kim C, Grady D, Segal M, Redberg R. Meta-analysis of exercise testing to detect coronary artery disease in women. *Am J Cardiol* 1999;83;660-6.
34. Galiuto L, Picano E. Stress echo in microvascular disease. In: Picano E. Stress echocardiography. 6th ed. Berlin: Springer International Publishing; 2015. p. 485-507.
35. Marwick TH, Picano E, Marraccini P, Schwitter J, Senior R. Comparison with other imaging techniques. In: Picano E. Stress echocardiography. 6th ed. Berlin: Springer International Publishing; 2015. p. 627-59.
36. Cavusoglu Y, Entok E, Timuralp B, Vardareli E, Kudaiberdieva G, Birdane A, Gorenek B, Unalir A, Goktekin O, Ata N. Regional distribution and extent of perfusion abnormalities, and the lung to heart uptake ratios during exercise thallium-201 SPECT imaging in patients with cardiac syndrome X. *Can J Cardiol* 2005;21:57-62.
37. Patel MB, Bui LP, Kirkeeide RL, Gould KL. Imaging microvascular dysfunction and mechanisms for female-male differences in CAD. *JACC Cardiovasc Imaging* 2016;9:465-82.
38. Thomson LE, Wei J, Agarwal M, Haft-Baradaran A, Shufelt C, Mehta PK, Gill EB, Johnson BD, Kenkre T, Handberg EM, Li D, Sharif B, Berman DS, Petersen JW, Pepine CJ, Bairey Merz CN. Cardiac magnetic resonance myocardial perfusion reserve index is reduced in women with coronary microvascular dysfunction. A National Heart, Lung, and Blood Institute-sponsored study from the Women's Ischemia Syndrome Evaluation. *Circ Cardiovasc Imaging* 2015;8:e002481.
39. Douglas PS, Hoffmann U, Patel MR, Mark DB, Al-Khalidi HR, Cavanaugh B, Cole J, Dolor RJ, Fordyce CB, Huang M, Khan MA, Kosinski AS, Krucoff MW, Malhotra V, Picard MH, Udelson JE, Velazquez EJ, Yow E, Cooper LS, Lee KL; PROMISE Investigators. Outcomes of anatomical versus functional testing for coronary artery disease. *N Engl J Med* 2015;372:1291-300.

Yaroustovsky *et al. Vessel Plus* 2017;1:49-60
DOI: 10.20517/2574-1209.2017.11

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Review **Open Access**

Blood purification in intensive care patients with multiple organ dysfunction syndrome and sepsis after cardiac surgery

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How to cite this article: Yaroustovsky M, Abramyan M, Komardina E, Nazarova H. Blood purification in intensive care patients with multiple organ dysfunction syndrome and sepsis after cardiac surgery. Vessel Plus 2017;1:49-60.

Prof. Michael Yaroustovsky, the Chief of the Department of Detoxication and Endoscopy at Bakoulev Scientific Center for Cardiovascular Surgery, Corresponding Member of RAS, is a well-known Russian scientist who has made significant contributions to the development of modern technologies of extracorporeal blood purification in the treatment of critical states. He owns the absolute priority in the development and the introduction into the clinical practice of Russian clinics of modern methods of correction and maintaining homeostasis, including albumin dialysis, combined plasma filtration and adsorption, selective hemoperfusion, etc. at extremely severe contingent of patients of different age groups having multiple organ failure and septic complications after cardiac surgery. He is the author of more than 270 scientific articles, including 7 monographs and the first Russian manual on Blood purification in Intensive Care.

Article history:
Received: 11-04-2017
Accepted: 08-06-2017
Published: 27-06-2017

Key words:
Extracorporeal blood purification,
acute kidney injury,
acute liver failure,
extracorporeal membrane oxygenation,
sepsis,
selective lipopolysaccharide-adsorption,
critical condition,
cardiovascular surgery

ABSTRACT

Extracorporeal blood purification is becoming increasingly important in intensive therapy for multiple organ dysfunction syndrome (MODS) and sepsis, considering all of their pathophysiological aspects. The results of treatment, particularly in children, considering their anatomical and physiological features, are related to the severity and progression of organ failure, the indications that are found, the choice of method, and the timely initiation of blood purification. Multiple organ support therapy is the aim of introducing and applying blood purification today. Various extracorporeal blood purification techniques directly affect the molecular and electrolyte composition of blood and influence all structures of the human body, which can allow us to correct, recover, replace and maintain homeostasis in MODS. The potential of new extracorporeal molecular technologies allows their successful use in severe cardiac and respiratory failure, acute kidney injury and hepatic dysfunction and in complex therapy for severe infections and sepsis and extreme metabolic violations. Adult and pediatric patients after cardiac surgery with cardiopulmonary bypass form a special cohort that often requires the application of various intra- and extracorporeal techniques due to the development of MODS, infections and sepsis in the postoperative period.

Quick Response Code:

INTRODUCTION

Extracorporeal blood purification is a modern medical direction based on the modification of blood components outside the patient's body aiming to change their properties or remove pathological substances that cause or support the disease. The use of these methods concerning the pathophysiological process allows us to achieve therapeutic effects, even when traditional methods are ineffective.

BLOOD PURIFICATION FOR ACUTE KIDNEY INJURYIN PATIENTS WITH MULTIPLE ORGAN DYSFUNCTION SYNDROME

Renal replacement therapy (RRT) is a routine method for intensive care patients with acute kidney injury (AKI). However, the incidence of isolated AKI in adult intensive care unit (ICU) patients does not exceed 5.7%;[1] up to 90% of the AKI in this group are part of a multiple organ dysfunction syndrome (MODS), indicating the severity of the patients' condition.[2,3] Similarly, AKI remains one of the serious complications after open heart surgery in children. The reported incidence of AKI after cardiopulmonary bypass (CPB) ranges from 23-52% with up to 17% of these patients require RRT.[4] It prolongs the duration of ICU stays up to 3-4 weeks and increases mortality up to 40-90%.[5] Isolated pediatric AKI corresponds to only 15%, and it is more common in MODS.[6]

The goal of extracorporeal blood purification in the ICU today should be the treatment of MODS considering the pathophysiological aspects of it rather than the classical RRT in AKI.[7,8] The choice of the optimal blood purification modality is based on the ability to correct water-electrolyte and metabolic imbalances and to decrease the manifestations of endo- and exotoxicosis, and other homeostatic disorders, thereby improving survival of critically ill patients. Multiple organ support therapy is an effective clinical approach. Various systems and procedures for extracorporeal blood purification including diffusion, convection, filtration, adsorption, and apheresis directly affect the molecular and electrolyte composition of the blood, allowing one to correct, recover and maintain homeostasis.

Dialysis, convection and filtration can be used today not only for RRT. Hemodialysis (HD), hemofiltration (HF) and hemodiafiltration (HDF) are pathogenically valid and effective methods in MODS. Pediatric AKI, especially in underweight children, usually requires the contemporary use of peritoneal dialysis (PD) and extracorporeal methods. Each technique has its advantages and limitations, which form an algorithm of therapeutic protocol choice.[9,10]

Cardiosurgery patients with MODS form an extremely serious group. First, one should pay attention to the left and right ventricle efficiency, level of preload, volume overload of pulmonary circulation and oxygenation index (PO_2/FiO_2). At the time of initiating extracorporeal therapy, such patients usually have severe gas exchange and hemodynamic disturbances, which require a multicomponent inotropic (epinephrine > 0.2 μg/kg/min, dobutamine > 5 μg/kg/min, dopamine > 7 μg/kg/min) and mechanical circulatory support [intra-aortic balloon counterpulsation and extracorporeal membrane oxygenation (ECMO)]. Patients with MODS have an increased preload [central venous pressure (CVP) > 18 mmHg, left atrial pressure (Pla) > 20 mmHg], low oxygenation index (PO_2/FiO_2 < 150), edematous syndrome, azotemia (creatinine > 350 μmol/L, urea > 25 mmol/L), and electrolyte (hyperkalemia) and metabolic (lactic acidosis) disorders. After the first several hours of dialysis, hemofiltration, hemodiafiltration (HD, HF, HDF), we observed preload reduction (CVP and Pla decreased > 10%) and hemodynamic stabilization [cardiac index, left ventricular ejection fraction (LVEF) and arterial pressure] against a background of inotropic support decline, up to 25% of the prescribed dose.

In the pediatric ICU, the use of acute PD is justified as the RRT method. PD does not adversely affect hemodynamics; it requires no systemic anticoagulation or vascular access, and it excludes the emergence of disequilibrium syndrome. Moreover, this method is simple, efficient and safe, and it does not require complex expensive equipment. The indications for PD in children after cardiac surgery are oligo/anuria, hypervolemia, edematous syndrome with cardiac and respiratory insufficiency progression, azotemia and hyperkalemia.[5,11] PD allows one to decrease circulatory insufficiency, in particular, to raise mean arterial pressure (MAP) and LVEF with inotropic support decline, to normalize ventricular filling pressure and preload and postload (CVP and Pla), to optimize the circulating blood volume and correct edema syndrome [Figure 1]. The favorable effect of slow and constant filtration in PD helps reduce tissue hyperhydration, improve pulmonary gas exchange and increase the oxygenation index. Individualized programs and dosages of PD and appropriate parenteral nutrition make it possible to predict the course of AKI, along with the stabilization and further decrease of azotemia.[12]

More "aggressive" blood purification in infants is

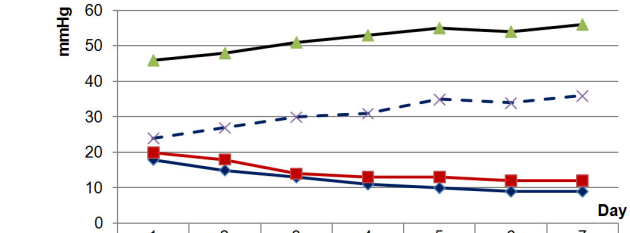
dictated by an inadequate PD dosing in metabolic and water-electrolyte disorders with severe edema syndrome, tissue hyperhydration and the preservation of azotemia. Carrying out constant HF/HD in infants makes it possible to achieve the necessary fluid balance, and decreases in CVP and Pla are accompanied by MAP and LVEF increases, which allow inotropic support reduction in some cases.[13]

We are in strong agreement with researchers who believe that hemodynamic and gas exchange improvements are associated with the optimization of circulating blood volume and plasma colloid osmotic pressure.[5] We also think that blood purification's positive effects are implemented by filtration of interstitial fluid excess and reduction of myocardial and pulmonary edema.[8]

The effectiveness of any blood purification method in cardiosurgery patients depends on the adequate correction of electrolyte (hyperkalemia, hyper- and/or hyponatremia) and metabolic (lactatemia, acidosis, alkalosis) disorders and azotemia. Hyperkalemia is caused by acute kidney injury, catabolism, rhabdomyolysis and reperfusion after crash syndrome, hemolysis and disseminated intravascular coagulation. It is often aggravated by concomitant metabolic acidosis and is an additional cardiotoxic factor.

The diffusion and filtration rates during continuous veno-venous hemofiltration (CVVHF) or CVVHF dialysis permit one to correct azotemia, hyperkalemia and metabolic parameters and control them later.[14] In contrast to PD, constant or intermittent extracorporeal therapy stabilizes and significantly reduces the azotemia level earlier, quickly corrects hyperkalemia and metabolic disorders, and provides clearance of exo- and endotoxins.

Extracorporeal blood purification in adult and pediatric MODS patients is used for both renal and extrarenal



	1	2	3	4	5	6	7
CVP	18	15	13	11	10	9	9
Pla	20	18	14	13	13	12	12
MAP	46	48	51	53	55	54	56
LVEF,%	24	27	30	31	35	34	36

Figure 1: Hemodynamics parameters during peritoneal dialysis. CVP: central venous pressure; Pla: left atrial pressure; MAP: mean arterial pressure; LVEF: left ventricle ejection fraction

indications. Its elimination of medium-molecular depressive factors, which are elevated in MODS and sepsis,[2,8] and correction of hyperkalemia and azotemia prevent the development of immune paralysis and uremic polyserositis. During extracorporeal procedures, it is possible to normalize body temperature and temperature balance as a whole.

The ability to perform the necessary volume of infusion-transfusion therapy in anuria and tissue hyperhydration without the risk of hypervolemia is very important.

Because of the high risk of both hypo- and hypercoagulation in cardiosurgery patients, choosing the anticoagulation mode carefully is also very important.

CVVHF IN CONJUNCTION WITH ECMO IN CHILDREN WITH CRITICAL HEART FAILURE AND ACUTE KIDNEY INJURY AFTER CARDIAC SURGERY

Due to the significant expansion of cardiac surgery worldwide, the number of ECMO patients in the near postoperative period has increased significantly. Severe cardiac and/or respiratory failure is accompanied by cardiogenic shock and hypoxia with MODS and AKI development. These patients have hypervolemia and electrolyte disorders (hyperkalemia, hypocalcemia, hypo- or hypernatremia, tissue hyperhydration), which can increase azotemia and metabolic disorders. Thus, continuous hemofiltration in conjunction with ECMO is a justified method of correcting the entire complex of homeostatic disorders.[15]

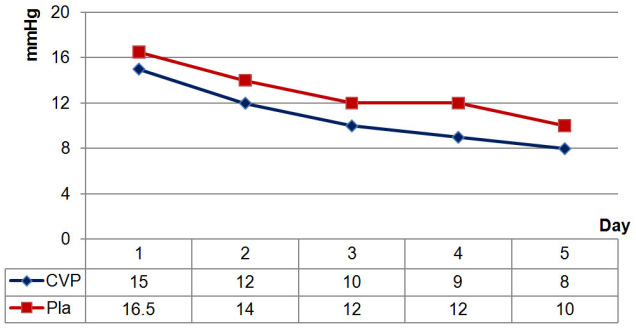
Many medical centers offer continuous RRT as the most manageable and effective method.[16] We chose CVVHF, which influences various aspects of the pathogenesis of MODS, inhibits its development and progression, and helps eliminate the medium molecular weight substances, thereby contributing to a decrease in inflammatory cascade activity.[17] In addition, the use of high volumes of replacement liquids (more than 30-35 mL/kg/h) can be of great importance for enhancing lymphatic transport between the intercellular space, tissues and blood and for reducing the activity of the inflammatory cascade.

The results of the treatment and its cost allow the conclusion that CVVHF connected to an ECMO circuit (single circuit) is relatively simple and has advantages for intensive care patients.[18,19]

We have shown the successful use of CVVHF in conjunction with ECMO in children (more than 80 patients) who have undergone correction of complex congenital heart defects.[20] This combination of different techniques in a single extracorporeal circuit allows the correction of water and electrolyte disturbances, metabolic disorders and azotemia from the first day of treatment. Moreover, the prescribed ultrafiltration volume is calculated and programmed according to the level of volemia in each specific case. The determining factors are as follows: CVP and Pla, pulmonary artery pressure, ventricular end-diastolic volumes, the volume of necessary infusion and transfusion therapy and nutritional support. Only one day after connecting CVVHF to an ECMO circuit, we observed a significant ($P < 0.05$) decrease in CVP and Pla to 15 (14-17) and 16.5 (14-18.75) mmHg, respectively. When CVP reached 8-12 mmHg and Pla 10-14 mmHg, the RRT mode was switched to isovolemic ultrafiltration [Figure 2].

Since a significant amount of liquid is exchanged daily during CVVHF in conjunction with ECMO, the use of automated volumetric control devices (infusomats) is a prerequisite to avoid possible errors and maintain clear liquid balance. It should be noted that passive (non-automated) ultrafiltration in CVVHF is always associated with an inaccurate calculation of the liquid balance and is dangerous, particularly in children weighing up to 10 kg.[20]

According to the proposed scheme (CVVHF + ECMO in single circuit), a substitute is administered in one of the patient's central veins (v. femoralis, v. subclavia or v. jugularis) by means of infusomat. The type of replacement solution is determined directly in each case, depending on the level of potassium and other blood electrolytes. As a substitute, we used crystalloid solutions of Duosol (BBRAUN, Germany) with bicarbonate buffer and variable potassium content (2 or 4 mmol/L). The hemofiltration "dose" is set at a rate of 20-40 mL/kg/h to ensure the required quality of the procedure. Anticoagulation was carried out for the combined ECMO and HF single circuit with the use of unfractionated heparin; the activated clotting time was maintained within 180-200 s.



	1	2	3	4	5
CVP	15	12	10	9	8
Pla	16.5	14	12	12	10

Figure 2: Dynamics of CVP and Pla during CVVHF in conjunction with ECMO. CVP: central venous pressure; Pla: left atrial pressure; CVVHF: continuous veno-venous hemofiltration; ECMO: extracorporeal membrane oxygenation

The circuit lifetime should not exceed 48 h. Since the aim of the CVVHF connection was to perform RRT, its duration was determined by the dynamics of the renal dysfunction and the clinical state of the child.

The use of methods of extracorporeal blood purification should be considered a "bridge" to the recovery of kidney function. Given the obvious need to control the electrolyte, acid-base and water balance in patients with AKI, the use of CVVHF may be considered a method of supporting kidney function that is similar to breathing assistance by mechanical ventilation or cardiac and respiratory support by ECMO.[19,21] The primary goal of RRT is to prevent undesirable additional effects by reducing uremic intoxication and maintaining the "internal environment" as close to the physiological state as possible, without adversely affecting the functions of the patient's vital organs and system.[20]

EXTRACORPOREAL BLOOD PURIFICATION IN INTENSIVE CARE PATIENTS WITH ACUTE LIVER FAILURE

Acute liver failure (ALF) is a rare but severe and life-threatening condition in patients after cardiac surgery. In most cases, ALF develops in the setting of MODS. The frequency of MODS in patients after cardiac surgery with CPB is relatively low, but the occurrence of liver dysfunction increases mortality to almost absolute values.[22-24] Despite the progress of conservative treatment for ALF and the development of new therapeutic recommendations, blood purification continues to play an important role in this case. Extracorporeal methods have the ability to eliminate both hydrophobic albumin-bound and water-soluble substances, thereby limiting the extent of hepatocyte damage and providing time for the organ's recovery or performing liver transplantation for the patient.[25,26] Extracorporeal therapy also helps achieve one of the most important goals in this case -- increasing the albumin binding capacity by eliminating albumin-bound toxic substances.

Currently, there are two groups of extracorporeal methods to support liver functions: the systems containing cells (human or animal hepatocytes), and techniques without biological substrates.

Extracorporeal methods based on patient's blood perfusion through exogenous hepatocytes were not widely used due to their high complexity and cost, the insufficient cell mass for liver regeneration (≥ 400 g would be needed) as well as low biocompatibility and infection risk.

Modern extracorporeal blood purification methods without using biological components comprise high-volume plasma exchange, albumin dialysis [Molecular Adsorbent Recirculating System (MARS®)], single-pass albumin dialysis (SPAD) and methods that combine plasma separation and adsorption [Fractionated Plasma Separation and Adsorption (FPSA) Prometheus®)].

In acute liver failure, plasma exchange provides a reduction in bilirubinemia, but this is achieved only at high exchange volumes (up to 10 L), and the lack of specificity and high risk of allergic and infectious complications reduce the benefits and limit the use of this method.[27,28] SPAD therapy uses standard equipment for kidney replacement therapy with a highly porous membrane dialyzers and albumin containing dialysis solutions to remove hydrophobic substances.

The emergence of new hi-tech techniques, such as albumin dialysis (MARS-therapy) and fractionated plasma separation and adsorption (Prometheus-therapy), allow optimistic results to be obtained with complex intensive therapy for this severe category of patients.[29]

The beneficial effect of these procedures on clinical and laboratory parameters is explained by the removal of vasoactive substances and toxins, leading to improvements in organ and tissue perfusion, hemodynamic parameters and kidney function and reducing portal hypertension, intracranial pressure and hepatic encephalopathy.[30,31] The experience from implementing this therapy in adults allows the use of extracorporeal liver support to be recommended in pediatric practice.[32-34]

MARS-therapy is a method of albumin dialysis that uses a filter permeable to substances with a molecular weight of up to 50 kDa. The toxins accumulated on the filter membrane are subsequently bound to the donor albumin used as a dialysate. Simultaneously, the albumin dialysate is purified by passing through activated carbon, anion exchange resin and a low-permeability dialyzer, which provides a link between the albumin circuit and the traditional bicarbonate-based dialysis solution. Thus, the simultaneous selective removal of albumin-bound and water-soluble substances has become possible.[35,36]

The principle of Prometheus-therapy is based on plasma separation on the AlbuFlow filter, the membrane of which is permeable to molecules of up to 250 kD, including albumin, clotting factors and fibrinogen. Separated plasma regenerates when it successively passes through absorbers with ion exchange and neutral resin. Removal of low molecular weight water soluble compounds is provided by the performance of HF or HD. This technique is the most effective for eliminating albumin-bound toxins such as unconjugated bilirubin and bile acids.[37,38]

In assessing the safety and efficacy of MARS and Prometheus therapies in adult patients with ALF after cardiac surgery, we analyzed the dynamics of hydrophobic and hydrophilic toxic substance concentrations as well as the severity of hepatocyte cytolysis and the impairment of liver synthetic function. A key aspect in the evaluation of MARS and Prometheus liver support system safety in patients after cardiac surgery was the absence of a negative influence on hemodynamics and lung oxygenation function. In evaluating the safety of these systems, monitoring the dynamics of serum albumin is recommended, which is important in the setting of initial dysproteinemia in ALF and the use of high permeability hemo- or plasma filters. According to several authors, the serum albumin concentration during MARS-therapy remains unchanged because the diameter of the filter pores (50 kDa) does not exceed the size of the albumin molecule (65 kDa).[26,38] However, the albumin loss during Prometheus-therapy can reach 3 g/L ($P = 0.055$).[31,39] We observed no significant negative dynamics of serum albumin [oscillations: 32 (30-35) and 30 (26-35) g/L, $P = 0.052$, before and after MARS-therapy; 33 (31-34) and 31 (28-36) g/L, $P = 0.051$, before and after Prometheus-therapy], which is probably due to the adequate routine correction of hypoproteinemia in patients with ALF.[39] Likewise, despite published data on the loss of coagulation factors during Prometheus-therapy,[40] we did not observe clinically significant hemorrhagic complications.

The use of both liver support systems in cardio surgical patients provided a significant reduction in the total bilirubin concentration, and Prometheus-therapy allowed the clearance of unconjugated bilirubin. During MARS-therapy, the initial level of total bilirubin was 230 (181-256) μmol/L whereas during Prometheus-therapy, it was 333 (189-450) μmol/L; the concentrations of unconjugated bilirubin were 103 (72-135) and 119 (74-166) μmol/L, respectively.

Extracorporeal liver support provided a statistically significant reduction in total bilirubin concentration (8.6% for MARS-therapy (P = 0.028) and 33% for Prometheus (P < 0.001)). Prometheus-therapy also provided significant clearance of unconjugated bilirubin (29%, P = 0.003).[39] These results agree with data from other researchers, who reported a total bilirubin reduction of 26-59% during Prometheus and of 23% during albumin dialysis.[41-43] Such differences in reducing the level of total bilirubin are primarily due to the technical characteristics of the membranes and the different rates of perfusion and albumin dialysate/plasma.

During our study, we noted positive dynamics of the serum aminotransferase levels, which reflect hepatocyte cytolysis. This trend indirectly indicated a decrease in the degree of liver parenchyma lesion. The decrease of ASAT and ALAT was 15.5% (P = 1.0) and 43% (P = 0.31) during MARS-therapy, respectively, and 8% (P = 0.79) and 26% (P = 0.0005) during Prometheus-therapy, respectively. According to foreign colleagues, Prometheus-therapy decreases serum aminotransferase to 40-55% in patients with predominantly toxic ALF.[44]

In our study, serum cholinesterase was the marker of normalization of liver synthetic function. Its growing concentration in the setting of extracorporeal liver support suggests a positive effect of the treatment. This conclusion is also confirmed by the observed growth of the initially low level of antithrombin III in patients enrolled in the study [MARS-therapy: 31% (30-33%), Prometheus-therapy: 51% (33-62%)]. Since most of the patients were receiving RRT at the time of enrollment, the baseline levels of azotemia and ammonia were low. However, in spite of the performed blood purification procedures, there were significant positive changes of urea and creatinine levels, which indicates that the efficiency of MARS and Prometheus methods in eliminating water-soluble substances was sufficiently high to allow their use in AKI and ALF in the setting of MODS.[39,44]

According to many authors, the impact of extracorporeal blood purification methods on survival rate in ALF was statistically insignificant. However, the results of multi-center prospective randomized studies for this issue were ambiguous.

In the HELIOS study including 145 patients with acute-on-chronic liver dysfunction receiving 8-10 Prometheus procedures, the increase in total survival rate was statistically insignificant. However, the subgroup of patients with the most severe condition, i.e. with hepatorenal syndrome type I and MELD score above 30, a statistically significant increase in survival rate was found.[45] Taking such results into account, we recommend to perform a differentiated evaluation of extracorporeal therapy effectiveness, including its effect on survival rate in specific patient subgroups.

At the A.N. Bakulev NSPCCS, the MARS-therapy method was successfully applied for the first time in children with MODS after the radical correction of congenital heart disease. Patients with MODS developed severe hepatic failure along with heart (EF < 40%, required inotropic support), respiratory and renal failure. We observed the failure of conservative therapy for hepatic insufficiency, progressive increases in bilirubin (up to 500 μmol/L) and cytolytic syndrome with an increase of ASAT and ALAT enzymes (more than 200 U/L). During MARS-therapy, positive dynamics were more evident in children than in adults. The reduction of jaundice was noted at the end of the procedure (duration: 16-18 h), which indicates the effective elimination of bilirubin and bile acids. This fact can be explained by the ratio of the child's body surface area to the sorption capacity of the columns with activated carbon and ion-exchange resin. For example, the total bilirubin decrease in a child weighing less than 6 kg (age: 4 months) at the end was approximately 75% and stayed constant over 12 h. The unconjugated bilirubin decrease in this patient amounted to 80% of the initial value, continued 12 h later and reached almost 90%. Conjugated bilirubin decreased less significantly: by 70% directly at the end of the procedure and by 55% in 12 h [Figure 3].

As observed for other types of blood purification, the liver support system's effects on the circulatory and respiratory systems are very important for using these methods in intensive therapy for children after cardiac surgery. By the end of MARS-therapy, MAP exceeded 25% of the baseline, but the need for inotropic support decreased.[32,33]

Due to the large heat loss during extracorporeal

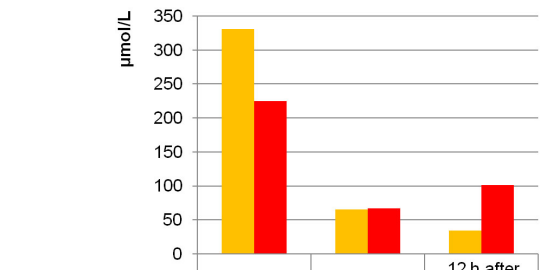


Figure 3: Dynamics of the fractions of bilirubin during molecular adsorbent recirculating system-therapy (4-month patient)

blood purification in children, it is important to prevent hypothermia. Hypothermia can lead to cardiac output decrease by peripheral vasospasm and an increase in TPVR and post-load. To avoid heat loss during MARS-therapy in children, it is necessary to use a heat exchanger and other physical heating means and to establish a high temperature (38-39 °C) on the bicarbonate line.[44]

EXTRACORPOREAL BLOOD PURIFICATION IN COMPLEX INTENSIVE THERAPY FOR SEPSIS IN ADULT AND PEDIATRIC PATIENTS AFTER OPEN-HEART SURGERY

Sepsis and septic shock are among the most common causes of death in critically ill patients in the ICU. Over the last decade, clinics worldwide have noted a trend towards a decrease in the frequency of Gram-positive bacteria and an increase in the frequency of Gram-negative bacteria within microbiological studies.

A structural component of the membrane of gram-negative microorganisms is bacterial lipopolysaccharide (or endotoxin), which is the main trigger of both sepsis and a cascade of inflammatory reactions.[46] When entering the systemic bloodstream from a focus of infection (e.g. pneumonia, mediastinitis) or natural reservoir (for example, the gastrointestinal tract, particularly after CPB), endotoxin interacts with competent cells of the immune system as a hormone and activates the synthesis of a wide range of mediators. The excess production of which determines the clinical manifestations of sepsis, such as systemic endothelial damage, decreased vascular tone, hypotension, cardiovascular hyporeactivity, fever, microcirculatory disorder, activation of hemostatic system factors, tissue hypoperfusion, hypoxia and multi-organ failure.[47,48] The development of microcirculatory disorders and tissue hypoxia in the gastrointestinal tract creates conditions for bacterial translocation. Thus, the vicious circle initiated by lipopolysaccharide is closed.[49,50]

The pathogenic chain of septic development and progression determines a need to develop and introduce new promising technologies.[47,51,52]

Modern methods of blood purification allow etiopathogenic therapy application for sepsis: to eliminate the etiotropic agent (endotoxin) by selective lipopolysaccharide (LPS)-adsorption and to remove the effector substances of systemic inflammation, replacing the functions of the affected organs and recovering the body's homeostasis by coupled plasmofiltration and adsorption (CPFA), convection and filtration techniques using ultrahigh permeable membranes.[53,54]

Many authors have observed that LPS-adsorption with PMX cartridges leads to a significant decrease in endotoxin activity levels after two consecutive procedures.[55] Positive dynamics of the endotoxin concentration (lowering by 38%) in patients with abdominal sepsis and septic shock were demonstrated in the European multicenter study "EUPHAS".[56] In the MEDIC study conducted in Europe and North America on ICU patients, selective LPS-adsorption led to a decrease in the mean endotoxin activity assay (EAA) level from 0.65 to 0.45 within 12 h after the procedure. According to the authors, the average reduction of the endotoxin concentration (26.1%) measured with the EAA is equivalent to a 50-100-fold decrease in the LPS level.[57]

In the European multicentre trial, the EUPHAS trial, 28-day survival after DHP-PMX was 53% and 32% in the study and control groups, respectively.[56] The assessment of retrospective data on using selective LPS adsorption in 306 patients with severe sepsis enrolled in the EUPHAS 2 trial indicated that 28-day mortality was 35% and 49% in the group with abdominal surgical pathology and in the group with non-abdominal pathology, respectively.[58] Similar results were observed in earlier published Japanese[59-61] and European studies[62] and in the meta-analysis data regarding immobilized Polymyxin B use efficacy during sepsis.[52,63]

Our experience with combined application of various techniques in a single circuit (LPS-adsorption + CPFA and LPS-adsorption + HD/HDF using filters with high cut-off membranes) showed their safety and pathogenic efficiency.[53,54] The application of convection and filtration techniques (CPFA, HDF using filters with high cut-off membranes) allows the correction of water-electrolyte and metabolic disorders and azotemia, which often accompany sepsis-associated MODS.[64-67]

LPS-adsorption using Toraymyxin-PMX-20R Polymyxin B immobilized fiber cartridges was found to be effective, simultaneously acting through sorption and apheresis on bacterial endotoxin, systemic inflammatory mediators and activated immune cells.[59-61,68,69]

CPFA performs an immunomodulatory role by acting on inflammatory mediators (interleukin-1β, -6, -8, -10, -18, tumor necrosis factor-α and others) via various mass transfer mechanisms such as diffusion,

convection and adsorption. The advantages of CPFA include the passage of plasma (and not blood) through the sorption column, which increases the contact time with the sorption surface and improves the efficiency of the procedure.[65,70]

HDF, which is carried out using filters with high cut-off membranes (EMiC2, Fresenius, Germany; 40 kDa cut-off), eliminates substances of low and medium molecular weight without albumin or coagulation factor loss. The permeability of these filters is similar to that of the kidney glomerular basement membrane, suggesting that the therapeutic effect of HDF is more physiological.[66,71-73]

Several studies have demonstrated the beneficial effect of selective LPS-adsorption, HD/HDF (with high cut-off membranes) and CPFA in sepsis.[58,64-66]

We proposed two new techniques of combined blood purification methods in single circuit: (1) LPS-adsorption + HDF using filters with high cut-off membranes; and (2) LPS-adsorption + CPFA. In the available literature, there was no information about a similar extracorporeal therapy protocol for sepsis in cardiac surgical patients. A study of adults enrolled in these protocols, carried out at the A.N. Bakulev NSPCCS in 2009-2016, showed the safety and efficacy of the combined therapies mentioned above.[53,54]

We observed that the combination of LPS-adsorption and HDF filters with high cut-off EMiC2 membranes led to a 34% MAP increase [from 76 (65-81) to 90 (85-102) mmHg, $P < 0.001$] with reduced vasopressor and inotropic support. The increase in MAP during LPS-adsorption with CPFA was 12% with the significant decrease in vasopressor support [the dose of noradrenaline was halved from 0.2 (0.15-0.3) to 0.1 (0.05-0.15) μg/kg/min, $P = 0.024$]. The improved oxygenation was confirmed by an increase in the oxygenation index increase (by 36% and 28% in LPS-adsorption with HDF in single circuit using EMiC2 and LPS-adsorption with CPFA in single circuit, respectively). One of the mechanisms that have a favorable effect on blood oxygenation is a decrease in the intensity of the infiltrative-inflammatory lung tissue process during blood purification.

We also evaluated the efficiency of blood purification in sepsis by monitoring the dynamics of EAA, procalcitonin level, bacteriological blood and sputum examination and the clinical state of patients during the procedures. The obtained results showed a decrease of 20% in EAA (from 0.74 to 0.59, $P = 0.03$) and of approximately 70% in procalcitonin (from 8.2 to 2.44 pg/mL, $P = 0.01$) in patients with sepsis after two procedures of combined LPS-adsorption and HDF with EMiC2. Similar dynamics of the infectious markers were observed when conducting LPS-adsorption with CPFA: EAA decreased by 30% (from 0.77 to 0.53, $P = 0.003$) and procalcitonin by 55% (from 6.23 to 2.83 pg/mL, $P = 0.005$). We also observed positive dynamics in leukocytosis and hyperthermia. Both variants of combined blood purification contributed to decreases in C-reactive protein (CRP) and pro-inflammatory cytokine levels, thus confirming the decrease in the intensity of the systemic inflammation response.[53,54]

Including combined blood purification methods into the complex therapy for sepsis allowed the results of treatment to be optimized. The 28-day survival was 65% in the LPS-adsorption + HDF with EMiC2 group and 75% in the LPS-adsorption + CPFA group.

The efficiency of the proposed concept of etiopathogenetic extracorporeal therapy for gram-negative sepsis is confirmed by the favorable results obtained in cardiac surgical patients characterized by their initially severe condition and more severe course of septic complications. The combined methods of blood purification can affect either the starting factor in the early development of sepsis by endotoxin removal or the subsequent release of inflammatory mediators after immune cell activation. These therapeutic methods also maintain vital organ function in MODS development.

Our experience from using selective LPS-adsorption in adult patients with sepsis allowed us to use this method for sepsis treatment in children. Blood purification in children with sepsis is limited to RRT technologies.[74] The principle of selective sorption use in children is absolutely innovative. No information was found in the database of the US National Library of Medicine National Institutes of Health on the use of selective LPS-adsorption for sepsis treatment in children after cardiac surgery.

The application of the smaller volume and sorption capacity of the Polymyxin B-immobilized cartridge (Toraymyxin-PMX-0.5R) is a new and promising extracorporeal technology that aims to reduce mortality from sepsis in children. Experience in selective endotoxin sorption is available only in Japan, where a study group enrolled children with abdominal pathology. The authors identified the safety and high efficiency of using Toraymyxin-PMX-0.5R cartridges.[75,76]

Today, the A.N. Bakulev NSPCCS has gained initial

Table 1: Changes of clinical and laboratory data before and after LPS-adsorptionin the children with sepsis

Indices	Before	After	*P*
MAP, mmHg	65.5 (63.5-74.8)	80.5 (76.3-83.5)	**0.012**
HR, beats/min	141 (126-146)	135 (126-141)	0.19
Epinephrine, μg/kg/min	0.05 (0.048-0.07)	0.05 (0.05-0.06)	0.92
Body temperature, °C	37.9 (36.2-38.5)	36.8 (36.6-37.1)	0.07
WBC, 10^9/L	13.3 (9.2-20.1)	11.5 (9.4-20.4)	0.65
PLT, 10^9/L	164 (81-257)	132 (67-253)	0.5
PCT, ng/mL	5.11 (2.48-19.48)	1.24 (0.76-2.14)	0.11
EAA	0.75 (0.68-0.97)	0.6 (0.33-0.8)	**0.013**
Presepsin, pg/mL	914 (673-2,812)	525 (288-3,343)	0.44
CRP, mg/dL	3.41 (1.37-5.95)	1.5 (1.04-3.67)	1.0

LPS: lipopolysaccharide; MAP: mean arterial pressure; HR: heart rate; WBC: white blood cell; CRP: C-reactive protein; PLT: platelet; PCT: procalcitonin; EAA: endotoxin activity assay

experience with selective LPS-adsorption for sepsis treatment in children after congenital heart disease correction with cardiopulmonary bypass. Selective endotoxin adsorption was carried out using Polymyxin B-immobilized cartridges (Toraymyxin-PMX-0.5R). Ten children aged 9-48 months and weighing 6.2-14 kg received this procedure. In 8 cases, the infection source was ventilator-associated pneumonia, and in 1 case, it was mediastinitis and pleural empyema, and in 1 patient infected by bacterial translocation from the gut after the ECMO procedures (duration of 5 days) and dysfunction of gut. Gram-negative etiology in all cases was confirmed by microbiological examination of blood and of bronchoalveolar lavage fluid (sputum). The decision to include selective LPS-adsorption in complex therapy was made on the basis of clinical and laboratory data by a council of physicians. This study was approved by the Local Ethics Committee of the A.N. Bakulev NSPCCS.

Children before LPS-adsorption have clinical and laboratory signs of sepsis, hyperthermia (38.7-39.5 °C), leukocytosis/leucopenia 3.3-21 × 10^9/L, subcompensated disseminated intravascular coagulation syndrome (D-dimer 530-1,580 ng/mL), procalcitonin 6.5-130 ng/mL, presepsin 415-1,300 pg/mL, EAA 0.6-1.0, and high levels of CRP. The cardiopulmonary component prevails in the structure of organ failure, which requires multicomponent inotropic and/or vasopressor support and mechanical ventilation with high positive end expiratory pressure. Two children required RRT, daily intermittent hemodialysis, which used polysulphone high-flux membrane filters (AVpaed, Fresenius, Germany).

The results of this pilot study showed an improvement in hemodynamic and oxygenation indices, a tendency for leukocytosis and body temperature to decrease, positive X-ray dynamics and negative microbiological examination results. After LPS-adsorption, we noted decreases in the endotoxin level, procalcitonin and CRP [Table 1]. Statistical analyses were performed with SPSS software, version 20 (SPSS, Inc., USA). The results are expressed as the median and interquartile range. The critical level of significance was set at 0.05. Nine patients were discharged, and one patient died of cerebral edema and MODS after repeated emergency surgery due to the development of an acute fistula of the prosthetic mitral valve.

Other authors also reported that this blood purification method in children has no side effects.[75,76] Our results suggest that selective LPS-adsorption in children from middle childhood is clinically effective and safe. Endotoxin adsorption using Polymyxin B cartridges is one of the promising methods in children.

In this article, we briefly discussed some aspects of blood purification in the ICU of a cardiac surgery hospital. Over the last several decades, there have been huge changes in extracorporeal therapy use for critically ill patients. This is due to the proven efficiency of these methods in many pathological conditions and the emergence of new promising technologies. The availability of appropriate equipment and trained medical staff limiting the wide use of extracorporeal blood purification, especially in pediatric intensive care where it seems so complicated, is a thing of the past. The only issue that preserves a degree of technical complexity is creating adequate vascular access in a child and providing the possibility of performing the full-dose extracorporeal therapy according to the protocol and the assigned clinical tasks. Of course, we await the results of new multicenter randomized trials and its application for practical goals of blood purification in specific pathological conditions and clinical situations, particularly in MODS. These results will allow the most appropriate time for the initiation of blood purification to be determined, valid indications to be developed and its efficiency to be proven (convective or diffusive, continuous or intermittent, sorption or apheresis) in critically ill patients after high-risk surgery.

Authors' contributions

Conception, design and methodology of the study, results analysing and manuscript writing: M.

Yaroustovsky, M. Abramyan
Collecting samples and providing clinical data: E. Komardina, H. Nazarova
Final manuscript approval: M. Yaroustovsky, M. Abramyan, E. Komardina, H. Nazarova

Financial support and sponsorship
None.

Conflicts of interest
There are no conflicts of interest.

Patient consent
All patients in this study consented to all of the approved methods of treatment before their heart operation. Extracorporeal therapy was conducted in conformity with the institutional guidelines.

Ethics approval
The related study was approved by the Local Ethics Committee of the A.N. Bakulev NSPCCS.

REFERENCES

1. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Ronco C; Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) Investigators. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA* 2005;294:813-8.
2. Bellomo R, Ronco C. Continuous renal replacement therapy: hemofiltration, hemodiafiltration or hemodialysis. In: Ronco C, Bellomo R, Kellum JA, editors. Critical Care Nephrology. 2nd ed. Philadelphia: Saunders Elsevier; 2009. p. 1354-8.
3. Hoste EA, Kellum JA. Incidence, classification, and outcomes of acute kidney injury. *Contrib Nephrol* 2007;156:32-8.
4. Blinder JJ, Goldstein SL, Lee VV, Baycroft A, Fraser CD, Nelson D, Jefferies JL. Congenital heart surgery in infants: effects of acute kidney injury on outcomes. *J Thorac Cardiovasc Surg* 2012;143:368-74.
5. Kaddourah A, Goldstein S. Renal replacement therapy in neonates. *Clin Perinatol* 2014;41:517-27.
6. Gulla KM, Sachdev A, Gupta D, Gupta N, Anand K, Pruthi PK. Continuous renal replacement therapy in children with severe sepsis and multiorgan dysfunction - a pilot study on timing of initiation. *Indian J Crit Care Med* 2015;19:613-7.
7. Palevsky PM. Renal replacement therapy in acute kidney injury. *Adv Chronic Kidney Dis* 2013;20:76-84.
8. Ronco C, Ricci Z, De Backer D, Kellum JA, Taccone FS, Joannidis M, Pickkers P, Cantaluppi V, Turani F, Saudan P, Bellomo R, Joannes-Boyau Olivier, Antonelli M, Payen D, Prowle JR, Vincent JL. Renal replacement therapy in acute kidney injury: controversy and consensus. *Crit Care* 2015;19:146.
9. Bridges BC, Askenazi DJ, Smith J, Goldstein SL. Pediatric renal replacement therapy in the intensive care unit. *Blood Purif* 2012;34:138-48.
10. Goldstein SL. Advances in pediatric renal replacement therapy for acute kidney injury. *Semin Dial* 2011;24:187-91.
11. Bojan M, Gioanni S, Vouhé PR, Journois D, Pouard P. Early initiation of peritoneal dialysis in neonates and infants with acute kidney injury following cardiac surgery is associated with a significant decrease in mortality. *Kidney Int* 2012;82:474-81.
12. Ponce D, Balbi AL, Amerling R. Advances in peritoneal dialysis in acute kidney injury. *Blood Purif* 2012;34:107-16.
13. Jander A, Tkaczyk M, Pagowska-Klimek I, Pietrzykowski W, Moll J, Krajewski W, Nowicki M. Continuous veno-venous hemodiafiltration in children after cardiac surgery. *Eur J Cardiothorac Surg* 2007;31:1022-8.
14. Iarustovskiĭ MB, Grigor'iants RG, Strakhov DG, Banketov IaV, Geptner RA, Abramian AV, Shipova EA. A present-day variant of intermittent replacement renal therapy in patients with multiple organ failure after cardiovascular surgery. *Anesteziol Reanimatol* 2002;(2):49-54. (in Russian)
15. Askenazi DJ, Selewski DT, Paden ML, Cooper DS, Bridges BC, Zappitelli M, Fleming GM. Renal replacement therapy in critically ill patients receiving extracorporeal membrane oxygenation. *Clin J Am Soc Nephrol* 2012;7:1328-36.
16. Hoover NG, Heard M, Reid C, Wagoner S, Rogers K, Foland J, Paden ML, Fortenberry JD. Enhanced fluid management with continuous venovenous hemofiltration in pediatric respiratory failure patients receiving extracorporeal membrane oxygenation support. *Int Care Med* 2008;34:2241-7.
17. Shaheen IS, Harvey B, Watson AR, Pandya HC, Mayer A, Thomas D. Continuous venovenous hemofiltration with or without extracorporeal membrane oxygenation in children. *Pediatr Crit Care Med* 2007;8:362-5.
18. Chen H, Yu R, Yin N, Zhou J. Combination of extracorporeal membrane oxygenation and continuous renal replacement therapy in critically ill patients: a systematic review. *Crit Care* 2014;18:675.
19. Fleming GM, Askenazi DJ, Bridges BC, Cooper DS, Paden ML, Selewski DT, Zappitelli M. A multicenter international survey of renal supportive therapy during ECMO: the Kidney Intervention During Extracorporeal Membrane Oxygenation (KIDMO) group. *ASAIO J* 2012;58:407-14.
20. Yaroustovsky MB, Abramyan MV, Nazarova EI, Komardina EV. Continuous hemofiltration in a single circuit with extracorporeal membrane oxygenation in children with critical heart failure after cardiac surgery. *Clin Experiment Surg Petrovsky J* 2017;5:59-67. (in Russian)
21. Gupta P, McDonald R, Chipman CW, Stroud M, Gossett JM, Imamura M, Bhutta AT. 20-year experience of prolonged extracorporeal membrane oxygenation in critically ill children with cardiac or pulmonary failure. *Ann Thorac Surg* 2012;93:1584-90.
22. Daas M, Plevak DJ, Wijdicks EF, Rakela J, Wiesner RH, Piepgras DG, Dunn WF, Steers JL. Acute liver failure: results of 5 years clinical protocol. *Liver Transpl Surg* 1995;1:210-9.
23. Cochran JB, Losek JD. Acute liver failure in children. *Pediatr Emerg Care* 2007;23:129-35.
24. Lee WS, McKiernan P, Kelly DA. Etiology, outcome and prognostic indicators of childhood fulminant hepatic failure in the United Kingdom. *J Pediatr Gastroenterol Nutr* 2005;40:575-81.
25. Saliba F, Camus C, Durand F, Mathurin P, Letierce A, Delafosse B, Barange K, Perrigault PF, Belnard M, Ichaï P, Samuel D. Albumin dialysis with a noncell artificial liver support device in patients with acute liver failure: a randomized, controlled trial. *Ann Intern Med* 2013;159:522-31.
26. Stange J, Hassanein TI, Mehta R, Mitzner SR, Bartlett RH. The molecular adsorbent recycling system as a liver support system based on albumin dialysis: a summary of preclinical investigations, prospective, randomized, controlled clinical trial and clinical experience from 19 centers. *Artif Organs* 2002;26:103-40.
27. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, Triantafyllou E, Bernal W, Auzinger G, Shawcross D, Eefsen M, Bjerring PN, Clemmesen JO, Hockerstedt K, Frederiksen HJ, Hansen BA, Antoniades CG, Wendon J. High-volume plasma

exchange in patients with acute liver failure: an open randomised controlled trial. *J Hepatol* 2016;64:69-78.
28. Davenport A. Extracorporeal support for patients with hepatic failure. *Hemodial Int* 2003;7:256-63.
29. Saliba F, Samuel D. Artificial liver support: a real step forward. *Minerva Med* 2015;106:35-43.
30. Bañares R, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, Saliba F, Sauerbruch T, Klammt S, Ockenga J, Pares A, Wendon J, Brünnler T, Kramer L, Mathurin P, de la Mata M, Gasbarrini A, Müllhaupt B, Wilmer A, Laleman W, Eefsen M, Sen S, Zipprich A, Tenorio T, Pavesi M, Schmidt HH, Mitzner S, Williams R, Arroyo V; RELIEF study group. Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: the RELIEF trial. *Hepatology* 2013;57:1153-62.
31. Kribben A, Gerken G, Haag S, Herget-Rosenthal S, Treichel U, Betz C, Sarrazin C, Hoste E, Van Vlierberghe H, Escorsell A, Hafer C, Schreiner O, Galle PR,Mancini E, Caraceni P, Karvellas CJ, Salmhofer H, Knotek M, Ginès P, Kozik-Jaromin J, Rifai K; HELIOS Study Group. Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure. *Gastroenterology* 2012;142:782-9.
32. Rosenthal P. Is the molecular adsorbent recirculating system the answer for children with acute liver failure? *Liver Transpl* 2015;21:277-8.
33. Schaefer B, Schmitt CP. The role of molecular adsorbent recirculating system dialysis for extracorporeal liver support in children. *Pediatr Nephrol* 2013;28:1763-9.
34. Yaroustovsky MB, Abramyan MV, Komardina EV. Methods of molecular transfusion in intensive care of critical states in pediatric postoperative cardiac surgery patients. *Ann Russ Acad Med Sci* 2016;71:341-9. (in Russian)
35. Mitzner SR, Stange J, Klammt S, Risler T, Erley CM, Bader BD, Berger ED, Lauchart W, Peszynski P, Freytag J, Hickstein H, Loock J, Löhr JM, Liebe S, Emmrich J, Korten G, Schmidt R. Improvement of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a prospective, randomized, controlled clinical trial. *Liver Transpl* 2000;6:277-86.
36. Vaid A, Chweich H, Balk EM, Jaber BL. Molecular adsorbent recirculating system as artificial support therapy for liver failure: a meta-analysis. *ASAIO J* 2012;58:51-9.
37. Laleman W, Wilmer A, Evenepoel P, Elst IV, Zeegers M, Zaman Z, Verslype C, Fevery J, Nevens F. Effect of Prometheus liver assist system on systemic hemodynamics in patients with cirrhosis: a randomized controlled study. *World J Gastroenterol* 2008;14:2065-71.
38. Oppert M, Rademacher S, Petrasch K, Jörres A. Extracorporeal liver support therapy with Prometheus in patients with liver failure in the intensive care unit. *Ther Apher Dial* 2009;13:426-30.
39. Yaroustovsky MB, Abramian MV, Komardina EV, Rep'eva EV, Nazarova EI, Krotenko NP, Geptner RA. Extracorporeal methods of hematological correction in patients with acute liver insufficiency after cardiac surgery. *Anesteziol Reanimatol* 2014;59:4-10. (in Russian)
40. Meijers BKI, Verhamme P, Nevens F, Hoylaerts MF, Bammens B, Wilmer A, Arnout J, Vanrenterghem Y, Evenepoel P. Major coagulation disturbances during fractionated plasma separation and adsorption. *Am J Transplant* 2007;7:2195-9.
41. Gong D, Ji D, Zhu D, Xu B, Liu Z. Efficient removal of serum bilirubin by a novel artificial liver support system using albumin convection: a pilot study. *Blood Purif* 2012;34:201-8.
42. Donati G, La Manna G, Cianciolo G, Grandinetti V, Carretta E, Cappuccilli M, Panicali L, Iorio M, Piscaglia F, Bolondi L, Colì L, Stefoni S. Extracorporeal detoxification for hepatic failure using molecular adsorbent recirculating system: depurative efficiency and clinical results in a long-term follow-up. *Artif Organs* 2014;38:125-34.
43. Tsipotis E, Shuja A, Jaber BL. Albumin dialysis for liver failure: a systematic review. *Adv Chronic Kidney Dis* 2015;22:382-90.
44. Yaroustovsky MB, Abramyan MV, Krotenko NP, Komardina EV. Methods of molecular transfusion in the intensive therapy of critical states. *Ann RussAcad Med Sci* 2016;71:281-7.
45. Rifai K, Kribben A, Gerken G, Haag S, Herget-Rosenthal S, Treichel U, Betz C, Sarrazin C, Van Vlierberghe H, Hoste E, Escorsell A, Gines P, Hafer C, Schuchmann M, Galle PR, Bernardi M, Caraceni P, Abeles D, Berr F, Knotek M, Kozik-Jaromin J. Extracorporeal liver support by fractionated plasma separation and adsorption (Prometheus) in patients with acute-on-chronic liver failure (HELIOS study): a prospective randomized controlled multicenter study. *J Hepatol* 2010;52:S3.
46. Marshall JC. Endotoxin in the pathogenesis of sepsis. *Contrib Nephrol* 2010;167:1-13.
47. Hanna W, Wong HR. Pediatric sepsis: challenges and adjunctive therapies. *Crit Care Clin* 2013;29:203-22.
48. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. *JAMA* 2014;311:1308-16.
49. Berthet J, Damien P, Hamzeh-Cognasse H, Arthaud CA, Eyraud MA, Zéni F, Pozzetto B, McNicol A, Garraud O, Cognasse F. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. *Clin Immun* 2012;145:189-200.
50. Bosmann M, Ward PA. The inflammatory response in sepsis. *Trends Immunol* 2013;34:129-36.
51. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb SA, Beale RJ, Vincent JL, Moreno R; Surviving Sepsis Campaign Guidelines Committee including the Pediatric Subgroup. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med* 2013;41:580-637.
52. Zhou F, Peng Z, Murugan R, Kellum JA. Blood purification and mortality in sepsis: a meta-analysis of randomized trials. *Crit Care Med* 2013;41:2209-20.
53. Yaroustovsky M, Abramyan M, Krotenko N, Popov D, Plyushch M, Rogalskaya E. A pilot study of selective lipopolysaccharide adsorption and coupled plasma filtration and adsorption in adult patients with severe sepsis. *Blood Purif* 2015;39:210-7.
54. Yaroustovsky M, Abramyan M, Krotenko N, Popov D, Plyushch M, Rogalskaya E, Nazarova H. Combined extracorporeal therapy for severe sepsis in patients after cardiac surgery. *Blood Purif* 2014;37:39-46.
55. Kojika M, Sato N, Yaegashi Y, Suzuki Y, Suzuki K, Nakae H, Endo S: Endotoxin adsorption therapy for septic shock using polymyxin B-immobilized fibers (PMX): evaluation by high-sensitivity endotoxin assay and measurement of the cytokine production capacity. *Ther Apher Dial* 2006;10:12-8.
56. Cruz DN, Antonelli M, Fumagalli R, Foltran F, Brienza N, Donati A, Malcangi V, Petrini F, Volta G, Bobbio Pallavicini FM, Rottoli F, Giunta F, Ronco C. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. *JAMA* 2009;301:2445-52.
57. Marshall JC, Foster D, Vincent JL, Cook DJ, Cohen J, Dellinger RP, Opal S, Abraham E, Brett SJ, Smith T, Mehta S, Derzko A, Romaschin A; MEDIC study. Diagnostic and prognostic implications of endotoxemia in critical illness: results of the MEDIC study. *J Infect Dis* 2004;190:527-34.
58. Cruz DN. Polymyxin B hemoperfusion: matching the cure to the disease. *Crit Care Med* 2014;42:1309-10.

59. Mitaka C, Tsuchida N, Kawada K, Nakajima Y, Imai T, Sasaki S. A longer duration of polymyxin B-immobilized fiber column hemoperfusion improves pulmonary oxygenation in patients with septic shock. *Shock* 2009;32:478-83.
60. Tanaka Y, Okano K, Tsuchiya K, Yamamoto M, Nitta K. Polymyxin B hemoperfusion improves hemodynamic status in patients with sepsis with both Gram-negative and non-Gram-negative bacteria. *J Clin Exp Nephrol* 2016;1:4.
61. Takahashi M, Takeuchi H, Kawakubo H, Nakamura R, Takahashi T, Wada N, Saikawa Y, Omori T, Kitagawa Y. Effectiveness of polymyxin B-direct hemoperfusion (PMX-DHP) therapy using a polymyxin B-immobilized fiber column in patients with post-esophagectomy sepsis. *Esophagus* 2014;11:189-96.
62. Vincent JL, Laterre PF, Cohen J, Burchardi H, Bruining H, Lerma FA, Wittebole X, De Backer D, Brett S, Marzo D, Nakamura H, John S. A pilot-controlled study of a polymyxin B-immobilized hemoperfusion cartridge in patients with severe sepsis secondary to intra-abdominal infection. *Shock* 2005;23:400-5.
63. Cruz DN, Perazella MA, Bellomo R, de Cal M, Polanco N, Corradi V, Lentini P, Nalesso F, Ueno T, Ranieri VM, Ronco C. Effectiveness of polymyxin B-immobilized fiber column in sepsis: a systematic review. *Crit Care* 2007;11:R47.
64. Berto P, Ronco C, Cruz D, Melotti RM, Antonelli M. Cost-effectiveness analysis of polymyxin-B immobilized fiber column and conventional medical therapy in the management of abdominal septic shock in Italy. *Blood Purif* 2011;32:331-40.
65. Berlot G, Agbedjro A, Tomasini A, Bianco F, Gerini U, Viviani M, Giudici F. Effects of the volume of processed plasma on the outcome, arterial pressure and blood procalcitonin levels in patients with severe sepsis and septic shock treated with coupled plasma filtration and adsorption. *Blood Purif* 2014;37:146-51.
66. Haase M, Bellomo R, Baldwin I, Haase-Fielitz A, Fealy N, Davenport P, Morgera S, Goehl H, Storr M, Boyce N, Neumayer HH. Hemodialysis membrane with a high-molecular-weight cut-off and cytokine levels in sepsis complicated by acute renal failure: a phase 1 randomized trial. *Am J Kidney Dis* 2007;50:296-304.
67. Rimmelé Th, Kellum J. Clinical review: blood purification for sepsis. *Crit Care* 2011;15:205.
68. Esteban E, Ferrer R, Alsina L, Artigas A. Immunomodulation in sepsis: the role of endotoxin removal by polymyxin B-immobilized cartridge. *Mediators Inflamm* 2013;2013:507539.
69. Yaroustovsky M, Abramyan M, Krotenko N, Popov D, Plyushch M, Popok Z. Endotoxin adsorption using polymyxin B immobilised fibre cartridges in severe sepsis patients following cardiac surgery. *Int J Artif Organs* 2014;37:299-307.
70. Hu D, Sun S, Zhu B, Mei Z, Wang L, Zhu S, Zhao W. Effects of coupled plasma filtration adsorption on septic patients with multiple organ dysfunction syndrome. *Ren Fail* 2012;34:834-9.
71. Lee D, Haase M, Haase-Fielitz A, Paizis K, Goehl H, Bellomo R. A pilot, randomized, double-blind, cross-over study of high cut-off versus high-flux dialysis membranes. *Blood Purif* 2009;28:365-72.
72. Morgera S, Haase M, Kuss T, Vargas-Hein O, Zuckermann-Becker H, Melzer C, Krieg H, Wegner B, Bellomo R, Neumayer HH. Pilot study of the effects of high cutoff hemofiltration on the need for norepinephrine in septic patients with acute renal failure. *Crit Care Med* 2006;34:2099-104.
73. Naka T, Haase M, Bellomo R. "Super high-flux" or "high cut-off" hemofiltration and hemodialysis. *Contrib Nephrol* 2010;166:181-9.
74. Liu JP, Wang XW, Qie LP. Disease indicators for sepsis and analysis of sepsis treatment in children using the continuous blood purification technique. *Genet Mol Res* 2015;4:5685-93.
75. Hirakawa E, Ibara S, Tokuhisa T, Hiwatashi S, Hayashida Y, Maede Y, Matsui T, Nakazawa Y, Inoue T, Kodaira Y. Septic neonate rescued by polymyxin B hemoperfusion. *Pediatr Int* 2013;55:e70-2.
76. Morishita Y, Kita Y, Ohtake K, Kusano E, Hishikawa S, Mizuta K, Kawarasaki H. Successful treatment of sepsis with polymyxin-B immobilized fiber hemoperfusion in a child after living donor liver transplantation. *Dig Dis Sci* 2005;50:757.

Bobronnikova. *Vessel Plus* 2017;1:61-7
DOI: 10.20517/2574-1209.2016.10

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Original Article **Open Access**

Galectin-3 as a potential biomarker of metabolic disorders and cardiovascular remodeling in patients with hypertension and type 2 diabetes

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How to cite this article: Bobronnikova L. Galectin-3 as a potential biomarker of metabolic disorders and cardiovascular remodeling in patients with hypertension and type 2 diabetes. Vessel Plus 2017;1:61-7.

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Article history:
Received: 26-10-2016
Accepted: 08-05-2017
Published: 27-06-2017

Key words:
Arterial hypertension,
type 2 diabetes mellitus,
insulin resistance,
cardiovascular remodeling,
galectin-3

ABSTRACT

Aim: To determine the impact of galectin-3 levels on the progression of metabolic disorders and structural-functional changes in the myocardium and blood vessels in patients with concomitant systemic arterial hypertension (AH) and type 2 diabetes mellitus (type 2 DM). **Methods:** Ninety-five patients with hypertension stage II grade 2 (53 males and 42 females) were examined. The patients were separated: group 1 (n = 32) patients with AH, without carbohydrate metabolism disorders; group 2 (n = 30) patients with AH and pre-diabetes; group 3 (n = 33) concomitant AH and type 2 DM. Carbohydrate metabolism, insulin resistance parameters and galectin-3 levels were measured. Echocardiography was performed to determine the structural-functional condition of the heart. Estimation of the intima-media complex thickness of the common carotid artery was performed by duplex ultrasound scanning. **Results:** Hypertrophy of the left ventricular myocardium is most evident in patients with AH and type 2 DM compared with groups 1 and 2, and the control group ($P < 0.05$). The levels of galectin-3 in the group with concomitant hypertension and type 2 DM were higher than in groups 1 and 2, and the control group ($P < 0.05$). A positive correlation was revealed between the level of galectin-3, indices of insulin resistance, and structural-functional cardiac and vessel remodeling. **Conclusion**: Galectin-3 levels in serum are significantly linked with indices of insulin resistance and cardiovascular remodeling.

Quick Response Code:

INTRODUCTION

Despite the appreciable advancement in arterial hypertension (AH) prevention and treatment, the disease remains to be one of the most urgent problems in cardiology.[1] Commonly, AH accompanies pre-diabetes and type 2 diabetes mellitus (type 2 DM), important risk factors for cardiovascular complications.

Chronic hyperglycemia in AH patients with glucose metabolism disorders and type 2 DM significantly contributes to the formation and progression of cardiovascular disease.[2] Carbohydrate metabolism disorders significantly increase risk of acute cardiovascular events, such as myocardial infarction.[3] A meta-analysis of large prospective studies encompassing a total of 450,000 selected patients demonstrated that the risk of coronary death is increased 2-3 fold in patients with diabetes.[4] Chronic hyperglycemia not only directly promotes myocardial lesions, but also exacerbates the negative effect of other risk factors for cardiovascular pathology.[5] It has been noted that the degree of impairment in myocardial diastolic properties directly depends on the level of glycated hemoglobin, which relates to the degree of myocardium protein glycation[6] and deposition of collagen in the myocardium with fibrosis.[7] Furthermore, it is known that patients with carbohydrate metabolism disorders have higher left ventricular (LV) mass, even in the absence of AH and heart disease.[8]

Recently, new biomarkers that may reflect the pathogenic process of insulin resistance and consequent cardiovascular events have been identified. One of those markers is galectin-3 (Gal-3).[9] It belongs to the β-galactoside-binding proteins family. Due to the presence of a collagenous domain in its structure, galectin can interact with a wide range of extracellular matrix proteins such as tenascin, fibronectin, and laminin.[10] Gal-3 is produced by numerous cells including neutrophils, macrophages, labrocytes, fibroblasts, and osteoclasts.[11] Gal-3 is found in lungs, stomach, intestine and uterus.[12] It was determined that Gal-3 is involved in proliferation, macrophage chemotaxis, phagocytosis, neutrophil transudation, oxidative stress, apoptosis, angiogenesis, and associated with the development of insulin resistance.[13,14] In experimental studies using different models of cardiovascular disease, Gal-3 has been implicated as an inducer of fibrosis and myocardial remodeling.[15,16]

Recently, a single-case study demonstrated Gal-3 as a mediator of development and progression of cardiac hypertrophy in patients with AH.[17] There are also data describing its impact on the formation of diastolic myocardial dysfunction.[18]

The role of Gal-3 has not yet been examined in large cohorts of AH and type 2 DM patients, but according to single-case studies the plasma Gal-3 levels correlate with the prevalence of type 2 DM, AH and obesity.[19,20]

Thus, the goal of determining the effect of this biomarker on the progression of dysglycemia and cardiovascular remodeling in patients with AH and comorbid type 2 DM is attracting considerable interest in clinical practice.

The experiments herein studied the effect of Gal-3 levels on the progression of metabolic disorders and structural-functional changes in the myocardium and blood vessels of patients with concomitant AH and type 2 DM.

METHODS

Ninety-five patients with hypertension stage II grade 2 (53 males and 42 females) were examined in the specialized Department of Arterial Hypertension and Kidney Disease of State Institution (National Institute of Therapy named after L.T. Malaya) of National Academy of Medical Sciences of Ukraine. The patients were distributed into the following groups: group 1 (n = 32) included patients with AH, without carbohydrate metabolism disorders; group 2 (n = 30), patients with AH and pre-diabetes; group 3 (n = 33), concomitant AH and type 2 DM. The control group (n = 20) was comparable by age and gender to each group of examined patients. All the patients signed an informed consent agreement to participate in the research.

The exclusion criteria included the presence of severe somatic diseases such as kidney, liver, heart, and respiratory failure, anamnestic evidence of stroke, myocardial infarction, oncological diseases, decompensated type 2 DM course according to World Health Organization criteria, female patients previously diagnosed with type 2 DM macrovascular complications, thyroid function disorders, primary familial hypercholesterolemia, secondary hypertensions, and pregnancy.

AH diagnostics were performed according to the recommendations of the European Society of Hypertension and the European Society of Cardiology (ESH/ESC, 2013), as well as the Ukrainian Association of Cardiology on prevention and treatment of hypertension (2013). Anthropometric measurements included the calculation of body mass index (BMI) and the degree of obesity according to the International Diabetes Federation criteria (2015). Type 2 DM was diagnosed according to the general recommendations of the European Association for the Study of Diabetes

(EASD, 2013).

Glycated hemoglobin (HbA1c) levels in whole blood were measured using the test-system produced by Reagent (Ukraine). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by the formula: HOMA-IR = insulin (fasting insulin, mcU/mL) × fasting glucose (mmol/L) / 22.5. At HOMA-IR > 2.77 patients were considered as having insulin resistance.[21] C-reactive protein (CRP) - DRG Elisa were determined with Enzyme-Linked ImmunoSorbent Assay (ELISA).

The concentrations of fasting blood glucose (FBG) levels was determined using glucose oxidase methodology. Fasting serum insulin levels were determined by ELISA with DRG kit (USA). In order to determine glucose tolerance, an oral glucose tolerance test was performed.

Gal-3 levels in the serum were determined by ELISA using the kit of Bender Med Systems (Austria).

Structural-functional cardiac parameters were determined by echocardiography using the diagnostic system (Philips IU, USA) in B- and M-modes by standard technique according to the general recommendations of the American Society of Echocardiography (2015). The determination of the interventricular septal wall thickness, LV posterior wall, end-systolic dimension (ESD), end-diastolic dimension (EDD), LV ejection fraction; end-systolic volume (ESV), end-diastolic volume (EDV); and analysis of the LV diastolic function were conducted during the registration of transtricuspid diastolic flow in the pulsed-wave Doppler mode. LV myocardial mass (LVMM) was calculated using the formula of Devereux R.B. (1986) and the index of LVMM (LVMMI) was determined as the ratio of LVMM to body surface area as described by Brown D.W. (2000).

To assess the structural-functional state of the vessels we performed ultrasound scanning of common carotid arteries with measurement of the intima-media complex thickness of the common carotid artery (IMT CCA) using an ultrasound diagnostic system (Philips IU, USA) with linear sensor at the frequency of at least 7 MHz in B-mode.

As soon as the distribution of indices were converted to the normal, statistical processing of obtained results was performed by parametric methods (with evaluation of mean, its standard error or standard deviation; Student *t*-test for independent samples by groups, Pearson correlation) using Statsoft Statistica 8.0 software package. The threshold of *P*-value of 0.05 was chosen; in case of multiple comparison a Bonferroni correction was made.

RESULTS

The results of trophological status analysis revealed characteristic features in the examined groups. Patients with a BMI of 18.5 to 24.9 kg/m^2 (n = 5) were identified in the group with isolated AH. However third-degree obesity (BMI > 40.0 kg/m^2) was observed in one patient with AH, in three patients with AH and pre-diabetes, and in 5 patients with concomitant AH and type 2 DM. The majority of patients with isolated and concomitant disease in groups 2 and 3 (64.2%, 54.3%, and 51.3% respectively) had a BMI of 30 to 34.9 kg/m^2. In patients with type 2 DM, those with a BMI of 30.0 to 34.9 kg/m^2 were predominately male (67.3%), while those with a BMI of 35.0 to 39.9 kg/m^2 were predominately female (74.5%).

FBG levels were significantly higher in patients with concomitant AH and type 2 DM compared with patients in group 1, group 2 and the control group (P < 0.05).

Maximal values of HOMA-IR, insulin and C-peptide in patients of group 3 compared to that of groups 1 and 2 [Table 1], describes the progression of IR associated with the presence of type 2 diabetes. HOMA-IR exceeded control values by 2.1-fold in the group of patients with AH, 2.4-fold in patients with AH and pre-diabetes, and by 2.7-fold higher in patients with concomitant AH and type 2 DM.

In groups 2 and 3, there was a positive correlation between insulin levels in the peripheral blood and LV hypertrophy (LVH) (r = 0.44, P < 0.01; and r = 0.42, P < 0.01, respectively) and IMT CCA (r = 0.36, P < 0.05; and r = 0.38, P < 0.05, respectively).

Examination of IMT CCA showed mean values in patients with AH (0.85 ± 0.05 mm, P < 0.05) in comparison with groups 2 and 3, and control group. In patients with AH and pre-diabetes this index was 0.9 ± 0.05 mm (P < 0.05). In patients with AH and type 2 diabetes IMT CCA was 0.95 ± 0.07 mm (P < 0.05). IMT CCA indices in groups 2 and 3 displayed reverse correlation with HOMA-IR (r = 0.36, P < 0.001).

An increase of IMT CCA ≥ 0.9 mm was observed in patients with AH in 46.8% of cases, 52.7% in patients with AH and pre-diabetes, and 59.2% in patients with concomitant disease.

Indices of IMT CCA were associated with age (P = 0.032), BMI (P = 0.044), waist measurements (P =

Table 1: Carbohydrate metabolism and IR indices in surveyed groups of patients (mean ± SD)

Indices	1. Control (n = 20)	2. AH (n = 32)	3. AH + pre-diabetes (n = 30)	4. AH + type 2 DM (n = 33)	P
HOMA-IR	1.66 ± 0.56	3.46 ± 2.70	4.46 ± 2.70	5.42 ± 3.10	P_{1-2} = 0.00001 P_{1-3} = 0.00001 P_{2-3} = 0.13
Insulin, mcU/mL	5.57 ± 2.10	10.90 ± 5.80	11.90 ± 5.80	13.80 ± 7.40	P_{1-2} = 0.0004 P_{1-3} = 0.0002 P_{2-3} = 0.049
CRP, ng/mL	0.48 ± 0.23	0.94 ± 0.53	1.02 ± 0.41	1.20 ± 0.73	P_{1-2} = 0.0003 P_{1-3} = 0.0001 P_{2-3} = 0.064
Glucose, mmol/L	4.25 ± 0.12	6.58 ± 1.12	7.60 ± 3.85	8.20 ± 1.23	P_{1-2} = 0.00002 P_{1-3} = 0.00003 P_{2-3} = 0.14
HbA1c (%)	4.60 ± 0.02	6.50 ± 0.01	7.10 ± 0.50	9.20 ± 0.60	P_{1-2} = 0.0006 P_{1-3} = 0.0003 P_{2-3} = 0.035
GTT, mmol/L	5.12 ± 0.04	6.16 ± 0.05	11.34 ± 0.40	14.80 ± 2.10	P_{1-2} = 0.0004 P_{1-3} = 0.0002 P_{2-3} = 0.054

SD: standard deviation; IR: insulin resistance; AH: arterial hypertension; type 2 DM: type 2 diabetes mellitus; HOMA-IR: homeostatic model assessment for insulin resistance; CRP: C-reactive protein; HbA1c: glycated hemoglobin; GTT: glucose tolerance test

Table 2: Hemodynamic parameters in surveyed groups of patients

Indexes	Control (n = 20)	AH (n = 32)	AH + pre-diabetes (n = 30)	AH + type 2 DM (n = 33)
SBP, mmHg	125.30 ± 4.40	158.70 ± 3.20*	173.90 ± 4.60*,#	185.40 ± 4.80$
DBP, mmHg	81.80 ± 5.60	90.20 ± 5.30*	98.50 ± 8.20*,#	105.20 ± 9.30$
LASPh, cm	2.72 ± 0.09	2.82 ± 0.90	3.25 ± 0.05*,#	3.64 ± 0.07$
EDV, cm^3	129.00 ± 1.16	135.24 ± 1.16	141.10 ± 1.15 *,#	144.20 ± 1.13#
ESV, cm^3	47.40 ± 0.30	48.10 ± 0.40	61.20 ± 0.80*,#	78.40 ± 0.60$
EDD, cm^3	4.62 ± 0.02	5.16 ± 0.04*	5.35 ± 0.06*,#	5.56 ± 0.08#
ESD LV, cm	4.12 ± 0.04	4.18 ± 0.04	3.55 ± 0.03*	3.96 ± 0.04#
Stroke volume, cm^3	75.50 ± 1.27	83.90 ± 1.36	91.10 ± 0.74*,#	97.20 ± 0.72#
Ejection fraction, %	65.40 ± 0.86	66.80 ± 0.74	52.90 ± 0.42*	54.80 ± 0.44#
Myocardium mass index LV, g/m^2	81.60 ± 0.02	98.60 ± 0.03	116.40 ± 1.42*,#	143.40 ± 1.36#,$

*P < 0.05 *vs.* control group; #P < 0.05 *vs.* AH patients; $$P$ < 0.05 *vs.* patients with AH and pre-diabetes. AH: arterial hypertension; type 2 DM: type 2 diabetes mellitus; SBP: systolic blood pressure; DBP: diastolic blood pressure; LASPh: left atrial systole phase; EDV: end-diastolic volume; ESV: end-systolic volume; EDD: end-diastolic dimension; ESD: end-systolic dimension; LV: left ventricular

0.046), and HOMA-IR (P = 0.044). Patients from group 2 in 12.3% of cases, and in 38.7% of cases from group 3 were marked as having atherosclerotic plaques with a degree of stenosis < 10%.

LVH was diagnosed in 92.3% of patients from group 3, 75.8% of patients from group 2, and 55.4% of patients from group 1, compared to the controls (P < 0.05). Patients with AH and type 2 DM were characterized by an increase of average LVMM (P < 0.05) and LVMMI values (P < 0.05) compared with patients in group 1, group 2, and the control group. Indices of Doppler echography intracardiac hemodynamics in patients with AH were characterized by a decrease in the early and late diastolic filling rate of LV [Table 2]. However, under the concomitant conditions of disease in groups 2 and 3, these indexes were significantly reduced compared to group 1 and the control group (P < 0.05). Similar patterns were observed in the ratio of the early and late diastolic filling velocities.

Also, the maximum ESD LV and EDD LV values were registered in patients of group 3 in comparison with group 1 and control (P < 0.05) and a pattern of increase in these indicators was observed in group 2. The same situation was observed with respect to ESV LV and EDV LV (P < 0.05). Patients in groups 2 and 3 with concomitant disease had a significant increase of MMI relative to group 1 (P < 0.05).

Levels of Gal-3 were heightened in patients concomitant AH and type 2 DM compared to groups 1 and 2, and the control group (34.2 ng/mL compared to 27.6 ng/mL, 25.8 ng/mL, 15.6 ng/mL respectively, P < 0.001).

In patients with AH and pre-diabetes we observed a trend towards a positive correlation between Gal-3, CRP, and HOMA-IR, which is most marked in patients

with AH and type 2 DM [Gal-3, CRP (r = 0.52, P < 0.01), and HOMA-IR (r = 0.62, P < 0.01)].

In patients with concomitant AH and type 2 DM there was a positive correlation between Gal-3 and the IMT CCA (r = 0.44, P < 0.001). It is should be noted that in patients with isolated AH and in patients of groups 2 and 3 having LVH, Gal-3 levels were significantly higher than in patients without LVH (P < 0.05).

DISCUSSION

The study found patients from group 3 to have the highest insulin and C-peptide values, suggesting that the progression of IR under hyperinsulinemia is associated with the presence of type 2 diabetes.

Identified correlations between insulin and LVH and IMT CCA in patients from groups 2 and 3, suggest that hyperinsulinemia is an important component for the development and progression of AH and contributes to the development of myocardial hypertrophy and smooth muscle elements of peripheral vessels.

Further observation is necessary in patients from groups 2 and 3 with identified atherosclerotic plaques of stenosis grade exceeding 10%. It should be noted that current results are concordant with the Insulin Resistance Atherosclerosis Study, which revealed a clear direct dependence between the degree of insulin resistance and the carotid artery wall thickness both in individuals without diabetes, and in patients with type 2 DM. With each unit of insulin resistance, IMT increased by 30 μm.[22] Similar conclusions were obtained in an analysis of the results of 11 studies, involving 1578 patients with type 2 DM, including 132 patients with pre-diabetes who developed IMT CCA index during the treatment.[23] It was found that un-treated patients with type 2 DM had an average content of HbA1c = 7.86%, and an increase in IMT CCA by 0.034 mm per year.[24] This reveals significant dependence between HbA1c levels and the rate of IMT CCA increase.[24]

Patients of group 2 with concomitant AH presented with structural-functional and intraventricular hemodynamic changes, changes that were most apparent in group 3.

As to the mechanisms of LVH in AH patients with type 2 DM, we should mention a complex of metabolic disorders which are typical for type 2 DM.[25] Those disorders primary include insulin resistance and hyperinsulinemia. Indeed, insulin resistance and hyperinsulinemia are triggers that induce a series of hormonal, neurohumoral and metabolic events that are the basis of early LVH in concomitant AH and type 2 DM.[26] This process is largely a result of activation of the sympathetic-adrenal system, a powerful stimulus for renin and angiotensin II excretion with a consequent increase of aldosterone production and development of the hyperkinetic, hyper-renin variant of hypertension.[27] Powerful hypertrophic and proliferative processes in the myocardium are triggered accompanied by volume overload of the heart, which leads to LVH in patients with AH and type 2 DM. The most important proliferative and hypertrophic factors involved in the processes of myocardial hypertrophy include a number of cytokines and other growth factors.[25]

It has been observed that Gal-3 levels are positively correlated with insulin resistance indices (CRP, HOMA) in all groups of patients. These data confirm that Gal-3 is involved in the formation of insulin resistance. In this study, higher levels of Gal-3 are associated with the development of type 2 DM. The obtained results substantiate the study of Seferovic *et al*.,[28] which describes elevated levels of Gal-3 in patients with type 2 DM that were strongly correlated with HbA1c levels. Yilmaz *et al*.[29] showed that Gal-3 may be an independent predictor of type 2 DM in general. Recent clinical studies have shown that high levels of Gal-3 correlate with gender, age, and the risk of cardiovascular pathology development.[30] On the other hand, the study of Ohkura *et al*.[31] found that low levels of serum Gal-3 are associated with insulin resistance in patients with type 2 DM, but it should be noted that a small sample of patients were examined with no comorbid pathology and a BMI within the normal range.

Gal-3 values were positively correlated with LVH and increased indices of IMT CCA. These data are consistent with available literature showing that Gal-3 increases its activity under the influence of such factors as increased levels of angiotensin II and pressure overload of the myocardial wall. References contain some data identifying a correlation of GAL-3 and the total number of atherosclerosis plaques in coronary arteries.[32] It can be assumed that Gal-3 levels may be a marker for the major pathogenetic mechanisms of development and progression of atherosclerosis, which can help the early detection of atherosclerosis before its clinical manifestations. Also, increasing serum Gal-3 levels may help to identify early heart failure with preserved LV ejection fraction, which often occurs in patients with hypertension, diabetes, and obesity.[33]

The above results indicate that elevated levels of Gal-3 in the blood plasma can be a predictor of type 2 DM, and factor in the development of structural and functional changes in the myocardium. Nevertheless, these findings require further study on a large sample

of patients, taking into account gender differences and duration of comorbid pathology.

In conclusion, chronic hyperglycemia and insulin resistance affected the cardiovascular remodeling of the myocardium and blood vessels, which was mostly observed in the concomitant AH and type 2 DM patients.

This study makes it possible to assume that Gal-3 is involved in the processes of insulin resistance progression and cardiovascular remodeling in patients with isolated hypertension and patients with hypertension and type 2 DM.

In order to gain a clearer understanding of the Gal-3 role in the pathogenesis of hypertension and type 2 DM, the larger clinical trial needs to be conducted.

Authors' contributions

L. Bobronnikova contributed solely to this paper.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

All the patients signed an informed consent agreement to participate in the research.

Ethics approval

The study protocol was approved by the Local Ethics Committee of the Kharkov National Medical University and was performed in accordance to the Declaration of Helsinki.

REFERENCES

1. Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F; Task Force Members. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2013;31:281-357.
2. Fox CS, Coady S, Sorlie PD, Levy D, Meigs JB, D'Agostino RB Sr, Wilson PW, Savage PJ. Trends in cardiovascular complications of diabetes. *JAMA* 2004;292:2495-9.
3. Aroor AR, Mandavia CH, Sowers JR. Insulin resistance and heart failure: molecular mechanisms. *Heart Fail Clin* 2012;8:609-17.
4. Huxley R, Barzi F, Woodward M. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. *BMJ* 2006;332:73-8.
5. Blacher J, Staessen JA, Girerd X. Pulse pressure not mean pressure determines cardiovascular risk in older hypertensive patients. *Arch Intern Med* 2000;160:1085-9.
6. Rydén L, Standl E, Bartnik M, Van den Berghe G, Betteridge J, de Boer MJ, Cosentino F, Jönsson B, Laakso M, Malmberg K, Priori S, Ostergren J, Tuomilehto J, Thrainsdottir I, Vanhorebeek I, Stramba-Badiale M, Lindgren P, Qiao Q, Priori SG, Blanc JJ, Budaj A, Camm J, Dean V, Deckers J, Dickstein K, Lekakis J, McGregor K, Metra M, Morais J, Osterspey A, Tamargo J, Zamorano JL, Deckers JW, Bertrand M, Charbonnel B, Erdmann E, Ferrannini E, Flyvbjerg A, Gohlke H, Juanatey JR, Graham I, Monteiro PF, Parhofer K, Pyörälä K, Raz I, Schernthaner G, Volpe M, Wood D; Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC); European Association for the Study of Diabetes (EASD). Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). *Eur Heart J* 2007;28:88-136.
7. Schillaci G, Verdecchia P, Porcellati C, Cuccurullo O, Cosco C, Perticone F. Continuous relation between left ventricular mass and cardiovascular risk in essential hypertension. *Hypertension* 2000;35:580-6.
8. Vakili BA, Okin PM, Devereux RB. Prognostic implications of left ventricular hypertrophy. *Am Heart J* 2001;141:334-41.
9. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG, Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol* 2012;60:1249-56.
10. de Boer RA, van Veldhuisen DJ, Gansevoort RT, Muller Kobold AC, van Gilst WH, Hillege HL, Bakker SJ, van der Harst P. The fibrosis marker galectin-3 and outcome in the general population. *J Intern Med* 2012;272:55-64.
11. van der Velde AR, Gullestad L, Ueland T, Aukrust P, Guo Y, Adourian A, Muntendam P, van Veldhuisen DJ, de Boer RA. Prognostic value of changes in galectin-3 levels over time in patients with heart failure: data from CORONA and COACH. *Circ Heart Fail* 2013;6:219-26.
12. Barrow H, Rhodes JM, Yu LG. Simultaneous determination of serum galectin-3 and -4 levels detects metastases in colorectal cancer patients. *Cell Oncol (Dordr)* 2013;36:9-13.
13. Vereecken P, Awada A, Suciu S, Castro G, Morandini R, Litynska A, Lienard D, Ezzedine K, Ghanem G, Heenen M. Evaluation of the prognostic significance of serum galectin-3 in American Joint Committee on Cancer stage III and stage IV melanoma patients. *Melanoma Res* 2009;19:316-20.
14. McCullough PA, Olobatoke A, Vanhecke TE. Galectin-3: a novel blood test for the evaluation and management of patients with heart failure. *Rev Cardiovasc Med* 2011;12:200-10.
15. Mensah-Brown EP, Al Rabesi Z, Shahin A, Al Shamsi M, Arsenijevic N, Hsu DK, Liu FT, Lukic ML. Targeted disruption of the galectin-3 gene results in decreased susceptibility to multiple low dose streptozotocin-induced diabetes in mice. *Clin Immunol* 2009;130:83-8.
16. Pejnovic NN, Pantic JM, Jovanovic IP, Radosavljevic GD, Milovanovic MZ, Nikolic IG, Zdravkovic NS, Djukic AL, Arsenijevic NN, Lukic ML. Galectin-3 deficiency accelerates high-fat diet-induced obesity and amplifies inflammation in adipose tissue and pancreatic islets. *Diabetes* 2013;62:1932-44.
17. Saksida T, Nikolic I, Vujicic M, Nilsson UJ, Leffler H, Lukic ML, Stojanovic I, Stosic-Grujicic S. Galectin-3 deficiency protects pancreatic islet cells from cytokine-triggered apoptosis in vitro. *J Cell Physiol* 2013;228:1568-76.
18. Darrow AL, Shohet RV, Maresh JG. Transcriptional analysis of the

endothelial response to diabetes reveals a role for galectin-3. *Physiol Genomics* 2011;43:1144-52.
19. Karlsen AE, Sørling ZM, Sparre T, Larsen MR, Mahmood A, Sørling J, Roepstorff P, Wrzesinski K, Larsen PM, Fey S, Nielsen K, Heding P, Ricordi C, Johannesen J, Kristiansen OP, Christensen UB, Kockum I, Luthman H, Nerup J, Pociot F. Immune-mediated beta-cell destruction in vitro and in vivo-a pivotal role for galectin-3. *Biochem Biophys Res Commun* 2006;344:406-15.
20. Pugliese G, Iacobini C, Ricci C, Blasetti Fantauzzi C, Menini S. Galectin-3 in diabetic patients. *Clin Chem Lab Med* 2014;52:1413-23.
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-9.
22. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998;339:229-34.
23. Yokoyama H, Katakami N, Yamasaki Y. Recent advances of intervention to inhibit progression of carotid intima-media thickness in patients with type 2 diabetes mellitus. *Stroke* 2006;37:2420-7.
24. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 2003;421:384-8.
25. Thakur V, Richards R, Reisin E. Obesity, hypertension, and the heart. *Am J Med Sci* 2001;321:242-8.
26. Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000;106:473-81.
27. du Cailar G, Pasquié JL, Ribstein J, Mimran A. Left ventricular adaptation to hypertension and plasma renin activity. *J Hum Hypertens* 2000;14:181-8.
28. Seferovic JP, Lalic NM, Floridi F, Tesic M, Seferovic PM, Giga V, Lalic K, Jotic A, Jovicic S, Colak E, Salerno G, Cardelli P, Di Somma S. Structural myocardial alterations in diabetes and hypertension: the role of galectin-3. *Clin Chem Lab Med* 2014;52:1499-505.
29. Yilmaz H, Cakmak M, Inan O, Darcin T, Akcay A. Increased levels of galectin-3 were associated with prediabetes and diabetes: new risk factor? *J Endocrinol Invest* 201;38:527-33.
30. de Boer RA, van Veldhuisen DJ, Gansevoort RT, Muller Kobold AC, van Gilst WH, Hillege HL, Bakker SJ, van der Harst P. The fibrosis marker galectin-3 and outcome in the general population. *J Intern Med* 2012;272:55-64.
31. Ohkura T, Fujioka Y, Nakanishi R, Shiochi H, Sumi K, Yamamoto N, Matsuzawa K, Izawa S, Ohkura H, Ueta E, Kato M, Miyoshi E, Taniguchi S, Yamamoto K. Low serum galectin-3 concentrations are associated with insulin resistance in patients with type 2 diabetes mellitus. *Diabetol Metab Syndr* 2014;6:106.
32. Ozturk D, Celik O, Satilmis S, Aslan S, Erturk M, Cakmak HA, Kalkan AK, Ozyilmaz S, Diker V, Gul M. Association between serum galectin-3 levels and coronary atherosclerosis and plaque burden/structure in patients with type 2 diabetes mellitus. *Coron Artery Dis* 2015;26:396-401.
33. Meluzín J, Tomandl J. Can biomarkers help to diagnose early heart failure with preserved ejection fraction? *Dis Markers* 2015;2015:426045.

Cordero *et al. Vessel Plus* 2017;1:68-76
DOI: 10.20517/2574-1209.2017.02

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Initial experience with bioresorbable vascular scaffolds for percutaneous revascularisation in patients with acute coronary syndrome

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How to cite this article: Cordero A, López-Palop R, Carrillo P, Frutos A, Gunturiz C, García-Carrilero M, Bertomeu-Martinez V. Initial experience with bioresorbable vascular scaffolds for percutaneous revascularisation in patients with acute coronary syndrome. Vessel Plus 2017;1:68-76.

Article history:
Received: 21-01-2017
Accepted: 22-03-2017
Published: 27-06-2017

Key words:
Bioresorbable vascular stents,
stents,
acute coronary syndrome,
revascularisation,
prognosis

ABSTRACT

Aim: Bioresorbable vascular scaffolds (BVS) have recently been introduced to minimise the long-term complications of metallic stents in acute coronary syndrome (ACS), but their benefits have not been well analysed. **Methods:** The authors studied all ACS patients treated with any kind of stent at a single centre between March 2013 (when the first BVS was implanted) and June 2016. **Results:** The study included 951 subjects, mean age 67.9 ± 13.3 years, mean Global Registry of Acute Coronary Events (GRACE) score 148.5 ± 44.8, 75.2% men and 38.2% with an ST-segment elevation myocardial infarction. The mean number of stents implanted was 1.3 ± 1.0 and 54 subjects (5.7%) received at least 1 BVS. Drug-eluting stents were implanted in 57.3% subjects, followed by bare-metal stents (19.0%). The subjects treated with BVS were younger and had lower GRACE scores compared to the rest. In-hospital mortality was 4.8% and no subject treated with BVS died before discharge. BVS-treated patients received dual antiplatelet therapy or new antiplatelet agents more frequently. During a median follow-up of 13 months, all-cause mortality was 7.8%, cardiovascular mortality was 6.1%, and at least 1 major cardiovascular event occurred in 26.4% of the subjects. Stent type did not affect prognosis. **Conclusion:** Coronary revascularisation using BVS in selected ACS patients is safe and effective.

INTRODUCTION

Cardiovascular disease is the leading cause of mortality in the world, and coronary heart disease is the primary contributor to that cardiovascular mortality.[1] Coronary heart disease is a progressive condition resulting from atherosclerosis that produces unstable coronary plaque, with episodes of erosion and intraluminal thrombosis that become manifest as an acute coronary syndrome (ACS),[2] ventricular fibrillation, asystole, and sudden death. Percutaneous coronary interventions are the cornerstone of ACS treatment, and coronary stents are utilised in almost all of these.[3-5] Bare-metal stents (BMS) were the first devices developed to improve the results of balloon angioplasty by the resolving post-balloon dissections,

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and preventing coronary wall recoil and constrictive remodelling.[6] Percutaneous coronary intervention (PCI) with stents was progressively adopted as the standard of care for interdiction of ACS, myocardial ischemia and infarction.[7] However, as this strategy was being adopted it became clear that stent restenosis was a frequent and potentially fatal complication in patients treated with BMS.[6] Drug-eluting stents (DES) largely overcame the restenosis problem with BMS but they were also linked to many long-term complications such as late stent-thrombosis, neo-atherosclerosis, side-branch jailing and/or preclusion of future surgical revascularisation at the same lesion.[8]

Bioresorbable vascular scaffolds (BVS) are newly adopted device for coronary stenting and were designed to minimise the long-term complications of stents[6] by providing an on-permanent scaffold.[9] The first BVS was designed and tested in humans at the end of last century and now shows promising results in terms of feasibility and complete reabsorption.[10] Several randomised clinical trials and meta-analyses have outlined the usefulness of BVS for coronary revascularisation,[11-13] although the clinical benefit for ACS patients has not been well analysed.[14,15] The objective of our study was to report the safety and utility of BVS compared to other stents in a cohort of ACS patients.

METHODS

Study design

In 2009, we initiated an ongoing prospective registry of all patients admitted for ACS in our institution. Several results have already been published.[5,16] For this study, we included all patients admitted with the diagnosis of ACS between January 2013 and March 2016, resulting in a cohort of 951 consecutive subjects. The patients were classified according to the stent type that was used and the cohort was divided in 4 groups: no stent, BMS, DES, or BVS. The interventional cardiologist made the treatment decisions and stent selection after considering the clinical situation. The DES implanted during the study period were all second- and third-generation devices. Intravascular ultrasound and optical coherence tomography were performed in most cases where a BVS was implanted but the final decisions were made according to the treating physicians' clinical judgements.

ACS was defined by the presence of typical clinical symptoms of unstable angina or impending myocardial infarction. ACS was classified as ST-elevation myocardial infarction (STEMI) and non-STEMI according to the electrocardiographic findings. The mortality risk was assessed by the Global Registry of Acute Coronary Events (GRACE) score; according to individual scores patients with categorised in low risk < 108), intermediate risk (109-130) and high-risk (> 140).[3] All patients with STEMI received primary PCI and patients with non-STEMI were referred for PCI, if appropriate as determined by a comprehensive medical evaluation. According to previous reports, incomplete coronary revascularisation was defined as when at least one of the main coronary arteries, or a secondary branch > 1.5 mm, with significant lesions (> 70%), was treated but not fully revascularised.[17,18]

Risk factors, clinical antecedents, treatments, complementary tests, and main diagnosis at discharge were tabulated for all subjects by trained medical staff.[5] For the diagnosis of previous coronary artery disease, subjects needed to have a clinical diagnosis of myocardial infarction, stable or unstable angina or known prior coronary revascularisation. Previous heart failure was determined based upon their prior clinical diagnosis of heart failure. Glomerular filtration rate was estimated from serum creatinine values with the Modification of Diet in Renal Disease Study equation. Overall estimation of comorbidities was assessed by the Charlson Index, adapted for patients with coronary heart disease;[19] patients with a Charlson score ≥ 4 were considered as having high-comorbidity risks. Following current recommendations, optimal medical treatment was codified when patients received prescriptions for four medical treatments: an antiplatelet agent, a statin, a beta-adrenergic blocker, and an angiotensin-converter enzyme inhibitor or angiotensin-receptor blocker.[20,21] Statin treatment was classified as low intensity, moderate intensity, or high intensity based on the current guidelines.[22,23] Ticagrelor and prasugrel were analysed together as a group of new antiplatelet agents in place of clopidogrel.

The post-discharge follow-up of all subjects followed a well-established protocol. The end-points analysed were cardiovascular and all-cause mortality as well as time to first major cardiovascular event (MACE) (ACS, heart failure hospitalisation, fatal or non-fatal stroke, or major bleeding). Two staff members reviewed clinical records, and (in absence of hospital contact), the electronic medical history was consulted for outpatient follow-up care. All physicians in the geographic area use a unified electronic medical record (EMR) that documents every contact with the health care system, for either medical or nursing visits. If electronic medical reports were lacking, one nurse who had been trained to acquire the needed data by telephone was directed to call the subject and assess all endpoints through a follow-up conversation. All emergency calls, visits

Table 1: Clinical features of the cohort according to the stent type

Characteristics	No stent	BMS	DES	BVS	*P*
Number	171 (18.0%)	181 (19.9%)	545 (57.3%)	54 (5.7%)	
Age (years), mean ± SD	71.4 ± 13.0	72.4 ± 13.8	66.5 ± 12.3	56.4 ± 12.7	< 0.01#
Age > 75 years	41.5%	50.3%	26.1%	5.6%	< 0.01
Males	63.2%	74.6%	78.0%	87.0%	< 0.01
Diabetes	32.7%	35.9%	29.7%	31.5%	0.47
Hypertension	70.8%	69.1%	59.8%	38.9%	< 0.01
Current smokes	19.9%	24.9%	36.5%	50.0%	< 0.01
Dyslipidemia	49.7%	50.3%	47.0%	42.6%	0.70
Previous HF	7.6%	2.8%	1.8%	0.0%	< 0.01
Previous CHD	25.1%	21.5%	22.4%	5.6%	0.02
Previous PCI	14.7%	10.1%	16.7%	3.7%	0.02
Previous CABG	7.1%	3.6%	4.0%	0.0%	0.13
Peripheral arterial disease	7.6%	7.2%	4.6%	5.6%	0.37
Atrial fibrillation	15.8%	14.4%	4.4%	0.0%	< 0.01
Previous stroke	10.5%	13.3%	5.1%	3.7%	< 0.01
COPD	9.4%	12.7%	10.3%	9.3%	0.73
STEMI	11.1%	46.4%	43.5%	42.6%	< 0.01
GFR (mL/min/1.72 m^2), mean ± SD	78.3 ± 38.5	70.7 ± 29.6	82.8 ± 31.3	82.9 ± 22.9	< 0.01#
GFR < 60 mL/min/1.72 m^2	26.3%	40.7%	16.7%	15.4%	< 0.01
GRACE score, mean ± SD	146.8 ± 50.6	162.6 ± 46.0	145.6 ± 42.3	124.8 ± 29.7	< 0.01#
GRACE > 140	42.9%	67.1%	51.4%	32.7%	< 0.01
Charlson index, mean ± SD	2.6 ± 2.9	2.7 ± 2.4	2.2 ± 1.8	2.1 ± 2.1	0.03*
Charlson index ≥ 4	23.4%	24.9%	18.2%	14.8%	0.12
LVEF (%), mean ± SD	54.6 ± 13.0	52.5 ± 12.4	53.3 ± 12.5	56.4 ± 8.8	0.07

#For comparisons between BVS and the rest; *for comparisons between BVS or DES and the rest. BMS: bare metal stent; DES: drug-eluting stents; BVS: bioresorvable vascular scaffold; HF: heart failure; CHD: coronary heart disease; PCI: percutaneous coronary intervention; CABG: coronary arterial bypass graft; COPD: chronic obstructive pulmonary disease; STEMI: ST-elevation myocardial infarction; GFR: glomerular filtration rate; GRACE: Global Registry of Acute Coronary Events; LVEF: left ventricle ejection fraction

to the emergency room of the hospital, or hospital readmissions also are registered in the single informatics application. Fatalities directly related to cardiac events, such as ACS, heart failure hospitalisation or sudden death were attributed to cardiovascular causes; non-cardiovascular mortality was coded when another concurrent process was thought to be the main cause of the fatality, representing mainly infections, cancer deaths or accidents. The ethics committee of the hospital approved the protocol for the study and for obtaining informed consent from the subjects.

Statistical analysis

Data were processed with IBM SPSS 22.0 and STATA 14-0 statistical packages for Mac computers. Quantitative variables are presented as mean ± standard deviation (SD) and differences were assessed by a Students *t*-test or Chi-squared tests. Qualitative variables are presented as percentages and differences were analysed by the analysis of variance (ANOVA) test. An analysis of interactions and collinearity between main clinical variables was performed and results were taken under consideration for further analysis. Statistical differences were accepted as significant if the *P* value was < 0.05.

Cox regression models performed survival analyses once the proportional risk tests were verified. The model was adjusted by all variables that obtained *P* values < 0.1 in the univariate analysis or could have plausible clinical implications; results are presented as the hazard ratio (HR) and 95% confidence intervals (CI). Stent type was analysed as a categorical variable in dummy models. The model's discriminative accuracy was assessed by the Harrell's C-statistic, and its calibration was verified by the Gronnesby and Borgan test. Analysis of recurrent cardiovascular events was performed by negative binomial regression, and results are presented as incidence rate ratio (IRR) and rates/100 patients/year.[24]

RESULTS

During the study period, a total of 951 patients were enrolled as subjects. They had a mean age of 67.9 (± 13.4) years, 75.2% were male, 38.2% were diagnosed with STEMI, and their mean GRACE score was 148.5 (± 44.8). The revascularisation strategies included DES (57.3%) and BMS (19.0%); 54 (5.7%) received at least 1 BVS [Table 1]. In 171 subjects (18.0%), no stents were placed after the angiography and diagnostic studies were done. BVS-treated patients were the younger, and had the highest percentage of current smokers but the lowest prevalence of known heart failure, atrial fibrillation, or stroke. No differences were observed in

Table 2: Procedure and coronary lesions characteristics

Characteristics	No stent	BMS	DES	BVS	*P*
Radial approach	93.0%	96.4%	98.1%	100.0%	0.01
No. of vessels with lesions, mean ± SD	0.8 ± 1.1	1.6 ± 0.8	1.7 ± 0.8	2.1 ± 0.8	< 0.01
No. of stents/patient, mean ± SD	0.0 ± 0.0	1.4 ± 0.6	1.6 ± 0.9	2.8 ± 1.5	< 0.01
Complete revascularization	49.0%	71.4%	76.3%	85.2%	< 0.01
Left main disease	7.1%	0.6%	6.1%	0.0%	< 0.01
Coronary lesions					< 0.01
1 vessel disease	13.1%	59.9%	47.4%	24.1%	
2 vessel disease	13.1%	22.2%	29.8%	42.6%	
3 vessel disease	5.7%	16.8%	16.5%	33.3%	
LM + 1 vessel disease	1.6%	0.0%	0.6%	0.0%	
LM + 2 vessel disease	0.8%	0.0%	2.3%	0.0%	
LM + 3 vessel disease	6.6%	0.6%	3.2%	0.0%	

BMS: bare metal stent; DES: drug-eluting stents; BVS: bioresorvable vascular scaffold; LM: left main

Table 3: Medical treatments recommended at discharge according to the stent received within hospital revascularization

Characteristics	No stent	BMS	DES	BVS	*P*
Aspirin	76.9%	96.4%	96.6%	98.1%	0.01
Clopidogrel	25.6%	64.3%	43.5%	29.6%	< 0.01
Ticagrelor	5.8%	14.9%	28.3%	31.5%	< 0.01
Prasugrel	0.0%	17.3%	25.6%	38.9%	< 0.01
Any new antiplatelet	5.8%	32.1%	53.9%	70.4%	< 0.01
DAPT	18.6%	92.9%	94.5%	98.1%	< 0.01
Oral anticoagulants	14.1%	10.1%	5.3%	0.0%	< 0.01
ARB/ACEI	71.2%	79.2%	82.0%	79.6%	0.03
Beta blockers	64.7%	83.3%	88.4%	90.7%	< 0.01
Diuretics	37.2%	26.8%	19.5%	11.1%	< 0.01
Statins	69.2%	95.2%	95.1%	96.3%	< 0.01
High dose statin	32.1%	70.8%	73.6%	83.3%	< 0.01
Moderate dose statin	29.5%	16.7%	16.9%	13.0%	< 0.01
Low dose statin	7.7%	6.5%	1.7%	0.0%	< 0.01
Nitrates	19.9%	6.5%	4.7%	1.9%	< 0.01
Fibrates	1.9%	3.0%	1.9%	1.9%	0.87
Ezetimibe	1.9%	0.6%	4.0%	1.9%	0.10
Optimal medical treatment	33.3%	63.7%	70.4%	66.7%	< 0.01

BMS: bare metal stent; DES: drug-eluting stents; BVS: bioresorvable vascular scaffold; DAPT: double antiplatelet treatment; ARB: angiotensin receptor blocker; ACEI: angiotensin-converter enzyme inhibitors

the prevalence of diabetes or dyslipidaemia according to the stent type received. BMS-treated patients had the highest prevalence of comorbidities, reflected by the highest mean Charlson index.

The details of the revascularisation outcomes are presented in Table 2. The radial artery approach was utilised in most cases and in all BVS-treated subjects. The mean number of coronary lesions was 1.6 (± 0.9) which was significantly higher in BVS-treated subjects; the mean number of stents per subject was 1.3 (± 1.0) and BVS-treated cases also had the highest rate. As a consequence, the rate of complete revascularisation was the highest in patients treated with BVS. No BVS implantations were attempted in the left main coronary artery.

In-hospital mortality was 4.8% (46 patients out of 951) and no patient treated with BVS died prior to discharge. The highest in-hospital mortality rate was observed in patients who were not (or could not be) stented (8.8%), followed by those treated with BMS (7.2%) and DES (3.3%). As shown in Table 3, BVS-treated patients received the highest rate of dual antiplatelet treatment and 70% received a new antiplatelet drug (prasugrel or ticagrelor). Generally, patients treated with DES or BVS received more intensive medical treatments.

Post-discharge follow-up was achieved in 95.0% of the cohort, with median time of 13 months (interquartile range 9.0 to 22.0). All-cause mortality was 7.8%, and cardiovascular mortality was 6.1%. Additionally, 26.4% subjects experienced at least one MACE. Kaplan-Meier curves are presented in Figure 1. All-cause mortality varied according to stent type: no stent (13.5%), BMS (10.1%), DES (5.7%), and BVS (5.6%); the differences were significant (log-rank test, $P < 0.01$). Cardiovascular mortality declined in the same order: no stent (10.3%),

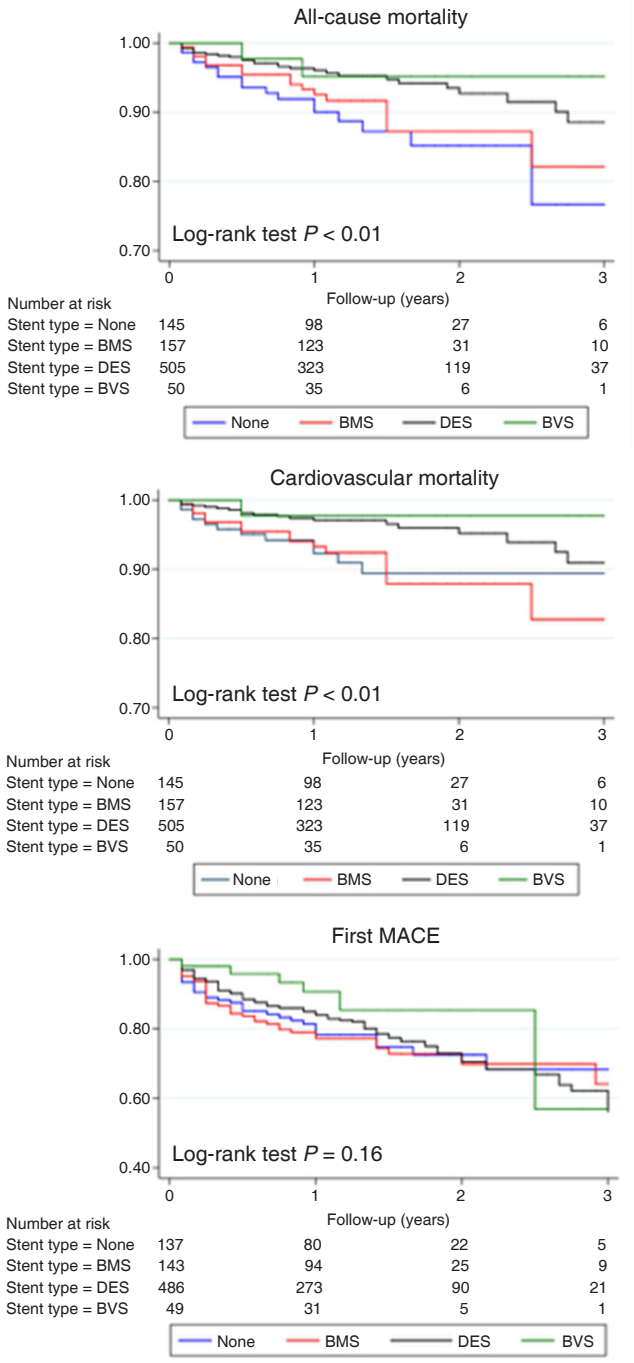

Figure 1: Kaplan-Myer curves presenting cardiovascular mortality, all-cause mortality and time to first major cardiovascular event (MACE) according to stent type. BMS: bare metal stent; DES: drug-eluting stent; BVS: bioresorbable vascular scaffold

BMS (9.5%), DES (4.0%), and BVS (3.7%) (log-rank test, $P < 0.01$). No differences were observed between DES and BVS in mortality rates. No differences were observed in the incidence of MACE according to stent type, although a tendency to lower incidence of time to first MACE was noted for BVS: no stent (28.8%), BMS (31.5%), DES (25.5%), and BVS (16.7%) ($P = 0.16$). The highest rate of MACEs/year/100 patients was

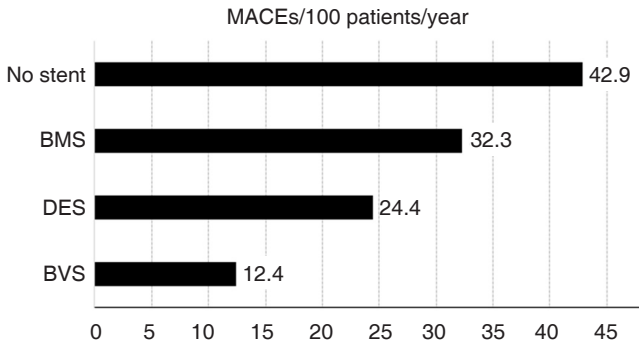

Figure 2: Major cardiovascular event (MACE) rates/100 patients/year according to stent type. BMS: bare metal stent; DES: drug-eluting stent; BVS: bioresorbable vascular scaffold

recorded in patients that received no stents, followed by BMS, DES, and BVS [Figure 2]. Multivariate analysis did not find any associations of mortality or cumulative MACEs with stent type [Table 4]. Diabetes, age > 75 years and GRACE score > 140 were associated with higher cardiovascular and all-cause mortality, whereas predictors of recurrent MACEs included age > 75 years, diabetes, and previous cardiovascular disease; revascularisation was negatively associated with recurrent events.

DISCUSSION

This single-centre experience with BVS supports their safety and effectiveness for revascularisation during ACS. BVS-treated patients had a lower risk profile despite the fact that they had more coronary lesions and were treated more aggressively, as they received a higher number of stents, more complete revascularisation, and more intensive antiplatelet and statin regimens.

Decision-making for stent use is influenced by many factors related to patient characteristics, coronary lesions, and other risks. DES were introduced to better control the rate of restenosis that occurs in patients treated with BMS. The superiority of DES has been largely demonstrated,[25] although mortality benefit was only clearly outlined with the later generation of DES.[8] BVS were conceived to avoid long-term complications of the metal structures by providing temporary structural integrity before being resorbed completely within the vessel wall.[9,26] The long-term incidence of cardiovascular events related to DES-treated vessels is around 2% to 3% per year for at least 5 years[27,28] and the contribution of permanent metallic devices in lumen target lesions has a relevant contribution.[29] The Absorb® BVS is a 150 μm thick bioresorbable polyl-lactide scaffold with a conformal bioresorbable coating (with a total thickness of 7 μm) that elutes everolimus.[30] Angiographic follow-up of BVS has

Table 4: Results of the multivariate analysis

Characteristics	Cardiovascular mortality		All-cause mortality		Cumulative major cardiovascular events	
	HR, 95% CI	*P*	HR, 95% CI	*P*	IRR, 95% CI	*P*
Age, > 75 years	3.67 (1.82-7.41)	< 0.01	3.17 (1.75-5.74)	< 0.01	1.73 (1.26- 2.39)	< 0.01
Diabetes	2.96 (1.63-5.36)	< 0.01	3.35 (2.00-5.62)	< 0.01	1.91 (1.45-2.53)	< 0.01
Revascularization	0.69 (0.25-1.89)	0.48	0.75 (0.32-1.77)	0.52	0.58 (0.33-1.00)	0.05
Previous CVD	1.01 (0.96-1.08)	0.55	1.03 (0.98-1.08)	0.23	1.05 (1.01-1.08)	< 0.01
GRACE score > 140	3.27 (1.76-6.09)	< 0.01	3.49 (2.04-5.99)	< 0.01	1.12 (0.82-1.53)	0.47

HR: hazard ratio; CI: confidence intervals; IRR: incidence rate ratio; CVD: cardiovascular disease; GRACE: Global Registry of Acute Coronary Events

clearly demonstrated the complete reabsorption of the scaffolds[9] and significant lumen gain.[31] The initial experience of BVS in our institution supports the clinical safety and efficacy of BVS in ACS patients with a significant post-discharge follow-up.

As with every innovation, concerns arose when data from randomised clinical trials and large numbers of patients treated with BVS became available. The pooled analysis of first studies showed the equivalence at 1-year of BVS compared with everolimus-DES although a non-significant trend (HR: 2.09, 95% CI: 0.92 to 4.75, *P* = 0.08) to higher late stent-thrombosis was already outlined.[30] In the ABSORB-II trial, BVS had similar rates of repeat revascularisation at 1 year of follow-up, despite inferior mid-term angiographic performance, in comparison with everolimus-eluting metallic stents.[11] Nonetheless, patients treated with a BVS had a three-fold increased risk of subacute stent thrombosis. These results have been verified in subsequent meta-analyses.[12,13] Stent thrombosis is a challenging clinical problem and related to many factors, including the different antiplatelet regimens, discontinuation of dual antiplatelet therapy, procoagulant states, stent malposition, polymer content, and many others.[32,33] Major cardiovascular events and mortality rates in the BVS-treated patients in our cohort were similar or even lower than in patients treated with other stents. Moreover, patients treated with BVS in our study had a higher number of vessels with significant lesions, and subsequently received more stents. This suggests that in well-selected ACS patients under intensive platelet treatment, the use of BVS can be a reasonable strategy of percutaneous revascularisation as has been proposed by other reports[14,15] and meta-analyses.[12,13]

BVS-treated patients in our cohort received dual antiplatelet therapy with clopidogrel or the newer antiplatelet drugs, prasugrel or ticagrelor, more frequently than the rest of patients. This could be influenced by many factors, such as the percutaneous coronary intervention characteristics, the number of stents, the younger age or absence of concomitant anticoagulation, but reflects that patients that received a BVS were treated more intensively. The effect of BVS for late adverse events could be especially important for young patients with ACS because they have an impaired vascular healing[34] and new antiplatelet agents could have similar effects.[35] The efficacy of BVS has been tested in only two randomised clinical trials[36,37] and most reports come from observational studies.[14,15,38] The primary endpoint in many of these studies was not cardiovascular mortality, and only the feasibility of the BVS implantation was assessed. We conducted an observational prospective study with the aim of providing mortality rates in a real-world cohort of patients. Moreover, we examined the cumulative incidence of recurrent events, which has been proposed as the best approach to monitor the actual course and prognosis of coronary heart disease.[24]

Coronary heart disease is a chronic inflammatory disease and it develops as a result of a progressive process.[39] Despite optimal medical treatment and revascularisation, recurrent events are common.[21,40] The most frequent statistical analyses for follow-up events are based in time-to-events, and therefore, patients are excluded from further analysis once they experience such events. Nonetheless, analysis of recurrent events has been proposed as a more accurate way to assess the actual life-long course and prognosis.[41] Optimal medical treatment has been demonstrated to provide benefit in patients with stable[42] and unstable[21] coronary heart disease regardless of revascularisation. Nonetheless, revascularisation has a much more critical role in ACS patients, and it has been identified as one of the major factors related to long-term ACS survival. Our analysis identified a negative association between revascularisation and recurrent events that provides additional support to its key role in the treatment of ACS patients. STEMI represents less than 40% of ACS and its emergent treatment requires many resources because acute phase mortality is much higher than in non-STEMI.[1] Nonetheless, the long-term mortality and medical costs are equivalent for both types of ACS. Non-STEMI patients comprise a heterogeneous group, and these patients are usually older and have more comorbidities. This can yield challenging decision-making with regard to revascularisation and medical treatment. As a result,

revascularisation rates are significantly lower in non-STEMI compared to STEMI patients, highlighting the unmet needs for validated and organised systems of care for non-STEMI patients.[43] Our results highlight the role of revascularisation for ACS and the recurrence of ischemic events. Stent type was not associated with any of these events although our follow-up suggested that revascularisation has higher impact than the other techniques used.

Our study has several limitations. The first is that a very limited number of patients were treated with BVS. Second, there may have been unmeasured confounders, details about the physician's decision-making, or patient factors that are not captured by the registry but account for the treatment differences observed. Furthermore, our analysis uses observational, non-randomised data, and thus, associations between various treatments and outcomes may be confounded by unmeasured variables. Finally, long-term outcomes could be modified by factors that were not captured by the follow-up protocol that was employed at our outpatient centres. Nonetheless, since clinical features and event rates are similar to previous reports,[4,36-38] we believe that our results should be representative of daily clinical practice and that our conclusions are probably valid.

In conclusion, our study supports the hypothesis that coronary revascularisation using BVS is safe and effective in selected ACS patients. Percutaneous coronary revascularisation with stents is the cornerstone of ACS treatment but there are long-term complications of metallic devices that can be relevant. The use of BVS is a novel strategy with promising results, and there is an expectation that they will prove useful in ACS selected patients. The long term prognosis of BVS-treated patients remains unknown, but given the growing number of patients being treated with these devices, further evidence regarding their net clinical benefit will likely emerge with additional time.

Authors' contributions

Manuscript's conception and writting: A. Cordero, R. López-Palop and P. Carrillo
Data base fulfilment: A. Cordero, C. Gunturiz, M. Garcia-Carrilero
Manuscript's revision: A. Frutos, V. Bertomeu-Martinez

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

All patients included in the registry provided the informed consent.

Ethics approval

The ethics committee of the hospital approved the study protocol and informed consent.

REFERENCES

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2016 Update: a report from the American Heart Association. *Circulation* 2016;133:e38-360.
2. Crea F, Liuzzo G. Pathogenesis of acute coronary syndromes. *J Am Coll Cardiol* 2013;61:1-11.
3. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, Bax JJ, Borger MA, Brotons C, Chew DP, Gencer B, Hasenfuss G, Kjeldsen K, Lancellotti P, Landmesser U, Mehilli J, Mukherjee D, Storey RF, Windecker S, Baumgartner H, Gaemperli O, Achenbach S, Agewall S, Badimon L, Baigent C, Bueno H, Bugiardini R, Carerj S, Casselman F, Cuisset T, Erol C, Fitzsimons D, Halle M, Hamm C, Hildick-Smith D, Huber K, Iliodromitis E, James S, Lewis BS, Lip GY, Piepoli MF, Richter D, Rosemann T, Sechtem U, Steg PG, Vrints C, Luis Zamorano J; Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2016;37:267-315.
4. Barrabes JA, Bardaji A, Jimenez-Candil J, del Nogal Saez F, Bodi V, Basterra N, Marco E, Melgares R, Cunat de la Hoz J, Fernandez-Ortiz A; investigators of DIOCLES study. Prognosis and management of acute coronary syndrome in Spain in 2012: the DIOCLES study. *Rev Esp Cardiol* 2015;68:98-106.
5. Cordero A, Lopez-Palop R, Carrillo P, Frutos A, Miralles S, Gunturiz C, Garcia-Carrilero M, Bertomeu-Martinez V. Changes in acute coronary syndrome treatment and prognosis after implementation of the infarction code in a hospital with a cardiac catheterization unit. *Rev Esp Cardiol (Engl Ed)* 2016;69:754-9.
6. Serruys PW, Garcia-Garcia HM, Onuma Y. From metallic cages to transient bioresorbable scaffolds: change in paradigm of coronary revascularization in the upcoming decade? *Eur Heart J* 2012;33:16-25b.
7. Ko DT, Tu JV, Samadashvili Z, Guo H, Alter DA, Cantor WJ, Hannan EL. Temporal trends in the use of percutaneous coronary intervention and coronary artery bypass surgery in New York State and Ontario. *Circulation* 2010;121:2635-44.
8. Windecker S, Stortecky S, Stefanini GG, da Costa BR, Rutjes AW, Di Nisio M, Silletta MG, Maione A, Alfonso F, Clemmensen PM, Collet JP, Cremer J, Falk V, Filippatos G, Hamm C, Head S, Kappetein AP, Kastrati A, Knuuti J, Landmesser U, Laufer G, Neumann FJ, Richter D, Schauerte P, Sousa Uva M, Taggart DP, Torracca L, Valgimigli

M, Wijns W, Witkowski A, Kolh P, Jüni P. Revascularisation versus medical treatment in patients with stable coronary artery disease: network meta-analysis. *BMJ* 2014;348:g3859.
9. Onuma Y, Serruys PW, Perkins LE, Okamura T, Gonzalo N, Garcia-Garcia HM, Regar E, Kamberi M, Powers JC, Rapoza R, van Beusekom H, van der Giessen W, Virmani R. Intracoronary optical coherence tomography and histology at 1 month and 2, 3, and 4 years after implantation of everolimus-eluting bioresorbable vascular scaffolds in a porcine coronary artery model: an attempt to decipher the human optical coherence tomography images in the ABSORB trial. *Circulation* 2010;122:2288-300.
10. Tamai H, Igaki K, Kyo E, Kosuga K, Kawashima A, Matsui S, Komori H, Tsuji T, Motohara S, Uehata H. Initial and 6-month results of biodegradable poly-l-lactic acid coronary stents in humans. *Circulation* 2000;102:399-404.
11. Serruys PW, Chevalier B, Sotomi Y, Cequier A, Carrie D, Piek JJ, Van Boven AJ, Dominici M, Dudek D, McClean D, Helqvist S, Haude M, Reith S, de Sousa Almeida M, Campo G, Iniguez A, Sabate M, Windecker S, Onuma Y. Comparison of an everolimus-eluting bioresorbable scaffold with an everolimus-eluting metallic stent for the treatment of coronary artery stenosis (ABSORB II): a 3 year, randomised, controlled, single-blind, multicentre clinical trial. *Lancet* 2016;388:2479-91.
12. Cassese S, Byrne RA, Ndrepepa G, Kufner S, Wiebe J, Repp J, Schunkert H, Fusaro M, Kimura T, Kastrati A. Everolimus-eluting bioresorbable vascular scaffolds versus everolimus-eluting metallic stents: a meta-analysis of randomised controlled trials. *Lancet* 2016;387:537-44.
13. Lu C, Filion KB, Eisenberg MJ. The safety and efficacy of Absorb bioresorbable vascular scaffold: a systematic review. *Clin Cardiol* 2016;39:48-55.
14. Gori T, Schulz E, Hink U, Wenzel P, Post F, Jabs A, Münzel T. Early outcome after implantation of Absorb bioresorbable drug-eluting scaffolds in patients with acute coronary syndromes. *EuroIntervention* 2014;9:1036-41.
15. Kočka V, Malý M, Toušek P, Buděšínský T, Lisa L, Prodanov P, Jarkovský J, Widimský P. Bioresorbable vascular scaffolds in acute ST-segment elevation myocardial infarction: a prospective multicentre study 'Prague 19'. *Eur Heart J* 2014;35:787-94.
16. Cordero A, Moreno-Arribas J, Bertomeu-González V, Agudo P, Miralles B, Masiá MD, López-Palop R, Bertomeu-Martinez V. Low levels of high-density lipoproteins cholesterol are independently associated with acute coronary heart disease in patients hospitalized for chest pain. *Rev Esp Cardiol* 2012;65:319-25.
17. Farooq V, Serruys PW, Garcia-Garcia HM, Zhang Y, Bourantas CV, Holmes DR, Mack M, Feldman T, Morice MC, Stahle E, James S, Colombo A, Diletti R, Papafaklis MI, de Vries T, Morel MA, van Es GA, Mohr FW, Dawkins KD, Kappetein AP, Sianos G, Boersma E. The negative impact of incomplete angiographic revascularization on clinical outcomes and its association with total occlusions: the SYNTAX (Synergy Between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery) trial. *J Am Coll Cardiol* 2013;61:282-94.
18. Garcia S, Sandoval Y, Roukoz H, Adabag S, Canoniero M, Yannopoulos D, Brilakis ES. Outcomes after complete versus incomplete revascularization of patients with multivessel coronary artery disease: a meta-analysis of 89,883 patients enrolled in randomized clinical trials and observational studies. *J Am Coll Cardiol* 2013;62:1421-31.
19. Sachdev M, Sun JL, Tsiatis AA, Nelson CL, Mark DB, Jollis JG. The prognostic importance of comorbidity for mortality in patients with stable coronary artery disease. *J Am Coll Cardiol* 2004;43:576-82.
20. Boden WE, O'Rourke RA, Teo KK, Hartigan PM, Maron DJ, Kostuk WJ, Knudtson M, Dada M, Casperson P, Harris CL, Chaitman BR, Shaw L, Gosselin G, Nawaz S, Title LM, Gau G, Blaustein AS, Booth DC, Bates ER, Spertus JA, Berman DS, Mancini GB, Weintraub WS; COURAGE Trial Research Group. Optimal medical therapy with or without PCI for stable coronary disease. *N Engl J Med* 2007;356:1503-16.
21. Iqbal J, Zhang YJ, Holmes DR, Morice MC, Mack MJ, Kappetein AP, Feldman T, Stahle E, Escaned J, Banning AP, Gunn JP, Colombo A, Steyerberg EW, Mohr FW, Serruys PW. Optimal medical therapy improves clinical outcomes in patients undergoing revascularization with percutaneous coronary intervention or coronary artery bypass grafting: insights from the synergy between percutaneous coronary intervention with TAXUS and cardiac surgery (SYNTAX) trial at the 5-year follow-up. *Circulation* 2015;131:1269-77.
22. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC Jr, Watson K, Wilson PW; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA Guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014;63:2889-934.
23. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Ž, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WM, Vlachopoulos C, Wood DA, Zamorano JL. 2016 ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) developed with the special contribution of the European Assocciation for Cardiovascular Prevention & Rehabilitation (EACPR). *Atherosclerosis* 2016;253:281-344.
24. Rogers JK, Pocock SJ, McMurray JJ, Granger CB, Michelson EL, Ostergren J, Pfeffer MA, Solomon SD, Swedberg K, Yusuf S. Analysing recurrent hospitalizations in heart failure: a review of statistical methodology, with application to CHARM-Preserved. *Eur J Heart Fail* 2014;16:33-40.
25. Windecker S, Kolh P, Alfonso F, Collet JP, Cremer J, Falk V, Filippatos G, Hamm C, Head SJ, Jüni P, Kappetein AP, Kastrati A, Knuuti J, Landmesser U, Laufer G, Neumann FJ, Richter DJ, Schauerte P, Sousa Uva M, Stefanini GG, Taggart DP, Torracca L, Valgimigli M, Wijns W, Witkowski A. 2014 ESC/EACTS Guidelines on myocardial revascularization: the Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). *Eur Heart J* 2014;35:2541-619.
26. Karanasos A, Simsek C, Gnanadesigan M, van Ditzhuijzen NS, Freire R, Dijkstra J, Tu S, Van Mieghem N, van Soest G, de Jaegere P, Serruys PW, Zijlstra F, van Geuns RJ, Regar E. OCT assessment of the long-term vascular healing response 5 years after everolimus-eluting bioresorbable vascular scaffold. *J Am Coll Cardiol* 2014;64:2343-56.
27. Stone GW, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N, Marso SP, Parise H, Templin B, White R, Zhang Z, Serruys PW; PROSPECT Investigators. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med* 2011;364:226-35.
28. Taniwaki M, Stefanini GG, Silber S, Richardt G, Vranckx P, Serruys PW, Buszman PE, Kelbaek H, Windecker S; RESOLUTE All-Comers Investigators. 4-year clinical outcomes and predictors of repeat revascularization in patients treated with new-generation drug-eluting stents: a report from the RESOLUTE All-Comers trial

(A Randomized Comparison of a Zotarolimus-Eluting Stent With an Everolimus-Eluting Stent for Percutaneous Coronary Intervention). *J Am Coll Cardiol* 2014;63:1617-25.
29. Tada T, Kadota K, Hosogi S, Miyake K, Ohya M, Amano H, Izawa Y, Kanazawa T, Kubo S, Ichinohe T, Hyoudou Y, Hayakawa Y, Sabbah MM, Otsuru S, Hasegawa D, Habara S, Tanaka H, Fuku Y, Katoh H, Goto T, Mitsudo K. Association between tissue characteristics assessed with optical coherence tomography and mid-term results after percutaneous coronary intervention for in-stent restenosis lesions: a comparison between balloon angioplasty, paclitaxel-coated balloon dilatation, and drug-eluting stent implantation. *Eur Heart J Cardiovasc Imaging* 2015;16:1101-11.
30. Stone GW, Gao R, Kimura T, Kereiakes DJ, Ellis SG, Onuma Y, Cheong WF, Jones-McMeans J, Su X, Zhang Z, Serruys PW. 1-year outcomes with the Absorb bioresorbable scaffold in patients with coronary artery disease: a patient-level, pooled meta-analysis. *Lancet* 2016;387:1277-89.
31. Lane JP, Perkins LE, Sheehy AJ, Pacheco EJ, Frie MP, Lambert BJ, Rapoza RJ, Virmani R. Lumen gain and restoration of pulsatility after implantation of a bioresorbable vascular scaffold in porcine coronary arteries. *JACC Cardiovasc Interv* 2014;7:688-95.
32. de la Torre-Hernández JM, Alfonso F, Hernández F, Elizaga J, Sanmartin M, Pinar E, Lozano I, Vazquez JM, Botas J, Perez de PA, Hernández JM, Sanchis J, Nodar JM, Gomez-Jaume A, Larman M, Diarte JA, Rodríguez-Collado J, Rumoroso JR, Lopez-Minguez JR, Mauri J; ESTROFA Study Group. Drug-eluting stent thrombosis: results from the multicenter Spanish registry ESTROFA (Estudio ESpanol sobre TROmbosis de stents FArmacoactivos). *J Am Coll Cardiol* 2008;51:986-90.
33. Baber U, Mehran R, Giustino G, Cohen DJ, Henry TD, Sartori S, Ariti C, Litherland C, Dangas G, Gibson CM, Krucoff MW, Moliterno DJ, Kirtane AJ, Stone GW, Colombo A, Chieffo A, Kini AS, Witzenbichler B, Weisz G, Steg PG, Pocock S. Coronary thrombosis and major bleeding after PCI with drug-eluting stents: risk scores from PARIS. *J Am Coll Cardiol* 2016;67:2224-34.
34. Nakazawa G, Finn AV, Joner M, Ladich E, Kutys R, Mont EK, Gold HK, Burke AP, Kolodgie FD, Virmani R. Delayed arterial healing and increased late stent thrombosis at culprit sites after drug-eluting stent placement for acute myocardial infarction patients: an autopsy study. *Circulation* 2008;118:1138-45.
35. Vilahur G, Gutierrez M, Casani L, Varela L, Capdevila A, Pons-Llado G, Carreras F, Carlsson L, Hidalgo A, Badimon L. Protective effects of ticagrelor on myocardial injury after infarction. *Circulation* 2016;134:1708-19.
36. Brugaletta S, Gori T, Low AF, Tousek P, Pinar E, Gomez-Lara J, Scalone G, Schulz E, Chan MY, Kocka V, Hurtado J, Gomez-Hospital JA, Münzel T, Lee CH, Cequier A, Valdés M, Widimsky P, Serruys PW, Sabaté M. Absorb bioresorbable vascular scaffold versus everolimus-eluting metallic stent in ST-segment elevation myocardial infarction: 1-year results of a propensity score matching comparison: the BVS-EXAMINATION Study (bioresorbable vascular scaffold-a clinical evaluation of everolimus eluting coronary stents in the treatment of patients with ST-segment elevation myocardial infarction). *JACC Cardiovasc Interv* 2015;8:189-97.
37. Sabaté M, Windecker S, Iñiguez A, Okkels-Jensen L, Cequier A, Brugaletta S, Hofma SH, Räber L, Christiansen EH, Suttorp M, Pilgrim T, Anne van Es G, Sotomi Y, García-García HM, Onuma Y, Serruys PW. Everolimus-eluting bioresorbable stent vs. durable polymer everolimus-eluting metallic stent in patients with ST-segment elevation myocardial infarction: results of the randomized ABSORB ST-segment elevation myocardial infarction-TROFI II trial. *Eur Heart J* 2016;37:229-40.
38. Capodanno D, Gori T, Nef H, Latib A, Mehilli J, Lesiak M, Caramanno G, Naber C, Di Mario C, Colombo A, Capranzano P, Wiebe J, Araszkiewicz A, Geraci S, Pyxaras S, Mattesini A, Naganuma T, Münzel T, Tamburino C. Percutaneous coronary intervention with everolimus-eluting bioresorbable vascular scaffolds in routine clinical practice: early and midterm outcomes from the European multicentre GHOST-EU registry. *EuroIntervention* 2015;10:1144-53.
39. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868-74.
40. Andrés E, Cordero A, Magán P, Alegría E, León M, Luengo E, Botaya RM, García Ortiz L, Casasnovas JA. Long-term mortality and hospital readmission after acute myocardial infarction: an eight-year follow-up study. *Rev Esp Cardiol* 2012;65:414-20.
41. Rogers JK, Yaroshinsky A, Pocock SJ, Stokar D, Pogoda J. Analysis of recurrent events with an associated informative dropout time: application of the joint frailty model. *Stat Med* 2016;35:2195-205.
42. Sedlis SP, Hartigan PM, Teo KK, Maron DJ, Spertus JA, Mancini GB, Kostuk W, Chaitman BR, Berman D, Lorin JD, Dada M, Weintraub WS, Boden WE; COURAGE Trial Investigators. Effect of PCI on long-term survival in patients with stable ischemic heart disease. *N Engl J Med* 2015;373:1937-46.
43. Cordero A, Carrillo P, López-Palop R. Letter by Cordero et al regarding article, "Regional systems of care demonstration project: American Heart Association Mission: Lifeline STEMI Systems Accelerator". *Circulation* 2016;134:e700-1.

Nardi *et al. Vessel Plus* 2017;1:77-83
DOI: 10.20517/2574-1209.2017.07

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The effect of postoperative malperfusion after surgical treatment of type A acute aortic dissection on early and mid-term survival

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How to cite this article: Nardi P, Olevano C, Bassano C, Bovio E, Cecchetti L, Forlani S, Ruvolo G. The effect of postoperative malperfusion after surgical treatment of type A acute aortic dissection on early and mid-term survival. Vessel Plus 2017;1:77-83.

Article history:
Received: 06-03-2017
Accepted: 06-04-2017
Published: 27-06-2017

Key words:
Acute type aortic dissection,
aortic surgery,
malperfusion,
survival

ABSTRACT

Aim: To evaluate whether postoperative malperfusion (PM) affected in-hospital and long-term survival in acute type A aortic dissection (AAAD) surgical patients and to identify risk factors for PM. **Methods:** Patients who underwent AAAD surgery at a single institution between January 2005 and March 2015 were retrospectively analyzed. **Results:** Two-hundred fourteen patients with complete data were identified. At presentation, 119 patients (55.6%) showed preoperative malperfusions: 68 (31.8%) were cerebral, 38 (17.7%) were renal, and 13 (6.1%) were mesenteric. PM was found in 55 patients (25.7%). In-hospital mortality was 47.3% (26/55) *vs.* 22.6% (36/159) in PM and non-PM patients, respectively ($P < 0.0001$). Independent predictors for in-hospital mortality included being 75 years or older [odds ratio (OR): 1.1, 95% confidence interval (CI): 1.03-1.13, $P < 0.001$] and having renal PM (OR: 53.5, 95% CI: 3.97-721.3, $P < 0.01$). Five-year survival was 78.6 ± 7.8% *vs.* 93.9 ± 3.4% in PM and non-PM patients, respectively ($P < 0.001$). Independent predictors for long-term survival were being at least 75 years old (OR: 3.7, 95% CI: 0.9-14.0, $P = 0.05$) and having renal PM (OR: 28.6, 95% CI: 1.8-462.0, $P = 0.01$). PM and intimal tears distal to the ascending aorta or the proximal aortic arch were also risk factors. **Conclusion:** PM, especially with renal involvement, is associated with in-hospital mortality and reduced long-term survival. AAAD surgeries reduced preoperative malperfusions. Sites of cannulation and interventions requiring circulatory arrest during cardiopulmonary bypass were not predictors of PM.

INTRODUCTION

Acute type A aortic dissection (AAAD) is a life-threatening condition and one of the most challenging diseases faced by cardiothoracic surgeons. Despite preventative measures including early surgical referrals for patients, preoperative care and improved surgical techniques, in-hospital mortality following surgery remains high, ranging from 10% to 30%.[1,2]

Malperfusion of systemic organs is a complication of aortic dissection caused by branch-vessel

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involvement. Occurrences can result in dangerous end-organ ischemic dysfunctions, especially when involving the brain. Clinical diagnoses are critical to the development of effective treatment strategies. Proper diagnoses also have important influence on immediate and long-term outcomes of treatment.[3-5]

Malperfusion following either type A or type B acute aortic dissection, is a fairly common complication. It can involve several arterial regions including the cerebral, renal, or mesenteric segments, as well as the upper or lower limbs. In the Stanford Classification Type A Dissection (De Bakey Classification Types I and II) surgical treatment can prevent this complication. In the event of malperfusion, distal aortic percutaneous fenestration can allow blood flow to return to the true lumen. Alternatively, when the distal aortic true lumen is completely obstructed, an endovascular stent can be inserted to recanalize the lower arteries (i.e. the celiac trunk, renal, superior mesenteric and iliac arteries). The International Registry of Acute Aortic Dissection (IRAD) does not include malperfusion as an independent predictor of mortality. Nonetheless, several studies have supported the relevance of this complication, given that it increased in-hospital mortality and adversely affected late survival.[6-9]

The aim of this study was to evaluate the effect of postoperative malperfusion (PM) on in-hospital mortality and long-term survival in patients undergoing surgery for AAAD in a single, high-volume aortic surgery center.

METHODS

Between January 2005 and December 2015, 227 patients (mean age 62.5 ± 12.6 years) underwent emergent operations for AAAD. The study was approved by the local Institutional Review Board, which waived the need for patient consent. The preoperative patients' characteristics are given in Table 1.

The diagnosis of malperfusion was based on clinical symptoms and/or imaging evidence, i.e. absence of organ perfusion as determined by computed tomography (CT) scan angiography.

Malperfusion was classified as: cerebral if there was presence of a focal or global stroke leading to brain function deterioration that persisted more than 24 h, or a transient ischemic attack; renal if there was an impairment of renal function (e.g. anuria requiring continuous veno-venous hemofiltration, or a two-fold increase of creatinine serum level); or mesenteric if there was evidence of tense abdominal or intestinal dysfunction, or increased serum levels of liver and/or pancreatic enzymes. The database queries were completely obtained from 214 patients.

Surgical techniques

Prior to operative procedures, patients were monitored with Swan-Ganz pulmonary artery catheters, arterial cannulations to ensure continuous arterial blood pressure measurements (i.e. radial or femoral measurements), and corporeal temperature measurements (TC) during surgery (i.e. rectal, esophageal, or tympanic measurements). Additionally, cerebral monitoring was performed with near-infrared spectroscopy (INVOS® System, Somanetics Corp., Troy, MI, USA) and transcranial Doppler measurements of blood flow velocities in the middle and/or anterior cerebral arteries of the Willis circle.

The heart was accessed through a complete median longitudinal sternotomy in all patients. Arterial access for cardiopulmonary bypass was through either the femoral artery (*n* = 96), the right axillary artery (*n* = 96), or direct aortic cannulation (*n* = 22). Aortic repair was performed in conditions of circulatory arrest and moderate hypothermia (25-28 °C) in 124 (58%) patients.

Cerebral perfusion was given in 118 (55.1%) cases. Fifty-three (24.8%) patients received unilateral selective antegrade perfusion across the right axillary artery in the right common carotid artery. Sixty-five (30.4%) patients received bilateral perfusion using the Kazui technique.[10,11]

Table 1: Preoperative characteristics, *n* (%)

Variable	Overall (*n* = 214)	non-PM (*n* = 159)	PM (*n* = 55)	*P* value
Age (years), mean ± SD	62.5 ± 12.6	62.3 ± 13.5	63.2 ± 10.6	NS
Male gender	156 (72.9)	114	42	NS
Clinical history				
Hypertension	188 (87.9)	138	50	NS
Smoke habit	69 (32.2)	49	20	NS
BMI (kg/m^2) > 30	48 (22.4)	33	15	NS
History of CAD	15 (7.0)	10	5	NS
Diabetes on insulin	11 (5.1)	7	4	NS
Previous cardiac surgery	9 (4.2)	6	3	NS
Dialisys-dependent renal failure	4 (1.8)	1	3	0.02
Malperfusion, *n* (%)				
Overall malperfusion	119 (55.6)			
Brain	68 (31.8)	47	21	NS
Kidney	38 (17.7)	26	12	NS
Visceral	13 (6.1)	8	5	NS
Entry tear aortic dissection				
Ascending aorta	115 (53.7)	91	24	NS
Aortic arch	33 (15.4)	23	10	NS
Descending aorta	8 (3.7)	3	5	0.02
Unknown	58 (27.1)	42	16	NS

PM: postoperative malperfusion; BMI: body mass index; CAD: coronary artery disease; NS: not significant

Table 2: Surgical variables

Variable	Overall (*n* = 214)	non-PM (*n* = 159)	PM (*n* = 55)	*P* value
Arterial cannulation, *n* (%)				
Right axillary	96 (44.9)	69	27	NS
Right or left femoral	96 (44.9)	72	24	NS
Central (into the aorta)	22 (10.2)	18	4	NS
Brain perfusion, *n* (%)				
Monolateral	53 (24.7)	38	15	NS
Bilateral	65 (30.4)	48	17	NS
Not performed	96 (44.9)	74	22	NS
Circulatory arrest, *n* (%)	125 (58.4)	90	35	NS
Circulatory arrest temperature (°C), mean ± SD	27 ± 2	26.9 ± 2.5	27.1 ± 1.9	NS
Surgical times (min), mean ± SD				
CPB	161 ± 82	160.9 ± 87.5	168.8 ± 67.6	NS
Aortic cross clamp	91 ± 47	92.2 ± 46.4	95.2 ± 46.4	NS
Circulatory arrest	38 ± 31	37.6 ± 32.4	39.5 ± 30.8	NS
Surgical procedures, *n* (%)				
AAR	72 (33.6)	53	19	NS
AAR + hemiarch replacement	50 (23.4)	34	16	NS
AAR + arch replacement	38 (17.8)	29	9	NS
Bentall	43 (20.1)	36	7	NS
Bentall + hemiarch replacement	11 (5.1)	7	4	NS

PM: postoperative malperfusion; CPB: cardiopulmonary bypass; AAR: ascending aorta replacement; NS: not significant

Three regions of the aorta (ascending, arch and proximal descending) were investigated in each patient to identify sites of intimal tearing; tears were resected whenever possible. Intimal tearing occurred in the ascending aorta in 115 (53.7%) patients, in the arch in 33 (15.4%) patients, and in the descending aorta in 8 (3.7%) patients. In 58 (27.1%) patients no entry tear was found. Seventy-two patients (33.6%) underwent isolated ascending aortic replacement, 50 (23.4%) had hemiarch resection and 38 (17.8%) had both total arch replacement and ascending aortic replacement. The aortic root was replaced in 43 (20.1%) patients using the modified button Bentall technique. Intraoperative data are given in Table 2.

Data collection

In-hospital mortality events included both intraoperative and postoperative mortality within 30 days after surgery. Clinical follow-up visits were performed every 12 months in our outpatient control unit; CT-angiography and/or echocardiographic data were collected. For patients living far from this institution who could not participate in regular follow-up visits to the department, clinical status was ascertained by personal interviews with the patients and their cardiologists, including the recording of noninvasive tests.

Statistical analyses

Statistical analyses were performed using StatView 4.5 programming (SAS Institute Inc., Abacus Concepts, Berkeley, CA, USA). Student's *t*-test for continuous variables and Chi-squared or Fisher's exact tests for categorical variables were used. To detect independent predictors of in-hospital mortality and PM logistic regression analyses were performed. Statistical significance by univariate analyses ($P < 0.10$) were required for entry into the multivariate models. Preoperatively analyzed variables included: age, gender, arterial hypertension, smoking status, body mass index, history of concomitant coronary artery disease, diabetes, chronic kidney disease, previous cardiac surgery, left ventricular dysfunction [e.g. left ventricular ejection fraction (LVEF) < 40%], presence of preoperative malperfusion, and the sites of entry tears. Intraoperatively analyzed variables included: cannulation sites, techniques for obtaining brain perfusions, surgical times, whether there was a need for circulatory arrest, and types of operations (i.e. isolated ascending aorta replacement, Bentall procedure, ascending aortic replacement extended to the hemiarch, or total arch replacement). Overall long-term survival (not including operative mortality) was expressed as a mean of the values plus or minus one standard deviation. Survival analyses were computed using the Kaplan-Meier method; Mantel-Cox log-rank tests were used to compare survival estimates between subgroups. Cox regression models were used to evaluate the influence of the variables on time to death in the entire cohort. A *P* value less than 0.05 was considered statistically significant.

RESULTS

At presentation, 119 (55.6%) patients exhibited preoperative malperfusion: 68 (31.8%) were cerebral with neurological symptoms, 38 (17.7%) were renal, and 13 (6.1%) were mesenteric. The most relevant

Table 3: Predictors of in-hospital mortality

Variable	Univariate	Multivariate		
	P value	OR	95% CI	*P* value
Age ≥ 75 years	0.0078	1.1	1.0-1.1	0.0004
Renal PM	< 0.0001	53.5	4.0-721.3	0.0027
Any preoperative malperfusion syndromes	< 0.0001			0.14
Preoperative cerebral preoperative malperfusion	0.0345			0.62
BMI > 30	0.0131			0.40
CABG	0.0353			0.15
CPB time	0.0008			0.12
Aortic cross clamp time	0.0225			0.90

PM: postoperative malperfusion; BMI: body mass index; CABG: coronary artery bypass grafting; CPB: cardiopulmonary bypass; OR:odds ratio; CI: confidence interval

clinical characteristics of the cohort, stratified according to the presence or absence of PM, were reported in Table 1.

Intraoperative mortality occurred in 14 (6.5%) patients and in-hospital mortality after surgery occurred in 48 (22.4%) patients. Using univariate analyses, significant risk factors for in-hospital mortality included: being at least 75 years old, having a body mass index of more than 30 kg/m^2, having preoperative overall or cerebral malperfusion, having longer cardiopulmonary bypass and aortic clamping times, needing concomitant coronary artery bypass grafting (CABG), having renal PM requiring continuous veno-venous hemofiltration, and have postoperative mechanical ventilation for up to 24 h. The multivariate analyses revealed that being 75 years old or older at the time of surgery ($P < 0.001$) and having renal PM ($P < 0.01$) were independent predictors of in-hospital mortality [Table 3].

Fifty-five (25.7%) patients showed clinical symptoms and/or imaging evidence of PM. In 42 cases, only 1 organ system was affected, including: the brain in 13 (6.5%) patients, the kidneys in 22 (10.3%) patients, and the viscera in 7 (3.3%) patients. In a subgroup of PM patients with just 1 affected organ system, the mortality rate was 40.5%. In 11 patients, PM occurred in 2 organ systems (5 in the brain and kidneys, 3 in the brain and viscera, 3 in the kidneys and viscera); the mortality rate in this subgroup was 63.6%. PM involving all 3 organ systems occurred in just 2 cases and both patients died. Overall, the in-hospital mortality rate in patients affected by any PM was 47.3% (26/55) *vs*. 22.6% (36/159) in patients without PM ($P < 0.001$). Univariate analyses identified that the risk factors for any PM included: having a preoperative LVEF of less than 40% ($P < 0.01$), having preoperative renal dysfunction ($P < 0.05$) and having an entry tear distal to the ascending aorta or to the proximal aortic arch ($P < 0.05$). Multivariate analyses determined that having a LVEF value of 40% or less was the only independent predictor of having a PM ($P < 0.05$) [Table 4].

Table 4: Predictors of postoperative malperfusion syndrome

Variable	Univariate	Multivariate
	P value	*P* value
Preoperative LVEF < 40%	0.007	0.04
Preoperative dialysis	0.03	NS
Entry tear distal to the ascending aorta - proximal aortic arch	0.04	NS

LVEF: left ventricular ejection fraction; OR: odds ratio; CI: confidence interval; NS: not significant

Preoperative cerebral malperfusion was an independent predictor of cerebral PM [odds ratio (OR): 2.5, 95% confidence interval (CI): 1.0-6.1, $P < 0.05$]. Interestingly, a LVEF of less than 40% was only a found to be a significant risk factor for renal PM when using univariate analysis techniques ($P < 0.0001$). Finally, an entry tear distal to the ascending aorta or to the proximal aortic arch requiring extensive repair and longer surgical time was a risk factor of mesenteric PM ($P < 0.05$, using univariate analyses).

Follow-up results

The mean duration of follow-up was 42.4 ± 23.7 months (median 46 months). All patients were followed until the end of the study period. One- and 5-year overall survival rates were 96.0 ± 1.6% and 90.8 ± 3.2%, respectively [Figure 1]. Cox regression analyses identified that independent predictors of long-term survival were: being at least 75 years old at the time of surgery (OR: 3.7, 95% CI: 0.9-14.0, $P = 0.05$) and having a renal PM (OR: 28.6, 95% CI: 1.8-462.0, $P = 0.01$) [Table 5]. When the survival probability was dichotomized by age, (with a threshold of 75 years old at the time of the surgery), the 5-year survival rates were 91.6 ± 3.5% for patients < 75 years old and 65.1 ± 19.5% for patients ≥ 75 years old ($P < 0.05$). The 5-year survival rate for patients without PM was 93.9 ± 3.4% *vs*. 78.6 ± 7.8% for those affected by PM (Log-rank test, $P < 0.01$).

DISCUSSION

Despite improvements in medical management and surgical techniques, acute type A aortic dissections still have high mortality and morbidity rates.[1,2] The IRAD revealed that the expected mortality rate for patients undergoing AAAD surgery ranges from 20% to 30%.[9] Our cardiac surgery division has extensive experience in the treatment of acute aortic dissection; we observed an in-hospital mortality rate of 29%. Several studies

Table 5: Predictors of late mortality

Variable	Univariate	Multivariate		
	P value	OR	95% CI	*P* value
Age ≥ 75 years	0.03	3.7	0.9-14.0	0.05
Renal PM	< 0.0001	28.9	1.8-462.0	0.017
Cerebral PM	0.001			NS
Visceral PM	0.09			NS
Any PM	0.001			NS
Entry tear (distal *vs.* proximal) of the dissection	0.02			NS

PM: postoperative malperfusion; OR:odds ratio; CI: confidence interval; NS: not significant

show that both patient characteristics and multi-organ involvement (i.e. affecting the brain, kidney, and mesenteric organs) play a key role in immediate and post-procedural outcomes. For example, Caus *et al*.[12] showed that being at least 70 years old at the time of operation was an independent predictor of worsened outcomes for AAAD treated patients; these authors reported a 5-year survival rate of 30%. These data are similar to the clinical experience reported here, in which patients who were at least 75 years old had lower actuarial survival rates than patients under 75 years of age (65.1 ± 19.5% *vs*. 91.6 ± 3.5%).

Malperfusion of organ systems remains a severe condition that is frequently associated with adverse outcomes in AAAD patients undergoing surgical procedures. Data from the German Registry for AAAD suggested that the number of organs involved in the malperfusion was associated with immediate outcomes of surgery. In fact, outcomes were substantially worsened in the presence of any type of malperfusion syndrome, which was exacerbated with increased numbers of affected organs. A 12.6% early mortality rate was observed in the absence of malperfusion versus 43.4% mortality in patients with three organ systems affected by malperfusion.[13] Here, preoperative clinical symptoms and/or imaging evidence of malperfusion occurred in 119 patients. After surgery, 55 (25.7%) patients had malperfusion syndrome. In-hospital mortality was significantly higher (47.3%, 26 patients) in this group compared to patients without PM (22.6%, 36 patients) ($P < 0.0001$). Despite the small sample size per group, a strong association between the number of malperfused organs and early mortality was observed. In fact, when 2 organs were affected operative mortality elevated to greater than 60%.

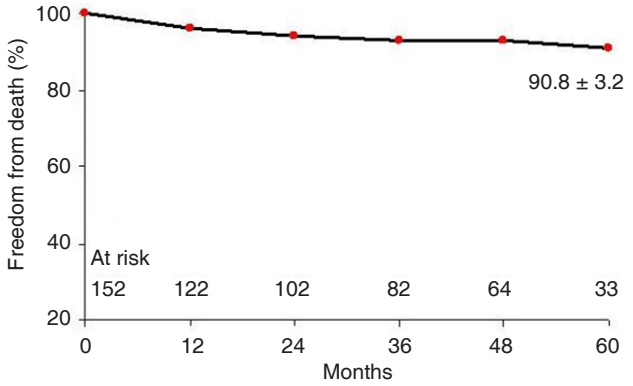

Figure 1: Overall late survival

Furthermore, these data show that PM-affected patients had lower survival probabilities when compared to those who did not develop this postoperative complication (78.6 ± 7.8% *vs*. 93.9 ± 3.4%). Correlations between the preoperative presence of malperfusion and mortality have been previously described.[14,15] Pacini *et al*.[14] found that patients presenting with any malperfusion syndrome had a mortality rate of 43.7%, compared to 15% in patients without malperfusion ($P = 0.001$); strikingly, mortality rates were 34.7%, 61.9% and 85.7% with involvement of 1, 2, or more than 2 organ malperfusions, respectively. Mesenteric malperfusion was identified as an independent predictor of operative mortality. Similarly, Geirsson *et al*.[15] reported a 30.5% operative mortality in the presence of any malperfusion syndrome; in this study cerebral malperfusion was detected as a risk factor for in-hospital mortality ($P < 0.001$) and reduced long-term survival ($P = 0.0002$).

In the present study, the most important independent risk factor of early and 5-year mortality was presence of a renal PM requiring continuous veno-venous hemofiltration. Previously, we identified in 100 consecutive patients receiving AAAD operations from 1995 to 2006, that renal failure, either chronic (OR: 0.3, $P = 0.04$) or developed acutely in the postoperative period (OR: 8.9, $P = 0.001$), was a predictor of operative mortality. However, renal failure was not a predictor of reduced 5-year survival.[8] In the same group of patients, preoperative LVEF values of less than 50% were also predictors of reduced survival ($P = 0.02$).

Another important issue is the surgical timing of aortic repairs. Previous authors have suggested delaying acute aortic dissection surgeries when patients experience preoperative malperfusion, particularly in the mesentery. This delayed treatment strategy involved early endovascular treatment with a complete or partial resolution of organ ischemia, followed by timely aortic surgeries.[16,17] While this management approach may be beneficial in a specific subpopulation,

patient AAAD survival outcomes have been shown to relate closely to the length of time between diagnoses and surgeries.[18,19] Given the high mortality of patients with mesenteric malperfusion (40-100%), initial management with an interventional procedure treating the condition should be considered.[20,21] In fact, previous data suggested that mesenteric malperfusion was associated with the highest mortality rates when compared to malperfusions occurring in any other organ systems. The surgical strategy presented here, consisting of immediate aortic dissection treatment, showed that incidence of preoperative malperfusion was reduced roughly in half; from 56% preoperatively to 25% in the immediate postoperative period.

Univariate analyses of preoperative variables determined that three risk factors predicted the occurrence of a PM in any organ system. These risk factors were: having a LVEF less than 40%, having renal impairment that required continuous hemofiltration, and having an entry tear distal to the ascending aorta or the proximal aortic arch. However, the only variable that maintained significance in the multivariate model was having a preoperative LVEF of less than 40%. Reduced ejection fraction likely associated with concomitant ischemic coronary disease, which could have increased the risk of a postoperative low cardiac function and subsequent PM. Juxtaposition of intimal tears distal to the ascending aorta or the proximal arch were non-significant factors in the multivariate analyses. However, these factors contributed risk to progression of aortic disease and PM. Patients with a primary entry tear in the descending aorta were at the highest risk of PM. These patients probably required additional extensive repairs compared to patients with primary entry tears in the ascending aorta. Some of these high-risk patients may benefit from a "frozen elephant trunk" procedure to address the entire pathology.[22]

Analysis of preoperative variables contributing risk for each type of PM revealed that only one variable independently predicted cerebral PM: preoperative cerebral malperfusion (OR: 2.5, 95% CI: 1.0-6.1, $P < 0.05$). Shortening the length of time between onset of cerebral symptoms and dissected aortic surgery was critical for improved outcomes in this subset of patients. Estrera *et al*.[23] reported improved outcomes in AAAD patients who underwent cardiac surgeries within 10 h of neurological symptom onsets.

With regard to arterial cannulation sites, some authors have suggested that cannulation of the axillary artery will ensure better brain protection during surgery. However, the experience reported here did not confirm this evidence. Nonetheless, many surgeons still limit the extent of surgery to the ascending aorta, even though limited repair has a higher probability of re-intervention on the remaining aortic segments at a later date. The primary aim of performing AAAD is as an emergent, life-saving procedure. If a center is only able to perform a limited repair technique, but still saves the life of the patient, then the primary intention of the procedure has been achieved.[24,25]

In this study, no independent predictors of renal and mesenteric PM were identified. However, using univariate analyses, having a LVEF value less than 40% was statistically relevant ($P < 0.0001$). Additionally, having entry tears distal to ascending aortic segments that required extensive repairs and longer surgical times was also recognized as a significant risk factor ($P < 0.05$).

This study had several limitations. First, it was a retrospective analysis of an experience at a single institution. Second, preoperative treatments to address organ malperfusions were not performed. Third, the possible effects of revascularization strategies for the treatment of PM were not explored. Revascularization techniques may improve long-term outcomes.

In conclusion, PM is a severe condition that is frequently associated with adverse immediate and long-term outcomes in surgical AAAD patients. At this institution, the incidence of PM after AAAD surgery was noteworthy, occurring in roughly 10% of patients. AAAD surgical procedures effectively reduced preoperative malperfusions in about half of cases. In fact, repairs to the ascending aorta and proximal arch, as well as removal of primary tears, significantly increased the true lumen flow and allowed treatment of a majority of malperfusion syndromes, including those in the cerebral, mesenteric, and renal systems. Postoperative malperfusion, especially involving the kidneys, was associated with high in-hospital mortality and reduced long-term survival. There was no evidence that the types of surgical techniques undertaken, the sites of cannulation, or the use of more complex interventions (requiring circulatory arrest during cardiopulmonary bypass) were risk factors contributing to PM.

Authors' contributions

Study design: P. Nardi, C. Olevano, C. Bassano, E. Bovio, G. Ruvolo
Development of methodology: P. Nardi, C. Bassano
Collection of data: C. Olevano, E. Bovio, L. Cecchetti
Analysis and/or interpretation of data: P. Nardi, C. Olevano, C. Bassano, E. Bovio
Writing (not revising) all or sections of the manuscript:

P. Nardi, C. Olevano, E. Bovio, S. Forlani
Manuscript review: P. Nardi, E. Bovio, L. Cecchetti
Supervision: G. Ruvolo

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

The local Institutional Review Board waived the need for patient consent.

Ethics approval

The study was approved by the local Institutional Review Board.

REFERENCES

1. Colli A, Carrozzini M, Galuppo M, Comisso M, Toto F, Gregori D, Gerosa G. Analysis of early and long-term outcomes of acute type A aortic dissection according to the new international aortic arch surgery study group recommendations. *Heart Vessels* 2016;31:1616-24.
2. Berretta P, Patel HJ, Gleason TG, Sundt TM, Myrmel T, Desai N, Korach A, Panza A, Bavaria J, Khoynezhad A, Woznicki E, Montgomery D, Isselbacher EM, Di Bartolomeo R, Fattori R, Nienaber CA, Eagle KA, Trimarchi S, Di Eusanio M. IRAD experience on surgical type A acute dissection patients: results and predictors of mortality. *Ann Cardiothorac Surg* 2016;5:346-51.
3. Orihashi K. Malperfusion in acute type A aortic dissection: unsolved problem. *Ann Thorac Surg* 2013;95:1570-6.
4. Fabre O, Guesnier L, Renaut C, Gautier L, Geronimi H, Jasaitis L, Strauch K. Current treatment of acute type A aortic dissection. Surgical treatment and treatment of malperfusion syndrome. *Ann Cardiol Angeiol (Paris)* 2005;54:332-8. (in French)
5. Immer FF, Grobéty V, Lauten A, Carrel TP. Does malperfusion syndrome affect early and mid-term outcome in patients suffering from acute type A aortic dissection? *Interact Cardiovasc Thorac Surg* 2006;5:187-90.
6. Zhang J, Jiang Y, Gao C, Feng J, Wang A. Risk factors for hospital death in patients with acute aortic dissection. *Heart Lung Circ* 2015;24:348-53.
7. Appoo JJ, Pozeg Z. Strategies in the surgical treatment of type A aortic arch dissection. *Ann Cardiothorac Surg* 2013;2:205-11.
8. Nardi P, Scafuri A, Pellegrino A, Bassano C, Zeitani J, Bertoldo F, Penta de Peppo A, Chiariello L. Surgery for type A aortic dissection: long-term results and risk factor analysis. *G Ital Cardiol (Rome)* 2007;8:580-5. (in Italian)
9. Rampoldi V, Trimarchi S, Eagle KA, Nienaber CA, Oh JK, Bossone E, Myrmel T, Sangiorgi GM, De Vincentiis C, Cooper JV, Fang J, Smith D, Tsai T, Raghupathy A, Fattori R, Sechtem U, Deeb MG, Sundt TM 3rd, Isselbacher EM; International Registry of Acute Aortic Dissection (IRAD) Investigators. Simple risk models to predict surgical mortality in acute type A aortic dissection: the International Registry of Acute Aortic Dissection score. *Ann Thorac Surg* 2007;83:55-61.
10. Bassano C, Mve Mvondo C, Bovio E, Chiariello L. Bilateral cerebral perfusion via right axillary artery cannulation alone in aortic arch surgery. *Thorac Cardiovasc Surg* 2013;61:584-6.
11. Kazui T, Kimura N, Yamada O, Komatsu S. Surgical outcome of aortic arch aneurysms using selective cerebral perfusion. *Ann Thorac Surg* 1994;57:904-11.
12. Caus T, Frapier JM, Giorgi R, Aymard T, Riberi A, Albat B, Chaptal PA, Mesana T. Clinical outcome after repair of acute type A aortic dissection in patient over 70 years-old. *Eur J Cardiothorac Surg* 2002;22:211-7.
13. Czerny M, Schoenhoff F, Etz C, Englberger L, Khaladj N, Zierer A, Weigang E, Hoffmann I, Blettner M, Carrel TP. The impact of pre-operative malperfusion on outcome in acute type A aortic dissection: results from the GERAADA registry. *J Am Coll Cadiol* 2015;65:2628-35.
14. Pacini D, Leone A, Belotti LM, Fortuna D, Gabbieri D, Zussa C, Contini A, Di Bartolomeo R; RERIC (Emilia Romagna Cardiac Surgery Registry) Investigators. Acute type A aortic dissection: significance of multiorgan malperfusion. *Eur J Cardiothorac Surg* 2013;43:820-6.
15. Geirsson A, Szeto WY, Pochettino A, McGarvey ML, Keane MG, Woo YJ, Augoustides JG, Bavaria JE. Significance of malperfusion syndromes prior to contemporary surgical repair for acute type A dissection: outcomes and need for additional revascularizations. *Eur J Cardiothorac Surg* 2007;32:255-62.
16. Perera N, Galvin SD, Seevanayagam S, Matalanis G. Optimal management of acute type A aortic dissection with mesenteric malperfusion. *Interact Cardiovasc Thorac Surg* 2014;19:290-4.
17. Yamashiro S, Arakaki R, Kise Y, Inafuku H, Kuniyoshi Y. Management of visceral malperfusion complicated with acute type A aortic dissection. *Interact Cardiovasc Thorac Surg* 2015;21:346-51.
18. Deeb GM, Williams DM, Bolling SF, Quint LE, Monaghan H, Sievers J, Karavite D, Shea M. Surgical delay for acute type A dissection with malperfusion. *Ann Thorac Surg* 1997;64:1669-75.
19. Tsagakis K, Pizanis N, Kamler M, Konorza T, Zoepf T, Erbel R, Jakob H. ICU controlled delay for acute type A aortic dissection repair after intervention for total visceral malpefusion: a way out of a dilemma? *Thorac Cardiovasc Surg* 2008;56:298-300.
20. Slonim SM, Miller DC, Mitchell RS, Semba CP, Razavi MK, Dake MD. Percutaneous balloon fenestration and stenting for life-threatening ischemic complications in patients with acute aortic dissection. *J Thorac Cardiovasc Surg* 1999;117:1118-26.
21. Fabre O, Vincentelli A, Willoteux S, Beregi JP, Prat A. Preoperative fenestration for type A acute aortic dissection with mesenteric malperfusion. *Ann Thorac Surg* 2002;73:950-1.
22. Shrestha M, Fleissner F, Ius F, Koigeldiyev N, Kaufeld T, Beckmann E, Martens A, Haverich A. Total aortic arch replacement with frozen elephant trunk in acute type A aortic dissections: are we pushing the limits too far? *Eur J Cardiothorac Surg* 2015;47:361-6.
23. Estrera AL, Garami Z, Miller CC, Porat EE, Achouh PE, Dhareshwar J, Meada R, Azizzadeh A, Safi HJ. Acute type A aortic dissection complicated by stroke: can immediate repair be performed safely? *J Thorac Cardiovasc Surg* 2006;132:1404-8.
24. Wong DR, Coselli JS, Palmero L, Bozinovski J, Carter SA, Murariu D, LeMaire SA. Axillary artery cannulation in surgery for acute or subacute ascending aortic dissections. *Ann Thorac Surg* 2010;90:731-7.
25. Tiwari KK, Murzi M, Bevilacqua S, Glauber M. Which cannulation (ascending aortic cannulation or peripheral arterial cannulation) is better for acute type A aortic dissection surgery? *Interact Cardiovasc Thorac Surg* 2010;10:797-802.

Shalimova. *Vessel Plus* 2017;1:84-90
DOI: 10.20517/2574-1209.2016.08

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Short Communication **Open Access**

Heart and vascular remodeling in essential hypertension and type 2 diabetes is dependent on genetic polymorphisms

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How to cite this article: Shalimova A. Heart and vascular remodeling in essential hypertension and type 2 diabetes is dependent on genetic polymorphisms. Vessel Plus 2017;1:84-90.

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Article history:
Received: 22-10-2016
Accepted: 29-12-2016
Published: 27-06-2017

Key words:
Essential hypertension,
type 2 diabetes comorbidity,
genetic polymorphism,
heart and vascular remodeling

ABSTRACT

Aim: To study heart and vascular remodeling in essential hypertension (EH) and concomitant type 2 diabetes mellitus (DM2) with respect to genetic polymorphism of the angiotensin II receptor type 1 (*AGTR1*) gene and peroxisome proliferator-activated receptor-γ2 (*PPARγ2*). **Methods:** Biochemical blood analysis, echocardiographic evaluation of mitral diastolic blood flow and tissue Doppler spectral modes, reactive hyperemia, color Doppler mapping. **Results:** Patients with *A/C* and *C/C* genotypes of the *AGTR1* gene had higher blood pressure, more pronounced metabolic disorders, a larger left ventricle (LV), higher myocardial mass index left ventricle, and a greater intima media thickness (IMT), with a lower rate of endothelium-dependent vasodilation (EDVD) compared to the *A/A* genotype. Patients with the *Pro/Pro* genotype of *PPARγ2* had higher levels of blood pressure, larger LVs, greater IMT, pulse wave velocity, and a lower rate of EDVD compared to the *Pro/Ala* and *Ala/Ala* genotypes. Patients with the *Pro/Pro* genotype had significantly more pronounced dyslipidemia and insulin resistance than patients with other *PPARγ2* genotypes. **Conclusion:** The polymorphism of genetic markers *AGTR1* and *PPARγ2* in patients with EH and concomitant DM2 was associated with the development of comorbidity. Different genotypes of specific genes alter the severity of cardiovascular remodeling and metabolic disorders.

Quick Response Code:

INTRODUCTION

There are a large number of patients with essential hypertension (EH) who also have type 2 diabetes mellitus (DM2), thus meriting further research into this problem. It is known that EH and DM2 have many common pathogenetic mechanisms that affect the development of comorbidity. According to many researchers, the most important predictors of EH and DM2 are hereditary risk factors.[1-3] However, there are controversial views on the role of gene expression and genetic polymorphisms in the development and course of diseases in different populations of patients, as well as their influence on the effectiveness of drug therapy.

Some studies have shown that the predisposing cause of EH can be mutational alleles of the angiotensin II receptor (*AGTR1*) gene; angiotensin is a powerful vasoconstrictor, thus playing a role in the pathogenesis of EH.[4,5] Angiotensin II (AT-II) receptor type 1, which is located on the vascular endothelium, mediates the main cardiovascular effects of angiotensin, including the induction of insulin-like growth factor and endothelin-1. The induction of cell growth is also mediated through *AGTR1*.[6] A number of investigations have reported that *AGTR1* polymorphisms may lead to changes in the regulation of vascular tone and proliferation of vascular wall elements.[7]

Hyperinsulinemia and insulin resistance (IR) are the factors that determine the frequency of cardiovascular complications in DM2.[8-10] Despite the fact that IR has a clearly identified genetic predisposition, its underlying genetic disorders have still not been identified.

There has been extensive research into the polymorphisms of peroxisome proliferator-activated receptors (*PPAR*), which are transcription factors that control the activity of many genes, as well as regulating lipid and carbohydrate metabolism.[11,12] Here we focused on *PPARγ2*, which is almost exclusively located in adipose tissue, and plays a vital role in controlling adipogenesis and circulation of fatty acids.

It has been established that genetic polymorphisms of *PPARγ2* are different in diverse populations, and data on the effect of *PPARγ2* on the development of IR are quite controversial. Therefore, there is a continued interest by scientists into the study of *PPARγ2* polymorphisms in the development of IR and other pathological processes, and the presence of conflicting data regarding its role in different populations justifies the ongoing research into the Ukrainian population of patients with comorbid disorders.

Our aim was to study heart and vascular remodeling in patients with EH and concomitant DM2 according to the genetic polymorphisms of *AGTR1* and *PPARγ2*.

METHODS

The authors examined 320 patients with EH stage II grade 2 and moderate, sub-compensated DM2 (the main group); 90 patients with EH stage II grade 2 without DM2 (the comparison group); and 31 healthy individuals (the control group).

In this study, using standard biochemical methods on the patients, we defined venous blood glucose concentration, glycosylated hemoglobin (HbA1c), and insulin levels. IR was determined using the homeostasis model assessment index (HOMA-IR). Ultrasound examinations were performed on a cardiac ultrasound scanner («ULTIMA RA» firm "RADMIR", Ukraine) in one-, two-dimensional and Doppler modes with color mapping by conventional methods. The following measurements were made: volumes of the left atrium (LA) and right atrium (RA); end-systolic diameter (ESD) and end-diastolic diameter (EDD) of the left ventricle (LV); end-diastolic pressure (EDP) in the LV; left ventricular ejection fraction (EF); index of relative wall thickness (IRWT); and the myocardial mass index (MMI) of the LV. Diastolic function of the LV was assessed by studying the blood flow in the pulmonary artery and transmitral diastolic flow in the pulsed Doppler mode with the following parameters: maximum rate of early LV filling (E); maximum speed of late (atrial) LV filling (A); the ratio of the maximum velocity of early and late LV filling (E/A); LV isovolumic relaxation time (IVRT); deceleration time (DT) of early diastolic flow velocity; average pulmonary artery pressure (PAP), according to Kitabatake; the ratio of peak E and e on the mitral valve in the spectral and tissue Doppler (E/e). For studying endothelial function, the degree of endothelium-dependent vasodilation (EDVD) in reactive hyperemia was determined in all patients. Investigations were carried out using a broadband linear transducer 5-12 MHz Doppler color mapping with three readings being taken arteries at 15-min intervals between samples on the left and right brachial arteries, according to the method of Celermajer DS (a modification of the method by Ivanova OV). Normally, the maximum vasodilation of the brachial artery should exceed 10% of the original diameter. Simultaneously, we measured the intima media thickness (IMT) of the carotid artery (CA 2 cm proximal to the bifurcation of the common carotid artery). Pulse wave velocity (PWV) of the CA was determined using the W-Track-method (method of phase tracking, patented scanner developers). Determining the PWV of the abdominal aortic (AA) (on the left subclavian artery to the femoral

artery) was performed using a phased transducer with a frequency of 2-4 MHz. An *A1166C* polymorphism of the *AGTR1* gene and a *Pro12Ala* polymorphism of the *PPARγ2* gene were assessed by the molecular genetic method. Three genotypes of the *AGTR1* gene (*A/A*, *A/C* and *C/C*) were identified, along with three genotypes of the *PPARγ2* gene (*Pro/Pro*, *Pro/Ala* and *Ala/Ala*). Processing of statistical data was performed using the software package "Statistics for Windows 6.0". The values are presented as the average value of parameters (M) and standard error (m).

The study protocol was approved by the Ethics Committee of the Kharkiv National Medical University. All participants were informed about the aim of the study and signed a written consent form.

RESULTS

Evaluation of the *A1166C* polymorphism of the *AGTR1* gene in patients with comorbidity of EH and DM2, compared to the distribution of alleles and genotypes in healthy individuals, and in patients with EH but without DM2, showed that 61.6% of patients with EH and DM2, and 57.8% of patients with EH without DM2, had *A/C* and *C/C* genotypes of *AGTR1*, which have been shown to be associated with cardiovascular complications by some researchers.[9,10] The genotypes of the main group ($P < 0.01$) and the comparison group ($P < 0.05$) were significantly different from the control group. The *C* allele was established in 33.1% of patients with EH and DM2, and 31.1% of patients with EH without DM2; in the control group, the *C* allele was significantly less present ($P < 0.05$) [Table 1].

In the next step of the study, hemodynamic and metabolic parameters in patients with comorbidity of EH and DM2, in different polymorphism variants of the *AGTR1* gene, were compared [Table 2].

Patients with *A/C* and *C/C* genotypes of the *AGTR1* gene had higher blood pressure ($P < 0.001$) compared to the *A/A* genotype. These patients also had significantly larger LV and myocardial mass index left ventricle (MMILV), and a greater IMT, with a lower rate of EDVD. Patients with the indicated genotypes also had significantly more pronounced metabolic disorders than patients with the *A/A* genotype. No significant differences in hemodynamic and metabolic parameters between the *C/C* and *A/C* genotypes were found [Table 2].

Given that *A/C* and *C/C* genotypes presented significantly different characteristics from the *A/A* genotype, with more severe disorders of echocardiographic and biochemical parameters, but were not significantly different from each other, in the next step of the study, patients with *A/C* and *C/C* genotypes were merged into a single group, namely the *A/C* + *C/C* genotype.

For establishing associations of *AGTR1* polymorphisms with cardiovascular remodeling, a comparative evaluation of echocardiographic parameters and indicators of the structural and functional state of the heart and blood vessels of the main group of patients, with different genotypes, was performed [Table 3]. It was found that patients with the *A/C* + *C/C* genotype had significantly larger LV compared to the *A/A* genotype ($P < 0.01$). Thus, patients with the *A/C* + *C/C* genotype also had significantly ($P < 0.05$) greater MMILV compared to the *A/A* genotype. In addition, the *A/C* + *C/C* genotype had significantly ($P < 0.05$) lower values of the diastolic function indicator E/A.

Assessment of the great vessels showed that IMT in the main group of patients with the genotype *A/C* + *C/C* was significantly ($P < 0.001$) greater than the genotype *A/A* [Table 3]. The study did not reveal any significant differences in PWV values in the carotid artery or the abdominal aorta in the diverse *AGTR1* genotypes. It should be noted that in patients with EH with concomitant DM2 and genotype *A/C* + *C/C*, the level of EDVD was significantly ($P < 0.001$) lower than the genotype *A/A*.

In the next step of the study, the *Pro12Ala* polymorphism of *PPARγ2* was estimated and compared with the distribution of alleles and genotypes in healthy individuals and in patients with EH without DM2 [Table 4].

It was found that, in all study groups, patients with the *Pro* allele were predominant (86.6% in the main group, 85.6% in the comparison group and 87.1% in

Table 1: The distribution of *AGTR1* alleles and genotypes in the patients, *n* (%)

Indices	Main group ($n = 320$)	Comparison group ($n = 90$)	Control group ($n = 31$)
A allele	214 (66.9)*	62 (68.9)*	25 (80.6)
C allele	106 (33.1)*	28 (31.1)*	6 (19.4)
A/A genotype	123 (38.4)*	38 (42.2)*	20 (64.5)
A/C genotype	182 (56.9)*	49 (54.5)*	10 (32.3)
C/C genotype	15 (4.7)	3 (3.3)	1 (3.2)

*$P < 0.05$ *vs.* the control group

Table 2: Comparative evaluation of hemodynamic and metabolic parameters in patients of the main group depending on genotypes of the *AGTR1* gene

Indices	Main group (*n* = 320)		
	***A/A* (*n* = 123)**	***A/C* (*n* = 182)**	***C/C* (*n* = 15)**
SBP, mmHg	166.041 ± 0.261	173.821 ± 0.221*	172.401 ± 0.621*
DBP, mmHg	99.461 ± 0.181	102.041 ± 0.231*	102.331 ± 0.851*
EDD LV, cm	4.911 ± 0.031	5.031 ± 0.031*	5.161 ± 0.095*
ESD LV, cm	3.221 ± 0.031	3.321 ± 0.031*	3.421 ± 0.091*
MMI LV, g/m	134.571 ± 2.391	143.931 ± 3.09*	146.611 ± 3.791*
E/A	0.950 ± 0.021	0.897 ± 0.015*	0.981 ± 0.021
E/e	6.223 ± 0.104	6.411 ± 0.251	6.661 ± 0.291
IMT, mm	0.914 ± 0.009	0.953 ± 0.007*	0.969 ± 0.024*
PWV of the CA, m/c	8.736 ± 0.097	8.870 ± 0.087	9.033 ± 0.333
PWV of the AA, m/c	8.866 ± 0.118	9.013 ± 0.099	9.109 ± 0.354
EDVD, %	6.657 ± 0.077	6.211 ± 0.065*	6.187 ± 0.242*
blood glucose, mmol/L	6.933 ± 0.052	7.046 ± 0.0291*	7.201 ± 0.025*
HbA1c, %	6.993 ± 0.044	7.011 ± 0.0361*	7.104 ± 0.015*
insulin, mcU/mL	23.249 ± 0.416	24.982 ± 0.291*	26.840 ± 0.994*
HOMA-IR	7.272 ± 0.130	7.991 ± 0.097*	8.276 ± 0.324*

*$P < 0.05$ *vs.* the *A/A* genotypes. SBP: systolic blood pressure; DBP: diastolic blood pressure; EDD LV: end-diastolic diameter of left ventricle; ESD LV: end-systolic diameter of left ventricle; MMI LV: myocardial mass index of left ventricle; E/A: ratio of the maximum velocity of early and late left ventricle filling; E/e: ratio of peak e and E on the mitral valve in the spectral and tissue Doppler; IMT: intima media thickness; PVW CA: pulse wave velocity by the carotid artery; PVW AA: pulse wave velocity by the abdominal aortic; EDVD: endothelium dependent vasodilation; HOMA-IR: homeostasis model assessment index

Table 3: Structural and functional state of heart and vessels in patients of the main group depending on genotypes of the *AGTR1* gene

Indices	Main group (*n* = 320)	
	***A/A* (*n* = 123)**	***A/C* + *C/C* (*n* = 197)**
EDD LV, cm	4.911 ± 0.031	5.040 ± 0.030*
ESD LV, cm	3.221 ± 0.031	3.326 ± 0.027*
MMI LV, g/m	134.571 ± 3.391	144.138 ± 2.965*
E/A	0.951 ± 0.021	0.903 ± 0.014*
E/e	6.223 ± 0.104	6.411 ± 0.151
IMT, mm	0.914 ± 0.009	0.952 ± 0.007*
PWV of the CA, m/c	8.736 ± 0.097	8.883 ± 0.084
PWV of the AA, m/c	8.866 ± 0.118	9.020 ± 0.095
EDVD, %	6.657 ± 0.077	6.180 ± 0.062*

*$P < 0.05$ *vs.* the *A/A* genotypes. EDD LV: end-diastolic diameter of left ventricle; ESD LV: end-systolic diameter of left ventricle; MMI LV: myocardial mass index of left ventricle; E/A: ratio of the maximum velocity of early and late left ventricle filling; E/e: ratio of peak e and E on the mitral valve in the spectral and tissue Doppler; IMT: intima media thickness; PVW CA: pulse wave velocity by the carotid artery; PVW AA: pulse wave velocity by the abdominal aortic; EDVD: endothelium dependent vasodilation

Table 4: Distribution of *PPARγ2* alleles and genotypes in the patients, *n* (%)

Indices	Main group (*n* = 320)	Comparison group (*n* = 90)	Control group (*n* = 31)
Pro allele	277 (86.6)	77 (85.6)	27 (87.1)
Ala allele	43 (13.4)	13 (14.4)	4 (12.9)
Pro/Pro genotype	242 (75.6)	67 (74.4)	24 (77.4)
Pro/Ala genotype	71 (22.2)	21 (23.3)	6 (19.4)
Ala/Ala genotype	7 (2.2)	2 (2.3)	1 (3.2)

the control group). It was also demonstrated that, in the main group and the comparison group, there were no significant differences in the frequency of different variants of the *PPARγ2* genotype. In both these patient groups, the *Pro/Pro* genotype was predominant, with a frequency of 75.6% and 74.4%, respectively. The homozygous genotype *Ala/Ala* was only found in 2.2% of the main group of patients and 2.3% of the comparison group of patients ($P > 0.05$). In the control group of patients, the *Pro/Pro* genotype was also prevalent (77.4% of cases); *Pro/Ala* and *Ala/Ala* genotypes were found in 19.4% and 3.2% of control patients, respectively. A similar distribution of *PPARγ* genotypes, according to other researchers[13] was inherent in the European population.

Comparison of hemodynamic and metabolic parameters of patients with EH and concomitant DM2, in different variants of *PPARγ2* polymorphisms, showed that patients with the *Pro/Pro* genotype of *PPARγ2* had significantly ($P < 0.01$) higher levels of blood pressure; larger LV sizes; greater IMT and PWV, with a lower EDVD degree, compared to the *Pro/Ala* and *Ala/Ala* genotypes [Table 5]. In addition, patients with the *Pro/Pro* genotype had significantly more pronounced dyslipidemia ($P < 0.01$) and IR ($P < 0.001$)

Table 5: Comparative evaluation of hemodynamic and metabolic parameters in patients of the main group depending on genotypes of *PPARγ2*

Indices	Main group (*n* = 320)		
	Pro/Pro	***Pro/Ala***	***Ala/Ala***
SBP, mmHg	172.372 ± 0.259	165.775 ± 0.351*	165.714 ± 1.017*
DBP, mmHg	101.599 ± 0.196	99.338 ± 0.232*	100.001 ± 1113
EDD LV, cm	5.024 ± 0.026	4.878 ± 0.038*	4.856 ± 0.084
ESD LV, cm	3.316 ± 0.024	3.198 ± 0.034*	3.149 ± 0.072
MMI LV, g/m	142.794 ± 2.551	136.553 ± 2.983	139.933 ± 7.664
E/A	0.924 ± 0.013	0.915 ± 0.027	0.943 ± 0.158
E/e	6.348 ± 0.097	6.108 ± 0.193	6.422 ± 0.866
IMT, mm	0.951 ± 0.006	0.900 ± 0.011*	0.849 ± 0.035*
PWV of the CA, m/c	8.910 ± 0.075	8.592 ± 0.122*	8.301 ± 0.301
PWV of the AA, m/c	9.020 ± 0.086	8.874 ± 0.146	7.779 ± 0.461*º
EDVD, %	6.155 ± 0.056	7.017 ± 0.079*	6.959 ± 0.241*
blood glucose, mmol/L	7.190 ± 0.022	6.968 ± 0.029*	6.643 ± 0.057*
HbA1c, %	7.103 ± 0.013	6.939 ± 0.058*	6.929 ± 0.042*
insulin, mcU/mL	25.182 ± 0.255	21.906 ± 1.526	22.814 ± 1.735
HOMA-IR	8.039 ± 0.083	6.767 ± 0.156*	6.722 ± 0.484*

*$P < 0.05$ *vs.* the *Pro/Pro* genotypes; º$P < 0.05$ *vs.* the *Pro/Ala* genotypes. SBP: systolic blood pressure; DBP: diastolic blood pressure; EDD LV: end-diastolic diameter of left ventricle; ESD LV: end-systolic diameter of left ventricle; MMI LV: myocardial mass index of left ventricle; E/A: ratio of the maximum velocity of early and late left ventricle filling; E/e: ratio of peak e and E on the mitral valve in the spectral and tissue Doppler; IMT: intima media thickness; PVW CA: pulse wave velocity by the carotid artery; PVW AA: pulse wave velocity by the abdominal aortic; EDVD: endothelium dependent vasodilation; HOMA-IR: homeostasis model assessment index

Table 6: Structural and functional state of the heart and vessels in the main group of patients depending on *PPARγ2* genotypes

Indices	Main group (*n* = 320)	
	***Pro/Pro* (*n* = 242)**	***Pro/Ala* + *Ala/Ala* (*n* = 78)**
EDD LV, cm	5.024 ± 0.026	4.876 ± 0.035*
ESD LV, cm	3.316 ± 0.024	3.194 ± 0.032*
MMI LV, g/m	142.794 ± 2.551	133.215 ± 2.799*
E/A	0.924 ± 0.013	0.917 ± 0.028
E/e	6.348 ± 0.097	6.136 ± 0.190
IMT, mm	0.951 ± 0.006	0.895 ± 0.011*
PWV of the CA, m/c	8.910 ± 0.075	8.566 ± 0.114*
PWV of the AA, m/c	9.020 ± 0.086	8.776 ± 0.143
EDVD, %	6.155 ± 0.056	7.012 ± 0.075*

*$P < 0.05$ *vs.* the *Pro/Pro* genotypes. EDD LV: end-diastolic diameter of left ventricle; ESD LV: end-systolic diameter of left ventricle; MMI LV: myocardial mass index of left ventricle; E/A: ratio of the maximum velocity of early and late left ventricle filling; E/e: ratio of peak e and E on the mitral valve in the spectral and tissue Doppler; IMT: intima media thickness; PVW CA: pulse wave velocity by the carotid artery; PVW AA: pulse wave velocity by the abdominal aortic; EDVD: endothelium dependent vasodilation

than patients with other *PPARγ2* genotypes.

However, the only significant difference in indicator levels, between the *Ala/Ala* and *Pro/Ala* genotypes was found in the PWV of the AA ($P < 0.05$). Given the fact that the *Pro/Ala* and *Ala/Ala* genotypes were significantly different from the *Pro/Pro* genotype, with the former genotypes collectively presenting less severe disorders of hemodynamic and metabolic indicators, but only differing from each other with respect to the PWV of the AA, and given the small percentage of patients with the homozygous *Ala/Ala* genotype, in the subsequent step, patients with the *Ala/Ala* and *Pro/Ala* genotypes were merged into a single group, namely the *Pro12Ala/Ala12Ala* genotype.

Analysis of the differences of indicators in the structural and functional state of the heart showed that patients with the *Pro12Ala/Ala12Ala* genotype had significantly smaller MMILV ($P < 0.05$) and LV sizes ($P < 0.01$) than patients with the *Pro/Pro* genotype [Table 6].

Considering previous data that *PPARγ2* affects gene expression in epithelial cells, vascular endothelium and macrophages, analysis of the state of blood vessels in different *PPARγ2* genotypes was conducted [Table 6]. Analyzing the major vessels in patients with EH and concomitant DM2 showed that IMT in patients with the *Pro12Ala/Ala12Ala* genotype was significantly less ($P < 0.001$) than in the *Pro/Pro* genotype. A significant difference ($P < 0.05$) was found in the PWV values of the CA depending on the *PPARγ2* genotype. It was also established that in the main group of patients with the *Pro/Pro* genotype, the EDVD was significantly lower ($P < 0.001$) than in the *Pro12Ala/Ala12Ala* genotype. Established features of the differences of indicators in *PPARγ2* genotypes confirm the association of *PPARγ2* polymorphisms with the severity of endothelial dysfunction and vascular remodeling in patients with comorbidity of EH and DM2.

DISCUSSION

Changes in echocardiographic parameters depending on genetic polymorphisms of the *AGTR1* gene can be regarded as a result of varying activation of AT1 receptors, leading to differential expression and proliferation of cardiomyocytes and myocardium remodeling.[14,15]

The involvement of polymorphisms of the *AGTR1* gene in the development and progression of atherosclerotic processes was demonstrated by significantly lower levels of anti-atherogenic high density lipoprotein cholesterol and significantly higher levels of glucose, HbA1c, insulin and HOMA-IR in patients with the *A/C + C/C* genotype compared to the *A/A* genotype. More pronounced IR in the *A/C + C/C* genotype can be explained by common mechanisms of hypertension and IR, including activation of the renin-angiotensin-aldosterone system, which affects the sensitivity of tissues to insulin and compensatory hyperinsulinemia.

The differences in blood pressure with respect to *PPARγ2* polymorphisms can be explained by the fact that the activity of *PPARγ2* receptors also depends on the production of proinflammatory and hypertensive cytokines by adipose tissue, which leads to hypertension.

The influence of *PPARγ2* polymorphisms in heart remodeling can be explained by the fact that *PPARγ2* act as modulators of gene expression in many tissues, including smooth muscle, thus the alteration of their activity due to polymorphisms contributes to the development and progression of cardiovascular disease.[16-20]

More pronounced metabolic disturbances in the *Pro/Pro* genotype of *PPARγ2* can be explained by the fact that *PPARγ2* control adipogenesis (including the production of free fatty acids, elevated levels of which are the cause of IR), and activity of *PPARγ2* affects production and circulation of lipoproteins and, as a consequence, the severity of atherosclerotic processes.

The modulating effect of polymorphisms of the genetic markers *AGTR1* and *PPARγ2* on the severity of cardiovascular remodeling in patients with comorbidity of EH and DM2 was, therefore, established.

In conclusion, polymorphisms of the genetic markers *AGTR1* and *PPARγ2* was associated with the development of comorbidity of EH and DM2. The *A/C* and *C/C* genotypes of the polymorphic marker *A1166C* of the *AGTR1* gene were characterized by significantly higher blood pressure and more pronounced cardiovascular remodeling compared to the *A/A* genotype. Patients with the *Pro/Pro* genotype of the *Pro12Ala* polymorphism of *PPARγ2* had more severe hemodynamic and metabolic disorders.

Authors' contributions

A. Shalimova contributed solely to this paper.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Each patient was informed the study and gave their consent.

Ethics approval

The study protocol was supported by the Ethics Committee of the Kharkiv National Medical University.

REFERENCES

1. Mettimano M, Romano-Spica V, Ianni A, Specchia M, Migneco A, Savi l. AGT and AT1R gene polymorphism in hypertensive heart disease. *In J Clin Pract* 2002;56:574-7.
2. Martínez-Gómez LE, Cruz M, Martínez-Nava GA, Madrid-Marina V, Parra E, García-Mena J, Espinoza-Rojo M, Estrada-Velasco BI, Piza-Roma LF, Aquilera P, Burguete-García AI. A replication study of the IRS1, CAPN10, TCF7L2, and PPARG gene polymorphisms associated with type 2 diabetes in two different populations of Mexico. *Ann Hum Genet* 2011;75:612-20.
3. Czarnecka D, Kawecka-Jaszcz K, Stolarz K, Olszanecka A, Kieć-Wilk B, Dembińska-Kieć A. Genetic factors in hypertension. Angiotensin-converting enzyme polymorphism. *Kardiol Pol* 2004;61:1-10.
4. Wang X, Zhu H, Dong Y, Treiber FA, Snieder H. Effects of angiotensinogen and angiotensin II type I receptor genes on blood pressure and left ventricular mass trajectories in multiethnic youth. *Twin Res Hum Genet* 2006;9:393-402.
5. Mettimano M, Romano-Spica V, Ianni A, Specchia M, Migneco A, Savi L. AGT and AT1R gene polymorphism in hypertensive heart diseas. *Int J Clin Pract* 2002;56:574-7.
6. Berry C, Brosnan MJ, Fennell J, Hamilton CA, Dominiczak AF. Oxidative stress and vascular damage in hypertension. *Curr Opin Nephrol Hypertens* 2001;10:247-55.
7. de Denus S, Zakrzewski-Jakubiak M, Dubé MP, Bélanger F, Lepage S, Leblanc MH, Gossard D, Ducharme A, Racine N, Whittom L, Lavoie J, Touyz RM, Turgeon J, White M. Effects of AGTR1 A1166C gene polymorphism in patients with heart failure treated with candesartan. *Ann Pharmacother* 2008;42:925-32.
8. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F; America Heart Association; National Heart, Lung, and Blood Institue. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institue Scientific Statement. *Circulation* 2005;112:2735-52.
9. Colwell JA. Type 2 diabetes, Pre-diabetes, and the metabolic syndrome. *JAMA* 2011;306:215.
10. Saad MF, Rewers M, Selby J, Howard G, Jinagouda S, Fahmi S,

Zaccaro D, Bergman RN, Savage PJ, Haffner SM. Insulin resistance and hypertension: the Insulin Resistance Atherosclerosis study. *Hypertention* 2004;43:1324-31.

11. Chen Z, Vigueira PA, Chambers KT, Hall AM, Mitra MS, Qi N, McDonald WG, Colca JR, Kletzien RF, Finck BN. Insulin resistance and metabolic derangements in obese mice are ameliorated by a novel peroxisome proliferator-activated receptor γ-sparing thiazolidinedione. *J Biol Chem* 2012;287:23537-48.
12. Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J, Shuldiner AR. Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 1997;241:270-4.
13. Palatini P, Ceolotto G, Dorigatti F, Mos L, Santonastaso M, Bratti P, Papparella I, Pessina AC, Semplicini A. Angiotensin II type 1 receptor gene polymorphism predicts development of hypertension and metabolic syndrome. *Am J Hypertens* 2009;22:208-14.
14. Lehtonen J, Paukku K, Daviet L, Kontula K. Angiotensin II type 1 receptor 1166 polymorphism A to C increases mRNA stability and steady-state levels. *Circulation* 2006;114:190.
15. Jiang Z, Zhao W, Yu F, Xu G. Association of angiotension II type 1 receptor gene polymorphism with essentional hypertension. *Chin Med J (Engl)* 2001;114:1249-51.
16. Motavallian A, Andalib S, Vaseghi G, Mirmohammad-Sadeghi H, Amini M. Association between PRO12ALA polymorphism of the PPAR-γ2 gene and type 2 diabetes mellitus in Iranian patients. *Indian J Hum Genet* 2013;19:239-44.
17. Ohshima K, Mogi M, Horiuchi M. Role of peroxisome proliferator-activated receptor-γ in vascular inflammation. *Int J Vasc Med* 2012;2012:508416.
18. He W. PPARγ2 polymorphism and human health. *PPAR Res* 2009;2009:849538.
19. Zhang F, Lu Y, Zheng S. Peroxisome proliferator-activated receptor-γ cross-regulation of signaling events implicated in liver fibrogenesis. *Cell Signal* 2012;24:596-605.
20. Gawrieh S, Marion MC, Komorowski R, Wallace J, Charlton M, Kissebah A, Langefeld CD, Olivier M. Genetic variation in the peroxisome proliferator activated receptor-gamma gene is associated with histologically advanced NAFLD. *Dig Dis Sci* 2012;57:952-7.

Harangi *et al. Vessel Plus* 2017;1:91-5
DOI: 10.20517/2574-1209.2017.08

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LDL apheresis or PCSK9 inhibition? Sometimes we have to combine them

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How to cite this article: Harangi M, Juhász L, Nádró B, Balla J, Paragh G. LDL apheresis or PCSK9 inhibition? Sometimes we have to combine them. Vessel Plus 2017;1:91-5.

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Article history:
Received: 26-03-2017
Accepted: 18-04-2017
Published: 27-06-2017

Key words:
Low density lipoprotein apheresis, PCSK9 inhibition, familial hypercholesterolemia, low-density lipoprotein, lipoprotein(a)

ABSTRACT

This study presents the case of a female patient with severe heterozygous familial hypercholesterolemia. Despite the combined maximum dose oral treatment with rosuvastatin and ezetimibe, we found markedly elevated lipid parameters. Therefore, we indicated monthly selective low density lipoprotein (LDL) apheresis treatment using the Direct Adsorption of Lipoproteins system. After more than 2 years the lipid levels of the patients were still above the therapeutic goals. Finally, we completed the treatment by the inhibitor evolocumab biweekly. Further LDL cholesterol (LDL-C) reduction was achieved resulting in lipid parameters on goals. However, administration of evolocumab without LDL apheresis could not reduce the LDL-C below 2.5 mmol/L. We concluded that both LDL apheresis and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor treatments were effective and well tolerated. None of them alone would be enough to achieve lipid goals in this patient. However, the combination of these potent treatments may normalize the lipid levels and prevent cardiovascular complications.

INTRODUCTION

Familial hypercholesterolemia (FH) is perhaps the most common single-gene variant causing premature morbidity and mortality. Heterozygous FH affects about 1 in 200 people.[1] Genetic loci involved in FH phenotype include LDLR (low-density lipoprotein receptor), APOB (apolipoprotein B-100), and PCSK9 (proprotein convertase subtilisin/kexin type 9).[2] The FH phenotype is based on family history, LDL cholesterol (LDL-C) levels, and presence of physical findings such as xanthomas, xanthelasma and corneal

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arcus. This allows for risk assessment and diagnosis when genetic testing is not available. Criteria that are most commonly used in the diagnosis of FH is the Dutch Lipid Network criteria.[3] Genetic testing is critical for cascade screening and genetic counseling.[1]

Total cholesterol levels of 350-550 mg/dL (9-14.2 mmol/L) are typical of heterozygous FH while total cholesterol levels of 650-1,000 mg/dL (16.8-25.9 mmol/L) are typical of homozygous FH.[4] In heterozygous patients LDL-C levels are usually > 190 mg/dL (> 4.9 mmol/L). In adults with homozygous FH, untreated LDL-C levels are generally, but not always, > 500 mg/dL (> 13 mmol/L). However, levels can be lower in children or in treated individuals. Thus, LDL-C levels are not sufficient to confirm a diagnosis.[5]

FH is associated with high risk of enhanced atherogenesis leading to the early development of coronary artery disease (CAD), carotid artery disease and peripheral artery disease. The European Atherosclerosis Society suggests LDL-C goals of less than 100 mg/dL (2.59 mmol/L) in all adults with FH, and less than 70 mg/dL (1.81 mmol/L) in adults with known CAD or diabetes mellitus.[3] Therefore, there is an ardent need for an aggressive therapeutic intervention based on high dose statin or statin and ezetimibe administration, selective LDL-apheresis or newly developed further therapeutic strategies including the inhibition of PCSK9.[6]

LDL apheresis techniques are artificial extracorporeal methods for LDL-C elimination. There are various methods, including cascade filtration or lipid filtration, immunoadsorption, heparin-induced LDL precipitation, dextran sulfate LDL adsorption, and the LDL hemoperfusion. All of these techniques are effective and well tolerated. The constant reduction of cholesterol is meant, above all, to prevent the progression or the development of atherosclerosis.[7]

Human monoclonal antibodies against PCSK9: alirocumab and evolocumab have recently been approved by the Food and Drug Administration. These agents target and inactivate PCSK9, a hepatic protease that attaches and internalizes LDL receptors into lysosomes hence promoting their destruction.[8] By preventing LDL receptor destruction, LDL-C levels can be lowered 50-60% above that achieved by statin therapy alone.[9] Although the data are deficient, PCSK9 inhibitors may reduce the frequency or even eliminate the need for LDL apheresis therapy. A recent study reported that switching from LDL apheresis to evolocumab maintained the LDL-lowering effect but did not decrease high-density lipoprotein cholesterol (HDL-C) levels in three patients with heterozygous FH and coronary artery disease.[10] Furthermore, findings from the ODYSSEY ESCAPE study suggest a role for alirocumab in the overall management of patients with heterozygous FH undergoing regular lipoprotein apheresis therapy, with the potential to avoid apheresis treatments or delay the requirement for such treatments.[11]

Here we demonstrate the efficacy of selective LDL apheresis, PCSK9 inhibitor evolocumab and the combination of the two treatment strategies in the case of a severe heterozygous FH patient.

CASE REPORT

The 48-year-old female patient (body weight: 85 kg, height: 176 cm, body mass index: 27.4 kg/m^2) with extremely high total cholesterol, LDL-C and lipoprotein(a) [Lp(a)] levels was treated by our Lipid ambulance. She had xanthelasmas on both eye lids [Figure 1], but neither xanthoma nor corneal arcus could be seen.

Carotid artery ultrasound proved significant stenosis on both sides. Electrocardiography, echocardiography and exercise electrocardiography did not show the signs of CAD. Using the Dutch Lipid Network Criteria the diagnosis of FH is “probable”. Sequencing of the LDL receptor gene we found a known pathogenic mutation in heterozygous form (c.1865 A>G, exon 13). Since the hypercholesterolemia, we found is unexpectedly severe in heterozygous FH, further genetic tests are in process. Although mildly elevated triglyceride (3.1 mmol/L) and lower HDL-C (1.1 mmol/L) levels are not usual in FH, the fact that the patient was overweight may explain this.

Despite the maximum dose of combined treatment (rosuvastatin 40 mg/day, ezetimibe 10 mg/day, fenofibrate 267 mg/day), lipid levels were continuously high above the goals (total cholesterol 11.7 mmol/L, LDL-C 9.1 mmol/L). Therefore, we indicated the selective LDL apheresis treatment once a month (financially supported by the Hungarian National Health Insurance Company). We performed the

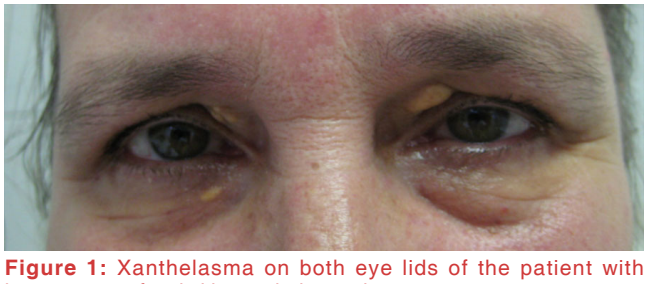

Figure 1: Xanthelasma on both eye lids of the patient with heterozygous familial hypercholesterolemia

treatments in the Extracorporeal Organ Replacement Center of University of Debrecen using the Direct Adsorption of Lipoproteins (DALI) system (Fresenius Medical Care). The DALI system can remove ApoB containing lipoproteins from the whole blood in one step without prior plasma separation. We used DALI 750 columns, 1:20 proportioned ACD-A (citrate solution) for anticoagulation. Average treated volume was 8,058 mL/treatment. The total cholesterol, LDL-C [Figure 2] and Lp(a) levels markedly decreased.

During the first two treatments we observed mild and transient bradykinin reactions with hypotension, dyspnea, atypical chest pain and flushing. After three treatments apheresis was suspended for three months at the patient's wish. Then, because of increasing lipid levels we continued apheresis treatment. Although between two treatments there was an increase in lipid levels, the time-average cholesterol (TAC) and LDL-cholesterol (TAL) levels calculated with a formula by Kroon *et al*.[12] decreased significantly (TAC: 6.44 mmol/L, TAL: 4.68 mmol/L). Monthly apheresis treatment decreased TAC by 45.7% and TAL by 48.6%. The average reduction of Lp(a) level was 72%. Despite the efficacy of monthly apheresis treatments, lipid levels were still above the goals. Theoretically, we could increase the frequency of apheresis treatments and administer it biweekly, however, our insurance company would not support it because of its high costs. One of the two PCSK9 inhibitor monoclonal antibodies available in Hungary since 2015 is evolocumab. We decided to start this new agent added to the LDL apheresis treatment. Evolocumab was administrated biweekly, subcutaneously in a dose of 140 mg/day. After two months of combined apheresis plus PCSK9 inhibitor treatments we detected further significant reductions in total cholesterol and LDL-C levels (62.1% and 66.1%, respectively), and finally, we achieved the lipid goals. Hoping that the PCSK9 inhibitor treatment would be effective without further apheresis treatments, we stopped the apheresis and continued the evolocumab therapy. Unfortunately, despite the impressive efficacy of the PCSK9 inhibition, after a further three months the patient was above the goals [Figures 2 and 3].

DISCUSSION

Adequate treatment of patients with FH is a challenge and an everyday problem for the therapists. Combination of high dose oral lipid lowering agents is in some severe cases not effective enough because of the extremely high initial lipid levels. Our demonstrated case is a good example for this situation. Selective LDL apheresis treatments are available and widely used since the 80s. Removal of the ApoB containing lipoprotein particles can reduce the total cholesterol and LDL-C levels by 50-75% acutely. The TAC shows inter-individual differences and is usually between 20% to 40%.[13] In our case the reduction was even higher.

Moreover, LDL apheresis can markedly reduce the Lp(a) levels even by 60-80%. Lp(a) is an independent risk factor for cardiovascular disease and is not lowered by oral lipid-lowering therapy apart from nicotinic acid.[14] In our case, LDL apheresis showed similar

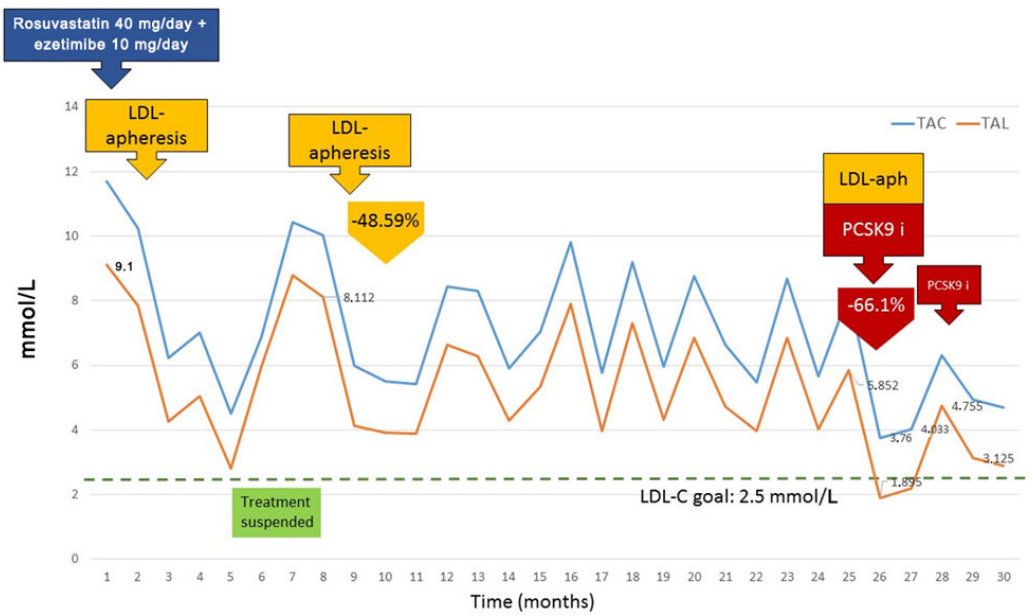

Figure 2: Total cholesterol/TAC and low-density lipoprotein cholesterol/TAL levels of the patient during the treatments. TAC = C_{min} + 0.73(C_{max} - C_{min}); TAL = LDL_{min} + 0.73(LDL_{max} - LDL_{min}). LDL-C: low density lipoprotein cholesterol; TAC: time average cholesterol; TAL: time average low-density lipoprotein cholesterol

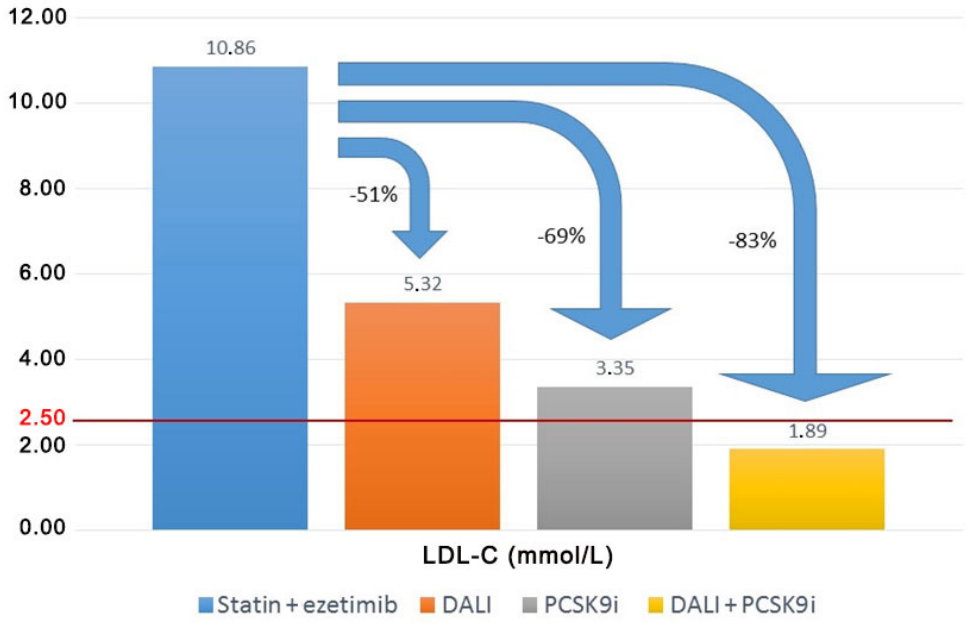

Figure 3: LDL-C levels during the various treatment strategies. The oral treatment was continued during treatment with PCSK9 and LDL apheresis. LDL-C: low density lipoprotein cholesterol; PCSK9: proprotein convertase subtilisin/kexin type 9

Lp(a) reduction. It must be noted that from the initiation of the apheresis treatments regular carotid ultrasound examinations did not show any progression and CAD was not diagnosed.

One hundred and forty mg biweekly administrated evolocumab can reduce the LDL-C level by 66%.[15] PCSK9 inhibition also results in significant (18-20%) reductions in plasma Lp(a). Unique efficacy of evolocumab in LDL-C reduction could be observed in our case. However, LDL apheresis treatment is superior in Lp(a) reduction.

So, which treatment strategy should we choose in our severe heterozygous FH patients? Would selective LDL apheresis or PCSK9 inhibition be better? Decades of clinical experience, results of long-term follow-up studies[16,17] and unique Lp(a) reducing efficacy supports selective LDL apheresis. Furthermore, LDL apheresis treatment significantly improves the oxidative, inflammatory, rheological and hemostasis status of the treated patients.[18] Nevertheless, superior efficacy of PCSK9 inhibitors in LDL-C reduction is in its favor. Both treatment options are well tolerated and safe, even in combination as was demonstrated in our case. It must be noted that further observations are needed to assess long-term side effects of this therapy.

This case report highlights the difficulties of the treatment of severe heterozygous FH and proves that in some special cases LDL apheresis and PCSK9 inhibition can be combined successfully and safely resulting in extreme LDL-C reduction. Combined treatment may give hope for FH patients whose quality of life and life expectancies are limited because of the ineffective or not tolerated lipid lowering treatment.

Selective LDL apheresis or PCSK9 inhibition? Sometimes we have to combine them.

Authors' contributions

Concept, data analysis, and manuscript preparation: M. Harangi
Data acquisition, and literature search: L. Juhász, B. Nádró
Manuscript editing, and manuscript review: J. Balla, G. Paragh

Financial support and sponsorship

This research was supported by a grant from the National Research, Development and Innovation (NFKI) (OTKA 115723) and by the GINOP-2.3.2-15-2016-00005 Project. The project is co-financed by the European Union under the European Regional Development Fund.

Conflicts of interest

There are no conflicts of interest.

Patient consent

The patient gave her consent form.

Ethics approval

The work is conforming to the guiding principles of the Declaration of Helsinki, and our patient gave informed consent.

REFERENCES

1. Gidding SS, Champagne MA, de Ferranti SD, Defesche J, Ito MK, Knowles JW, McCrindle B, Raal F, Rader D, Santos RD, Lopes-Virella M, Watts GF, Wierzbicki AS; American Heart Association Atherosclerosis, Hypertension, and Obesity in Young Committee of Council on Cardiovascular Disease in Young, Council on Cardiovascular and Stroke Nursing, Council on Functional Genomics and Translational Biology, and Council on Lifestyle and Cardiometabolic Health. The agenda for familial hypercholesterolemia: a scientific statement from the American Heart Association. *Circulation* 2015;132:2167-92.
2. Ahmad Z, Adams-Huet B, Chen C, Garg A. Low prevalence of mutations in known loci for autosomal dominant hypercholesterolemia in a multiethnic patient cohort. *Circ Cardiovasc Genet* 2012;5:666-75.
3. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Ž, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WM, Vlachopoulos C, Wood DA, Zamorano JL; Authors/Task Force Members; Additional Contributor. 2016 ESC/EAS guidelines for the management of dyslipidaemias. *Eur Heart J* 2016;37:2999-3058.
4. Repas TB, Tanner JR. Preventing early cardiovascular death in patients with familial hypercholesterolemia. *J Am Osteopath Assoc* 2014;114:99-108.
5. Cuchel M, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, Kuivenhoven JA, Nordestgaard BG, Descamps OS, Steinhagen-Thiessen E, Tybjærg-Hansen A, Watts GF, Averna M, Boileau C, Borén J, Catapano AL, Defesche JC, Hovingh GK, Humphries SE, Kovanen PT, Masana L, Pajukanta P, Parhofer KG, Ray KK, Stalenhoef AF, Stroes E, Taskinen MR, Wiegman A, Wiklund O, Chapman MJ; European Atherosclerosis Society Consensus Panel on Familial Hypercholesterolaemia. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur Heart J* 2014;35:2146-57.
6. Page MM, Watts GF. Emerging PCSK9 inhibitors for treating dyslipidaemia: buttressing the gaps in coronary prevention. *Expert Opin Emerg Drugs* 2015;20:299-312.
7. Bambauer R, Bambauer C, Lehmann B, Latza R, Schiel R. LDL-apheresis: technical and clinical aspects. *Sci World J* 2012;2012:314283.
8. Duff CJ, Scott MJ, Kirby IT, Hutchinson SE, Martin SL, Hooper NM. Antibody-mediated disruption of the interaction between PCSK9 and the low-density lipoprotein receptor. *Biochem J* 2009;419:577-84.
9. Chaudhary R, Garg J, Shah N, Sumner A. PCSK9 inhibitors: a new era of lipid lowering therapy. *World J Cardiol* 2017;9:76-91.
10. Lappegård KT, Enebakk T, Thunhaug H, Hovland A. Transition from LDL apheresis to evolocumab in heterozygous FH is equally effective in lowering LDL, without lowering HDL cholesterol. *Atherosclerosis* 2016;251:119-23.
11. Moriarty PM, Parhofer KG, Babirak SP, Cornier MA, Duell PB, Hohenstein B, Leebmann J, Ramlow W, Schettler V, Simha V, Steinhagen-Thiessen E, Thompson PD, Vogt A, von Stritzky B, Du Y, Manvelian G. Alirocumab in patients with heterozygous familial hypercholesterolaemia undergoing lipoprotein apheresis: the ODYSSEY ESCAPE trial. *Eur Heart J* 2016;37:3588-95.
12. Kroon AA, van't Hof MA, Demacker PN, Stalenhoef AF. The rebound of lipoproteins after LDL-apheresis. Kinetics and estimation of mean lipoprotein levels. *Atherosclerosis* 2000;152:519-26.
13. Yokoyama S, Hayashi R, Satani M, Yamamoto A. Selective removal of low density lipoprotein by plasmapheresis in familial hypercholesterolemia. *Arteriosclerosis* 1985;5:613-22.
14. Emerging Risk Factors Collaboration, Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA* 2009;302:412-23.
15. Raal F, Scott R, Somaratne R, Bridges I, Li G, Wasserman SM, Stein EA. Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation* 2012;126:2408-17.
16. Bosch T, Gahr S, Belschner U, Schaefer C, Lennertz A, Rammo J; DALI Study Group. Direct adsorption of low-density lipoprotein by DALI-LDL-apheresis: results of a prospective long-term multicenter follow-up covering 12,291 sessions. *Ther Apher Dial* 2006;10:210-8.
17. Mabuchi H, Koizumi J, Shimizu M, Kajinami K, Miyamoto S, Ueda K, Takegoshi T. Long-term efficacy of low-density lipoprotein apheresis on coronary heart disease in familial hypercholesterolemia. Hokuriku-FH-LDL-Apheresis Study Group. *Am J Cardiol* 1998;82:1489-95.
18. Mellwig KP, Pulawski E, Horstkotte D, van Buuren F. Lipid apheresis: oxidative stress, rheology, and vasodilatation. *Clin Res Cardiol Suppl* 2012;7:45-9.

Myasoedova *et al. Vessel Plus* 2017;1:96-7
DOI: 10.20517/2574-1209.2017.22

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Topic: Atherosclerosis and Related Diseases **Open Access**

A prominent role of D-dimer in inflammation and atherosclerosis

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How to cite this article: Myasoedova VA, Poggio P, Parolari A. A prominent role of D-dimer in inflammation and atherosclerosis. *Vessel Plus* 2017;1:96-7.

Article history: Received: 15 Jun 2017 Accepted: 9 Aug 2017 Published: 26 Sep 2017

Plasma D-dimer (D-d) is a product of plasmin fibrinolysis of cross-linked fibrin that leads to increased fibrin turnover. D-d is a known biomarker of coagulation and is commonly used for quick diagnosis of deep venues thrombosis, pulmonary embolism and acute aortic syndrome, with high sensitivity and rather low specificity.[1] Recent data suggest that D-d is helpful for both diagnostic and prognostic purposes.

Mori *et al.*[2] evaluated the potential of D-d and C-reactive protein (CRP) levels, measured at hospital admission, to predict long-term adverse events after acute aortic dissection. They showed that patients with the worst outcomes had both biomarkers elevated compared to patients with no or minor adverse events.

In another study by Gorla *et al.*,[3] the results obtained by evaluation of inflammatory imaging markers with flurodeoxyglucose positron emission tomography - computed tomography (FDG PET CT) and adverse outcomes, during a 3-year follow-up after acute aortic syndrome, suggested that all-cause mortality and major adverse events were higher in the PET-positive patients. Interestingly, these differences were more evident when PET results were combined with D-d levels.

These data suggest that, after acute cardiac states, the combination of D-d and CRP levels appears to be a strong predictor of cardiovascular events and overall mortality.

The role of systemic inflammation in all stages of atherosclerosis is well known. Based on D-d association with inflammation, we evaluated the role of this circulating biomarker in patients that underwent coronary artery bypass grafting (CABG).[4] The specific aim of this study was to assess if D-d level, before surgery, was able to predict graft occlusion. Eighteen months after CABG, we evaluated graft patency by CT scanning. Notably, pre-operative D-d level was an independent predictor for both arterial and venous grafts occlusion.

D-d was identified also as a predictor of atherosclerotic lesion severity and of overall outcomes in 2209 angiographically-proven patients with coronary artery disease (CAD).[5] In addition, in a large prospective study, D-d measured at baseline was shown to

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predict future acute myocardial infarction (odds ratio of 2.5) and cardiovascular death (odds ratio of 4.0).[6] Interestingly, D-d level was also independently associated with cardiac events incidence (odds ratio of 2.0) in post-menopausal women.[6]

A recent review by Soomro *et al*.[7] indicated the role of D-d as a prominent circulating biomarker. Indeed, in patients with peripheral aortic disease (PAD), D-d level linearly increased with severity of the disease, both in males and females. In addition to PAD progression, D-d was proven to predict also future coronary events. The increment of D-d in patients with atherosclerosis could be explained by the fact that the atherosclerotic process may lead to fibrinolysis.

In conclusion, the accumulating evidence suggests that D-d level can be helpful for diagnostic and risk stratification of patients with acute cardiac states as well as atherosclerosis. We need to focus our attention to the relationship between inflammation and hemostasis to identify D-d biological mechanisms and effects. Moreover, further studies are needed in order to understand the real potential of this biomarker. Finally, specific studies, aimed to identify a D-d plausible cut-off in patients with atherosclerosis, will allow its use in clinical practice.

DECLARATIONS

Authors' contributions

Did literature review and composed first draft of article: V.A. Myasoedova, P. Poggio

Reviewed data and worked on the subsequent revisions: A. Parolari

Reviewed progress of the article at each step, and finalized the manuscript: V.A. Myasoedova, P. Poggio, A. Parolari

Financial support and sponsorship

This work was supported by the Fondazione Gigi e Pupa Ferrari ONLUS.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. Parolari A, Tremoli E, Songia P, Pilozzi A, Di Bartolomeo R, Alamanni F, Mestres CA, Pacini D. Biological features of thoracic aortic diseases. Where are we now, where are we heading to: established and emerging biomarkers and molecular pathways. *Eur J Cardiothorac Surg* 2013;44:9-23.
2. Mori K, Tamune H, Tanaka H, Nakamura M. Admission values of D-dimer and C-reactive protein (CRP) predict the long-term outcomes in acute aortic dissection. *Intern Med* 2016;55:1837-43.
3. Gorla R, Erbel R, Kuehl H, Kahlert P, Tsagakis K, Jakob H, Mahabadi AA, Schlosser T, Bockisch A, Eggebrecht H, Bossone E, Janosi RA. Prognostic value of (18)F-fluorodeoxyglucose PET-CT imaging in acute aortic syndromes: comparison with serological biomarkers of inflammation. *Int J Cardiovasc Imaging* 2015;31:1677-85.
4. Parolari A, Cavallotti L, Andreini D, Myasoedova V, Banfi C, Camera M, Poggio P, Barili F, Pontone G, Mussoni L, Centenaro C, Alamanni F, Tremoli E; Coronary Bypass Grafting: Factors Related to Late Events and Graft Patency (CAGE) study investigators. D-dimer is associated with arterial and venous coronary artery bypass graft occlusion. *J Thorac Cardiovasc Surg* 2017; doi: 10.1016/j.jtcvs.2017.04.043.
5. Gong P, Yang SH, Li S, Luo SH, Zeng RX, Zhang Y, Guo YL, Zhu CG, Xu RX, Li JJ. Plasma d-Dimer as a useful marker predicts severity of atherosclerotic lesion and short-term outcome in patients with coronary artery disease. *Clin Appl Thromb Hemost* 2016;22:633-40.
6. Itakura H, Sobel BE, Boothroyd D, Leung LL, Iribarren C, Go AS, Fortmann SP, Quertermous T, Hlatky MA; Atherosclerotic Disease, Vascular Function and Genetic Epidemiology Advance (ADVANCE) Study. Do plasma biomarkers of coagulation and fibrinolysis differ between patients who have experienced an acute myocardial infarction versus stable exertional angina? *Am Heart J* 2007;154:1059-64.
7. Soomro AY, Guerchicoff A, Nichols DJ, Suleman J, Dangas GD. The current role and future prospects of D-dimer biomarker. *Eur Heart J Cardiovasc Pharmacother* 2016;2:175-84.

Samaha *et al. Vessel Plus* 2017;1:98-106
DOI: 10.20517/2574-1209.2017.18

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Topic: Atherosclerosis and Related Diseases **Open Access**

Associations of lifestyle and dietary habits with hyperlipidemia in Lebanon

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How to cite this article: Samaha AA, Zouein F, Gebbawi M, Fawaz M, Houjayri R, Samaha R, Baydoun S, Eid AH. Associations of lifestyle and dietary habits with hyperlipidemia in Lebanon. *Vessel Plus* 2017;1:98-106.

Article history:
Received: 8 Jun 2017
Accepted: 20 Jul 2017
Published: 26 Sep 2017

Key words:
Lipidemia,
cardiovascular disease,
diet,
Lebanon

ABSTRACT

Aim: This study was designed to evaluate the effects of dietary and lifestyle habits on several blood lipid parameters in the Lebanese population. **Methods:** This was a cross-sectional study for 2,000 individuals, of whom 1,003 completed the survey about their dietary and lifestyle habits. Anthropometric measurements and blood tests were performed and recorded. **Results:** Up to 53.2% of the population was hypercholesterolemic. Gender and age contributed to the prevalence of high levels of low density lipoprotein cholesterol (LDL-C) or triglycerides. Prevalence of hypercholesterolemia, hypertriglyceridemia and high LDL-C levels was higher in smokers, physically inactive or those who consume fatty meat or eggs. Prevalence of hypercholesterolemia was not affected by consumption of whole milk, skimmed milk or fruits and vegetables. However, the prevalence of hypertriglyceridemia and high LDL-levels was higher in individuals who consumed whole milk, and lower in those who consumed skimmed or fruits and vegetables. **Conclusion:** Hyperlipidemia affects more than half of the Lebanese population. The finding that the majority of the individuals were unaware of their lipid profile mandates warrant efforts for both patient and public education.

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality and morbidity in both developed and developing countries.[1] Although modifiable, hyperlipidemia remains a major risk factor for many diseases including coronary artery disease (CAD) and atherosclerosis.[2,3] Although increased levels of high density lipoprotein cholesterol (HDL-C) may play a protective role against CVD,[4,5] there is a positive correlation between increased serum levels of cholesterol, triglycerides (TG), or low density lipoprotein

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cholesterol (LDL-C) and CAD or atherosclerosis.[4-6] Thus, it is not surprising that the major consequence of hypercholesterolemia is atherosclerosis.[7,8] The major hallmark of atherosclerosis is the buildup of lipids, mainly cholesterol, in the vascular wall.

Atherosclerosis is the most common CVD, and conditions associated with it, such as stroke and acute myocardial infarction (AMI), continue to be the leading causes of morbidity throughout the world.[9,10] By causing a reduction in the vessel lumen, atherosclerotic plaques may dramatically diminish blood supply to tissues, which could have deleterious effects on the nourishment and oxygen supply of these tissues.[11]

Hypercholesterolemia remains a leading risk factor in both developed and developing countries.[12] Several studies have reported that a decrease in the serum levels of total cholesterol (TC) and LDL-C leads to a reduction in the risk of coronary events and is thus an effective strategy in secondary prevention.[13-17] It is also well documented that hyperlipidemia plays a major role in the development of many CVDs including atherosclerosis.[2,4,5]

There are many risk factors for atherosclerosis, such as gender, diabetes, stress, and hypertension.[18] However, hypercholesterolemia in particular is critical for the genesis of the disease as it plays a permissive role in the development of other pathogenic factors such as insulin resistance and vascular inflammation.[19] Notably, the relationship between atherosclerosis and hypercholesterolemia is affected by lifestyle factors, including physical activity and smoking.

Lebanon is considered a third-world country. Alarmingly, no large studies have been conducted to determine the prevalence of hyperlipidemia in the Lebanese population. There are no national studies showing the impact of lifestyle-related factors, such as diet, physical activity, alcohol consumption, or smoking on blood levels of lipids. Despite the findings that the Mediterranean diet may help reduce CVD risk and mortality,[20] nothing is known about this relation in Lebanon, a small Mediterranean country. Interestingly, there has been a change in the dietary habits, particularly in the 20-30 years old age group living in large cities in Lebanon.

This study was therefore undertaken to first determine the prevalence and awareness of hyperlipidemia in Lebanon. It was also designed to examine the influence of diet and lifestyle factors on lipidemia in the Lebanese population.

METHODS

Study population

This cross-sectional study recruited 2,000 participants; 997 of whom were later excluded because of the existence of illness with the potential to modulate lipid profiles, such as diabetes mellitus or the use of lipid altering medications. Diabetes was defined as having a fasting glucose level of ≥ 126 mg/dL or by the use of glucose-lowering drugs. Demographically, the remaining 1,003 individuals included in the study were distributed over the different geographic regions of Lebanon. Of these subjects, 501 were females and 502 males, all reported to be healthy with unremarkable previous medical history. Surprisingly, more than 70% of the subjects were unaware of their lipids profiles. Subjects were grouped in 20-year intervals. All participants above 55-year-old were assigned to one-age interval.

Data collection and definitions

Questionnaires were distributed with instructions for self-administration and collected one week later. Incomplete questionnaires were completed during short personal interviews. Information concerning height, weight, alcohol intake, smoking, and physical activity were recorded. Measurements of height and weight were taken with the participants wearing no shoes and very light clothing. Height was measured to the nearest 0.5 cm using a standard physician's height stadiometer. Body weight was measured to the nearest 0.1 kg using a portable balance scale. Body mass index (BMI; kg/m^2) was calculated as weight (kg) divided by the square of the height (m).

Hypercholesterolemia was defined as having blood total cholesterol of ≥ 200 mg/dL.

Participants not undergoing regular physical exercise for at least 30 min twice a week were considered sedentary.

Smoking was defined as smoking an average of one or more cigarettes per day.

Nutritional data were collected and pertained to the frequency of consumption of fatty meat, entire dairy products, skimmed milk, eggs, as well as fruits and vegetables.

Informed consent was obtained for all participants, either directly (for those 18 years or older) or through the parents/legal guardians (for subjects under 18 years of age).

The protocols followed for data collection, handling,

and analyzing were approved by the Research Ethics Committee at the Lebanese University.

Blood sampling and laboratory

Selected participants were asked to undergo a 12-h-fasting period, after which 5 mm of blood were collected by clean venipuncture into dry tubes (BD Vacutainer®). Plasma was separated by centrifugation (1,000 *g*, 4 ℃, 20 min). Plasma samples were frozen until analysis, which was carried out not later than 4 h after phlebotomy. Since seasonal variation in plasma cholesterol levels is well documented,[21] all blood samples were collected in the same month. Plasma lipids concentrations were measured at the Clinical Biochemistry Laboratory of Rayak Hospital, Lebanon. Total cholesterol, LDL and triglycerides levels were measured using automated enzymatic-calorimetric techniques (COBAS MIRA). LDL was calculated according to Friedewald formula as the total cholesterol minus HDL minus one-fifth triglycerides level. Final results are reported in mg/dL concentrations. The used reagents were supplied by SPINREAC.S.A.

Statistical analysis

All analyses were conducted using SPSS program by stepwise multiple regression analysis. The difference in mean values between two groups (like males versus females) was tested by Student's *t*-test. A level of 5% for the *P* value was considered significant.

RESULTS

Anthropometric measurements of the individuals who participated in this study are shown in Table 1. The age of the participants ranged from 15 to 87 years old, with a mean of 41.64 (± 15.9; SD) years. There was no significant difference between the mean age of males and females (41.8 *vs*. 41.48 years, respectively.) The mean weight of the participants was 74.15 (± 13.9; SD) kg, ranging from 36 kg to 150 kg. Their heights ranged from 142 cm to 195 cm, with a mean height of 166.46 (± 7.76; SD) cm. Both the height and weight of males were significantly higher than the females' [Table 1]. The range of the BMI was 14.2 to 49 kg/m^2, with a mean of 26.703 ± 4.42 kg/m^2. Age-adjusted BMI was found to be significantly higher in females than males ($P < 0.05$).

In this population, the means for serum levels of cholesterol, LDL-C, HDL-C and triglycerides were respectively 208.5 ± 49.5, 133.2 ± 46.1, 43.1 ± 8.9, and 163.6 ± 111.6 mg/dL [Table 2]. Overall, an alarming 53.24% of the population had a cholesterol levels above 200 mg/dL, and thus can be considered hypercholesterolemic.[22]

Importantly, cholesterol levels of the study participants were found to conform to a normal distribution with a mean of 208.5 ± 49.9 mg/dL, and a range of 110.4 mg/dL to 306.2 mg/dL [Figure 1]. Conformity of the distribution was tested by classical statistical analysis of the variance (q^2) with confidence

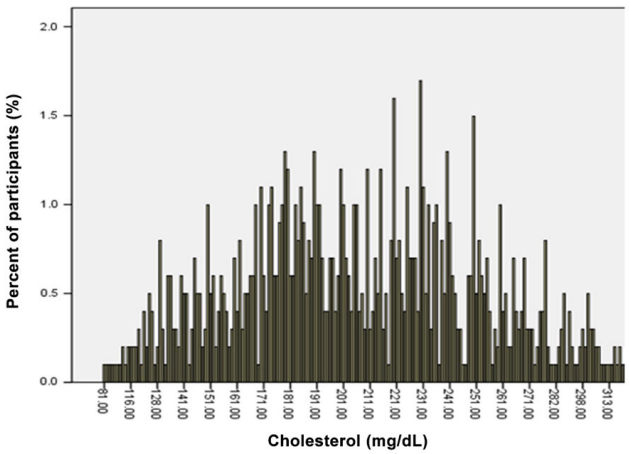

Figure 1: Distribution of cholesterol level in the population studied. The percent of the population with a measured level of cholesterol (mg/dL) is plotted

Table 1: Characteristics of the study participants

	Males (502)			Females (501)			Total (1,003)			*P*-value
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	
Age	41.8	15.9	71	41.5	15.8	71	41.6	15.9	72	0.34
Height	169.1	7.5	53	163.8	7.1	48	166.6	7.8	53	< 0.001
Weight	77.6	13.1	100.8	70.9	13.8	110	74.2	13.9	53	< 0.001
BMI	27.5	4.1	34.9	26.3	4.7	32.7	26.7	4.4	34.9	#

P value denotes significant difference between genders. #denotes $P < 0.05$ for the age-adjusted body mass index (BMI)

Table 2: Means of lipid profile parameters according to sex distribution

	Males (502)	Females (501)	Total (1,003)	*P*-value
Cholesterol	205.7 ± 47.8	211.2 ± 51.9	208.5 ± 49.9	0.08
LDL-C	123.2 ± 46.3	140.7 ± 44.6	133.2 ± 46.1	< 0.001
HDL-C	43.4 ± 8.7	42.9 ± 9.1	43.1 ± 8.9	0.465
TG	177.6 ± 123.4	150.8 ± 98.7	163.6 ± 111.6	< 0.001

Data presented as mean ± SD. LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglycerides

interval of 95% (q^2_c = 0.3693, q^2_α = 5.991, α = 0.05).

Table 2 also shows that the mean levels of cholesterol and HDL-C did not significantly differ between both genders. It is interesting to note that whereas mean values of LDL-C were significantly higher in females than males (140 *vs*. 123; *P* < 0.001), TG exhibited an opposite profile, being higher in males than females (177.6 *vs*. 150.8; *P* < 0.001).

Because gender did not significantly contribute to the levels of total cholesterol or HDL-C, we then focused our analysis on TG and LDL-C levels and sought to determine if age contributes to the observed gender differences.

Interestingly, LDL-C as well as TG levels showed a statistically significant increase with age in both males and females (*P* < 0.05 for TG and *P* < 0.001 for LDL-C). TG levels were higher in males in the two age groups [15-35] and [35-55], whereas LDL-C were higher in females in these two age groups. Interestingly, in the ≥ 55 age group, there was no significant difference in either of LDL-C or TG levels [Table 3].

The prevalence of high levels of cholesterol, TG or LDL-C in the different dietary or lifestyle-related groups is shown in Table 4.

Table 3: Means of serum levels of cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides in males and females according to different age groups

Age interval (years)	Gender/number	Triglycerides level	LDL level
[15-35]	Male/189	132.9 ± 117.2	109.7 ± 37.3
	Female/197	96.4 ± 48.6	123.9 ± 36.4
	P value	< 0.001	0.0001
[35-55]	Male/211	183.1 ± 114.6	118.4 ± 39.5
	Female/190	159.0 ± 89.0	147.6 ± 45.5
	P value	< 0.001	< 0.001
≥ 55	Male/102	228.5 ± 125.9	142.9 ± 54.8
	Female/114	210.3 ± 122.1	151.2 ± 46.4
	P value	0.283	0.23

Data presented as mean ± SD. LDL: low density lipoprotein; HDL: high density lipoprotein

Table 4: Prevalence of hypercholesterolemia, high triglyceridemia and high LDL-C among individuals with different lifestyle and dietary habits

	Percent of population (%)	Prevalence of hypercholesterolemia (%)	Prevalence of high triglyceride (%)	Prevalence of high LDL (%)
Gender				
Male	50.05	51.3	56.6	36
Female	49.99	48.7	43.3	64
		P < 0.01	*P* < 0.01	*P* < 0.01
Cigarette				
Non-smokers	62.4	45.12	40.6	36.6
Smokers	37.6	70.02	50.4	63.4
		P < 0.01	*P* = 0.002	*P* < 0. 01
Physical activity				
Sedentary	68.4	64.4	89.4	79.1
Active	31.6	32.9	10.6	20.9
		P < 0. 01	*P* < 0.001	*P* < 0.001
Fatty meat				
≥ 3 servings/day	50.1	72.1	60.2	53.3
< 3 servings/day	49.3	37.4	39.8	46.5
		P < 0.01	*P* < 0.001	*P* = 0.01
Whole milk				
≥ 1 servings/day	91.9	55.8	98.2	84.7
< 1 serving/day (on average)	8.1	46.9	1.8	15.3
		P = 0.122	*P* < 0.001	*P* < 0.001
Skimmed milk				
≥ 1 serving/day	10	55.3	4.5	15.3
< 1 serving/day	90	45.9	95.5	84.7
		P = 0.087	*P* < 0.001	*P* < 0.001
Eggs				
≥ 4-7 servings/week	74	58.1	80.5	77.9
< 3 servings/week	26	43.9	19.5	22.1
		P < 0.001	*P* < 0.001	*P* < 0.001
Fruits and vegetables				
≥ 3 servings/day	98	45.5	1.8	3.5
< 3 servings/day	2	56.4	98.2	96.5
		P = 0.33	*P* < 0.001	*P* < 0.001

LDL-C: low density lipoprotein cholesterol

The prevalence of hypercholesterolemia and triglyceridemia was significantly higher in males than females (both $P < 0.01$), but prevalence of high LDL-C was higher in females ($P < 0.01$).

Cigarette smokers have significantly higher prevalence of high cholesterol ($P < 0.01$), TG ($P = 0.002$) and LDL-C levels ($P < 0.001$). Likewise, sedentary individuals exhibited higher prevalence of all the three hyperlipidemias ($P < 0.01$ for cholesterol and $P < 0.001$ for TG and LDL-C).

With respect to the dietary factors, consumption of 3 or more servings of either fatty meat or eggs was significantly associated with higher prevalence of the three hyperlipidemias. Consumption of whole or skimmed milk did not significantly affect the prevalence of hypercholesterolemia. However, participants who reportedly consumed 1 or more servings of skimmed milk had significantly lower incidence of high TG or LDL-C levels ($P < 0.001$). The exact opposite is noted for those who consumed 1 or more servings of whole milk; these individuals had significantly higher prevalence of high TG and LDL-C levels ($P < 0.001$).

The number of serving of fruits/vegetables per week did not seem to contribute to the prevalence of hypercholesterolemia. However, those who consumed 3 or more servings of fruits/vegetables had a significantly lower prevalence of high TG and LDL-C levels ($P < 0.001$).

DISCUSSION

To the best of our knowledge, this is the first study to determine the prevalence of hypercholesterolemia in Lebanon. In fact, it is also the largest and most comprehensive study examining a health-related issue in Lebanon, a small country with a total population of around 4 million people. The relatively large number of participants and their wide demographic distribution covering all parts of Lebanon are two strengths of this study.

In this study, we first determined the prevalence of TC, LDL-C, HDL-C and TG in the Lebanese population. We then evaluated how age, gender, some dietary and lifestyle-related habits modulated the lipid profile. We herein report that 53% of the Lebanese population is hypercholesterolemic. Alarmingly, most of these subjects were unaware of their lipid profile. This mandates concerted efforts to educate the public about the danger of dyslipidemia as well as its relation to various cardiovascular diseases. Unfortunately, no large-scale studies have determined the CVD-associated mortality and morbidity in Lebanon. Moreover, given the lack of sufficient available literature, we could not find a study that analyzed the effect of gender or dietary/lifestyle habits on the prevalence of atherosclerosis in the Lebanese population. This has hindered us from making a meaningful conclusion on the interplay between these parameters and atherosclerosis.

Compared with other countries, the prevalence of hypercholesterolemia in Lebanon is higher than that in Turkey,[23] Saudi Arabia,[24] India,[25] Guadeloupe,[26] but lower than that in England,[27] and similar to that in Finland,[28] and the USA.[29]

Gender appeared to play an important role in the prevalence of the lipidemia studied. Although there was no difference in mean cholesterol levels between males and females, more males suffered from hypercholesterolemia. In addition, prevalence of high TG was higher in males than females. This lower prevalence in females may suggest that female sex hormones, particularly estrogen, offers a protective effect against the elevation of cholesterol or TG levels in the Lebanese population. Indeed, such a protective role for estrogen has been previously reported, but remains controversial.[30-33] Interestingly, in the female population included in this study, the levels of cholesterol and TG increased with age, even beyond menopause. On the other hand, it is possible that estrogen contributes to the increased levels of TG. While this would be consistent with other reports where estrogen was shown to precipitate hypertriglyceridemia,[34] it remains inconsistent with other studies suggesting that menopausal women have higher levels of LDL-C and TC compared with pre-menopausal women.[35,36] Moreover, estrogen injection has been shown to reduce hepatic cholesterol synthesis thereby leading to decreased blood levels of LDL-C.[36,37]

It is also possible that the male hormone, testosterone, predisposes men for hypercholesterolemia and triglyceridemia. Indeed, some reports suggest that testosterone levels are inversely proportional to serum lipid levels in males.[38] Moreover, animal studies show that testosterone-deficient male mice exhibit higher cholesterol levels, clearly suggesting a favorable role for testosterone in regulating blood lipid levels.[39] However, testosterone is unlikely to explain the higher prevalence of hypercholesterolemia in males, particularly because the population average of cholesterol did not differ between males and females.

Because it increased the prevalence of hypercholesterolemia and triglyceridemia, smoking

may explain the higher prevalence of these dyslipidemias in males versus females. This would be supported by our observation that smoking is more common among men than women in the study participants. This is not surprising since smoking is still not socially very acceptable for women in some regions of Lebanon, particularly in some rural areas. Surprisingly, when we adjusted for smoking, females were found to be at higher risk of developing hyperlipidemias. However, it is important to note that the prevalence of hyperlipidemia is not always higher in smokers versus non-smokers. For instance, the prevalence of hyperlipidemia among smokers was not significantly higher than that in non-smokers in an Asian population.[40] On the contrary, in a Romanian population, current smokers appear to have a worse lipid profile in both men and women.[41]

Contrary to cholesterol's and TG's prevalence, LDL-C was more prevalent in females than males. This may be due to the higher BMI in females [Table 1]. Indeed, we observed that high levels LDL-C are more prevalent in individuals with higher BMI (data not shown). This is consistent with the well-known notion that high BMI correlates with unfavorable lipid profile.[42,43]

Levels of LDL-C and TG increased with age, in both males and females. However, the sex difference in the values of LDL-C and TG becomes insignificant in the oldest age group (≥ 55 years). This could be explained by the aforementioned presumed effects of estrogen and/or testosterone, since both of these hormones dramatically decrease with age. Importantly and perhaps not unexpectedly, the vast majority (> 89%) of individuals within this age group were sedentary. We report herein that prevalence of high TG or LDL-C levels is lower in individuals with a physically active lifestyle. Taken together, these findings suggest that the sedentary lifestyle in this age group may account, at least in part, for the insignificant differences between both genders.

The levels of HDL-C remained relatively constant in all age groups, regardless of the gender (data not shown). This is consistent with what is previously reported in other population groups.[23,44]

Data on the impact of egg consumption on lipid profile is inconsistent. Some report a positive association between egg consumption and unfavorable lipid profile; while others suggest a negative or no association.[45-49] Because of this inconsistency, we investigated whether egg consumption affects the prevalence of different hyperlipidemias. The prevalence of hypercholesterolemia, hypertriglyceridemia and elevated LDL-C was higher in the group where egg consumption was ≥ 4 servings/week. Therefore, in the Lebanese population, consuming an average of one or more eggs per day is likely to negatively impact lipid profile. It is important to note that egg consumption itself is reported to increase intake of total fat or cholesterol.[50] Further studies are warranted to determine if this effect is modulated by other factors, like gender, age, or other dietary and lifestyle related factors.

Previous studies have suggested that consumption of skimmed milk does not modulate serum cholesterol level.[51] However, another study showed lower levels of serum cholesterol and LDL-C after isocaloric substitution of whole with skimmed milk.[52] In this report, we found that consumption of whole milk or skimmed milk did not affect the prevalence of hypercholesterolemia. Nonetheless, consumption of skimmed milk decreased the prevalence of high TG and high LDL-C but whole milk consumption increased this prevalence.

Consumption of a diet rich in fruits and vegetables is reported to favorably modulate the lipid profile in humans.[53] Indeed, high fruit intake is significantly associated with reduced odds of hypertriglyceridemia.[54] This is further supported by a recent study in the Korean population showing strong association between fruit intake and reduced prevalence of hypertriglyceridemia.[55] Interestingly, this study reported no association between the consumption of vegetables and blood lipid levels.[55] However, several other studies report little or no effects of fruits and vegetable consumption on lipid profile.[56-58] More recently, soya products were shown to favorably modulate lipid levels of TC, TG, and LDL-C and HDL-C.[59] These seemingly paradoxical results could be the result of different study designs as well as the data analysis.[60] It may also depend on whether the consumed vegetables are cooked/boiled or not, as boiling reduces some of the bioactive phytochemicals.[61] In this study, we found that the prevalence of hypertriglyceridemia and elevated LDL-C was lower in the group with the high consumption of fruits and vegetables.

It was strikingly surprising that most of the participants were unaware of their lipid profiles. This is perhaps owing to the absence of effective patient and public education. This is also in line with other reports indicating that some types of dyslipidemias are both underdiagnosed and under-treated.[62] It is hoped that this study will increase the awareness of the Lebanese community with respect to hyperlipidemia and its detrimental consequences. It is recommended that

patient education as well as national programs be put in place to further reduce the major risk factors of CVD.

DECLARATIONS

Authors' contributions

Contributed to the collection of data: A.A. Samaha, M. Gebbawi, M. Fawaz, R. Houjayri, R. Samaha, S. Baydoun
Analyzed the results: A.H. Eid, F. Zouein
Wrote the manuscript: A.H. Eid

Acknowledgments

The authors would like to thank all those who helped in dealing with the patients, specimen collection, *etc*. for their assistance.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Informed consent was obtained for all participants, either directly (for those 18 years or older) or through the parents/legal guardians (for subjects under 18 years of age).

Ethics approval

The protocols followed for data collection, handling, and analyzing were approved by the Research Ethics Committee at the Lebanese University and Makassed General Hospital.

REFERENCES

1. World Health Organization. Cardiovascular diseases (CVDs). In: Fact Sheets: World Health Organization; 2017.
2. Wilson P, D'Agostino R, Levy D, Belanger A, Silbershatz H, Kannel W. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998;97:1837-47.
3. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Borén J, Catapano AL, Descamps OS, Fisher E, Kovanen PT, Kuivenhoven JA, Lesnik P, Masana L, Nordestgaard BG, Ray KK, Reiner Z, Taskinen MR, Tokgözoglu L, Tybjærg-Hansen A, Watts GF; European Atherosclerosis Society Consensus Panel. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J* 2011;32:1345-61.
4. National Cholesterol Education Program (NCEP) Expert Panel on Detection E, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143-421.
5. Gordon D, Probstfield J, Garrison R, Neaton J, Castelli W, Knoke J, Jacobs DJ, Bangdiwala S, Tyroler H. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989;79:8-15.
6. Musunuru K, Kathiresan S. Surprises from genetic analyses of lipid risk factors for atherosclerosis. *Circ Res* 2016;118:579-85.
7. Daugherty A, Tabas I, Rader DJ. Accelerating the pace of atherosclerosis research. *Arterioscler Thromb Vasc Biol* 2015;35:11-2.
8. Melendez QM, Krishnaji ST, Wooten CJ, Lopez D. Hypercholesterolemia: the role of PCSK9. *Arch Biochem Biophys* 2017;625-626:39-53.
9. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Judd SE, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Mackey RH, Magid DJ, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Mussolino ME, Neumar RW, Nichol G, Pandey DK, Paynter NP, Reeves MJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation* 2014;129:e28-e292.
10. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. *Circ Res* 2016;118:535-46.
11. Saleh Al-Shehabi T, Iratni R, Eid AH. Anti-atherosclerotic plants which modulate the phenotype of vascular smooth muscle cells. *Phytomedicine* 2016;23:1068-81.
12. World Health Organization. World Health Report 2002. Available from: http://www.who.int/whr/2002/en/. [Last accessed on Jul 31, 2017]
13. Haskell WL, Alderman EL, Fair JM, Maron DJ, Mackey SF, Superko HR, Williams PT, Johnstone IM, Champagne MA, Krauss RM. Effects of intensive multiple risk factor reduction on coronary atherosclerosis and clinical cardiac events in men and women with coronary artery disease. The Stanford Coronary Risk Intervention Project (SCRIP). *Circulation* 1994;89:975-90.
14. Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ; Coordinating Committee of the National Cholesterol Education Program. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. *J Am Coll Cardiol* 2004;44:720-32.
15. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, Langendorfer A, Stein EA, Kruyer W, Gotto AM Jr. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA* 1998;279:1615-22.
16. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med* 1996;335:1001-9.
17. Pedersen TR, Kjekshus J, Berg K, Haghfelt T, Faergeman O, Faergeman G, Pyörälä K, Miettinen T, Wilhelmsen L, Olsson AG, Wedel H; Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). 1994. *Atheroscler Suppl* 2004;5:81-7.
18. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2000;20:1262-75.

19. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868-74.
20. Keys A, Aravanis C. Seven countries : a multivariate analysis of death and coronary heart disease. Cambridge: Harvard Univ. Press; 1980.
21. Råstam L, Hannan PJ, Luepker RV, Mittelmark MB, Murray DM, Slater JS. Seasonal variation in plasma cholesterol distributions: implications for screening and referral. *Am J Prev Med* 1992;8:360-6.
22. Gupta R, Rao RS, Misra A, Sharma SK. Recent trends in epidemiology of dyslipidemias in India. *Indian Heart J* 2017;69:382-92.
23. Erem C, Hacihasanoglu A, Deger O, Kocak M, Topbas M. Prevalence of dyslipidemia and associated risk factors among Turkish adults: Trabzon lipid study. *Endocrine* 2008;34:36-51.
24. al-Nuaim A, al-Rubeaan K, al-Mazrou Y, al-Attas O, al-Daghari N. Prevalence of hypercholesterolemia in Saudi Arabia, epidemiological study. *Int J Cardiol* 1996;54:41-9.
25. Singh R, Sharma J, Rastogi V, Raghuvanshi R, Moshiri M, Verma S, Janus E. Prevalence of coronary artery disease and coronary risk factors in rural and urban populations of north India. *Eur Heart J* 1997;18:1728-35.
26. Foucan L, Kangambega P, Koumavi Ekouévi D, Rozet J, Bangou-Brédent J. Lipid profile in an adult population in Guadeloupe. *Diabetes Metab* 2000;26:473-80.
27. Primatesta P, Poulter N. Lipid concentrations and the use of lipid lowering drugs: evidence from a national cross sectional survey. *BMJ* 2000;321:1322-5.
28. Vartiainen E, Jousilahti P, Alfthan G, Sundvall J, Pietinen P, Puska P. Cardiovascular risk factor changes in Finland, 1972-1997. *Int J Epidemiol* 2000;29:49-56.
29. Ford E, Li C, Pearson W, Zhao G, Mokdad A. Trends in hypercholesterolemia, treatment and control among United States adults. *Int J Cardiol* 2010;140:226-35.
30. Kavanagh K, Davis M, Zhang L, Wilson M, Register T, Adams M, Rudel L, Wagner J. Estrogen decreases atherosclerosis in part by reducing hepatic acyl-CoA:cholesterol acyltransferase 2 (ACAT2) in monkeys. *Arterioscler Thromb Vasc Biol* 2009;29:1471-7.
31. Godsland I. Effects of postmenopausal hormone replacement therapy on lipid, lipoprotein, and apolipoprotein (a) concentrations: analysis of studies published from 1974-2000. *Fertil Steril* 2001;75:898-915.
32. Li H, Mani S, Wu L, Fu M, Shuang T, Xu C, Wang R. The interaction of estrogen and CSE/H2S pathway in the development of atherosclerosis. *Am J Physiol Heart Circ Physiol* 2017;312:H406-14.
33. Oh Y, Jin Y, Park Y. Synergic hypocholesterolaemic effect of n-3 PUFA and oestrogen by modulation of hepatic cholesterol metabolism in female rats. *Br J Nutr* 2015;114:1766-73.
34. Lee J, Goldberg I. Hypertriglyceridemia-induced pancreatitis created by oral estrogen and in vitro fertilization ovulation induction. *J Clin Lipidol* 2008;2:63-6.
35. Phan BA, Toth PP. Dyslipidemia in women: etiology and management. *Int J Womens Health* 2014;6:185-94.
36. Moorthy K, Yadav UC, Mantha AK, Cowsik SM, Sharma D, Basir SF, Baquer NZ. Estradiol and progesterone treatments change the lipid profile in naturally menopausal rats from different age groups. *Biogerontology* 2004;5:411-9.
37. Nigro M, Santos AT, Barthem CS, Louzada RA, Fortunato RS, Ketzer LA, Carvalho DP, de Meis L. A change in liver metabolism but not in brown adipose tissue thermogenesis is an early event in ovariectomy-induced obesity in rats. *Endocrinology* 2014;155:2881-91.
38. Zhang N, Zhang H, Zhang X, Zhang B, Wang F, Wang C, Zhao M, Yu C, Gao L, Zhao J, Guan Q. The relationship between endogenous testosterone and lipid profile in middle-aged and elderly Chinese men. *Eur J Endocrinol* 2014;170:487-94.
39. Hatch NW, Srodulski SJ, Chan HW, Zhang X, Tannock LR, King VL. Endogenous androgen deficiency enhances diet-induced hypercholesterolemia and atherosclerosis in low-density lipoprotein receptor-deficient mice. *Gend Med* 2012;9:319-28.
40. Kim DE, Lee KB, Jang IM, Roh H, Ahn MY, Lee J. Associations of cigarette smoking with intracranial atherosclerosis in the patients with acute ischemic stroke. *Clin Neurol Neurosurg* 2012;114:1243-7.
41. Popa SG, Mota M, Mihaltan FD, Popa A, Munteanu I, Mota E, Serafinceanu C, Guja C, Hancu N, Catrinoiu D, Lichiardopol R, Bala C, Mihai B, Radulian G, Roman G, Timar R. Associations of smoking with cardiometabolic profile and renal function in a Romanian population-based sample from the PREDATORR cross-sectional study. *Eur J Gen Pract* 2017;23:164-70.
42. Donahue R, Orchard T, Kuller L, Drash A. Lipids and lipoproteins in a young adult population. The Beaver County Lipid Study. *Am J Epidemiol* 1985;122:458-67.
43. Yang Z, Ding X, Liu J, Duan P, Si L, Wan BH, Tu P. Associations between anthropometric parameters and lipid profiles in Chinese individuals with age ≥40 years and BMI <28kg/m2. *Plos One* 2017;12:e0178343.
44. Facchini FS, Hollenbeck CB, Jeppesen J, Chen YD, Reaven GM. Insulin resistance and cigarette smoking. *Lancet* 1992;339:1128-30.
45. Herron K, McGrane M, Waters D, Lofgren I, Clark R, Ordovas J, Fernandez M. The ABCG5 polymorphism contributes to individual responses to dietary cholesterol and carotenoids in eggs. *J Nutr* 2006;136:1161-5.
46. Chakrabarty G, Manjunatha S, Bijlani R, Ray R, Mahapatra S, Mehta N, Lakshmy R, Vashisht S, Manchanda S. The effect of ingestion of egg on the serum lipid profile of healthy young Indians. *Indian J Physiol Pharmacol* 2004;48:286-92.
47. Fernandez M. Dietary cholesterol provided by eggs and plasma lipoproteins in healthy populations. *Curr Opin Clin Nutr Metab Care* 2006;9:8-12.
48. Chakrabarty G, Bijlani R, Mahapatra S, Mehta N, Lakshmy R, Vashisht S, Manchanda S. The effect of ingestion of egg on serum lipid profile in healthy young free-living subjects. *Indian J Physiol Pharmacol* 2002;46:492-8.
49. Ballesteros M, Cabrera R, Saucedo MS, Fernandez M. Dietary cholesterol does not increase biomarkers for chronic disease in a pediatric population from northern Mexico. *Am J Clin Nutr* 2004;80:855-61.
50. Choi Y, Chang Y, Lee JE, Chun S, Cho J, Sung E, Suh BS, Rampal S, Zhao D, Zhang Y, Pastor-Barriuso R, Lima JA, Shin H, Ryu S, Guallar E. Egg consumption and coronary artery calcification in asymptomatic men and women. *Atherosclerosis* 2015;241:305-12.
51. Hussi E, Miettinen T, Ollus A, Kostiainen E, Ehnholm C, Haglund B, Huttunen J, Manninen V. Lack of serum cholesterol-lowering effect of skimmed milk and butter milk under controlled conditions. *Atherosclerosis* 1981;39:267-72.
52. Estévez-González M, Saavedra-Santana P, Betancor-León P. Reduction of serum cholesterol and low-density lipoprotein cholesterol levels in a juvenile population after isocaloric substitution of whole milk with a milk preparation (skimmed milk enriched with oleic acid). *J Pediatr* 1998;132:85-9.
53. Smith-Warner SA, Elmer PJ, Tharp TM, Fosdick L, Randall B, Gross M, Wood J, Potter JD. Increasing vegetable and fruit intake: randomized intervention and monitoring in an at-risk population. *Cancer Epidemiol Biomarkers Prev* 2000;9:307-17.
54. Hong S, Song Y, Lee KH, Lee HS, Lee M, Jee SH, Joung H. A fruit and dairy dietary pattern is associated with a reduced risk of metabolic syndrome. *Metabolism* 2012;61:883-90.
55. Yuan C, Lee HJ, Shin HJ, Stampfer MJ, Cho E. Fruit and vegetable consumption and hypertriglyceridemia: Korean National Health and Nutrition Examination Surveys (KNHANES) 2007-2009. *Eur J Clin Nutr* 2015;69:1193-9.
56. Broekmans W, Klöpping-Ketelaars W, Kluft C, van den Berg H, Kok

F, van Poppel G. Fruit and vegetables and cardiovascular risk profile: a diet controlled intervention study. *Eur J Clin Nutr* 2001;55:636-42.
57. Zino S, Skeaff M, Williams S, Mann J. Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. *BMJ* 1997;314:1787-91.
58. Djuric Z, Ren J, Mekhovich O, Venkatranamoorthy R, Heilbrun L. Effects of high fruit-vegetable and/or low-fat intervention on plasma micronutrient levels. *J Am Coll Nutr* 2006;25:178-87.
59. Tokede OA, Onabanjo TA, Yansane A, Gaziano JM, Djousse L. Soya products and serum lipids: a meta-analysis of randomised controlled trials. *Br J Nutr* 2015;114:831-43.
60. Dauchet L, Amouyel P, Dallongeville J, Medscape. Fruits, vegetables and coronary heart disease. *Nat Rev Cardiol* 2009;6:599-608.
61. Moreno DA, Lopez-Berenguer C, Garcia-Viguera C. Effects of stir-fry cooking with different edible oils on the phytochemical composition of broccoli. *J Food Sci* 2007;72:S064-8.
62. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, Wiklund O, Hegele RA, Raal FJ, Defesche JC, Wiegman A, Santos RD, Watts GF, Parhofer KG, Hovingh GK, Kovanen PT, Boileau C, Averna M, Borén J, Bruckert E, Catapano AL, Kuivenhoven JA, Pajukanta P, Ray K, Stalenhoef AF, Stroes E, Taskinen MR, Tybjærg-Hansen A; European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease. *Eur Heart J* 2013;34:3478-90a.

Alipov *et al. Vessel Plus* 2017;1:107-15
DOI: 10.20517/2574-1209.2017.20

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Topic: Atherosclerosis and Related Diseases **Open Access**

Chemical composition of circulating native and desialylated low density lipoprotein: what is the difference?

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How to cite this article: Alipov VI, Sukhorukov VN, Karagodin VP, Grechko AV, Orekhov AN. Chemical composition of circulating native and desialylated low density lipoprotein: what is the difference? *Vessel Plus* 2017;1:107-15.

Article history:
Received: 10 Jun 2017
Accepted: 25 Aug 2017
Published: 26 Sep 2017

Key words:
Desialylation,
desialylated low density lipoprotein,
modified low density lipoprotein,
sialic acid,
atherosclerosis

ABSTRACT

Atherosclerosis and related cardiovascular disorders remain the leading global cause of morbidity and mortality. Modified low density lipoprotein (LDL) is considered to play a crucial role in atherosclerosis development. During the past decades, several types of atherogenic LDL modification have been discovered. Desialylation was one of the atherogenic modifications observed in circulating atherogenic LDL *in vivo*. Sialic acid level negatively correlates with triglyceride and cholesterol contents. Desialylated LDL is small, dense and highly susceptible to oxidation, as reported for hyperlipidemic conditions. This atherogenic modification leads to increased cholesterol intake by macrophages and smooth-muscle cells, and is also associated with other pathologies, such as diabetes mellitus. Moreover, these conditions provoke damage and desialylated LDL particles may trigger autoimmune reactions in macrophages and B-cells.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. According to the American Heart Association, nearly 787,000 people in the US died from heart disease, stroke or other CVDs in 2011.[1] Atherosclerosis underlies most of the cardiovascular events in adults. Atherosclerotic plaque formation involves accumulation of cholesterol and its esters in the arterial intima, which results in migration and proliferation of various cell types (smooth muscle cells, macrophages, lymphocytes, neutrophils and dendritic cells) and inflammation, followed by necrosis and calcification.[2] Elevated blood pressure, diabetes mellitus, hyperlipidemia, family history and smoking are the major risk factors of atherosclerosis. These conditions provoke damage and lipid penetration into the arterial wall. According to current understanding, plasma low density lipoprotein (LDL) plays a crucial role in the pathogenesis of atherosclerosis because of its ability to deliver cholesterol from the liver to peripheral tissues, including the arterial wall. On the other hand, high density lipoprotein (HDL) negatively correlates with CVD and has protective effects.[3,4]

Currently, a high level of LDL cholesterol (LDL-C) is considered as a risk factor for CVD in clinical practice, and various treatment options (e.g. statins) are used to decrease it.[5,6] However, simple reduction of blood cholesterol level is not sufficient for effective atherosclerosis prevention. Moreover, this approach was demonstrated not to be efficient in several clinical studies.[7,8] The main drawback of statin therapy is the presence of several severe side-effects, such as statin-associated muscle symptoms, diabetes mellitus, and central nervous system disorders.[9] During the last decade, molecular mechanisms of atherosclerosis have become a subject of intensive research aimed at improving the clinical outcomes and developing novel therapies.

MODIFIED LDL AND ATHEROSCLEROSIS

Numerous studies have revealed that LDL subtypes form a heterogeneous group with different chemical and physical properties. Several types and subclasses of circulating LDL have different atherogenic effects. According to the widely accepted classification, the following LDL subtypes can be distinguished: small (dense), medium and large LDL. Dense LDL [with density (d) 1.044-1.060 g/mL] is considered to be the most atherogenic. Particles of this LDL subtype differ in size from 15 to 20 nm. For large LDL (d. 1.019-1.034 g/mL), mean particle size is 22 nm (up to 30 nm). Medium LDL (d. 1.034-1.044 g/mL) has a particle size in between small and large LDL.[10] An early study by Filipovic[11] and co-authors showed that LDL modification enhanced cholesterol intake by cultured cells. Subsequently, naturally modified LDL types were found in human blood.[11,12] During the past decades, numerous studies confirmed that LDL modifications, such as oxidation, desialylation and enzymatic processing, play a key role in increasing cholesterol intake, and the level of multiple modified LDL correlates with the risk of CVD.[13,14]

Other types of lipoproteins that are distinguished in some studies are electronegative LDL [LDL(-)] and lipoprotein (a) [Lp(a)]. The former subclass includes modified LDL with increased negative charge, which accounts for 3-5% of the total LDL in normolipidemic subjects. Several studies on LDL(-) showed that it represents a heterogeneous group of particles with various chemical modifications (oxidation, glycosylation, non-esterified fatty-acid enrichment, desialylation, and enzymatic modification) that share the common feature of increased electronegativity. Electronegative LDL is characterized by an enhanced ability to aggregate and is more susceptible to oxidation than native LDL (nLDL).[15,16] It was found that LDL(-) could accumulate in endothelial cells, monocytes, and lymphocytes through binding to scavenger receptors, such as platelet-activating factor receptor (PAF), lectin-like oxidized LDL receptors (LOX-1), and scavenger receptor A (SRA).[17,18] T-lymphocyte receptors (TCR) and CD14 are also involved in conveying LDL(-) biological effects.[19] It's worth mentioning that nLDL binding with oxidized forms of hemoglobin may cause changes in conformation and chemical composition of nLDL apolipoproteins.[20] High intracellular lipid level and activation of receptor pathways may result in cytotoxicity and the release of inflammatory cytokines.[21-24] On the other hand, recent studies showed that LDL(-) had an ability to induce anti-inflammatory cytokines [e.g. interleukin-10 (IL-10)] and counteract inflammatory effects promoted by lipopolysaccharides.[19,25] In that regard, the atherogenic role of LDL(-) needs further investigation. However, multiple studies confirmed that high LDL(-) level was a risk factor for CVD, which might be connected with other LDL(-) modifications, such as desialylation and oxidation.[16,26-28]

Lipoprotein (a) [Lp(a)] differs from LDL only by the presence of apoplipoprotein (a) bound to apolipoprotein B-100 (apoB-100) via a disulfide bridge. Lp(a) is normally present in the blood, and its plasma concentrations range from 1 to 1,000 mg/dL. High levels of Lp(a) are associated with some pathologies. For instance, Lp(a) level increased within 24 h after acute myocardial infarction, and its transient increase accompanied acute and chronic inflammatory processes.[29,30] Lp(a) gene polymorphism was associated with the incidence of cerebral vascular accident of large vessels, peripheral arterial disease, and abdominal aorta aneurysm.[31] Lp(a) level also correlated with IL-6, tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), and monocyte chemoattractant protein (MCP-1) levels.[32] In Korean population, patients with high Lp(a) level had higher CVD risk and worse disease course.[33] A Danish prospective study of 9,000 subjects revealed that extremely high plasma Lp(a) level (over 120 mg/dL) increased CVD risk 4-fold.[34] On the other hand, multiple prospective studies showed that a high Lp(a) level was not an independent risk factor for cardiovascular or cerebrovascular diseases.[29,35]

CHEMICAL COMPOSITION OF LDL

Non-modified, or nLDL, particle contains apolipoprotein B-100 (apoB-100) molecule, about 90 molecules of other regulatory proteins, a phospholipid monolayer, and a hydrophobic core, which accounts for 75% of LDL particle weight.[36] LDL contains proteins regulating apoB-100 metabolism and lipid transport [apolipoprotein C-II (apoC-II), apoC-III, apoE, apoA-I,

apoA-IV, and apoF], associated with inflammation (apoD, apoJ, apoM, serum amyloid A4, paraoxonase 1, prenylcysteine oxidase 1, migration inhibitory factor-related protein 8, and retinol binding protein), related with thrombosis (fibrinogen alpha chain) and components of the innate immunity system (lysozyme C, alpha-1 antitripsin, apoL-1, and transthyretin).[10] ApoB-100 is a large glycoprotein, which stabilizes and maintains LDL structure and composition. ApoB-100 has 24 potential N-glycosylation sites, with up to 16 asparagine residues actually glycosylated. Carbohydrates, including neutral and acidic carbohydrate chains, account for 5-9% of apoB-100 molecular weight. All chains contain N-acetylglucosamine and mannose residues. Acidic chains contain terminal sialic acid residues followed by galactose [Figure 1].[36-38] Loss of the terminal sialic acid residue results in exposure of galactose residues. It was suggested that almost all nLDL particles are partially monodesialylated because they have galactose ending chains.[39]

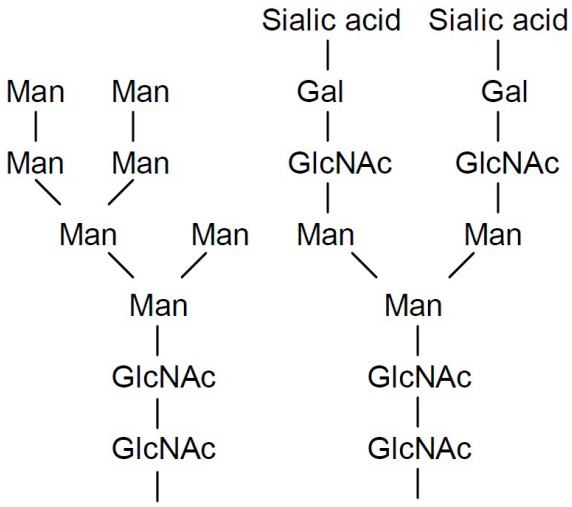

Figure 1: Carbohydrate chains in apoB-100. Both chains have mannose base (Man) connected to polypeptide chain by N-acetylglucosamine (GlcNAc). Acidic one has terminal sialic acid residues connected to galactose (Gal) molecules.[36-38]

The phospholipid monolayer contains phosphatidylcholine, sphingomyelin, lysophosphatidylcholine, phosphatidyl-ethanolamine, ceramide, and diacylglycerol. The hydrophobic core contains various lipid classes: non-esterified cholesterol, cholesterol esters, and triglycerides. Non-esterified cholesterol is also located on the surface of the LDL particle. nLDL transports 66% of serum gangliosides. Gangliosides are sialic-acid-rich glycosphingolipids and are thought to contain all the sialic acid residues associated with the LDL lipids.[36] The lipid part of nLDL also contains other monosaccharides: galactosamine and glucose.[40]

First studies of LDL carbohydrate composition revealed little or no variation in glucosamine, galactose and mannose values, but a marked variation in sialic acid levels [Table 1].[41] Further studies showed that in patients with coronary artery disease (CAD), LDL had a decreased sialic acid content. Isolated LDL from these patients, as well as *in vitro* desialylated LDL, caused atherogenic changes in cultured cells.[13,42,43] The most comprehensive study on chemical composition of LDL in patients with and without atherosclerosis was performed in 1993.[40] Carbohydrate content of LDL from patients with atherosclerosis was almost the same, except for sialic acid level, which was significantly (1.6 times) lower in patient LDL ($P < 0.05$) [Figure 2].[40] There was no significant difference in the levels of galactose, N-acetyl glucosamine and mannose between nLDL and modified LDL from healthy donors, as well as from patients with atherosclerosis. Sialic acid content in modified LDL was 30% lower than in nLDL from healthy subjects. Sialic acid level in modified LDL from patients was 2 to 3 fold lower than in nLDL. Comparison of nLDL obtained from healthy subjects and patients with atherosclerosis revealed no significant differences in carbohydrate contents. Modified LDL had a significantly lower level of sialic acid ($P < 0.05$). There was also a significant difference in the sialic acid content between modified LDL from healthy donors and from patients with atherosclerosis, $P < 0.05$.

Levels of all lipid-associated carbohydrates were 1.5 to 2 fold lower in LDL samples obtained from atherosclerosis patients in comparison to those from healthy subjects. Native and modified LDL obtained from patients and healthy subjects also differed by lipid composition. Modified LDL had decreased levels of cholesterol, cholesterol esters, triglycerides, phosphatidylcholine,

Table 1: Carbohydrate content of LDL according to early studies (percent dry weight)

	Sialic acid	Glucosamine	Galactose	Mannose
Schultze and Heide[87] (1960)	1.5	2.0	2.7	2.7
Ayrault-Jarrier[88] (1961)	1.3	1.2	-	-
Marshall and Kummerow[89] (1962)	0.35	1.2	3.23 (together)	
Kwiterovich *et al.*[90] (1974)	0.6	0.9	1.8	3.7
Swaminathan and Aladjem[41] (1976)	1.73	0.94	2.13	4.88

LDL: low density lipoprotein; "-": not measured

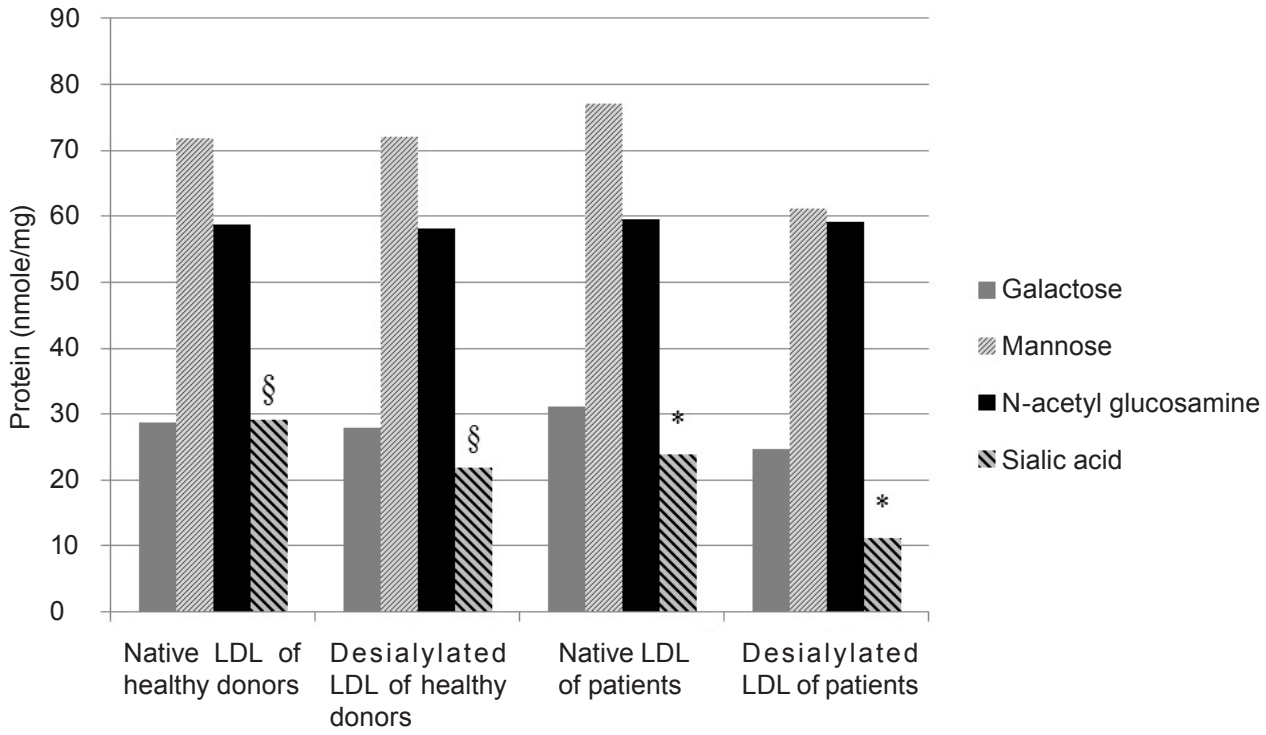

Figure 2: Mean content of carbohydrates in native and desialylated LDL (dLDL) of healthy donors and patients with atherosclerosis; §: significant difference in sialic acid content between native and desialylated LDL of healthy donors, $P<0.05$; *: significant difference in sialic acid content between native and dLDL in patients with atherosclerosis, $P<0.05$

and phosphatidylethanolamine, with higher levels of free fatty acids, mono- and diglycerides. At the same time, sphingomyelin content of modified LDL obtained from atherosclerosis patients was significantly decreased.[40] Further studies reported similar results.[42-44] Noteworthy, LDL desialylation positively correlated with particle density and negatively with particle size.[14,40,43-46] At the same time, several studies found no difference in LDL sialic acid content between subjects with and without atherosclerosis.[47-49]

To solve this dilemma, Lindbohn with co-authors suggested that the controversial results could be explained for a large part by the choice of study population, which was almost exclusively male. Another study was conducted on 22 middle-aged women with CAD and 11 control subjects. Patients' LDL had significantly lower sialic acid-to-apoB-100 ratio compared with the control group. A negative correlation was observed between sialic acid ratio and cholesterol, phospholipid and triglyceride concentration.[50] Recent studies confirmed previous results in CAD patients and revealed the crucial role of LDL desialylation in various other pathologies, such as aortic valve sclerosis, different types of hereditary hyperlipidemia and diabetes mellitus.[51-55]

OXIDIZED LDL

Studies conducted on cellular models demonstrated that *in vitro* oxidation caused increased uptake of LDL-C by cultured cells. Macrophages could not consume non-oxidized LDL because of receptor-dependent limitations. Oxidized LDL (oxLDL) can bind to various receptors for modified LDL [for example lectin-like oxidized LDL receptor (LOX-1)], which leads to increased cholesterol uptake and foam cell formation.[56] High levels of oxLDL were found in patients with atherosclerosis and, together with NO levels, were used as biomarkers of endothelial dysfunction.[56,57] To date, the precise LDL oxidation mechanism is not fully understood. Activated monocytes, macrophages and endothelial cells generate reactive oxygen species (ROS) and produce lipoxygenase, hypochlorous acid (HOCl) and myeloperoxidase. These substances, along with metal ions (Fe^{3+}, Cu^{2+}) are involved in LDL oxidation. It was shown that HOCl and hypothiocyanous acids can cause oxidation of the apoB-100 molecule.[58] Macrophages can recognize oxLDL with various receptors, including CD36, toll-like receptor 4 (TLR4), LOX-1, and receptor for advanced glycation end-products (RAGE).[59-61] Cholesterol and lipid accumulation in macrophages leads to the release of pro-inflammatory cytokines (e.g. TNF-α), which results in inflammation and recruitment of immune cells. OxLDL enter the endothelial cells through binding to LOX-1 receptors.[61] High lipid concentration results in IL-8 secretion, which stimulates inflammation and migration of smooth-muscle cells from the tunica media to the intima.

Chemical composition of oxLDL is characterized by 1.5 to 2 fold decreased levels of antioxidants, such as coenzyme Q10, tocopherols, β-carotene, and lycopene, and increased content of oxidation products. Intense oxidation of fatty acids, cholesterol and other lipids leads to accumulation of 13-hydroperoxylinoleic acid and other peroxides, hydroxides (e.g. 13-hydroxylinoleic acid), prostaglandin derivatives (isoprostanes), various aldehydes (malondialdehyde, oxovaleryl phosphatidylcholine, hexanal, *etc*.), lysophosphatidylcholine, 7-keto-cholesterol, various hydrocarbons, including pentane, and modified phosphatidyl ethanolamine/serine products. Products of protein oxidation include: protein carbonyls, non-enzymatic proteolyzed fragments, arginine, cysteine, modified cysteine, lysine, histidine, methionine, tyrosine, and tryptophan, protein cross-linking products due to tyrosine cross-links and bifunctional aldehydes, lipid-protein adducts which can be classified as ceroids (lipofuscins). Many of the above mentioned modifications, as well as conformational changes, might lead to increased antigenicity.[62] Lack of antioxidants makes oxLDL susceptible to further oxidation and apolipoprotein degradation. In the bloodstream, oxLDL is characterized by high density and increased negative charge. A controlled study of LDL structural changes due to *in vitro* oxidation with copper ions showed similar results. Small-angle X-ray scattering and dynamic light scattering techniques revealed high density, electrical charge, and increased degree of flexibility of the apoB-100.[63] However, oxidation should not be considered as the key modification leading to LDL electronegativity because the concentration of oxLDL in normolipidemic plasma is orders of magnitude lower than LDL(-) concentration.[17]

ELECTRONEGATIVE LDL

LDL(-) chemical composition is characterized by decreased sialic acid and antioxidant content, increased triglycerides, nonesterified fatty acids (NEFA), lysophosphatidylcholine, and ceramide levels compared to nLDL.[63,64] LDL(-) is also distinguished by phospholipolytic activities and abnormal apoB-100 conformation.[65] In nLDL, apoB-100 has a pentameric structure with alternating alpha helixes and beta pleated sheets. In LDL(-), apoB-100 has less alpha helixes and more beta sheets, as well as an altered pattern of exposed lysine residues that are involved in lipoprotein receptor binding interactions. Changes in apoB-100 structure may be caused by oxidation and nitration.[65] These chemical changes and presence of electronegative charge in desialylated LDL makes it possible to suggest that these two fractions are identical.[16]

GLYCATED LDL

Glycation of LDL occurs due to non-enzymatic reaction of glucose and its metabolites with free amino groups of apoB-100 lysine. This process is highly intensive in patients with diabetes mellitus and metabolic syndrome because of the high glucose blood level.[66] In non-diabetic patients, 4.8% of apoB-100 is glycated compared to 14.8% of total apoB glycated in patients with type II diabetes. It was demonstrated that small-dense LDL is more susceptible to glycation in patients with metabolic syndrome and type II diabetes than nLDL.[67] Glycation makes LDL more sensitive to oxidation. Formation of glycated LDL and other advanced glycation end products (AGEs) increases atherogenic properties of LDL and enhances lipid uptake by cultured aortic smooth-muscle cells. High concentration of AGEs leads to activation of the RAGE receptor pathway, which results in enhanced expression and NF-κB-dependent release of pro-inflammatory molecules. That, in turn, promotes vessel wall damage, endothelial dysfunction, monocyte and macrophage migration and recruitment to the vascular intima followed by oxidative stress, vascular wall remodeling and atherosclerotic lesion progression.[68] However, recent studies on diabetic patients showed that glycated LDL level was not an independent risk factor for CVD. At the same time, patients with type I and II diabetes had a high level of small dense desialylated LDL particles with oxidative modifications.[54] Therefore, glycation makes nLDL more susceptible to oxidation and enzymatic changes and may be the first step atherogenic modification of LDL in diabetic patients.

DESIALYLATION IMPACT ON ATHEROSCLEROSIS DEVELOPMENT

Under normal conditions, LDL lipid intake is controlled by lipoprotein receptors. Modification of LDL, such as oxidation and desialylation, allows LDL particles to escape this limitation and enter arterial cells via different pathways. Sialic acid provides LDL with negative charge, which protects the particle from binding to arterial proteoglycans. The increased ability of enzymatically desialylated LDL to interact with proteoglycans was confirmed by Millar *et al*.[45] However, small dense desialylated LDL are electronegative and can interact with macrophage lectin receptors, therefore mediating the lipid uptake.[68] Increased cholesterol accumulation may also result from macrophage scavenger receptor-mediated uptake followed by foam cell formation and macrophage cytokine release, which causes inflammation and monocyte migration in the intima.[70-72] Inhibition of Acyl-coenzyme A: cholesterol acyltransferase activity by desialylated LDL is also

considered as a possible mechanism of macrophage down-regulation.[14]

While some studies found no differences in lipid peroxide content between native and desialylated LDL,[73] Others reported that desialylation may cause both an increase and decrease in susceptibility to oxidation depending on LDL density and hyperlipidemia type.[74,75] Small dense LDL in type IIa hyperlipidemia was the most susceptible to oxidation.[75] Increased lipid peroxidation was found in desialylated LDL.[76] Another study showed that LDL sialic acid levels negatively correlates with thiobarbituric acid reactive substances and suggested that reactive oxygen substances may affect enzymatic desialylation *in vivo*.[77] It was suggested that a plasma enzyme called trans-sialidase is the possible cause of LDL desialylation in blood plasma.[78]

DESIALYLATION AND IMMUNE RESPONSE

Sialic acids belong to a group of *N*- or *O*- derivatives of neuraminic acid. N-acetylneuraminic acid (Neu5AC) is the most common type found in humans. Neu5AC is typically found at the terminal position of ganglioside glycan chains in the cellular glycocalyx. Sialic acids are involved in cell-cell interactions, including those between immune cells. Neu5AC refers to a self-associated molecular patterns (SAMPs) group because of their ability to suppress innate and adaptive autoimmune response.[79] Sialic-acid-binding immunoglobulin-like lectins (Siglecs) form a group of immune cell receptors that participate in the discrimination of “self” and “non-self” through recognition of cell glycan ligands. Macrophages have sialoadhesin (CD169), so-called Siglec-1, and B-cells have CD22, so-called Siglec-2. Studies on human immune cells discovered 14 members of the Siglec family. Siglec receptor binding with host-specific sialic acid provides negative regulation or even apoptosis in immune cells. For example, in B-cells activation of CD22 pathway leads to activation of Src homology region 2 domain-containing phosphatase-1 (SHP-1), which suppresses the activation of B-cell receptor (BCR).[80] Recent study showed that sialic acid binding domain mutations of Siglec-G resulted in decreased B-cell activation threshold.[81] Dysfunction of Siglec receptor interactions with sialic acid is associated with various autoimmune diseases.[79,80] Lack of sialoadhesin in macrophages causes activation of scavenger receptors and phagocytosis.[79-82] In atherosclerosis, decreased sialic acid content in desialylated LDL might result in increased cholesterol intake and inflammation through macrophage and B-cell activation.

Patients with various CVD have antibodies against modified LDL and lipoprotein-containing immune complexes (LDL-CIC) in the plasma. Immunoglobulin G (IgG) antibodies with high affinity for *in vitro* desialylated and malondialdehyde-modified LDL were detected in patients with angiographically assessed coronary atherosclerosis. On the other hand these antibodies have low affinity for native, glycated, acetylated, and LDL with other chemical modifications.[83] It was shown that IgG (subclasses G1, G3) against modified LDL have pro-atherogenic properties, while IgM antibodies are atheroprotective.[14,84] In 2013 Montano[85] and colleagues showed that monoclonal anti-oxLDL IgM (E06) inhibited oxLDL binding to macrophages in a dose dependent manner. Studies on LDL-CIC discovered that LDL in these complexes had atherogenic modifications, particularly LDL were small dense and had decreased sialic acid content. LDL-CIC stimulated lipid accumulation in cultured cells unlike nLDL.[86] Recent studies showed that IgG and LDL-CIC removal from patient sera reduced its atherogenic activity.[83] Level of LDL-CIC is used in diagnosis, prognosis and in several therapeutic approaches in CVD patients.[14,83,84]

CONCLUSION

Sialic acid level is decreased in atherogenic LDL and negatively correlates with triglyceride and cholesterol level in LDL. Desialylated LDL are small, dense and highly susceptible to peroxidation in several hyperlipidemia types. Desialylation results in atherogenic changes because of increased cholesterol intake in macrophages and smooth-muscle cells and is also associated with other pathologies, such as diabetes mellitus.

DECLARATIONS

Authors’ contributions

Analysis of literature, writing a draft: V.I. Alipov
Editing, writing a draft: V.N. Sukhorukov
Table and figures: V.P. Karagodin
Consultation: A.V. Grechko
English improvement: A.N. Orekhov

Financial support and sponsorship

This work was supported by Russian Science Foundation (Grant # 14-15-00112).

Conflicts of interest

The authors declare that they have no competing interests.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2015 update: a report from the american heart association. *Circulation* 2015;131:e29-322.
2. Aluganti Narasimhulu C, Fernandez-Ruiz I, Selvarajan K, Jiang X, Sengupta B, Riad A, Parthasarathy S. Atherosclerosis--do we know enough already to prevent it? *Curr Opin Pharmacol* 2016;27:92-102.
3. Parhofer KG. Increasing HDL-cholesterol and prevention of atherosclerosis: a critical perspective. *Atheroscler Suppl* 2015;18:109-11.
4. Ridker PM. LDL cholesterol: controversies and future therapeutic directions. *Lancet* 2014;384:607-17.
5. Ference BA, Yoo W, Alesh I, Mahajan N, Mirowska KK, Mewada A, Kahn J, Afonso L, Williams KA Sr, Flack JM. Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. *J Am Coll Cardiol* 2012;60:2631-9.
6. Soran H, Dent R, Durrington P. Evidence-based goals in LDL-C reduction. *Clin Res Cardiol* 2017;106:237-48.
7. Rahman A, Ahmed MU, Islam AK, Karim A, Sarker SA. A young male with familial hypercholesterolemia. *J Saudi Heart Assoc* 2012;24:261-4.
8. Norata GD, Ballantyne CM, Catapano AL. New therapeutic principles in dyslipidaemia: focus on LDL and Lp(a) lowering drugs. *Eur Heart J* 2013;34:1783-9.
9. Thompson PD, Panza G, Zaleski A, Taylor B. Statin-associated side effects. *J Am Coll Cardiol* 2016;67:2395-410.
10. Diffenderfer MR, Schaefer EJ. The composition and metabolism of large and small LDL. *Curr Opin Lipidol* 2014;25:221-6.
11. Filipovic I, Schwarzmann G, Mraz W, Wiegandt H, Buddecke E. Sialic-acid content of low-density lipoproteins controls their binding and uptake by cultured cells. *Eur J Biochem* 1979;93:51-5.
12. Tertov VV, Bittolo-Bon G, Sobenin IA, Cazzolato G, Orekhov AN, Avogaro P. Naturally occurring modified low density lipoproteins are similar if not identical: more electronegative and desialylated lipoprotein subfractions. *Exp Mol Pathol* 1995;62:166-72.
13. Harada LM, Carvalho MD, Passarelli M, Quintão EC. Lipoprotein desialylation simultaneously enhances the cell cholesterol uptake and impairs the reverse cholesterol transport system: in vitro evidences utilizing neuraminidase-treated lipoproteins and mouse peritoneal macrophages. *Atherosclerosis* 1998;139:65-75.
14. Orekhov AN, Bobryshev YV, Sobenin IA, Melnichenko AA, Chistiakov DA. Modified low density lipoprotein and lipoprotein-containing circulating immune complexes as diagnostic and prognostic biomarkers of atherosclerosis and type 1 diabetes macrovascular disease. *Int J Mol Sci* 2014;15:12807-41.
15. Sánchez-Quesada JL, Villegas S, Ordóñez-Llanos J. Electronegative low-density lipoprotein. A link between apolipoprotein B misfolding, lipoprotein aggregation and proteoglycan binding. *Curr Opin Lipidol* 2012;23:479-86.
16. Ivanova EA, Bobryshev YV, Orekhov AN. LDL electronegativity index: a potential novel index for predicting cardiovascular disease. *Vasc Health Risk Manag* 2015;11:525-32.
17. Estruch M, Sánchez-Quesada JL, Ordóñez Llanos J, Benítez S. Electronegative LDL: a circulating modified LDL with a role in inflammation. *Mediators Inflamm* 2013;2013:181324.
18. Chu CS, Wang YC, Lu LS, Walton B, Yilmaz HR, Huang RY, Sawamura T, Dixon RA, Lai WT, Chen CH, Lu J. Electronegative low-density lipoprotein increases C-reactive protein expression in vascular endothelial cells through the LOX-1 receptor. *PLoS One* 2013;8:e70533.
19. Estruch M, Bancells C, Beloki L, Sanchez-Quesada JL, Ordóñez-Llanos J, Benitez S. CD14 and TLR4 mediate cytokine release promoted by electronegative LDL in monocytes. *Atherosclerosis* 2013;229:356-62.
20. Sevanian A, Asatryan L, Ziouzenkova O. Low density lipoprotein (LDL) modification: basic concepts and relationship to atherosclerosis. *Blood Purif* 1999;17:66-78.
21. Lee AS, Wang GJ, Chan HC, Chen FY, Chang CM, Yang CY, Lee YT, Chang KC, Chen CH. Electronegative low-density lipoprotein induces cardiomyocyte apoptosis indirectly through endothelial cell-released chemokines. *Apoptosis* 2012;17:1009-18.
22. Chen CY, Hsu HC, Lee AS, Tang D, Chow LP, Yang CY, Chen H, Lee YT, Chen CH. The most negatively charged low-density lipoprotein L5 induces stress pathways in vascular endothelial cells. *J Vasc Res* 2012;49:329-41.
23. Mogilenko DA, Kudriavtsev IV, Trulioff AS, Shavva VS, Dizhe EB, Missyul BV, Zhakhov AV, Ischenko AM, Perevozchikov AP, Orlov SV. Modified low density lipoprotein stimulates complement C3 expression and secretion via liver X receptor and Toll-like receptor 4 activation in human macrophages. *J Biol Chem* 2012;287:5954-68.
24. Chan HC, Ke LY, Chu CS, Lee AS, Shen MY, Cruz MA, Hsu JF, Cheng KH, Chan HC, Lu J, Lai WT, Sawamura T, Sheu SH, Yen JH, Chen CH. Highly electronegative LDL from patients with ST-elevation myocardial infarction triggers platelet activation and aggregation. *Blood* 2013;122:3632-41.
25. Kannan Y, Sundaram K, Aluganti Narasimhulu C, Parthasarathy S, Wewers MD. Oxidatively modified low density lipoprotein (LDL) inhibits TLR2 and TLR4 cytokine responses in human monocytes but not in macrophages. *J Biol Chem* 2012;287:23479-88.
26. Niccoli G, Bacà M, De Spirito M, Parasassi T, Cosentino N, Greco G, Conte M, Montone RA, Arcovito G, Crea F. Impact of electronegative low-density lipoprotein on angiographic coronary atherosclerotic burden. *Atherosclerosis* 2012;223:166-70.
27. Hirowatari Y, Homma Y, Yoshizawa J, Homma K. Increase of electronegative-LDL-fraction ratio and IDL-cholesterol in chronic kidney disease patients with hemodialysis treatment. *Lipids Health Dis* 2012;11:111.
28. Hsu JF, Chou TC, Lu J, Chen SH, Chen FY, Chen CC, Chen JL, Elayda M, Ballantyne CM, Shayani S, Chen CH. Low-density lipoprotein electronegativity is a novel cardiometabolic risk factor. *PLoS One* 2014;9:e107340.
29. Maranhão RC, Carvalho PO, Strunz CC, Pileggi F. Lipoprotein (a): structure, pathophysiology and clinical implications. *Arq Bras Cardiol* 2014;103:76-84.
30. Enkhmaa B, Anuurad E, Zhang W, Abbuthalha A, Li XD, Dotterweich W, Pollard RB, Asmuth DM, Berglund L. HIV disease activity as a modulator of lipoprotein(a) and allele-specific apolipoprotein(a) levels. *Arterioscler Thromb Vasc Biol* 2013;33:387-92.
31. Helgadottir A, Gretarsdottir S, Thorleifsson G, Holm H, Patel RS, Gudnason T, Jones GT, van Rij AM, Eapen DJ, Baas AF, Tregouet DA, Morange PE, Emmerich J, Lindblad B, Gottsäter A, Kiemeny LA, Lindholt JS, Sakalihasan N, Ferrell RE, Carey DJ, Elmore JR, Tsao PS, Grarup N, Jørgensen T, Witte DR, Hansen T, Pedersen

O, Pola R, Gaetani E, Magnadottir HB, Wijmenga C, Tromp G, Ronkainen A, Ruigrok YM, Blankensteijn JD, Mueller T, Wells PS, Corral J, Soria JM, Souto JC, Peden JF, Jalilzadeh S, Mayosi BM, Keavney B, Strawbridge RJ, Sabater-Lleal M, Gertow K, Baldassarre D, Nyyssönen K, Rauramaa R, Smit AJ, Mannarino E, Giral P, Tremoli E, de Faire U, Humphries SE, Hamsten A, Haraldsdottir V, Olafsson I, Magnusson MK, Samani NJ, Levey AI, Markus HS, Kostulas K, Dichgans M, Berger K, Kuhlenbäumer G, Ringelstein EB, Stoll M, Seedorf U, Rothwell PM, Powell JT, Kuivaniemi H, Onundarson PT, Valdimarsson E, Matthiasson SE, Gudbjartsson DF, Thorgeirsson G, Quyyumi AA, Watkins H, Farrall M, Thorsteinsdottir U, Stefansson K. Apolipoprotein(a) genetic sequence variants associated with systemic atherosclerosis and coronary atherosclerotic burden but not with venous thromboembolism. *J Am Coll Cardiol* 2012;60:722-9.

32. Riches K, Porter KE. Lipoprotein(a): cellular effects and molecular mechanisms. *Cholesterol* 2012;2012:923289.
33. Kwon SW, Lee BK, Hong BK, Kim JY, Choi EY, Sung JM, Rhee JH, Park YM, Ma DW, Chung H, Mun HS, Lee SJ, Park JK, Min PK, Yoon YW, Rim SJ, Kwon HM. Prognostic significance of elevated lipoprotein(a) in coronary artery revascularization patients. *Int J Cardiol* 2013;167:1990-4.
34. Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG. Extreme lipoprotein(a) levels and improved cardiovascular risk prediction. *J Am Coll Cardiol* 2013;61:1146-56.
35. Gurdasani D, Sjouke B, Tsimikas S, Hovingh GK, Luben RN, Wainwright NW, Pomilla C, Wareham NJ, Khaw KT, Boekholdt SM, Sandhu MS. Lipoprotein(a) and risk of coronary, cerebrovascular, and peripheral artery disease: the EPIC-Norfolk prospective population study. *Arterioscler Thromb Vasc Biol* 2012;32:3058-65.
36. Fernández-Higuero JA, Salvador AM, Martín C, Milicua JC, Arrondo JL. Human LDL structural diversity studied by IR spectroscopy. *PLoS One* 2014;9:e92426.
37. Segrest JP, Jones MK, De Loof H, Dashti N. Structure of apolipoprotein B-100 in low density lipoproteins. *J Lipid Res* 2001;42:1346-67.
38. Bartlett AL, Stanley KK. All low density lipoprotein particles are partially desialylated in plasma. *Atherosclerosis* 1998;138:237-45.
39. Tertov VV, Orekhov AN, Sobenin IA, Morrisett JD, Gotto AM Jr, Guevara JG Jr. Carbohydrate composition of protein and lipid components in sialic acid-rich and -poor low density lipoproteins from subjects with and without coronary artery disease. *J Lipid Res* 1993;34:365-75.
40. Swaminathan N, Aladjem F. The monosaccharide composition and sequence of the carbohydrate moiety of human serum low density lipoproteins. *Biochemistry* 1976;15:1516-22.
41. Orekhov AN, Tertov VV, Mukhin DN, Mikhailenko IA. Modification of low density lipoprotein by desialylation causes lipid accumulation in cultured cells: discovery of desialylated lipoprotein with altered cellular metabolism in the blood of atherosclerotic patients. *Biochem Biophys Res Commun* 1989;162:206-11.
42. Ruelland A, Gallou G, Legras B, Paillard F, Cloarec L. LDL sialic acid content in patients with coronary artery disease. *Clin Chim Acta* 1993;221:127-33.
43. Lindbohm N. Sialic acid in lipoproteins: with a special reference to low density lipoproteins. Available from: https://helda.helsinki.fi/handle/10138/22467. [Last accessed on Sep 6, 2017]
44. Barbosa DS, Maranhão RC, Araújo FB, Chang YH, Hirata MH, Abdalla DS. Sialic acid and oxidizability of low density lipoprotein subfractions of hyperlipidemic patients. *Clin Biochem* 1995;28:435-41.
45. Millar JS, Anber V, Shepherd J, Packard CJ. Sialic acid-containing components of lipoproteins influence lipoprotein-proteoglycan interactions. *Atherosclerosis* 1999;145:253-60.
46. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko AV, Orekhov AN. Small dense low-density lipoprotein as biomarker for atherosclerotic diseases. *Oxid Med Cell Longev* 2017;2017:1273042.
47. Melajärvi N, Gylling H, Miettinen TA. Sialic acids and the metabolism of low density lipoprotein. *J Lipid Res* 1996;37:1625-31.
48. Lindbohm N, Gylling H, Miettinen TE, Miettinen TA. Sialic acid content of LDL and lipoprotein metabolism in combined hyperlipidemia and primary moderate hypercholesterolemia. *Clin Chim Acta* 1999;285:69-84.
49. Chappey B, Beyssen B, Foos E, Ledru F, Guermonprez JL, Gaux JC, Myara I. Sialic acid content of LDL in coronary artery disease: no evidence of desialylation in subjects with coronary stenosis and increased levels in subjects with extensive atherosclerosis and acute myocardial infarction: relation between desialylation and in vitro peroxidation. *Arterioscler Thromb Vasc Biol* 1998;18:876-83.
50. Lindbohm N, Gylling H, Rajaratnam RA, Miettinen TA. Sialic acid content of low-density lipoprotein in women with coronary artery disease. *J Lab Clin Med* 2000;136:110-5.
51. Orekhov AN, Ivanova EA, Bobryshev YV. Chapter 2: Naturally occurring multiple-modified low density lipoprotein. In: Ruiz M, editor. Blood Lipids and Lipoproteins. New York: Nova Science Publishers Inc; 2015. p. 13-54.
52. Twardowski L, Cheng F, Michaelsen J, Winter S, Hofmann U, Schaeffeler E, Müller S, Sonnenberg M, Steuer K, Ott G, Schwab M, Franke UF, Torzewski M. Enzymatically modified low-density lipoprotein is present in all stages of aortic valve sclerosis: implications for pathogenesis of the disease. *J Am Heart Assoc* 2015;4:e002156.
53. Guo S, Sang H, Yang N, Kan Y, Li F, Li Y, Li F, Qin S. Measurement of sialic acid from lipoproteins and human plasma by liquid chromatography-tandem mass spectrometry. *Se Pu* 2014;32:1197-200.
54. Sobenin IA, Galitsyna EV, Grechko AV, Orekhov AN. Small dense and desialylated low density lipoprotein in diabetic patients. *Vessel Plus* 2017;1:29-37.
55. Zakiev ER, Sukhorukov VN, Melnichenko AA, Sobenin IA, Ivanova EA, Orekhov AN. Lipid composition of circulating multiple-modified low density lipoprotein. *Lipids Health Dis* 2016;15:134.
56. Trpkovic A, Resanovic I, Stanimirovic J, Radak D, Mousa SA, Cenic-Milosevic D, Jevremovic D, Isenovic ER. Oxidized low-density lipoprotein as a biomarker of cardiovascular diseases. *Crit Rev Clin Lab Sci* 2015;52:70-85.
57. Gradinaru D, Borsa C, Ionescu C, Prada GI. Oxidized LDL and NO synthesis--biomarkers of endothelial dysfunction and ageing. *Mech Ageing Dev* 2015;151:101-13.
58. Delporte C, Van Antwerpen P, Vanhamme L, Roumeguère T, Zouaoui Boudjeltia K. Low-density lipoprotein modified by myeloperoxidase in inflammatory pathways and clinical studies. *Mediators Inflamm* 2013;2013:971579.
59. Rios FJ, Ferracini M, Pecenin M, Koga MM, Wang Y, Ketelhuth DF, Jancar S. Uptake of oxLDL and IL-10 production by macrophages requires PAFR and CD36 recruitment into the same lipid rafts. *PLoS One* 2013;8:e76893.
60. Yang K, Wang X, Liu Z, Lu L, Mao J, Meng H, Wang Y, Hu Y, Zeng Y, Zhang X, Chen Q, Liu Y, Shen W. Oxidized low-density lipoprotein promotes macrophage lipid accumulation via the toll-like receptor 4-Src pathway. *Circ J* 2015;79:2509-16.
61. Zeya B, Arjuman A, Chandra NC. Lectin-like oxidized low-density lipoprotein (LDL) receptor (LOX-1): a chameleon receptor for oxidized LDL. *Biochemistry* 2016;55:4437-44.
62. Parthasarathy S, Raghavamenon A, Garelnabi MO, Santanam N. Oxidized low-density lipoprotein. *Methods Mol Biol* 2010;610:403-17.
63. Oliveira CL, Santos PR, Monteiro AM, Figueiredo Neto AM. Effect of oxidation on the structure of human low- and high-density lipoproteins. *Biophys J* 2014;106:2595-605.

64. Estruch M, Sanchez-Quesada JL, Beloki L, Ordoñez-Llanos J, Benitez S. The induction of cytokine release in monocytes by electronegative low-density lipoprotein (LDL) is related to its higher ceramide content than native LDL. *Int J Mol Sci* 2013;14:2601-16.
65. Ke LY, Stancel N, Bair H, Chen CH. The underlying chemistry of electronegative LDL's atherogenicity. *Curr Atheroscler Rep* 2014;16:428.
66. Sánchez-Quesada JL, Pérez A. Modified lipoproteins as biomarkers of cardiovascular risk in diabetes mellitus. *Endocrinol Nutr* 2013;60:518-28.
67. Younis NN, Soran H, Pemberton P, Charlton-Menys V, Elseweidy MM, Durrington PN. Small dense LDL is more susceptible to glycation than more buoyant LDL in Type 2 diabetes. *Clin Sci (Lond)* 2013;124:343-9.
68. Del Turco S, Basta G. An update on advanced glycation endproducts and atherosclerosis. *Biofactors* 2012;38:266-74.
69. Grewal T, Bartlett A, Burgess JW, Packer NH, Stanley KK. Desialylated LDL uptake in human and mouse macrophages can be mediated by a lectin receptor. *Atherosclerosis* 1996;121:151-63.
70. Bhakdi S, Dorweiler B, Kirchmann R, Torzewski J, Weise E, Tranum-Jensen J, Walev I, Wieland E. On the pathogenesis of atherosclerosis: enzymatic transformation of human low density lipoprotein to an atherogenic moiety. *J Exp Med* 1995;182:1959-71.
71. Klouche M, Gottschling S, Gerl V, Hell W, Husmann M, Dorweiler B, Messner M, Bhakdi S. Atherogenic properties of enzymatically degraded LDL: selective induction of MCP-1 and cytotoxic effects on human macrophages. *Arterioscler Thromb Vasc Biol* 1998;18:1376-85.
72. Klouche M, May AE, Hemmes M, Messner M, Kanse SM, Preissner KT, Bhakdi S. Enzymatically modified, nonoxidized LDL induces selective adhesion and transmigration of monocytes and T-lymphocytes through human endothelial cell monolayers. *Arterioscler Thromb Vasc Biol* 1999;19:784-93.
73. Tertov VV, Sobenin IA, Gabbasov ZA, Popov EG, Jaakkola O, Solakivi T, Nikkari T, Smirnov VN, Orekhov AN. Multiple-modified desialylated low density lipoproteins that cause intracellular lipid accumulation. Isolation, fractionation and characterization. *Lab Invest* 1992;67:665-75.
74. Dousset N, Dousset JC, Taus M, Ferretti G, Curatola G, Soléra ML, Valdiguié P. Effect of desialylation on low density lipoproteins: comparative study before and after oxidative stress. *Biochem Mol Biol Int* 1994;32:555-63.
75. Barbosa DS, Maranhão RC, Araújo FB, Chang YH, Hirata MH, Abdalla DS. Sialic acid and oxidizability of low density lipoprotein subfractions of hyperlipidemic patients. *Clin Biochem* 1995;28:435-41.
76. Tertov VV, Sobenin IA, Kaplun VV, Orekhov AN. Antioxidant content in low density lipoprotein and lipoprotein oxidation in vivo and in vitro. *Free Radic Res* 1998;29:165-73.
77. Oztürk Z, Sönmez H, Görgün FM, Ekmekçi H, Bilgen D, Ozen N, Sözer V, Altuğ T, Kökoğlu E. The relationship between lipid peroxidation and LDL desialylation in experimental atherosclerosis. *Toxicol Mech Methods* 2007;17:265-73.
78. Tertov VV, Kaplun VV, Sobenin IA, Boytsova EY, Bovin NV, Orekhov AN. Human plasma trans-sialidase causes atherogenic modification of low density lipoprotein. *Atherosclerosis* 2001;159:103-15.
79. Mahajan VS, Pillai S. Sialic acids and autoimmune disease. *Immunol Rev* 2016;269:145-61.
80. Macauley MS, Crocker PR, Paulson JC. Siglec-mediated regulation of immune cell function in disease. *Nat Rev Immunol* 2014;14:653-66.
81. Hutzler S, Özgör L, Naito-Matsui Y, Kläsener K, Winkler TH, Reth M, Nitschke L. The ligand-binding domain of Siglec-G is crucial for its selective inhibitory function on B1 cells. *J Immunol* 2014;192:5406-14.
82. O'Neill AS, van den Berg TK, Mullen GE. Sialoadhesin - a macrophage-restricted marker of immunoregulation and inflammation. *Immunology* 2013;138:198-207.
83. Sobenin IA, Salonen JT, Zhelankin AV, Melnichenko AA, Kaikkonen J, Bobryshev YV, Orekhov AN. Low density lipoprotein-containing circulating immune complexes: role in atherosclerosis and diagnostic value. *Biomed Res Int* 2014;2014:205697.
84. Burut DF, Karim Y, Ferns GA. The role of immune complexes in atherogenesis. *Angiology* 2010;61:679-89.
85. Montano EN, Boullier A, Almazan F, Binder CJ, Witztum JL, Hartvigsen K. Development and application of a nonradioactive binding assay of oxidized low-density lipoprotein to macrophage scavenger receptors. *J Lipid Res* 2013;54:3206-14.
86. Tertov VV, Sobenin IA, Orekhov AN, Jaakkola O, Solakivi T, Nikkari T. Characteristics of low density lipoprotein isolated from circulating immune complexes. *Atherosclerosis* 1996;122:191-9.
87. Schultze HE, Heide K. Carbohydrate content of different blood components. *Med Grundlogenforsch* 1960;3:357.
88. Ayrault-Jarrier M. Action des detergents sur une β-lipoproteine lumaine isoleé: etude de l'extractibilité des lipids. *Bull Soc Chim Biol (Paris)* 1961;43:153-65. (in French)
89. Marshall WE, Kummerow FA. The carbohydrate constituents of human serum-lipoprotein: galactose, mannose, glucosamine, and sialic acid. *Arch Biochem Biophys* 1962;98:271-3.
90. Kwiterovich P, Bachorik P, Simon A. Isolation and partial characterization of the glycopeptides from human plasma low density lipoproteins. *Circ Suppl III* 1974;49-50:267.

Calvo *et al. Vessel Plus* 2017;1:116-28
DOI: 10.20517/2574-1209.2017.14

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Review **Open Access**

Omega-3 polyunsaturated fatty acids and cardiovascular health: a molecular view into structure and function

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How to cite this article: Calvo MJ, Martínez MS, Torres W, Chávez-Castillo M, Luzardo E, Villasmil N, Salazar J, Velasco M, Bermúdez V. Omega-3 polyunsaturated fatty acids and cardiovascular health: a molecular view into structure and function. *Vessel Plus* 2017;1:116-28.

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Article history:
Received: 8 May 2017
Accepted: 23 Jun 2017
Published: 26 Sep 2017

Key words:
Cardiovascular disease, omega-3 polyunsaturated fatty acids, eicosapentaenoic acid, docosahexaeonic acid

ABSTRACT

Given the notorious impact of cardiovascular disease (CVD) as the current leading cause of mortality worldwide, the prevention, identification and management of CV risk factors represents a priority in daily clinical practice. Several studies have shown the beneficial effects of dietary omega-3 polyunsaturated fatty acids (PUFAs) on CV health. Their derivatives, eicosapentaenoic acid and docosahexaenoic acid, intervene in multiple metabolic pathways, including: regulation of the inflammatory response, by reducing the synthesis of pro-inflammatory cytokines; regulation of platelet aggregation, activation and adhesion, by modulating thromboxane A2 and plasminogen activator inhibitor-1 activity; regulation of the coagulation pathways, by reducing the carboxylation of vitamin K-dependent coagulation factors; improvement of endothelial function, given their effects on prostaglandin synthesis and endothelial nitric oxide synthase; reduction of serum lipids, through their effects on the hepatic synthesis of triacylglycerides, beta-oxidation of fatty acids and lipoprotein catabolism; and improvement of myocardial function via their membrane-stabilizing effects, and an increase in fluidity, size and distribution of membrane lipid rafts. Nevertheless, these effects appear to vary according to the type of PUFA ingested, dietary sources, daily dosing and individual factors inherent to the subject. Therefore, further studies are required to determine the ideal supplementation for each kind of patient and their particular CV profiles.

Quick Response Code:

INTRODUCTION

Cardiovascular disease (CVD) represents an ongoing global epidemic. In 2014, 27.6 million people were diagnosed with CVD worldwide,[1] and by 2030, it may be responsible for up to 23.5 million deaths yearly.[2] These trends are common in all westernized countries, including Latin America. In Venezuela, 29.47% of all mortality was attributed to CVD in 2012.[3] Given the heavy burden CVD represents for public health systems, prevention has become a key component in clinical practice and research, oriented to the identification and management of several risk factors, both modifiable and non-modifiable.[4] Regarding modifiable risk factors, westernized dietary patterns, notable for their high intake of dairy products, refined carbohydrates and saturated fats, have been strongly linked with the development of not only CVD, but also hypertension (HTN), obesity and type 2 diabetes mellitus.[5-7]

In 1980, Bang *et al*.[8] studied the diet of the Eskimo population of Greenland, characterized by high intake of foods rich in long chain polyunsaturated fatty acids (LC-PUFAs), and paradoxically found a low incidence of CVD in these individuals.[9] From these pioneer studies, different epidemiological and interventional investigations have backed the cardioprotective role of n-3 LC-PUFAs.[10,11]

Although many beneficial CV effects have been ascribed to PUFAs, including hypolipidemic, antithrombotic, antihypertensive and antiarrhythmic properties,[12,13] the underlying molecular mechanisms remain to be elucidated. This review aims to offer an integrated state-of-the-art vision into the structure of PUFAs and their functions in the CV system.

GENERAL OVERVIEW OF PUFAS

Structure and classification

Fatty acids are molecules consisting of a long linear hydrocarbon chain that generally contains a pair number of carbon atoms between 12 and 24, with a carboxyl (-COOH) group in one end and a methyl (-CH3) group in the other.[14] They are termed saturated fatty acids when only simple bonds exist between the carbon atoms, while those that have one or more double bonds are known as unsaturated fatty acids.[15] The latter include widely recognized nutritionally essential molecules for humans and other animal species, including linoleic acid (LA) and α-linoleic acid (ALA).[16]

Fatty acids with more than one double bond in their chain are called PUFAs, which are classified in 2 main subgroups: n-6 long chain PUFAs (n-6 LC-PUFAs) and n-3 long chain PUFAs (n-3 LC-PUFAs), which are commonly referred to as omega-6 and omega-3 PUFAs, respectively.[17] The former are LA derivatives with 2 double bonds, which are located 6 carbons away from the methyl end (18:2Ω6); whereas n-3 LC-PUFAs derive from ALA and have 3 double bonds, with the first one being in the third carbon of the chain (18:3Ω3)[18,19] [Figure 1].

Metabolism and general biologic functions of essential PUFAs

The metabolism of both types of PUFAs ends in the formation of eicosanoids, which are biologically active compounds including prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs).[18] As shown in Figure 1, arachidonic acid (AA) is synthetized from LA (n-6), and is converted by the action of cyclooxygenase (COX) and lipoxygenase (LOX) into 2-series PGs and TXs and 4-series LTs and lipoxines. Although these mediators intervene in both the establishment and resolution of the inflammatory response, their net effect is predominantly pro-inflammatory.[19,20] In contrast, ALA (n-3) is a precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), from which 3-series PGs and TXs, as well as 5-series LTs, lipoxins, resolvins and neuroprotectins are derived. These compounds have chiefly anti-inflammatory effects,[17] which is why current nutritional guidelines are oriented towards an increase in n-3 PUFAs intake.[21-24] Furthermore, the products of both series mediate the regulation of other physiological processes, such as the maintenance of cell membrane architecture, especially through their arrangement in lipid rafts,[25,26] and play a role in hemostasis and vasoconstriction, which are further explained ahead.[18,27]

Role of PUFAs in cell membrane maintenance

The long hydrocarbon chains and double bonds in EPA and DHA exert modifications on the cell membrane due to their length and degree of unsaturation.[28] These molecules have been demonstrated to increase membrane fluidity and modify the size and distribution of lipid rafts in aortic endothelial cells and rat lymphocyte cultures.[29,30] Lipid rafts are dynamic membrane microdomains containing sterols, enriched sphingolipids and specific binding proteins, which attain a metastable resting state through a constellation of lipid-lipid, protein-lipid and protein-protein bonds.[31] Incorporation of n-3 PUFAs in lipid rafts results in decreased cholesterol and sphingolipids in these microdomains.[32] This has been confirmed by systematic studies in membrane models that suggest

cholesterol to be incompatible with environments rich in highly unsaturated lipids, as observed in phospholipid bilayers containing DHA.[33]

Most of the research on n-3 PUFAs in membrane models has centered around DHA.[34] This molecule is deemed unique because it contains six double bonds and is very flexible, with quick rearrangements amidst multiple conformational states.[29] Spectrometry studies have revealed DHA-containing phospholipids to form their own domains with a different arrangement in presence of sphingolipids and cholesterol, excluding saturated acyl chains from their structure.[35] In addition, because n-3 PUFAs tend to reject cholesterol, DHA-containing phospholipids tend to create non-raft domains which may be physically separated on cell membranes. This allows proteins to more readily occupy a space according to its requirements in a specific domain or in amplified rafts.[36] An alternative model points out that n-3 PUFAs are probably incorporated in the rafts as nanodomains, forcing cholesterol out of rafts.[26] This model is also applicable to proteins within lipid rafts, where incorporation of n-3 PUFAs into lipid rafts forces proteins to relocate to non-raft domains.[26] Further research is required to fully understand the biologic importance and mechanisms underlying the lateral organization of lipid microdomains in cell membranes, as well as the modulatory effects of n-3 PUFAs in this context.[32]

MOLECULAR MECHANISMS OF PUFAS IN CARDIOVASCULAR HEALTH

Chronic pro-inflammatory states

The anti-inflammatory effects of n-3 PUFAs have been widely reported.[37-40] One of the central mechanisms is the down-regulation of the synthesis of pro-inflammatory cytokines such as tumor necrosis factor alpha, interleukin 6 and monocyte chemoattractant protein-1 (MCP-1)[41-44] in adipose tissue. This occurs when EPA and DHA bind to the G-protein coupled receptor (GPR120) in macrophages and adipocytes, causing its activation and internalization with β-arrestin-2, and forming the GPR120/β-arrestine-2 complex.[45] This complex is then dissociated into the transforming growth factor beta (TGF-β) activated kinase 1 binding protein 1 (TAB1) that results in the inhibition of TGF-β activated kinase 1 (TAK1), and thus the down-regulation of the nuclear factor kappa B (NF-κB) and the inhibition of its function.[44] Besides, the incorporation of DHA to the lipid membrane disrupts the signaling of toll-like receptor 4 (TLR-4) by impeding its translocation to the lipid raft, and inhibiting the signaling pathway of MD2/TRIAP-MyD88/IRAK-TRAF6/IKKβ[41,46,47] [Figure 2]. Also, EPA and DHA cause the down-regulation of nicotinamide adenine dinucleotide phosphateoxidase, which induces the production of reactive oxygen species, a requirement for TLR-4 signaling.[41,42] These pathways converge in the inhibition of NF-κB, diminishing the inflammatory

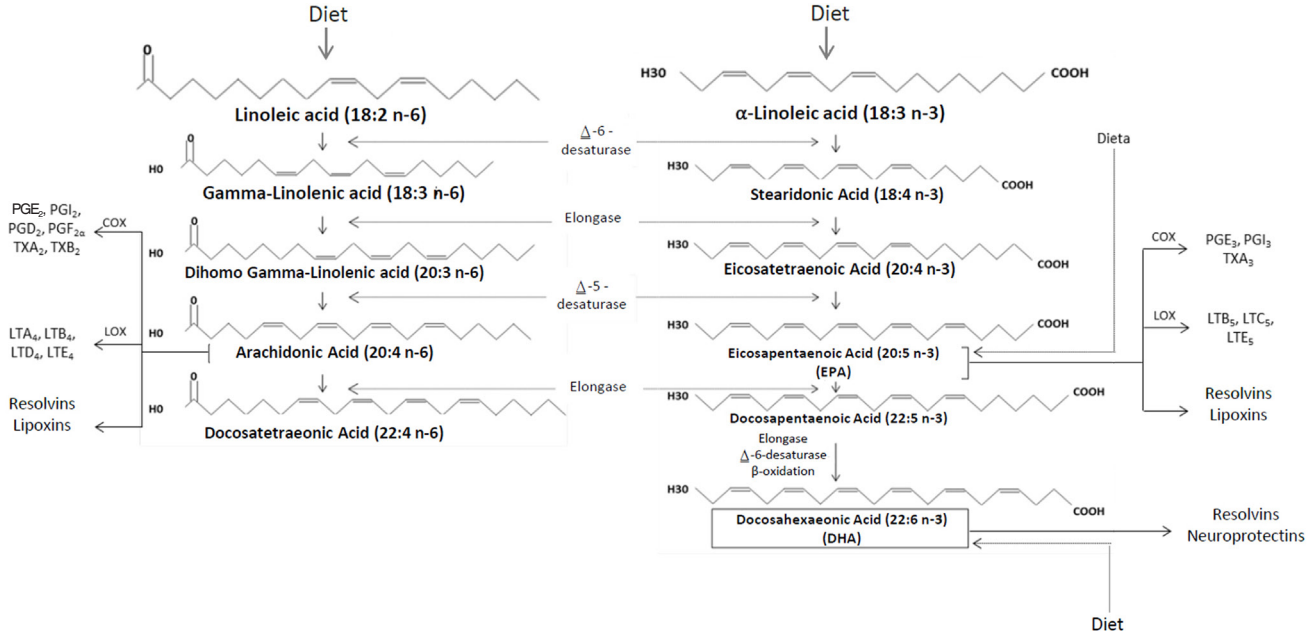

Figure 1: Metabolism of n-6 and n-3 polyunsaturated fatty acids. n-3 and n-6 polyunsaturated fatty acids are derived from linoleic acid (LA) and α-linoleic acid (ALA). Through various enzymatic reactions, LA is converted into arachidonic acid, responsible of the formation of mainly proinflammatory PG and TX. On the other hand, ALA is converted into EPA and DHA, which derive into mostly antiinflammatory PG and TX. PGE2: prostaglandin E2; PGD2: prostaglandin D2; PGF2α: prostaglandin F2α; PGI2: prostacyclin I2; PGE3: prostaglandin E3; PGI3: prostacyclin I3; TX: thromboxanes; LT: leukotrienes

response.[44,45] In addition n-3 PUFAs may also prevent macrophage infiltration in adipose tissue.[41]

Thrombogenesis

The antithrombotic properties of PUFAs have been described since the 1980s, owing to the pioneer studies by Bang and Dyerberg.[48] These studies demonstrated that the Eskimo diet, characterized by high intake of seafood rich in n-3 PUFAs (mainly fish, seal and whale), was associated with a low incidence of CVD, as well as a decrease in thrombogenesis, evident by high incidence of hemorrhages.[49,50]

Even though these effects have been described in populations of different latitudes,[51-53] the inverse relation between n-3 PUFAs intake, platelet aggregation, coagulation and fibrinolysis is still not completely elucidated;[54] however, both *in vitro* and *in vivo* studies have reported that n-3 PUFAs supplementation reduces TXA2 synthesis, platelet activation and adhesion,[55] and decreases plasminogen activator inhibitor-1 (PAI-1) activity and concentration.[56]

The mechanisms by which n-3 PUFAs decrease thrombogenesis have been extensively studied, especially in platelets. High n-3 PUFA intake, especially EPA and DHA, appears to favor the replacement of AA in cell membrane phospholipids, decreasing the binding rate of AA to COX-1, resulting in reduced TXA2 synthesis, a vasoconstriction and platelet aggregation-promoting molecule.[57] On the other hand, this secondarily increases the production of TXA3, which exerts a significantly lower biological activity than TXA2.[58] Another mechanism observed with *in vivo* studies is the capacity of n-3 PUFAs to act as TXA2 and PG H2 antagonists, through the synthesis of protectin DX, a product of DHA dihydroxylation obtained by the action of LOX.[59] This compound also has the capacity to inhibit both COX-1 and COX-2 in platelets and neutrophils, significantly decreasing both platelet activation and aggregation [Figure 3].[60,61]

In contrast, views on the mechanisms underlying the anticoagulant effects of n-3 PUFAs remain controversial. Some studies suggest these molecules may interfere in the carboxylation of vitamin K-dependent coagulation factors II, VII, IX and X;[62,63] while other studies attribute more relevance to a modification in serum fibrinogen levels.[64] Similarly, the role of n-3 PUFAs in fibrinolysis remains unclear,[65] however it has been proposed that by unknown mechanisms, they alter PAI-1 synthesis through a genetic pathway.[66]

Dyslipidemia

The effects of n-3 PUFAs on serum lipids were also first ascertained by Bang and Dyerberg[67] in their emblematic study on the Eskimo population. Results of this study showed that individuals who stayed in their birthplace had lower levels of triacylglycerides (TAG), very low-density lipoproteins (VLDL-C) and low-density lipoproteins (LDL-C), whereas those who later migrated to Denmark showed a serum lipid profile similar to

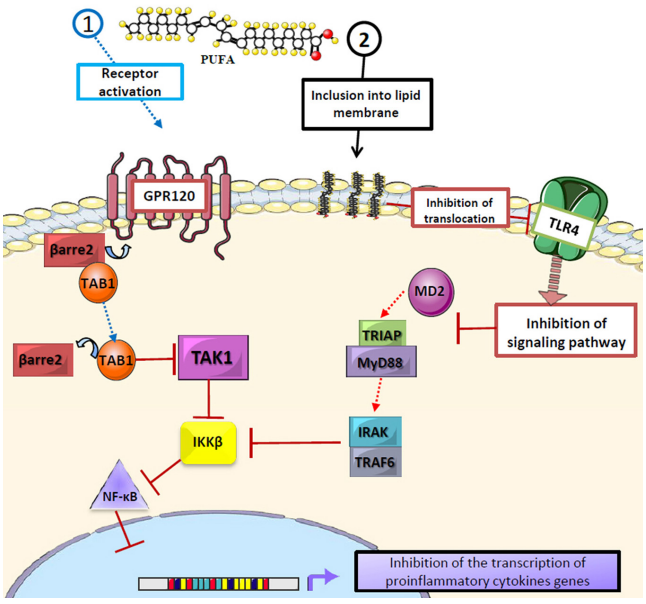

Figure 2: Role of polyunsaturated fatty acids in proinflammatory cytokine synthesis. EPA and DHA inhibit the production of proinflammatory cytokines through different mechanisms: (1) binding of EPA and DHA to the G protein-coupled receptor (GPR120) leads to its activation and binding to β arrestin-2, which then dissociates into TAB1 and inhibits TAK1, thus interrupting the IKKβ/NF-κB cascade; (2) the inclusion of EPA and DHA into the lipid bilayer, which modifies lipid rafts and interrupts the translocation of TLR-4 and the MD2/TRIAP-MyD88/IRAK-TRAF6/IKKβ/NF-κB pathway, thus inhibiting the production of cytokines, showing the antiinflammatory action of EPA and DHA

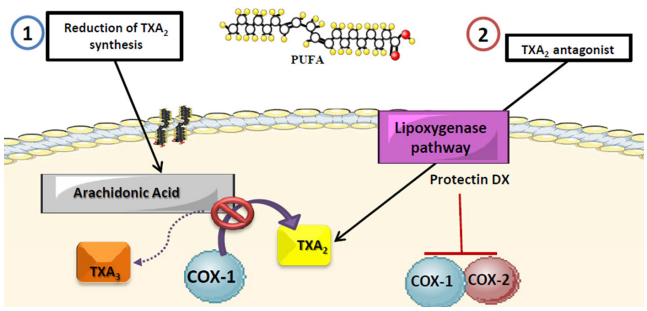

Figure 3: Role of polyunsaturated fatty acids in thrombogenesis. n-3 PUFAs exert their antithrombotic effect on platelets via two main processes: (1) replacement of arachidonic acid in the platelet membrane, which causes a decrease in the action of COX-1 on arachidonic acid, diminishing TXA2 synthesis and favoring the synthesis of TXA3; (2) activity as TXA2 antagonists through the synthesis of protectin DX, a molecule from the lipoxygenase pathway, which inhibitis COX-1 and COX-2. PUFA: polyunsaturated fatty acids; TXA2: thromboxane A2; TXA3: thromboxane A3; COX-1: cyclooxygenase 1; COX-2: cyclooxygenase 2

that of the Danish population. Thus, environmental factors - namely dietary n-3 PUFA intake - may exert a preponderant impact on serum lipids.[68]

Among these actions on lipid metabolism, the TAG-lowering effect has been found to be the most robust in large epidemiological studies;[69] however, it appears to be largely modifiable by the overall dietary composition, as elevated carbohydrate and saturated fat intake may surpass the effect of n-3 PUFAs due to increased TAG synthesis and storage.[69,70] In addition, the magnitude of the lipid-lowering effect of n-3 PUFAs depends on each subject's basal serum lipid levels, as greater decreases are observed in subjects with higher TAG levels.[70,71]

The mechanisms by which n-3 PUFAs achieve these effects on TAG are related to the decrease of their hepatic synthesis via competitive inhibition of the enzymes involved, especially 1,2 diglyceride acyltransferase, which catalyzes the conversion of diacylglycerides into TAG.[72] In addition, PUFAs have high affinity for several peroxisome proliferator-activated receptor (PPAR) subtypes, particularly PPAR-α, a nuclear transcription factor highly expressed in adipose tissue and skeletal muscle.[73] When PPAR-α is activated by specific substrates like PUFAs, it favors the synthesis of enzymes involved in lipid catabolism.[74] Therefore, n-3 PUFA intake promotes the β-oxidation of fatty acids in peripheral tissues, which contributes to the catabolism of circulating TAG in chylomicrons and VLDL-C. This results in diminished traffic of non-esterified fatty acids to hepatocytes, causing an additional reduction in the input of substrates for TAG synthesis, further decreasing the hepatic production of VLDL-C.[72]

Additionally, PUFAs downregulate the sterol regulatory element-binding protein 1c (SREBP1c), which modulates the expression of genes involved in the synthesis of fatty acids and TAG.[75,76] PUFAs inhibit SREBP1c activity in the liver by antagonizing the liver X receptor alpha, a nuclear receptor found in hepatocytes that regulates the synthesis of SREBP and the SREBP inhibitor protein.[77,78] Another reported genetic mechanism is the ability of PUFAs to inhibit the hepatic maturation of the carbohydrate-responsive element-binding protein, a transcription factor related to the expression of enzymes involved in TAG synthesis[79] [Figure 4].

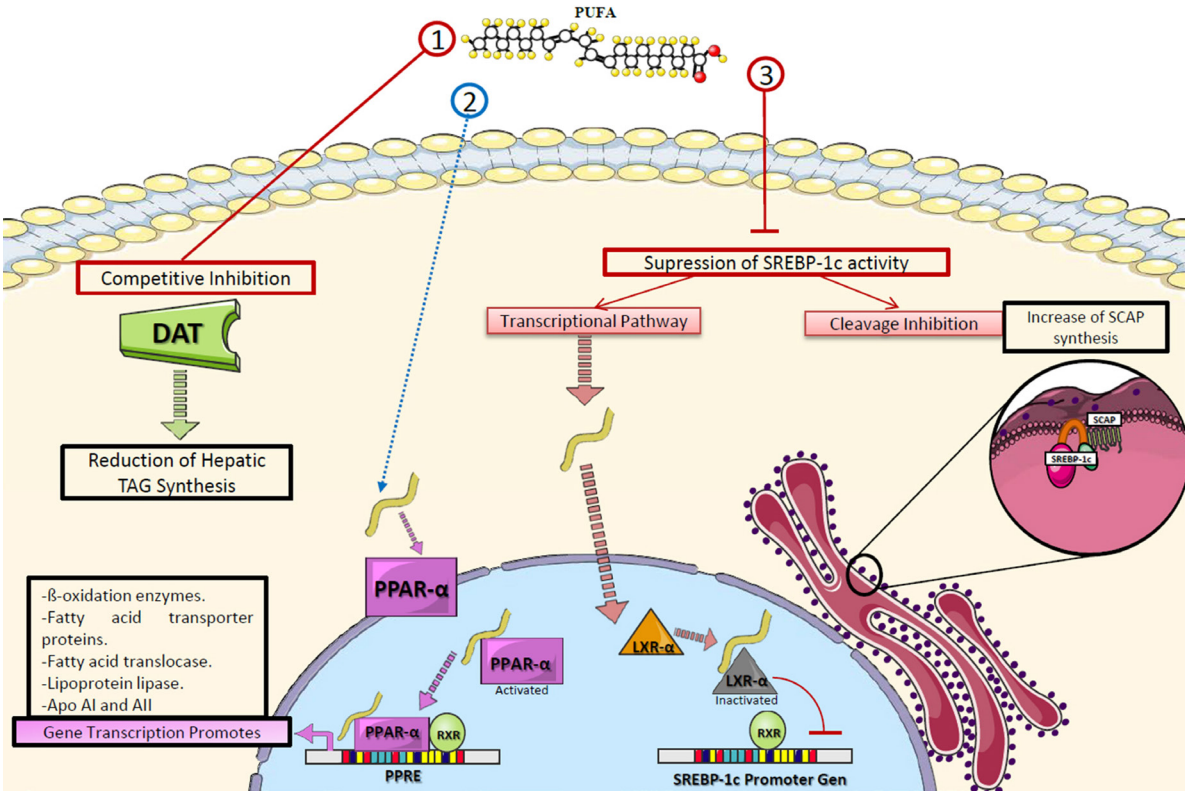

Figure 4: Role of polyunsaturated fatty acids in triacylglyceride metabolism. PUFAs decrease TAG through various mechanisms: (1) competitive inhibition of DAT; (2) activating PPAR-α, which promotes the transcription of enzymes involved in lipolysis and fatty acid transport; (3) suppressing the activity of SREBP-1c which regulates the expression of genes involved in fatty acid and TAG synthesis. PUFA: polyunsaturated fatty acid; DAT: 1,2 diglyceride acyltransferase; TAG: triacylglycerides; PPAR-α: peroxisome-proliferator activated receptor alpha; RXR: retinoid X receptor; LXR-α: liver X receptor alpha; SREBP-1c: sterol regulatory element-binding protein 1c; SCAP: SREBP inhibitor protein; PPRE: PPAR response elements

These TAG-lowering mechanisms are accompanied by a secondary decrease of VLDL-C: *in vitro* studies have described PUFA-mediated activation of non-proteosomal degradation of apolipoprotein B (Apo B). This protein, found in the membrane of chylomicrons, LDL-C and VLDL-C, allows the transport of lipids towards peripheral tissues.[80] Activation of this pathway results in the selective degradation of the Apo B which would have been integrated into naïve VLDL, thus reducing liver secretion of this lipoprotein.[81,82]

Several randomized studies have shown that PUFAs also contribute to a slight increase of high density lipoprotein (HDL-C), ranging between 2.3-7.9% for EPA, and 2.9-18.3% for DHA.[83,84] PUFA-mediated reduction of cholesteryl ester transfer protein (CETP) activity may be accountable for this effect, as it would lead to a decrease in the net flow of cholesterol esters from HDL-C to LDL-C and VLDL-C. Recent *in vitro* studies also suggest PUFAs to be regulators of CETP and apolipoprotein A1 gene expression.[85,86]

On the other hand, the effects that PUFAs exert on LDL-C are yet to be defined. A study on adult women by Ooi *et al*.[87] where the effects of diets with high and low fish intake (1.23 g/day and 0.27 g/day of EPA and DHA, respectively) were assessed showed a non-significant decrease in serum LDL-C levels for both diets. However, significantly lower Apo-B100 concentration, greater LDL-C Apo-B100 production rate and higher conversion percentage of TAG-rich lipoproteins (TRL) into LDL-C were reported in high fish intake diets. Other studies have reported that EPA and DHA supplement intake considerably increases LDL-C levels when compared to placebo and EPA-exclusive intake.[88] However, recent evidence suggest that EPA as opposed to DHA has more prominent effects on LDL in patients with hyper-TG due to its antioxidant properties in various Apo B-containing proteins,[89] improving secondarily endothelial function and inflammatory profile.[90] Studies in animals suggest that very high intake of PUFAs of marine origin increases the union of the TRL to the endothelial LPL, prolonging the interaction period and thus resulting in a boost of LDL-C formation. Furthermore, very high intake of n-3 PUFAs has been found to boost the production of smaller TRL with less amount of Apo-E, which show a tendency to convert into LDL-C;[91] as well as increase the expression of hepatic LDL-C receptors, amplifying its catabolism.[92] Indeed, the effects of PUFAs on LDL-C levels appear to significantly vary across specific PUFA types and quantity.[88,89]

Hypertension

The role of HTN as one of the main independent risk factors for CVD has been widely recognized along with the role of n-3 PUFAs on its management.[7] However, recent findings have shown that maintaining normal blood pressure (BP) levels even in non-hypertensive individuals significantly lowers the incidence of CVD, representing important evidence for the use of n-3 PUFAs as a powerful preventive intervention.[93] A recent study by Huang *et al*.[94] on 1,154 Chinese adults found hypertensive subjects to have lower plasma PUFA concentrations when compared to healthy counterparts. This echoes the results of a previous study on 447 Eskimo people, where both high dietary intake and elevated plasma levels of n-3 PUFAs were associated with lower levels of diastolic blood pressure.[95]

PUFAs may regulate BP through various mechanisms, most powerfully through conversion into vasodilator PG and promotion of renin release from the kidney.[96] Moreover, it has been demonstrated that a diet rich in n3-PUFAs suppresses the activity of the angiotensin-converting enzyme, reduces the formation of angiotensin II, improves the generation of eNO (endothelial nitric oxide) and suppresses the expression of TGF-β.[97] Recently, in murine models with angiotensin II-dependent HTN, the combination of a soluble epoxide hydrolase inhibitor along with a diet rich in n3-PUFAs was tested, showing higher levels of EPA and DHA epoxides and a reduction of inflammatory markers in the kidney (PGs and MCP-1), contributing to a decrease in systolic BP and inflammation.[98]

Various cytochrome P450 (CYP) isoforms have also been identified in the physiological production of active metabolites of AA, EPA and DHA as alternative substrates.[99] In this context, Agbor *et al*.[100] demonstrated the contribution of isoform CYP1A1 tothe metabolism of n3-PUFAs, and the activation of endothelial nitric oxide synthase and consequent increase innitric oxide (NO) bioavailability associated with a diet rich in n3-PUFAs [Figure 5].

Furthermore, a study by Hoshi *et al*.[101] exposed the activation of large conductance Ca^{2+}-activated K^+ channels by DHA, through a fast and reversible stimulus independent of Ca^{2+} concentration. However, the exact bonding site remains to be identified. The activation of these channels in smooth muscle cells allows passive K^+ efflux, which translates into the hyperpolarization of the cell membrane and thus its hypotensive effect.[102]

On the other hand, modifications in fatty acid composition in the lipid matrix of the cell membrane play an important role in the pathogenesis of hypertension.[103] A decrease of PUFAs in the cell

membrane of erythrocytes leads to a decrease in the negative charge of the membrane, with reduced phospholipid fluidity, activation of the synthesis of proinflammatory eicosanoids, and increased sensitivity of arterial smooth muscle cells to vasoconstrictive effects.[103,104]

Myocardial function

Reports from different human and animal models have demonstrated that n-3 PUFAs improve left ventricular inotropic function, without causing hypertrophy or increase in blood pressure.[105] The underlying mechanism involves an increase in the activity of myosin ATPase and Na^+/K^+ ATPase, and the expression of Ca^{2+} ATPase in the sarcoplasmic reticulum, which are associated with positive inotropism, and maintenance of intra-sarcoplasmic reticulum calcium concentration and the sodium calcium exchanger (NCX).[106,107] Furthermore, an indirect effect is achieved through an increase in ventricular efficiency, which is defined as the production of the highest ejection volume with the lowest possible oxygen consumption, and the decrease in blood pressure.[108] This is possible due to the incorporation of DHA in the cell membrane,[109] influencing the eicosanoids mechanism and modulating cellular Ca^{2+} and its signaling pathways.[110] On the other hand, it has also been attributed to the shortening in the monophasic action potential due to the suppression of ATP-dependent K^+ channels in the sarcolemma.[111]

Another proposed mechanism is the increase in the Na^+/K^+ ATPase activity, which boosts Na^+ concentrations, diminishing the intracellular Ca^{2+} concentrations due to its effect in NCX activity in the cell membrane.[112] In addition, it has been demonstrated that the Na^+/K^+ ATPase activation modulates the function of L-type Ca^{2+} channels, which causes a greater release of calcium by the sarcoplasmic reticulum and higher intracellular Ca^{2+} gradients during systole, increasing contraction strength.[113]

Cardiac arrhythmia

Several studies have reported an association between n-3 PUFA intake and a lower risk of CVD-related death, specifically from ischemic events, where the myocardium is more prone to suffer irregularities in its electric activity that can lead to sudden death.[114,115]

Myocardial cells at the border of the ischemic zone have a relatively depolarized resting potential and can potentially generate ventricular fibrillation because of how easily they can be excited.[114] Because of this, an elevation in n-3 PUFAs stabilizes the high excitability of these partially depolarized cells in the ischemic myocardium. This prevents spontaneous or premature depolarization,[116] resulting in a longer refractory period and an increase in the voltage needed for the cellular depolarization.[117-120] More specifically, n-3 PUFAs can inhibit voltage-dependent Na^+, K^+ and Ca^{2+} channels, as well as Na^+/Ca^{2+} exchangers and Ca^{2+}-activated K^+ channels.[121] Consequently, these changes lower membrane excitability,[122] translating to a net membrane-stabilizing effect.[116,123]

Finally, an antiarrhythmic mechanism has been implicated in the role that n-3 PUFAs play in autonomic control, by increasing the vagal tone.[124,125] Recent

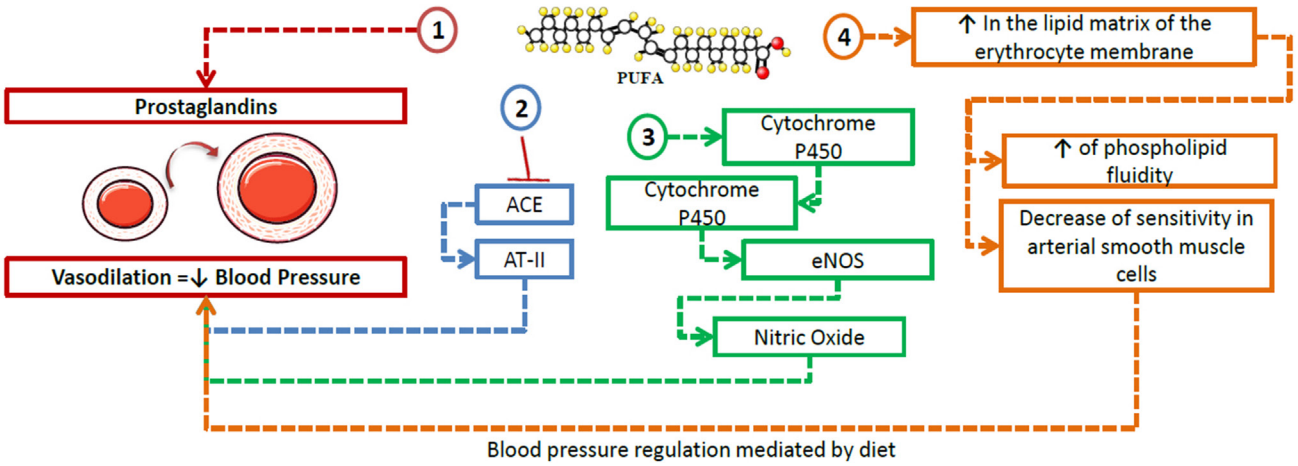

Figure 5: Role of polyunsaturated fatty acids in hypertension. n3-PUFAs intervene in blood regulation through the following pathways: (1) conversion into prostaglandins via the cyclooxygenase pathway, causing vasodilation of the smooth muscle in arterial walls; (2) inhibition of ACE, reducing the synthesis of AT-II, thus leading to a decrease in blood pressure; (3) promotion of cytochrome P450 isoforms such as CYP1A1, which contributes to the activation of eNOS, increasing the bioavailability of nitric oxide and thus causing vasodilation; (4) incorporation into the lipid matrix of the erythrocyte membrane, where they lead to a an increase, and a decrease in the sensitivity of arterial smooth muscle cells to vasoconstrictive effects. ACE: angiotensin-converting enzyme; AT-II: angiotensin II; eNOS: endothelial nitric oxide synthase; PUFA: polyunsaturated fatty acids

evidence highlights that the effect n-3 PUFAs exert on the electrophysiology of the ventricles and atria relies on their favorable action on cell-cell connections by modulating the expression and phosphorylation of connexin-43[125,126] [Figure 6].

Ischemia/reperfusion

Although the restoration of blood flow in the ischemic myocardium is essential for tissue survival during acute myocardial infarction, its reperfusion may directly accelerate the ischemic process or increase the myocardial injury in a phenomenon known as "reperfusion injury".[127-129] Important studies have reported that this event is responsible for up to 50% of the final infarction size.[130] PUFAs appear to be associated with reduced ischemic/reperfusion injury and thus with a better recovery after a coronary event.[131]

During ischemia/reperfusion, increased n-3 PUFA content in the mitochondrial membrane may contribute to stabilization and thus lower myocardial oxygen consumption (MVO_2), thereby attenuating the thermodynamic inefficiency caused by hypoxia.[132,133] In addition, a lower MVO_2 could diminish vulnerability to arrhythmia through the energetic maintenance of transmembrane potentials during episodes of ischemia.[133]

A study on 211 patients with ST segment elevation myocardial infarction who underwent reperfusion by percutaneous coronary intervention found patients with higher levels of n-3 PUFAs (EPA + DHA ≥ 155 mg/mL) had a lower incidence of reperfusion injury than those with lower levels of n-3 PUFAs (EPA + DHA < 155 mg/mL).[134] Although the antiarrhythmic effect may exert the most potent impact in ischemia/reperfusion injury,[135] other supplementary actions may also intervene, including antithrombotic, anti-inflammatory and vasoactive effects.[136-138]

CONCLUSION

As has been described in review, n-3 PUFAs boast several beneficial effects in CV physiology and pathophysiology [Figure 7]. Notwithstanding current available evidence supporting the administration of n-3 PUFAs as a therapeutic intervention in CVD, further research is required to better characterize the underlying molecular mechanisms, as well as refine recommendations for their clinical use. Indeed, one

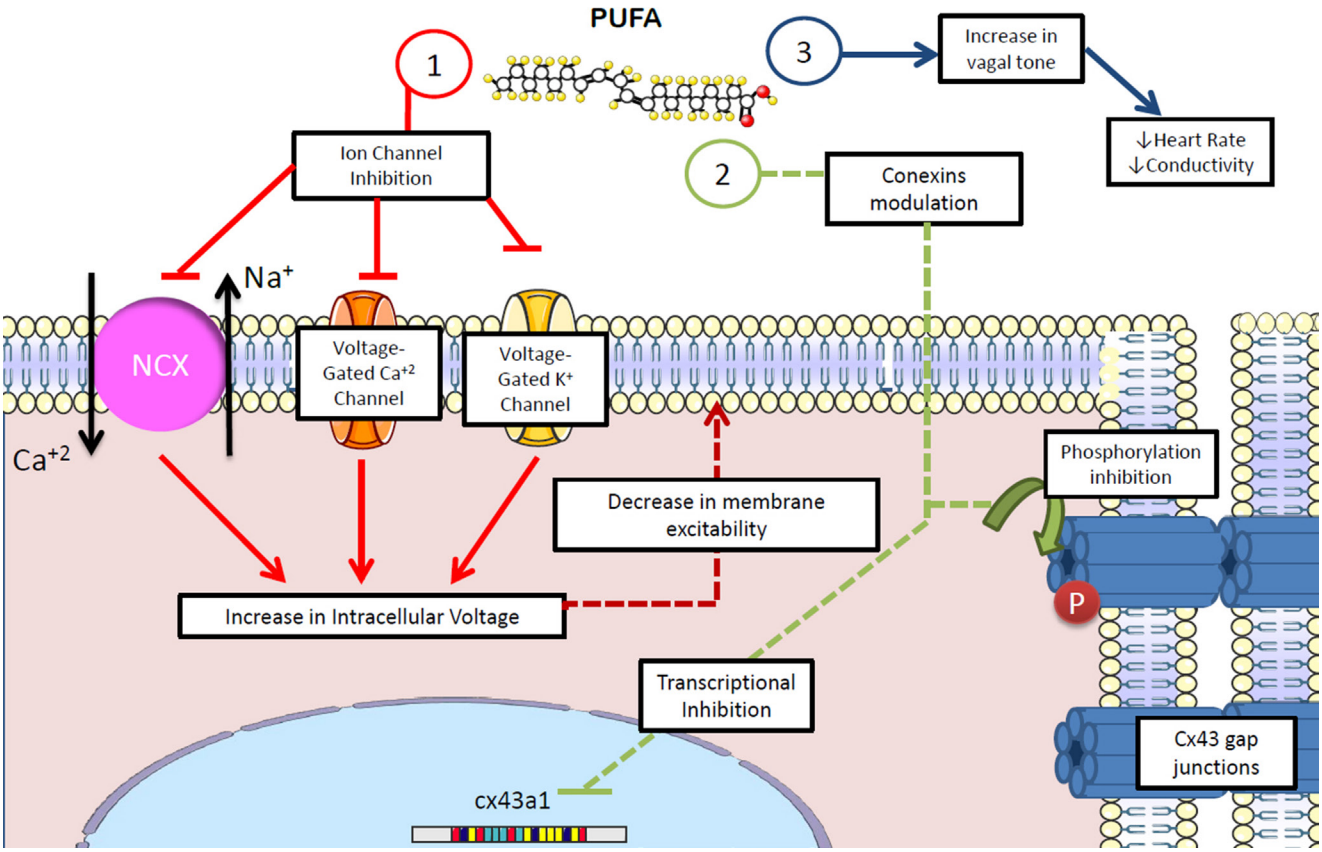

Figure 6: Antiarrhythmic effects of polyunsaturated fatty acids. n-3 PUFAs show several potential antiarrhythmic properties: (1) membrane potential stabilization by decreasing transmembrane io traffic; (2) inhibition of the activity and expression of connexins, decreasing the conductivity of myocardial tissue; (3) increase in vagal tone, decreasing heart rate, conductivity and myocardial excitability. NCX: sodium-calcium exchanger; Cx43: connexin-43; PUFA: polyunsaturated fatty acids

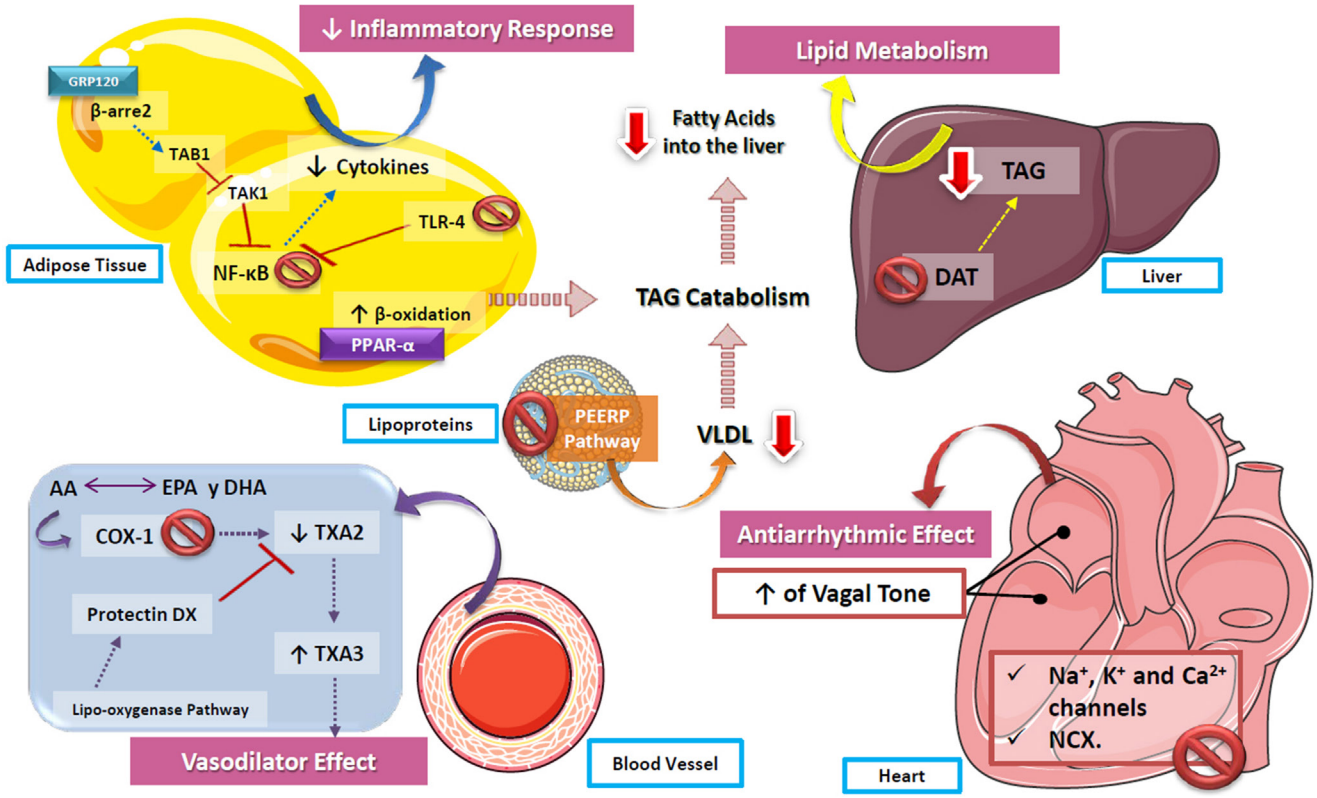

Figure 7: Role of polyunsaturated fatty acids in cardiovascular function. The actions of n3-PUFAs are diverse, including the decrease of the inflammatory response via NF-kB inhibition, as well as an increase of β-oxidation, causing the catabolism of triaclyglycerides and contributing to the decrease of lipids stored both in the liver and vessel walls. In addition, by increasing the production of TXA3 in vessel walls, PUFAs decrease vascular resistance, reducing blood pressure. On the other hand, one of the most described effects of n3-PUFAs is their action on cardiac arrhythmia, by inhibiting voltage-gated ion channels and exchangers, as well as increasing the vagal tone of the atria and ventricles, which leads to a lower heart rate. PUFA: polyunsaturated fatty acids; TXA2: thromboxane A2; TXA3: thromboxane A3; COX-1: cyclooxygenase 1; DAT: 1,2 diglyceride acyltransferase; TAG: triacylglycerides; NF-kB: nuclear factor kappa B; TLR-4: toll-like receptor 4; VLDL: very low-density lipoproteins

of the most pressing issues is the assessment of potential adverse effects linked to the therapeutic implementation of n3-PUFAs, along with the determination of adequate dosing and sources for these molecules in a myriad of specific clinical scenarios.

Authors' contributions

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Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. Center for Disease Control and Prevention. Summary Health Statistics: National Health Interview Survey, 2014. Avalaible from: https://ftp.cdc.gov/pub/Health_Statistics/NCHS/NHIS/SHS/2014_SHS_Table_A-1.pdf. [Last accessed on 29 Jun 2017]
2. World Health Organization. Global status report on non-communicable disease. 2010. Available from: http://whqlibdoc.who.int/publications/2011/9789240686458_eng.pdf. [Last accessed on 29 Jun 2017]
3. Anuario de Mortalidad 2013. Ministerio del Poder Popular para la Salud de la República Bolivariana de Venezuela. Available from: http://www.ovsalud.org/descargas/publicaciones/documentos-oficiales/Anuario-Mortalidad-2013.pdf. [Last accessed on 30 Jun 2017]
4. American Heart Association & American Stroke Association. Cardiovascular disease: a costly burden for America. Projections through 2035. Available from: https://www.heart.org/idc/groups/heart-public/@wcm/@adv/documents/downloadable/ucm_491543.

pdf. [Last accessed on 29 Jun 2017]
5. Sweazea KL. Compounding evidence implicating Western diets in the development of metabolic syndrome. *Acta Physiol* 2014;211:471-3.
6. Heinonen I, Petteri R, Ruohonen ST, Ruohonen S, Ahotupa M, Savontaus E. The effects of equal caloric high fat and western diet on metabolic syndrome, oxidative stress, and vascular endothelial function in mice. *Acta Physiol* 2014;211:515-27.
7. Willett WC. Dietary fats and coronary heart disease. *J Intern Med* 2012;272:13-24.
8. Bang H, Dyerberg J, Sinclair H. The composition of the Eskimo food in north western Greenland. *Am J Clin Nutri* 1980;23:2657-61.
9. Bjerregaard P. Disease pattern in Greenland: studies on morbidity in Upernavik 1979-1980 and mortality in Greenland 1968-1985. *Arctic Med Res* 1991;50 Suppl 4:1-62.
10. Kromhout D, Bosschieter E, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary disease. *N Engl J Med* 1985;312:1205-9.
11. Kris-Etherton P, Eckel RH, Howard BV, St Jeor S, Bazzarre TL; Nutrition Committee Population Science Committee and Clinical Science Committee of the American Heart Association. AHA Science Advisory: Lyon Diet Heart Study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association Step I Dietary Pattern on Cardiovascular Disease. *Circulation* 2001;103:1823-5.
12. Maehre HK, Jensen IJ, Elvevoll EO, Eilertsen KE. ω-3 fatty acids and cardiovascular diseases: effects, mechanisms and dietary relevance. *Int J Mol Sci* 2015;16:22636-61.
13. Torres N, Guevara-Cruz M, Velazquez-Villegas LA, Tovar AR. Nutrition and atherosclerosis. *Arch Med Res* 2015;46:408-26.
14. Abozid MM, Ayimba E. Effect of omega 3 fatty acids family in human health. *Int J Adv Res* 2014;2:202-11.
15. Jump D. The biochemistry of n-3 polyunsaturated fatty acids. *J Biol Chem* 2002;277:8755-8.
16. Graham C, Burdge C, Philip C. Conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* 2005;45:581-97.
17. Cicconel MM, Scicchitano P, Gesualdo M, Zito A, Carbonara S, Ricci G, Cortese F, Giordano P. The role of omega-3 polyunsatured fatty acid supplementation in childhood: a review. *Recent Pat Cardiovasc Drug Discov* 2013;8:42-55.
18. Innis S. Polyunsaturated fatty acids, inflammation and immunity. *Eur J Clin Nutr* 2002;56:S14-9.
19. Patterson E, Wall R, Fitzgerald G, Ross R, Stanton C. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J Nutr Metab* 2012;2012:539426.
20. Frolov A, Yang L, Dong H, Hammock BD, Crofford LJ. Anti-inflammatory properties of prostaglandin E2: deletion of microsomal prostaglandin E synthase-1 exacerbates non-inmune inflammatory arthritis in mice. *Prostaglandins Leukot Essent Fatty Acids* 2013;89:351-8.
21. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney MT, Corrà U, Cosyns B, Deaton C, Graham I, Hall MS, Hobbs FD, Løchen ML, Löllgen H, Marques-Vidal P, Perk J, Prescott E, Redon J, Richter DJ, Sattar N, Smulders Y, Tiberi M, van der Worp HB, van Dis I, Verschuren WM; Authors/Task Force Members. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* 2016;37:2315-81.
22. Luis D, Zlatkis K, Comenge B, García Z, Navarro JF, Lorenzo V, Carrero JJ. Dietary quality and adherence to dietary recommendations in patients undergoing hemodialysis. *J Ren Nutr* 2016;26:190-5.
23. Dawson D, Branch-Mays G, Gonzalez O, Ebersole J. Dietary modulation of the inflammatory cascade. *Periodontol 2000* 2014;64:161-97.
24. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J* 2010;8:1461.
25. Turk H, Chapkin R. Membrane lipid raft organization is uniquely modified by n-3 polyunsaturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2013;88:43-7.
26. Shaikh S. Biophysical and biochemical mechanisms by which dietary n-3 polyunsaturated fatty acids from fish oil disrupt membrane lipid rafts. *J Nutr Biochem* 2012;23:101-5.
27. Zanetti M, Grillo A, Losurdo P, Panizon E, Mearelli F, Cattin L, Barazzoni R, Carretta R. Omega-3 polyunsaturated fatty acids: structural and functional effects on the vascular wall. *Biomed Res Int* 2015;2015:791978.
28. Mozaffarian D, Wu JH. (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? *J Nutr* 2012;142:S614-25.
29. Hussein JS. Cell membrane fatty acids in health. *Int J Phram Pharm Sci* 2013;5:38-46.
30. Hashimoto M, Hossain MS, Yamasaki H, Yazawa K, Masumura S. Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. *Lipids* 1999;34:1297-304.
31. Owen DM, Magenau A, Williamson D, Gaus K. The lipid raft hypothesis revisited -- new insights on raft composition and function from super-resolution fluorescence microscopy. *Bioessays* 2012;34:739-47.
32. Ma DW, Seo J, Switzer KC, Fan YY, McMurray DN, Lupton JR, Chapkin RS. n-3 PUFA and membrane microdomains: a new frontier in bioactive lipid research. *J Nutr Biochem* 2004;15:700-6.
33. Jaureguiberry MS, Tricerri MA, Sanchez SA, Finarelli GS, Montanaro MA, Prieto ED, Rimoldi OJ. Role of plasma membrane lipid composition on cellular homeostasis: learning from cell line models expressing fatty acid desaturases. *Acta Biochim Biophys Sin (Shanghai)* 2014;46:273-82.
34. Hou TY, McMurray DN, Chapkin RS. Omega-3 fatty acids, lipid rafts, and T cell signaling. *Eur J Pharmacol* 2016;785:2-9.
35. Williams JA, Batten SE, Harris M, Rockett BD, Shaikh SR, Stillwell W, Wassall SR. Docosahexaenoic and eicosapentaenoic acids segregate differently between raft and nonraft domains. *Biophys J* 2012;103:228-37.
36. Levental KR, Lorent JH, Lin X, Skinkle AD, Surma MA, Stockenbojer EA, Gorfe AA, Levental I. Polyunsaturated lipids regulate membrane domain stability by tuning membrane order. *Biophys J* 2016;110:1800-10.
37. Cicero AF, Rosticci M, Morbini M, Cagnati M, Grandi E, Parini A, Borghi C. Lipid-lowering and anti-inflammatory effects of omega 3 ethyl esters and krill oil: a randomized, cross-over, clinical trial. *Arch Med Sci* 2016;12:507-12.
38. Raphael W, Sordillo LM. Dietary polyunsaturated fatty acids and inflammation: the role of phospholipid byosynthesis. *Int J Mol Sci* 2013;14:21167-88.
39. Tortosa-Caparros E, Navas-Carrillo, Marin F, Orenes-Piñero E. Anti-inflammatory effects of omega 3 and omega 3 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. *Crit Rev Food Sci Nutr* 2017;57:3421-9.
40. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol*

2013;75:645-62.
41. Puglisi MJ, Hasty AH, Saraswathi V. The role of adipose tissue in mediating the beneficial effects of dietary fish oil. *J Nutr Biochem* 2011;22:101-8.
42. Nakahira K, Kim HP, Geng XH, Nakao A, Wang X, Murase N, Borghi C. Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. *J Exp Med* 2006;203:2377-89.
43. Rockett BD, Salameh M, Carraway K, Morrison K, Shaikh SR. n-3 PUFA improves fatty acid composition, prevents palmitate-induced apoptosis, and differentially modifies B cell cytokine secretion in vitro and ex vivo. *J Lipid Res* 2010;51:1284-97.
44. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, Olefsky JM. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 2010;142:687-98.
45. Oh DY, Walenta E. The role of omega-3 fatty acid receptor GPR120 in insulin resistance. *Int J Obes Suppl* 2014;4:S14-6.
46. Oh DY, Walenta E. Omega-3 fatty acids and FFAR4. *Front Endocrinol (Lausanne)* 2014;5:115.
47. Stulnig TM, Huber J, Leitinger N, Imre EM, Angelisova P, Nowotny P, Waldhausl W. Polyunsaturated eicosapentaenoic acid displaces proteins from membrane rafts by altering raft lipid composition. *J Biol Chem* 2001;276:37335-40.
48. Bang HO, Dyerberg J. Lipid metabolism and ischemic heart disease in Greenland Eskimos. In: Draper HH, editor. Advances in Nutritional Research. New York, NY: Plenum Press; 1980. p. 1-22.
49. Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *Lancet* 1979;2:433-5.
50. Bang HO, Dyerberg J. The bleeding tendency in Eskimos. *Dan Med Bull* 1980;27:202-5.
51. Larson MK, Tormoen GW, Weaver LJ, Luepke KJ, Patel IA, Hjelmen CE. Exogenous modification of platelet membranes with the omega-3 fatty acids EPA and DHA reduces platelet procoagulant activity and thrombus formation. *Am J Physiol Cell Physiol* 2013;304:C273-9.
52. von Schacky C, Siess W, Fischer S, Weber PC. A comparative study of eicosapentaenoic acid metabolism by human platelets in vivo and in vitro. *J Lipid Res* 1985;26:457-64.
53. Hirai A, Hamazaki T, Terano T, Nishikawa T, Tamura Y, Kamugai A, Jajiki J. Eicosapentaenoic acid and platelet function in Japanese. *Lancet* 1980;2:1132-3.
54. Mohebi-Nejad A, Bikdeli B. Omega-3 supplements and cardiovascular diseases. *Tanaffos* 2014;13:6-14.
55. Lagarde M, Calzada C, Guichardant M, Vericel E. In vitro and in vivo bimodal effects of docosahexaenoic acid supplements on redox status and platelet function. *Prostaglandins Leukot Essent Fatty Acids* 2016;108:1-4.
56. Phang M, Lincz LF, Garg ML. Eicosapentaenoic and docosahexaenoic acid supplementations reduce platelet aggregation and hemostatic markers differentially in men and women. *J Nutr* 2013;143:457-63.
57. Walker CG, West AL, Browning LM, Madden J, Gambell JM, Jebb SA, Calder PC. The pattern of fatty acids displaced by EPA and DHA following 12 months supplementation varies between blood cell and plasma fractions. *Nutrients* 2015;7:6281-93.
58. Ayala J, Lopez C, Azueg H, Oberto C, Paiva A, Lares M. Effects of polyunsaturated fatty acids (omega-3) on the platelet aggregation. *Rev Latinoam Hiperte* 2009;4:71-8. (in Portuguese)
59. Parent CA, Lagarde M, Venton DL, Le Breton GC. Selective modulation of the human platelet thromboxane A2/prostaglandin H2 receptor by eicosapentaenoic and docosahexaenoic acids in intact platelets and solubilized platelet membranes. *J Biol Chem* 1992;267:6541-7.
60. Liu M, Boussetta T, Makni-Maalej K, Fay M, Driss F, El-Benna J, Lagarde M, Guichardant M. Protectin DX, a double lipoxygenase product of DHA, inhibits both ROS production in human neutrophils and cyclooxygenase activities. *Lipids* 2014;49:49-57.
61. Lagarde M, Calzada C, Guichardant M, Véricel E. Dose-effect and metabolism of docosahexaenoic acid. Pathophysiological relevance in blood platelets. *Prostaglandins Leukot Essent Fatty Acids* 2013;88:49-52.
62. Leray C, Wiesel ML, Freund M, Cazenave JP, Gachet C. Long-chain n-3 fatty acids specifically affect rat coagulation factors dependent on vitamin K. *Arterioscler Thromb Vasc Biol* 2001;21:459-65.
63. Vanschoonbeek K, Wouters K, van der Meijden PE, van Gorp PJ, Feijge MA, Herfs M, Schurgers LJ, Hofker MH, de Maat MP, Heemskerk JW. Anticoagulant effect of dietary fish oil in hyperlipidemia: a study of hepatic gene expression in APOE2 knock-in mice. *Arterioscler Thromb Vasc Biol* 2008;28:2023-9.
64. Vanschoonbeek K, Feijge MA, Paquay M, Rosing J, Saris W, Kluft C, Giesen PL, de Maat MP, Heemskerk JW. Variable hypocoagulant effect of fish oil intake in humans: modulation of fibrinogen level and thrombin generation. *Arterioscler Thromb Vasc Biol* 2004;24:1734-40.
65. Finnegan YE, Howarth D, Minihane AM, Kew S, Miller GJ, Calder PC, Williams CM. Plant and marine derived (n-3) polyunsaturated fatty acids do not affect blood coagulation and fibrinolytic factors in moderately hyperlipidemic humans. *J Nutr* 2003;133:2210-3.
66. Montegaard C, Tulk HM, Lauritzen L, Tholstrup T, Robinson LE. Acute ingestion of long-chain (n-3) polyunsaturated fatty acids decreases fibrinolysis in men with metabolic syndrome. *J Nutr* 2010;140:38-43.
67. Bang HO, Dyerberg J. Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. *Acta Med Scand* 1972;192:85-94.
68. Dyerberg J, Bang HO, Hjorne N. Plasma cholesterol concentration in Caucasian Danes and Greenland west coast Eskimos. *Dan Med Bull* 1977;24:52-5.
69. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis* 2006;189:19-30.
70. Balk E, Chung M, Lichtenstein A, Chew P, Kupelnick B, Lawrence A, DeVine D, Lau J. Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease. *Evid Rep Technol Assess (Summ)* 2004;(93):1-6.
71. Kelley DS, Siegel D, Vemuri M, Mackey BE. Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am J Clin Nutr* 2007;86:324-33.
72. Harris WS, Bulchandani D. Why do omega3 fatty acids lower serum triglycerids? *Curr Opin Lipidol* 2006;17:387-93.
73. Tillander V, Bjørndal B, Burri L, Bohov P, Skorve J, Berge RK, Alexson SE. Fish oil and krill oil supplementations differentially regulate lipid catabolic and synthetic pathways in mice. *Nutr Metab (Lond)* 2014;11:20.
74. Walczak R, Tontonoz P. PPARadigms and PPARadoxes: expanding rolesfor PPARg in the control of lipid metabolism. *J Lipid Res* 2002;43:177-86.
75. Shao W, Espenshade PJ. Expanding roles for SREBP in metabolism. *Cell Metab* 2012;16:414-9.
76. Landa V, Zídek V, Mlejnek P, Šimáková M, Šilhavý J, Trnovská J, Kazdová L, Pravenec M. Sterol regulatory element binding protein 2 overexpression is associated with reduced adipogenesis and ectopic fat accumulation in transgenic spontaneously hypertensive rats. *Physiol Res* 2014;63:587-90.
77. Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R, Goldstein JL, Brown MS. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 2002;110:489-500.

78. Watanabe M, Uesugi M. Small-molecule inhibitors of SREBP activation - potential for new treatment of metabolic disorders. *Med Chem Comm* 2013;4:1422-33.
79. Dentin R, Benhamed F, Pégorier JP, Foufelle F, Viollet B, Vaulont S, Girard J, Postic C. Polyunsaturated fatty acids suppress glycolytic and lipogenic genes through the inhibition of ChREBP nuclear protein translocation. *J Clin Invest* 2005;115:2843-54.
80. Packard CJ, Demant T, Stewart JP, Bedford D, Caslake MJ, Schwertfeger G, Bedynek A, Shepherd J, Seidel D. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J Lipid Res* 2000;41:305-18.
81. Fisher EA, Pan M, Chen X, Wu X, Wang H, Jamil H, Sparks JD, Williams KJ. The triple threat to nascent apolipoprotein B. Evidence for multiple, distinct degradative pathways. *J Biol Chem* 2001;276:27855-63.
82. Pan M, Cederbaum AI, Zhang YL, Ginsberg HN, Williams KJ, Fisher EA. Lipid peroxidation and oxidant stress regulate hepatic apolipoprotein B degradation and VLDL production. *J Clin Invest* 2004;113:1277-87.
83. Jacobson TA, Glickstein SB, Rowe JD, Soni PN. Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review. *J Clin Lipidol* 2012;6:5-18.
84. Maggi F, Sirtori CR, Baldassarre D, Rise P, Galli C. Correlations of n-3 (omega-3) fatty acid levels with lipoproteins: negative correlation with HDL-C after an oral load. *Nutrafods* 2013;12:13-6.
85. Raposo HF, Patrício PR, Simões MC, Oliveira HC. Fibrates and fish oil, but not corn oil, up-regulate the expression of the cholesteryl ester transfer protein (CETP) gene. *J Nutr Biochem* 2014;25:669-74.
86. Chan D, Watts GF, Nguyen MN, Barrett PH. Factorial study of the effect of n-3 fatty acid supplementation and atorvastatin on the kinetics of HDL apolipoproteins A-I and A-II in men with abdominal obesity. *Am J Clin Nutr* 2006;84:37-43.
87. Ooi E, Lichtenstein A, Millar J, Diffenderfer M, Lamon-Fava S, Rasmussen H, Welty FK, Barrett PH, Schaefer EJ. Effects of therapeutic lifestyle change diets high and low in dietary fish-derived FAs on lipoprotein metabolism in middle-aged and elderly subjects. *J Lipid Res* 2012;53:1958-67.
88. Fialkow J. Omega-3 fatty acid formulations in cardiovascular disease: dietary supplements are not substitutes for prescription products. *Am J Cardiovasc Drugs* 2016;16:229-39.
89. Mason R, Sherratt S, Jacob R. Eicosapentaenoic acid inhibits oxidation of ApoB-containing lipoprotein particles of different size in vitro when administered alone or in combination with atorvastatin active metabolite compared with other triglyceride-lowering agents. *J Cardiovasc Pharmacol* 2016;68:33-40.
90. Borow K, Nelson J, Mason R. Biologic plausibility, cellular effects, and molecular mechanisms of eicosapentaenoic acid (EPA) in atherosclerosis. *Atherosclerosis* 2015;242:357-66.
91. Yamada N, Shames DM, Stoudemire JB, Havel RJ. Metabolism of lipoproteins containing apolipoprotein B-100 in blood plasma of rabbits: heterogeneity related to the presence of apolipoprotein E. *Proc Natl Acad Sci U S A* 1986;83:3479-83.
92. Fernandez ML, McNamara DJ. Dietary fat-mediated changes in hepatic apoprotein B/E receptor in the guinea pig: effect of polyunsaturated, monounsaturated, and saturated fat. *Metabolism* 1989;38:1094-102.
93. McInnes GT. Lowering blood pressure for cardiovascular risk reduction. *J Hypertens Suppl* 2005;23:S3-8.
94. Huang T, Shou T, Cai N, Wahlqvist ML, Li D. Associations of plasma n-3 polyunsaturated fatty acids with blood pressure and cardiovascular risk factors among Chinese. *Int J Food Sci Nutr* 2012;63:667-73.
95. Ebbesson SO, Risica PM, Ebbesson LO, Kennish JM, Tejero ME. Omega-3 fatty acids improve glucose tolerance and components of the metabolic syndrome in Alaskan Eskimos: the Alaska Siberia project. *Int J Circumpolar Health* 2005;64:396-408.
96. Rasmussen BM, Vessby B, Uusitupa M, Berglund L, Pedersen E, Riccardi G, Rivellese AA, Tapsell L, Hermansen K; KANWU Study Group. Effects of dietary saturated, monounsaturated, and n-3 fatty acids on blood pressure in healthy subjects. *Am J Clin Nutr* 2006;83:221-6.
97. Cabo J, Alonso R, Mata P. Omega-3 fatty acids and blood pressure. *Br J Nutr* 2012;107 Suppl 2:S195-200.
98. Ulu A, Harris TR, Morisseau C, Miyabe C, Inoue H, Schuster G, Dong H, Iosif AM, Liu J, Weiss RH, Chiamvimonvat N, Imig JD, Hammock BD. Anti-inflammatory effects of omega-3 polyunsaturated fatty acids and soluble epoxide hydrolase inhibitors in angiotensin-II dependent hypertension. *J Cardiovasc Pharmacol* 2013;62:285.
99. Shao Z, Fu Z, Stahl A, Joyal JS, Hatton C, Juan A, Hurst C, Evans L, Cui Z, Pei D, Gong Y, Xu D, Tian K, Bogardus H, Edin ML, Lih F, Sapieha P, Chen J, Panigrahy D, Hellstrom A, Zeldin DC, Smith LE. Cytochrome P450 2C8 ω3-long-chain polyunsaturated fatty acid metabolites increase mouse retinal pathologic neovascularization--brief report. *Arterioscler Thromb Vasc Biol* 2014;34:581-6.
100. Agbor LN, Wiest EF, Rothe M, Schunck WH, Walker MK. Role of CYP1A1 in modulating the vascular and blood pressure benefits of omega-3 polyunsaturated fatty acids. *J Pharmacol Exp Ther* 2014;351:688-98.
101. Hoshi T, Wissuwa B, Tian Y, Tajima N, Xu R, Bauer M, Heinemann SH, Hou S. Omega-3 fatty acids lower blood pressure by directly activating large-conductance Ca2+-dependent K+ channels. *Proc Natl Acad Sci U S A* 2013;110:4816-21.
102. Wakle-Prabagaran M, Lorca RA, Ma X, Stamnes SJ, Amazu C, Hsiao JJ, Karch CM, Hyrc KL, Wright ME, England SK. BKCa channel regulates calcium oscillations induced by alpha-2-macroglobulin in human myometrial smooth muscle cells. *Proc Natl Acad Sci U S A* 2016;113:2335-44.
103. Novgorodtseva TP, Karaman YK, Gvozdenko TA, Antonyuk MV, Knyshova VV. Modification of fatty acids composition in erythrocytes lipids in arterial hypertension associated with dyslipidemia. *Health* 2013;5:73-7.
104. Klein-Platat C, Drai J, Oujaa M, Schlienger JL, Simon C. Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. *Am J Clin Nutr* 2005;82:1178-84.
105. Nodari S, Triggiani M, Campia U, Manerba A, Milesi G, Cesana BM. Effects of n-3 polyunsaturated fatty acids on left ventricular function and functional capacity in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 2011;57:870-9.
106. del Monte F, Harding SE, Schmidt U, Matsui T, Kang ZB, Dec GW, Gwathmey JK, Rosenzweig A, Hajjar RJ. Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation* 1999;100:2308-11.
107. Ribeiro Junior RF, Fernandes AA, Meira EF, Batista PR, Siman FD, Vassallo DV, Padilha AS, Stefanon I. Soybean oil increases SERCA2a expression and left ventricular contractility in rats without change in arterial blood pressure. *Lipids Health Dis* 2010;9:53.
108. Liu J, Conklin S, Manuck S, Yao J, Muldoon M. Long-chain omega-3 fatty acids and blood pressure. *Am J Hypertens* 2011;24:1121-6.
109. McLennan P. Myocardial membrane fatty acids and the antiarrhythmic actions of dietary fish oil in animal models. *Lipids* 2001;36:S111-4.
110. Mori T, Bao D, Burke V, Puddey I, Beilin J. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension* 1999;34:253-60.
111. Tsuburaya R, Yasuda S, Ito Y, Shiroto T, Gao J, Ito K, Shimokawa H. Eicosapentaenoic acid reduces ischemic ventricular fibrillation

via altering monophasic action potential in pigs. *J Mol Cell Cardiol* 2011;51:329-36.
112. Schwinger RH, Bundgaard H, Muller-Ehmsen J, Kjeldsen K. The Na+, K+- ATPase in the failing human heart. *Cardiovasc Res* 2003;57:913-20.
113. Lee DI, Klein MG, Zhu W, Xiao RP, Gerzanich V, Xu KY. Activation of (Na+- K+)-ATPase modulates cardiac L-type Ca2+ channel function. *Mol Pharmacol* 2009;75:774-81.
114. Leaf A, Kang J, Xiao Y, Billman G. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. *Circulation* 2003;107:2646-52.
115. Kromhout D, Yasuda S, Geleijnse J, Shimokawa H. Fish oil and omega 3 fatty acids in cardiovascular disease: do they really work? *Eur Heart J* 2012;33:436-43.
116. Xiao Y, Sigg D, Ujhelyi M, Wilhelm J, Richardson E, Iaizzo P. Pericardial delivery of omega-3 fatty acid: a novel approach to reducing myocardial infarct sizes and arrhythmias. *Am J Physiol Heart Circ Physiol* 2008;294:2212-8.
117. Xiao YF, Sigg DC, Leaf A. The antiarrhythmic effect of n-3 polyunsaturated fatty acids: modulation of cardiac ion channels as a potential mechanism. *J Membr Biol* 2005;206:141-54.
118. Endo J, Arita M. Cardioprotective mechanism of omega-3 polyunsaturated fatty acids. *J Cardiol* 2016;67:22-7.
119. Xiao Y, Ma L, Wang S, Josephson M, Wang G, Morgan J, Leaf A. Potent block of inactivation deficient Na+ channels by n-3 polyunsaturated fatty acids. *Am J Physiol Cell Physiol* 2006;290:362-70.
120. Li G, Sun H, Zhang X, Cheng L, Chiu S, Tse H, Lau CP. Omega-3 polyunsaturated fatty acids inhibit transient outward and ultra-rapid delayed rectifier K+ currents and Na+ current in human atrial myocytes. *Cardiovasc Res* 2009;81:286-93.
121. Moreno C, Macías Á, Prieto Á, de la Cruz A, González T, Valenzuela C. Effects of n-3 polyunsaturated fatty acids on cardiac ion channels. *Front Physiol* 2012;3:245.
122. Kang JX, Xiao YF, Leaf A. Free, long-chain, polyunsaturated fatty acids reduce membrane electrical excitability in neonatal rat cardiac myocytes. *Proc Natl Acad Sci U S A* 1995;92:3997-4001.
123. Piñeiro-Corrales G, Rivero N, Culebras F. Role of omega-3 fatty acids in cardiovascular disease prevention. *Nutr Hosp* 2013;28:1-5.
124. Marai I, Massalha S. Effect of omega-3 polyunsaturated fatty acids and vitamin d on cardiovascular diseases. *Isr Med Assoc J* 2014;16:117-21.
125. Christensen J, Schmidt E. Autonomic nervous system, heart rate variability and n-3 fatty acids. *J Cardiovasc Med (Hagerstown)* 2007;8:S19-22.
126. Dlugosova K, Okruhlicova L, Mitasikova M, Sotnikova R, Bernatova I, Weismann P, Slezak J, Tribulova N. Modulation of connexin-43 by omega-3 fatty acids in the aorta of old spontaneously hypertensive rats. *J Physiol Pharmacol* 2009;60:63-9.
127. Fischer R, Dechend R, Qadri F, Markovic M, Feldt S. Dietary n-3 polyunsaturated fatty acids and direct renin inhibition improve electrical remodeling in a model of high human renin hypertension. *Hypertension* 2008;51:540-6.
128. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007;357:1121-35.
129. Hausenloy DJ, Duchen MR, Yellon DM. Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischemia-reperfusion injury. *Cardiovasc Res* 2003;60:617-25.
130. Bei R, Frigiola A, Masuelli L, Marzocchella L, Tresoldi I, Modesti A, Galvano F. Effects of omega-3 polyunsaturated fatty acids on cardiac myocyte protection. *Front Biosci (Landmark Ed)* 2011;16:1833-43.
131. Pepe S, Tsuchiya N, Lakatta EG, Hansford RG. PUFA and aging modulate cardiac mitochondrial membrane lipid composition and Ca2_ activation of PDH. *Am J Physiol* 1999;276:149-58.
132. Pepe S, McLennan P. Cardiac membrane fatty acid composition modulates myocardial oxygen consumption and postischemic recovery of contractile function. *Circulation* 2002:105:2303-8.
133. Abdukeyum G, Owen A, Larkin T, McLennan P. Up-regulation of mitochondrial antioxidant superoxide dismutase underpins persistent cardiac nutritional-preconditioning by long chain n-3 polyunsaturated fatty acdis in the rat. *J Clin Med* 2016;5:E32.
134. Arakawa K, Himeno H, Kirigaya J, Otomo F, Matsushita K, Nakahashi H, Shimizu S, Nitta M, Yano H, Endo M, Kimura K, Umemura S. Impact of n-3 polyunsaturated fatty acids in predicting ischemia/reperfusion injury and progression of myocardial damage after reperfusion in patients with ST-segment elevation acute myocardial infarction. *J Cardiol* 2015;66:101-7.
135. Hausenloy D, Yellon D. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest* 2013;123:92-100.
136. Ibañez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. *J Am Coll Cardiol* 2015;65:1454-71.
137. Neri M, Riezzo I, Pascale N, Pomara C, Turillazzi E. Ischemia/reperfusion injury following acute myocardial infarction: a critical issue for clinicians and forensic pathologists. *Mediators Inflamm* 2017;2017:7018393.
138. Farías JG, Carrasco-Pozo C, Carrasco Loza R, Sepúlveda N, Álvarez P, Quezada M, Quiñones J, Molina V, Castillo RL. Polyunsaturated fatty acid induces cardioprotection against ischemia-reperfusion through the inhibition of NF-kappaB and induction of Nrf2. *Exp Biol Med (Maywood)* 2017;242:1104-14.

Gowdar *et al. Vessel Plus* 2017;1:129-36
DOI: 10.20517/2574-1209.2017.12

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Review **Open Access**

Probable protective role of diabetes mellitus in takotsubo cardiomyopathy: a review

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How to cite this article: Gowdar S, Syal S, Chhabra L. Probable protective role of diabetes mellitus in takotsubo cardiomyopathy: a review. *Vessel Plus* 2017;1:129-36.

Article history:
Received: 18 Apr 2017
Accepted: 24 Jul 2017
Published: 26 Sep 2017

Key words:
Diabetes mellitus,
stress cardiomyopathy,
takotsubo cardiomyopathy

ABSTRACT

Takotsubo cardiomyopathy (TC) is a syndrome that predominantly affects postmenopausal women and is characterized by transient regional systolic dysfunction of the left ventricle which occurs in the absence of angiographic evidence of significant obstructive coronary artery disease. It is often but not always triggered by emotional or physical stressful stimuli. In most cases, the regional ventricular dysfunction extends beyond a single epicardial coronary artery territory. It typically involves the apex, with rare atypical presentations involving the base and right ventricle. Although the pathophysiological underpinnings of TC have not been completely elucidated, possible mechanisms include catecholamine overactivity, diffuse multivessel coronary spasm, microvascular dysfunction and estrogen deficiency. The prevalence of diabetes mellitus has been noted to be low in multiple studies of patients with TC. In this review, the authors discuss the association between diabetes mellitus and TC, with a special emphasis on the possible protective effect of diabetes mellitus in development of TC.

INTRODUCTION

Takotsubo cardiomyopathy (TC), also known as apical ballooning syndrome or broken heart syndrome, is a syndrome characterized by transient regional systolic dysfunction of the left ventricle that occurs in the absence of angiographic evidence of significant obstructive coronary artery disease (CAD).[1,2] It is usually but not always triggered by emotional or physical stressful stimuli. It is predominantly seen in postmenopausal women. The syndrome may acutely mimic as an acute coronary syndrome; however the regional left ventricular dysfunction extends often beyond a single epicardial coronary artery territory. The first case was reported by Sato in Japan in 1990,[3] and since then, this syndrome has been increasingly recognized around the globe. The typical and most common presentation consists of apical dysfunction leading to apical ballooning appearance on coronary angiography, with basal hypercontractility. Atypical variants/presentations of TC are rare, and include transient dysfunction of basal, mid ventricular or right ventricular myocardium, with apical sparing. Although the pathophysiological underpinnings of TC have not been completely elucidated, possible mechanisms include catecholamine over-activity causing myocardial

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stunning, diffuse multi-vessel coronary spasm, microvascular dysfunction and estrogen deficiency.[4]

An increased catecholamine surge leading to exaggerated cardiac sympathetic stimulation has long been thought to be a plausible mechanism of TC pathogenesis. Animal studies using rat models have provided further evidence that left ventricular apical or midventricular dysfunction in TC patients could be a result of catecholamine mediated effects on cardiac beta receptors, particularly the pleiotropic β2 receptors. This is related to epinephrine and isoprenaline mediated switching of beta receptor activation from cardiostimulation to cardioinhibitory pathways, via the activation of Gi proteins, instead of Gs proteins.[5] Exaggerated sympathetic stimulation has been inferred from markedly elevated plasma catecholamine levels, both epinephrine and norepinephrine, in TC patients.[5] The specific mechanism of catecholamine mediated myocardial stunning is unclear, with possible mechanisms being decreased myocardial blood flow or direct catecholamine myocardial toxicity. Coronary microvascular dysfunction leading to impaired microcirculatory perfusion and myocardial stunning has also been thought to play a significant role in the development of TC.[4,6,7] Human and animal studies have illustrated the primary role of autonomic nerves innervating the heart and a secondary role of the adrenal medulla with sympathetic and parasympathetic influences on neurovisceral myocardial injury.[5] Neural disconnection between the brain and the heart in brain death and cardiac transplantation have been shown to blunt neuromyocardial damage and cause an amelioration of electrocardiographic abnormalities.[5] Similarly, diabetes mellitus (DM) is associated with autonomic neuropathy and thus may exert an independent potential influence on the pathogenesis of TC.[1] This article aims to review the association between DM and TC, based on the knowledge gained from the recent studies.

DATA

We searched PubMed/Medline, Scopus and Google Scholar for original articles published between 1990 and 2016, focusing on TC and DM.[1] The keywords used to conduct the relevant literature search, alone and/or in combination, were “takotsubo cardiomyopathy”, “stress cardiomyopathy”, “apical ballooning syndrome”, “diabetes”, “diabetes mellitus”, “prevalence”, “incidence” and “association”. All articles published in the English language were then independently reviewed. Papers included were original research, review, case reports and relevant correspondences. After a comprehensive review of the articles selected, we used literature relevant to our current discussion.

Retrospective studies

A large international collaborative systematic review sought to evaluate the prevalence of comorbidities, cardiovascular and others, in TC patients.[8] The authors evaluated 19 large case series between 2007 and 2013 which included 1,109 patients with TC. Among the 1,109 patients with TC (86% females), hypertension was present in 54% (range 27-83%), dyslipidemia in 32% (range 7-59%), but DM in only 17% (prevalence ranging between 4-34%). Of all the 19 studies, 11 demonstrated DM prevalence to be < 14%, with 6 studies demonstrating a prevalence rate of DM < 10%. Although there was no control group for comparison of the TC patients to the general population, or to the population of acute myocardial infarction (MI) patients, the authors concluded that traditional cardiovascular risk factors are commonly prevalent in TC patients, with frequencies similar to those seen in acute MI patients.[8] However, the prevalence of DM in this study appeared to correlate with the rates demonstrated by a large metanalysis,[5] which led to support the hypothesis of DM being a protective factor in TC patients.

A Spanish study comprising 328 TC patients compared the “primary” (265 patients) and “secondary” (63 patients) forms of TC.[9] Primary TC was described as that triggered by a psychic stress or without stressful stimulus. Additionally, secondary TC was described as the one triggered by the presence of physical stressors such as sepsis, intracranial hemorrhage or cerebrovascular accident, severe trauma, bronchospasm, surgery or other critical illnesses. Patients with secondary TC forms demonstrated more hospital complications, higher major adverse cardiac events, higher mortality and higher rates of recurrence. The prevalence of hypertension was 68.3% in the primary TC cohort and 60.3% in the secondary TC cohort. Dyslipidemia was seen in 44.9% and 41.2% in the primary and secondary forms of TC respectively. The prevalence of DM was overall low (13.1%), and the prevalence in the primary and secondary forms of TC, was 12.8% and 14.2%, respectively. Hypertension was slightly more and DM was slightly less prevalent in the “primary” TC group compared to the “secondary” TC group.[9,10] Thus, this study further underscores the low rates of DM in TC patients.

In another small retrospective study, the role of coronary microvascular function in patients with TC was compared with controls without CAD, using invasive angiography and TIMI frame counts.[11] The prevalence of DM was low (only 6.25%), again suggesting that the presence of DM may confer a protective effect in the

development of TC.

In this retrospective study based on the National Inpatient Samples (NIS) Database 2008 to 2009, a total of 24,701 patients with takotsubo cardiomyopathy were identified (mean age was 66.9 ± 30.7 years with patients aged > 64 years comprising 59.6% of the group).[12] Among the patients with TC, 89% were females and 11% males. Although the primary outcome of the study was in-patient mortality but other associated chronic comorbidities were also reported. The prevalence of hypertension and hyperlipidemia was found to be 58.4% and 37.5% respectively. And the prevalence of diabetes mellitus was 18.9%, which was lower than the prevalence of diabetes in the general population group aged ≥ 65 years (25.9% in general population aged > 65 years, National Diabetes Statistics Report 2014).

In a retrospective study and analysis of the electronic medical records of patients diagnosed with apical ballooning syndrome or stress cardiomyopathy at Mayo Clinic, a total of 224 patients were included (94.6% were females and mean age 71.7 ± 10.4 years).[13] Hypertension was present in 70.9% of the patients while diabetes was present in only a small percentage of the patients (13.8%). Again, this study supports the protective role of diabetes in TC.

In a different retrospective descriptive study reviewing patients with the discharge diagnosis of TC between 2003 and 2014 at Einstein Medical Center in Philadelphia, a total of 206 TC patients were identified.[14] Overall mean age was 67.8 years. Out of the 206 patients, 179 (87%) were females and 41 (19%) had diabetes. As compared to the low prevalence of diabetes in these patients, the prevalence of hypertension was seen to be much higher (68.4%).

In a retrospective study of 5,484 patients referred to the coronary care unit for acute coronary syndrome (ACS) between 2001 and 2013, the clinical records were studied and reviewed.[15] Out of this, 90 patients were found to have TC. The mean age of patients with TC was 71.9 ± 12.7 years and 97% were females. While the prevalence of hypertension was comparable in both groups (46% and 47% in TC and ACS group), DM was present in only 9% of TC patients as compared to 17% of patients with ACS. A lower prevalence of DM in TC again signifies its protective effect in the disease.

In another study utilizing the NIS Database data 2007 to 2012, Khera *et al*.[16] identified 22,005 patients who were discharged with a primary diagnosis of TC in the US. The number of hospitalizations was seen to have increased nearly 3-fold, from 1,642 cases in 2007 to 5,480 cases in 2012. The mean age of patients with TC was 65.7 years and more than 9 out of 10 (92.0%) were women. Whereas hypertension was present in 64.2% patients (ranging 59.2-67.6%), uncomplicated DM was seen in only 18% of the patients (ranging 15.6-19.3%). On the other hand, complicated DM was found to be present in even a lower percentage of TC patients (only 2.6%, ranging 1.4-3.2%). This study strongly supports the role of diabetic autonomic neuropathy/end-organ damage protecting the heart from catecholamine damage in TC.

Prospective studies

In a multi-center study conducted prospectively in Europe and North America between January 2005 and October 2010, 256 patients with TC were included and assessed at initial presentation as well as 1-6 months after the acute event (mean age 69 years and 89% females).[17] Whereas hypertension was noted in 73% of the patients, DM was noted in only 19% of the patients. This data also depicts a low prevalence of DM in TC.

Another prospective study comprising 100 patients (between 2002-2010) was conducted to determine the incidence of TC and associated risk factors for the development of heart failure in TC.[18] The mean age was 68 years and 89% were post-menopausal women. While hypertension was present in 68% of the patients with TC, DM was present in only 18% of the patients. As seen in other studies, this study also suggests a protective role of DM in TC.

Registries

In a large multicenter prospective registry (RETAKO registry, Spain), 202 patients with TC were studied (90% females, mean age 70 years).[19] The incidence of DM was 15% while the prevalence of other standard cardiac risk factors including hypertension and dyslipidemia were 69% and 41% respectively. This study again suggested a relative low prevalence of DM in TC.

A more recent study utilizing the data from the International Takotsubo registry compared 455 patients with TC to age and gender matched patients with ACS (mean age 67.7 ± 12.5 years and 68.7 ± 12.3 years, respectively and 90.3% women in both cohorts).[20] DM prevalence was 12.8% in the TC group, and 26.6% in the ACS cohort. Prevalence of hypertension was comparable among both the cohorts (about 65% in each cohort).[20,21] Thus, this study further strengthens the hypothesis of DM being possibly protective against the development of TC.

In this partial retrospective and partial prospective study, 190 patients enrolled in the Takotsubo Italian Network Registry were selected (mean age 66.0 ± 11.4 years).[22] Out of these 190 patients, 175 were female (92%). As compared to the prevalence of hypertension, which was found to be 48.4%, the prevalence of diabetes was found to be very low (5.7%). Thus, this study also favors the notion of DM playing a protective role in TC.

Case control studies

In a case-control study utilizing the NIS 2008-2009, Falola *et al*.[23] identified 1,724 patients with TC (average age 65.3 years) as cases. When cases were compared with controls [patients admitted with ST-Elevation myocardial infarction (STEMI) for initial care], it was seen that females comprised 90.2% of the patients in the TC group *vs*. only 35.7% in the STEMI (control) group. The prevalence of hypertension was noted to be 58.6% and 66% among the cases and control groups respectively. On the other hand, diabetes was considered as a protective factor for TC as the prevalence of diabetes in TC cases was quite low (1.6%). DM prevalence was significantly lower in TC patients when compared to those with STEMI (odds ratio 0.3, $P < 0.0001$).[23]

In another case control study, a total of 505 TC patients were identified from the Swedish Angiography and Angioplasty Registry between 2009 and 2013 using the Mayo Clinic criteria.[24] The TC patients were matched for age and gender with controls with and without CAD. All the patients presented with an acute event/chest pain and underwent coronary angiography to identify the underlying cause. Among the 505 patients with TC, 442 (87.5%) were women with a mean age of 67 ± 10 years. Only 33 patients with TC (6.5%) had DM as compared to 200 patients (19.8%) with CAD, which further supports that DM plays a protective role in TC.

In another Polish case-control study, 101 patients hospitalized with TC were studied.[25] The control group consisted of 101 female patients diagnosed with anterior myocardial infarction with STEMI. The mean age was 67.6 ± 14.2 years in the TC group *vs*. 72.1 ± 13.1 years in the control group. Females comprised 89.5% of the TC group. Whereas the prevalence of hypertension was comparable in both the groups (63.2% in the TC group and 68.3% in the control group), DM prevalence was significantly lower in those with TC (12.6% in the TC group and 29.7% in the control group).

Meta-analysis

In a meta-analysis of 959 papers including 794 single or multiple patient case reports and 165 case series, comprising a total of 33,894 TC patients, the prevalence of DM and hypertension was evaluated.[5] The identification of the prevalence of DM was the primary purpose of this analysis, with the prevalence of hypertension used as an index of representativeness of the TC patients to the general population. Five sub-analysis were performed in this study. In the first analysis, which included all TC patients including single case reports and small and large case series, comprising 33,894 patients (mean age 67.3 years and 89% females), 57.4% had hypertension, while the prevalence of DM was only 16.8%. In the second analysis (included only data from single patient case reports or patient series with patient data reported individually), comprising 1,085 patients (mean age 61.7 years and 86.3% females), 42.8% had hypertension and only 10.2% had DM. In the third analysis, using patient case series with patient data reported collectively and excluding individually reported patients and comprising 32,809 patients (mean age 67.3 years, 89% females), 57.9% had hypertension while the prevalence of DM was 17%. In the fourth analysis (TC patients aged > 60 years, from case reports and case series with patient data reported individually) of 687 patients (mean age 72.2 years and 89% females), 50.4% had hypertension while the prevalence of DM was 11.9%. Finally, in the fifth analysis (TC patients > 65 years from singe patient case reports and case series in which data on patients were reported individually), comprising 550 TC patients (mean age 74.6 years and 91.1% females), 52.2% had hypertension while only 12.5% had DM. All five analyses in this study thus consistently indicated a high prevalence of hypertension (> 50%), similar to the global and US rates of hypertension prevalence (prevalence of age adjusted hypertension was 65.4% for those > 60 years in the National Health and Nutrition Evaluation Survey or NHANES study and 60-65% globally). Given that hypertension prevalence was used an index of representativeness of the TC patients to the general population in this meta-analysis, it was assumed that the TC patient population was a comparable representative of the general population. Conversely, in all 5 analyses, DM had a low prevalence, with prevalence rates of 16.8%, 10.2%, 17%, 11.9% and 12.5%, respectively. These prevalence rates were significantly lower than prevalence rates of DM in similar age matched populations. Furthermore, this study was a global analysis, using TC patients from the entire world literature of TC. Global estimates of DM prevalence in the elderly is around 20%, and in this study, was 11.9% and 12.5% in the individual case analyses of patients over 60 and 65 years of age, respectively. Thus, the prevalence of DM in the TC patients was approximately half the expected prevalence rate of DM. In addition, in an analysis of 14 case series, with each case series comprising over

100 TC patients, the prevalence of DM ranged from 1.6% to 25.5%. The prevalence of DM in 9 out of the 14 patient series was < 16%, lower than the rates in the US and the global population. In addition, the prevalence of DM in 5 of the 14 patient series was < 9%, which is only one third the prevalence of DM in the general population over 65 years of age. The prevalence of hypertension ranged from 27.0% to 73.0%, and was over 50% in 10 of the 14 series, which is similar to the rates of hypertension prevalence in the US and the global population. In conclusion, results of all sub-analyses of the study demonstrated a compelling evidence to suggest a much lower prevalence of DM in TC and are suggestive of a potential protective effect of DM in the development of TC [Table 1].

Table 1: Diabetes mellitus prevalence in studies of takotsubo cardiomyopathy patients

Authors	No. of patients	Age (years), mean ± SD or range, (% of female)	DM prevalence (%)
Pelliccia *et al.*[8]*	1,109	59-76 (86)	17*
Núñez-Gil *et al.*[9]	328	69.7 ± 12.6 (90.2)	13.1
Khalid *et al.*[11]	16	68.3 ± 10.9 (100)	6.25
Brinjikji *et al.*[12]	24,701	66.9 ± 30.7 (89.0)	18.9
Patel *et al.*[13]	224	71.7 ± 10.4 (94.6)	13.8
Dias *et al.*[14]	206	67.8 (87)	19.0
Auzal *et al.*[15]	90	71.9 ± 12.7 (97)	9.0
Khera *et al.*[16]	22,005	65.7 (92.0)	18.0^, 2.6°
Eitel *et al.*[17]	256	69 ± 12 (89)	19.0
Núñez-Gil *et al.*[18]	100	68 (89)	18.0
Núñez-Gil *et al.*[19]	202	70 ± 12.5 (90.1)	15.3
Templin *et al.*[20]	1750	66.4 ± 13.1 (89.8)	14.2
Citro *et al.*[22]	190	66 ± 11.4 (92)	5.7
Falola *et al.*[23]	1,724	65.3 (90.2)	1.6
Tornvall *et al.*[24]	505	67 ± 10 (87.5)	6.5
Zalewska-Adamiec *et al.*[25]	101	67.6 ± 4.2 (89.5)	12.6
Madias[5]†			
Analysis 1	33,894	67.3 ± 6.0 (88.9)	16.8
Analysis 2	1,085	61.7 ± 16.7 (86.3)	10.2
Analysis 3	32,809	67.3 ± 6.0 (89.0)	17.0
Analysis 4	687	72.2 ± 7.7 (89.5)	11.9
Analysis 5	550	74.6 ± 6.6 (91.1)	12.5
Sara et al.[33]	1,439	51.1 (65.1)	8.8

*Systematic review including 19 studies; †analysis 1 - all patients included in meta-analysis, analysis 2 - individual patient cases, analysis 3 - patient case series, analysis 4 - individual patient cases > 60 years, analysis 5 - individual patient cases > 65 years; ^prevalence of uncomplicated diabetes; °prevalence of complicated diabetes. DM: diabetes mellitus

DISCUSSION

This data raises a possibility that sympathetic blockade and sympathectomy may be effective in preventing stress related cardiac dysfunction.[26,27] The protective effect of DM in TC may be secondary to autonomic neuropathy and/or hypo secretion of catecholamines in diabetic patients.[26-30] Diabetic neuropathy can affect up to 50% of patients with both type-1 and type-2 DM, with reduction of counter regulatory catecholamine secretion.[5] The pathogenesis of TC is thought to involve an autonomic or catecholamine storm, with primarily locally released catecholamines and blood borne systemic catecholamines. Since cardiac autonomic innervation is extensive, the catecholamine toxicity results in neurocardiac deleterious effects and myocardial stunning. Autonomic neuropathic changes in diabetics result in neuropathic changes in splanchnic autonomic sympathetic nerves or in adrenal chromaffin cells which are innervated by these autonomic nerves, and result in hyposecretion of epinephrine by the adrenals. Diabetic patients may also have autonomic neuropathy of cardiac sympathetic nerves. Clinical studies have documented reduced norepinephrine release in cardiac tissue in patients with type-2 DM and this has also been seen in rat models.[5] Thus, DM may serve as a protective factor in the development of TC with blunting of cardiac and splanchnic autonomic nervous system effects, with reduced local cardiac norepinephrine release and reduced systemic epinephrine release [Figure 1].[28,29] Animal studies showing the beneficial effects of sympathetic blockade on prevention of development of TC may further support the sympathetic/catecholamine surge model of pathogenesis of TC.[31]

One may thus speculate that diabetic patients with more severe disease or prolonged disease duration may be comparatively more immune towards the development of TC.[10,29,30] However, almost certainly, an interplay of factors such as DM, magnitude of the stressful stimulus (physical and emotional), presence and severity of the associated comorbid conditions and illnesses, would collaboratively play a role in the pathogenesis of TC. Thus, severe stressful triggers, physical and emotional and/or severe illness such as sepsis could still precipitate TC even in patients with severe or prolonged DM. This is supported by the fact that cases of development of TC have been previously reported in diabetic patients with severe sepsis and those with situations of overwhelming stress such as diabetic ketoacidosis,[32] suggesting that these conditions may overpower the protective effect of DM.

Coronary microvascular dysfunction is another mechanism which has been increasingly recognized as a significant factor in the pathogenesis of TC.[4,6,7] The importance of coronary microvascular dysfunction is also well established in Syndrome X. In a recent study of 1,439 patients (mean age 51 years, 65.1% women) with chest pain and non-obstructive CAD on coronary angiography, the presence of microvascular dysfunction was assessed.[33] Intracoronary Doppler

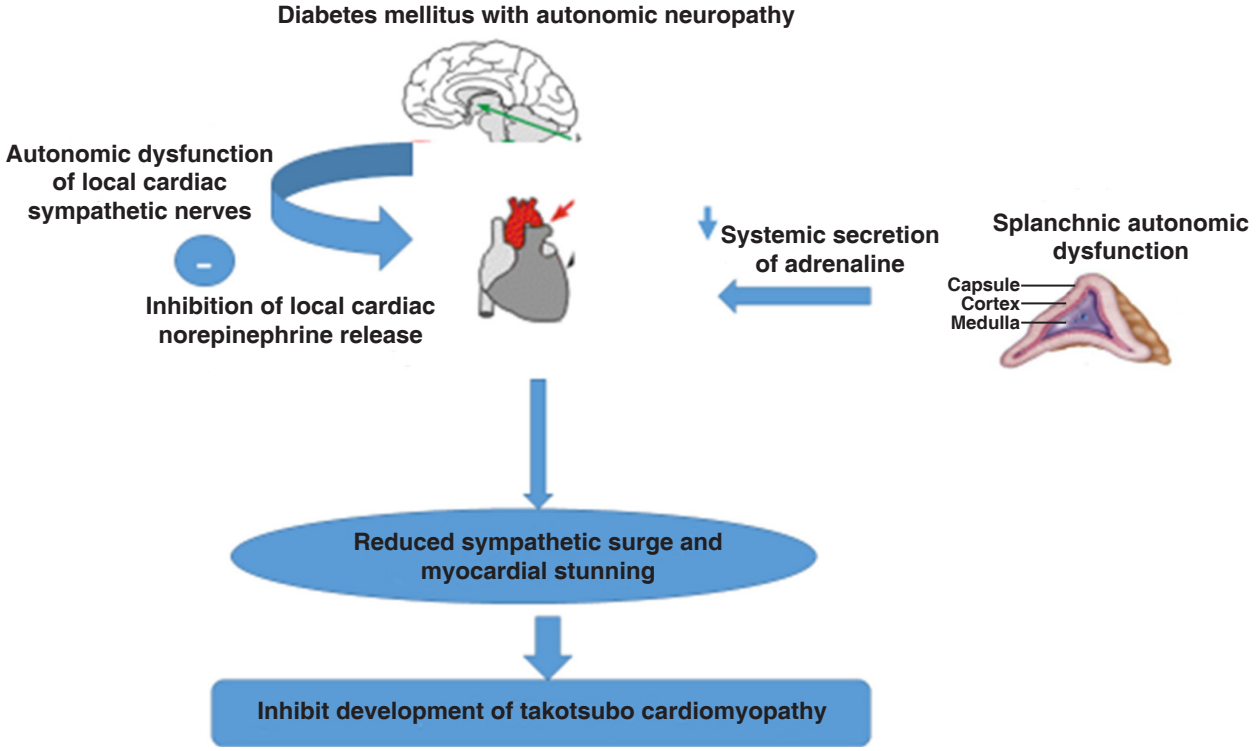

Figure 1: Pathophysiologic mechanism of protective effect of diabetes mellitus on development of takotsubo cardiomyopathy

measurements of hemodynamics in response to acetylcholine and adenosine were used for evaluation. Microvascular dysfunction was noted to be present in a large proportion of patients (64%). Evaluation of underlying cardiac risk factors showed that hyperlipidemia and hypertension were common, with hyperlipidemia seen in up to 61% and hypertension in up to 46% of patients. A noteworthy finding was that DM was quite uncommon in all patient groups, with the prevalence of DM only ranging from 7% to 12%. Since microvascular dysfunction has been increasingly thought to be one of the pivotal factors in the pathogenesis of TC, it somewhat supports an interesting association since diabetics also have a lower incidence of TC.[31] On the contrary, some studies have also suggested increasing prevalence of coronary microvascular dysfunction in diabetics.[31,34,35] Given that microvascular dysfunction is also an important pathogenic factor in TC, this discordance currently has no clear explanation and needs further evaluation. Future studies will strengthen our understanding on this subject.

CONCLUSION

Knowledge about the pathogenesis and risk factors associated with TC is significantly accelerating. Multiple studies have reported low prevalence and rates of DM in patients with TC. This is lower than the prevalence rates of diabetes in the general population, and in contrast to higher prevalence rates of DM in ACS patients. DM may confer a protective effect on the evolution of TC. An increasingly plausible explanation is that autonomic neuropathy from DM leads to cardiac sympathetic autonomic dysfunction and splanchnic autonomic dysfunction resulting in reduced local norepinephrine release and reduced systemic epinephrine release from chromaffin cells in the adrenal medulla, respectively. This autonomic neuropathy and catecholamine hyposecretion may lead to significant blunting and amelioration of cardiomyocyte injury and myocardial stunning, resulting from the catecholamine storm/surge thought to occur in the pathogenesis of TC. Further retrospective and prospective studies on TC should ensure accurate reporting of the underlying prevalence of comorbid conditions, including characterizing the particulars of diabetes mellitus in this population, to further define the postulated protective role of diabetes in these patients.

LIMITATIONS AND FUTURE DIRECTIONS

The data currently available on the subject is quite limited. The prevalence of DM in most of the studies remains relatively low. Dedicated studies on TC are needed incorporating patients with DM to explore the relationship further between DM and TC. Since the studies currently available are mainly retrospective and case-control studies, further prospective studies are needed to strengthen our understanding of

the pathophysiological basis of TC. It would be extremely informative if future case series, case reports and TC registries maintain and report more detailed information about the diabetes status of TC patients. Details like type of DM, chronicity, use of oral hypoglycemic agents, use of insulin, HbA1c values, uncomplicated, complicated by dysautonomia, chronic kidney disease, microvascular disease and presence of end organ damage need to be collected to further study the association between TC and DM. Future studies also need to highlight the presence of other co-morbities like hypertension, dyslipidemia and coronary microvascular dysfunction and their interaction with diabetes to better elucidate the effect of DM in the pathogenesis of TC.

DECLARATIONS

Authors' contributions

Did literature review and composed the first draft of the article: S. Gowdar
Reviewed data and worked on the subsequent revisions: S. Syal
Conceptualized the study design, reviewed progress of the article at each step, and finalized the manuscript of the review article: L. Chhabra

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. Parodi G, Del Pace S, Carrabba N, Salvadori C, Memisha G, Simonetti I, Antoniucci D, Gensini GF. Incidence, clinical findings, and outcome of women with left ventricular apical ballooning syndrome. *Am J Cardiol* 2007;99:182-5.
2. Akashi YJ, Goldstein DS, Barbaro G, Ueyama T. Takotsubo cardiomyopathy: a new form of acute, reversible heart failure. *Circulation* 2008;118:2754-62.
3. Sato H, Tateishi H, Uchida T. Takotsubo-type cardiomyopathy due to multivessel spasm. In: Kodama K, Haze K, Hon M, editors. Clinical Aspect of Myocardial Injury: From Ischemia to Heart Failure. Tokyo, Japan: Kagakuhyouronsha; 1990. p. 56-64.
4. Gowdar S, Chhabra L. Microvascular dysfunction in takotsubo cardiomyopathy. *ICF J* 2016;5:40-9.
5. Madias JE. Low prevalence of diabetes mellitus in patients with Takotsubo syndrome: a plausible 'protective' effect with pathophysiologic connotations. *Eur Heart J Acute Cardiovasc Care* 2016;5:164-70.
6. Khalid N, Chhabra L. Takotsubo cardiomyopathy and microcirculatory dysfunction. *Nat Rev Cardiol* 2015;12:497.
7. Khalid N, Ahmad SA, Umer A. Coronary flow reserve assessment via invasive and noninvasive means in Takotsubo cardiomyopathy. *Int J Cardiol* 2016;202:573.
8. Pelliccia F, Parodi G, Greco C, Antoniucci D, Brenner R, Bossone E, Cacciotti L, Capucci A, Citro R, Delmas C, Guerra F, Ionescu CN, Lairez O, Larrauri-Reyes M, Lee PH, Mansencal N, Marazzi G, Mihos CG, Morel O, Nef HM, Nunez Gil IJ, Passaseo I, Pineda AM, Rosano G, Santana O, Schneck F, Song BG, Song JK, Teh AW, Ungprasert P, Valbusa A, Wahl A, Yoshida T, Gaudio C, Kaski JC. Comorbidities frequency in Takotsubo syndrome: an international collaborative systematic review including 1109 patients. *Am J Med* 2015;128:654.e11-9.
9. Núñez-Gil IJ, Almendro-Delia M, Andrés M, Sionis A, Martin A, Bastante T, Córdoba-Soriano JG, Linares JA, González Sucarrats S, Sánchez-Grande-Flecha A, Fabregat-Andrés O, Pérez B, Escudier-Villa JM, Martin-Reyes R, Pérez-Castellanos A, Rueda Sobella F, Cambeiro C, Piqueras-Flores J, Vidal-Perez R, Bodí V, García de la Villa B, Corbí-Pascua M, Biagioni C, Mejía-Rentería HD, Feltes G, Barrabés J; RETAKO investigators. Secondary forms of Takotsubo cardiomyopathy: a whole different prognosis. *Eur Heart J Acute Cardiovasc Care* 2016;5:308-16.
10. Madias JE. Diabetes mellitus and Takotsubo syndrome in two Spanish cohorts. *Eur Heart J Acute Cardiovasc Care* 2016;5:297.
11. Khalid N, Iqbal I, Coram R, Raza T, Fahsah I, Ikram S. Thrombolysis in myocardial infarction frame count in takotsubo cardiomyopathy. *Int J Cardiol* 2015;191:107-8.
12. Brinjikji W, El-Sayed AM, Salka S. In-hospital mortality among patients with takotsubo cardiomyopathy: a study of the National Inpatient Sample 2008 to 2009. *Am Heart J* 2012;164:215-21.
13. Patel SM, Chokka RG, Prasad K, Prasad A. Distinctive clinical characteristics according to age and gender in apical ballooning syndrome (takotsubo/stress cardiomyopathy): an analysis focusing on men and young women. *J Card Fail* 2013;19:306-10.
14. Dias A, Franco E, Rubio M, Koshkelashvili N, Bhalla V, Amanullah S, Hebert K, Figueredo VM. Takotsubo syndrome: does it matter if you have diabetes mellitus? *Int J Cardiol* 2016;224:398-9.
15. Auzel O, Mustafic H, Pillière R, El Mahmoud R, Dubourg O, Mansencal N. Incidence, characteristics, risk factors, and outcomes of takotsubo cardiomyopathy with and without ventricular arrhythmia. *Am J Cardiol* 2016;117:1242-7.
16. Khera R, Light-McGroary K, Zahr F, Horwitz PA, Girotra S. Trends in hospitalization for takotsubo cardiomyopathy in the United States. *Am Heart J* 2016;172:53-63.
17. Eitel I, von Knobelsdorff-Brenkenhoff F, Bernhardt P, Carbone I, Muellerleile K, Aldrovandi A, Francone M, Desch S, Gutberlet M, Strohm O, Schuler G, Schulz-Menger J, Thiele H, Friedrich MG. Clinical characteristics and cardiovascular magnetic resonance findings in stress (takotsubo) cardiomyopathy. *JAMA* 2011;306:277-86.
18. Núñez-Gil IJ, Molina M, Bernardo E, Ibañez B, Ruiz-Mateos B, García-Rubira JC, Vivas D, Feltes G, Luaces M, Alonso J, Zamorano J, Macaya C, Fernández-Ortiz A. Tako-tsubo syndrome and heart failure: long-term follow-up. *Rev Esp Cardiol (Engl Ed)* 2012;65:996-1002.
19. Núñez Gil IJ, Andrés M, Almendro Delia M, Sionis A, Martín A, Bastante T, Córdoba Soriano JG, Linares Vicente JA, González Sucarrats S, Sánchez-Grande Flecha A; RETAKO investigators. Characterization of Tako-tsubo cardiomyopathy in Spain: results from the RETAKO National Registry. *Rev Esp Cardiol (Engl Ed)*

2015;68:505-12.
20. Templin C, Ghadri JR, Diekmann J, Napp LC, Bataiosu DR, Jaguszewski M, Cammann VL, Sarcon A, Geyer V, Neumann CA, Seifert B, Hellermann J, Schwyzer M, Eisenhardt K, Jenewein J, Franke J, Katus HA, Burgdorf C, Schunkert H, Moeller C, Thiele H, Bauersachs J, Tschöpe C, Schultheiss HP, Laney CA, Rajan L, Michels G, Pfister R, Ukena C, Böhm M, Erbel R, Cuneo A, Kuck KH, Jacobshagen C, Hasenfuss G, Karakas M, Koenig W, Rottbauer W, Said SM, Braun-Dullaeus RC, Cuculi F, Banning A, Fischer TA, Vasankari T, Airaksinen KE, Fijalkowski M, Rynkiewicz A, Pawlak M, Opolski G, Dworakowski R, MacCarthy P, Kaiser C, Osswald S, Galiuto L, Crea F, Dichtl W, Franz WM, Empen K, Felix SB, Delmas C, Lairez O, Erne P, Bax JJ, Ford I, Ruschitzka F, Prasad A, Lüscher TF. Clinical features and outcomes of takotsubo (stress) cardiomyopathy. *N Engl J Med* 2015;373:929-38.
21. Madias JE. Baroreceptor dysfunction, diabetes mellitus, and takotsubo syndrome: an intricate triangle needing exploration. *Int J Cardiol* 2015;184:517-8.
22. Citro R, Rigo F, Previtali M, Ciampi Q, Canterin FA, Provenza G, Giudice R, Patella MM, Vriz O, Mehta R, Baldi C, Mehta RH, Bossone E. Differences in clinical features and in-hospital outcomes of older adults with tako-tsubo cardiomyopathy. *J Am Geriatr Soc* 2012;60:93-8.
23. Falola M, Fonbah W, McGwin G Jr. Takotsubo cardiomyopathy versus ST-elevation myocardial infarction in a large case-control study: proposing a new mechanism. *Int J Cardiol* 2013;167:1079-81.
24. Tornvall P, Collste O, Ehrenborg E, Järnbert-Petterson H. A case-control study of risk markers and mortality in takotsubo stress cardiomyopathy. *J Am Coll Cardiol* 2016;67:1931-6.
25. Zalewska-Adamiec M, Bachorzewska-Gajewska H, Tomaszuk-Kazberuk A, Nowak K, Drozdowski P, Bychowski J, Krynicki R, Musial WJ, Dobrzycki S. Takotsubo cardiomyopathy: serious early complications and two-year mortality - a 101 case study. *Neth Heart J* 2016;24:511-19.
26. Boland TA, Lee VH, Bleck TP. Stress-induced cardiomyopathy. *Crit Care Med* 2015;43:686-93.
27. Khalid N, Ahmad SA, Umer A, Chhabra L. Role of microcirculatory disturbances and diabetic autonomic neuropathy in takotsubo cardiomyopathy. *Crit Care Med* 2015;43:e527.
28. Khalid N, Chhabra L, Ahmad SA, Sareen P, Spodick DH. Autonomic dysfunction and takotsubo cardiomyopathy. *Am J Med* 2015;128:e45-6.
29. Chhabra L. Brain-heart disconnection: a protective effect of diabetes mellitus in takotsubo cardiomyopathy. *Am J Cardiol* 2016;117:1858.
30. Chhabra L, Sareen P. Takotsubo cardiomyopathy and microvascular dysfunction. *Int J Cardiol* 2015;196:107.
31. Chhabra L, Kowlgi NG. Low incidence of diabetes mellitus in coronary microvascular dysfunction: an intriguing association. *JACC Cardiovasc Interv* 2016;9:395-6.
32. Ohigashi-Suzuki S, Saito Y, Tatsuno I. Takotsubo cardiomyopathy associated with sepsis in type 2 diabetes mellitus. *Am J Emerg Med* 2007;25:230-2.
33. Sara JD, Widmer RJ, Matsuzawa Y, Lennon RJ, Lerman LO, Lerman A. Prevalence of coronary microvascular dysfunction among patients with chest pain and nonobstructive coronary artery disease. *JACC Cardiovasc Interv* 2015;8:1445-53.
34. Kawata T, Daimon M, Miyazaki S, Ichikawa R, Maruyama M, Chiang SJ, Ito C, Sato F, Watada H, Daida H. Coronary microvascular function is independently associated with left ventricular filling pressure in patients with type 2 diabetes mellitus. *Cardiovasc Diabetol* 2015;14:98.
35. Sucato V, Evola S, Quagliana A, Novo G, Andolina G, Assennato P, Novo S. Comparison of coronary artery flow impairment in diabetic and hypertensive patients with stable microvascular angina. *Eur Rev Med Pharmacol Sci* 2014;18:3687-9.

Wu *et al. Vessel Plus* 2017;1:137-44
DOI: 10.20517/2574-1209.2017.15

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Perivascular mast cells promote neointimal elastin deposition and suppress chronic vein graft restenosis in hyperlipidaemic mice

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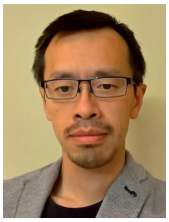
How to cite this article: Wu J, Lawrence C, Wadsworth RM, Kennedy S. Perivascular mast cells promote neointimal elastin deposition and suppress chronic vein graft restenosis in hyperlipidaemic mice. *Vessel Plus* 2017;1:137-44.



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Article history:
Received: 11 May 2017
Accepted: 23 Jun 2017
Published: 26 Sep 2017

Key words:
Mast cells,
elastin,
chronic restenosis,
neointima,
vein graft

ABSTRACT

Aim: Mast cells are versatile innate immune cells and are reported to promote vascular inflammation and neointimal lesion formation, thereby contributing to the development of vascular stenosis and atherosclerosis. However, it is not clear whether mast cells also regulate vascular matrix remodelling in established neointima. This study addressed the hypothesis that perivascular mast cells regulate neointimal matrix remodelling using a mouse vein graft model. **Methods:** The impact of mast cells on neointimal remodelling was investigated using mast cell-deficient animals in both normolipidaemic ($Kit^{W\text{-}sh/W\text{-}sh}$) and hyperlipidaemic ($apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$) conditions. The effect of perivascular mast cells on vascular matrix remodelling, including collagen and elastin deposition, was investigated using a local mast cell reconstitution method that selectively repopulated mast cells around the carotid artery (where the vein graft was inserted) in $Kit^{W\text{-}sh/W\text{-}sh}$ mice. **Results:** In normolipidaemic vein grafts ($Kit^{W\text{-}sh/W\text{-}sh}$ *vs.* the wild type control C57BL/6J), collagen synthesis was not affected by mast cell deficiency at 4 weeks. In contrast, neointimal elastin was reduced by 6.5-fold in mast cell-deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice, which was prevented by perivascular mast cell reconstitution. Mast cell deficiency induced a similar reduction in neointimal elastin in hyperlipidaemic mice ($apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$ *vs.* $apoE^{-/-}$), with a significant increase in cell proliferation and neointimal area at 4 weeks. **Conclusion:** Mast cells appear to promote elastin deposition in vein grafts and this may lead to further suppression of cell proliferation and neointimal thickening under hyperlipidaemic conditions.

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INTRODUCTION

Mast cells are versatile innate immune cells, well-known for their role in inflammation and innate immunity.[1,2] Despite the wide distribution of mast cells in arterial adventitia and perivascular connective tissue, our understanding of the influence of mast cells in vascular disease is limited. In recent years, accumulating evidence suggests that mast cells promote vascular inflammation and contribute to the progression of a number of vascular diseases including atherosclerosis, aortic aneurysm and vein graft neointima hyperplasia.[3-6] Systemic activation of mast cells using dinitrophenyl-albumin increased plaque size in apoE$^{-/-}$ mice, whilst selective stimulation of perivascular mast cells had no impact on plaque formation but destabilised established plaque with increased intra-plaque haemorrhage.[7] Compound 48/80, another mast cell activator, demonstrated a similar effect to promote atherosclerosis development, which was inhibited by the mast cell stabiliser cromolyn.[8,9] In addition to pharmacological manipulation of mast cell function, studies using genetic mast cell deficiency (as a consequence of spontaneous mutation in the promoter region of the c-kit gene[10]) also confirmed the detrimental effect of mast cells in atherosclerosis.[3] Mechanistic studies revealed that activated mast cells synthesised and released a wide range of pro-inflammatory factors including interleukin (IL)-6 and IL-8, interferon gamma, tumournecrosis factor alpha, histamine, chymase and tryptase. Consequently, mast cells exacerbated vascular inflammation with increased intra-plaque leukocyte infiltration, lipid uptake, vascular matrix degradation and subsequent plaque expansion and destabilisation.[6]

In vein graft disease, neointimal hyperplasia is the major cause of restenosis.[11,12] The neointima formation in vein grafts is mainly driven by acute vascular inflammation. In a mouse vein graft model, the acute inflammation usually lasts less than two weeks following grafting surgery.[13,14] The neointima thereafter undergoes a remodelling stage where neointimal cells differentiate into smooth muscle-like cells and synthesise large amount of vascular matrix including collagen and elastin as a process of arterialisation.[12,15] The remodelling process stabilises neointimal cells and prevents chronic neointimal thickening. We and others have found that perivascular mast cells boost acute vein graft inflammation via an increase in cytokine secretion and activation of the complement system.[14,16] This leads to a significant rise in cell proliferation in the acute inflammation stage (one week after vein graft implantation) and more neointima formation. However, it is not clear whether perivascular mast cells also regulate vascular matrix remodelling after resolution of acute inflammation. This is particularly important under pro-atherosclerotic conditions as vascular smooth muscle cells from pro-atherosclerotic animals are more susceptible to chronic proliferation in response to mitogenic stimuli.[17] In the present study, we performed interpositional vein grafting in mast cell deficient mouse lines bred on both a normolipidaemic and hyperlipidaemic genetic background (Kit$^{W\text{-}sh/W\text{-}sh}$ and apoE$^{-/-}$Kit$^{W\text{-}sh/W\text{-}sh}$) to address this question.

METHODS

Animals

All experiments which involved animals conformed to Directive 2010/63/EU of the European Parliament and also to the UK Home Office Animal (Scientific Procedures) Act 1986 and were performed under Project Licence PPL 60/4114. In addition, ethical permission for the study had been granted by the University of Strathclyde ethical review committee.

Mice carrying the Kit$^{W\text{-}sh/W\text{-}sh}$ mutation were used in this study as a mast cell deficiency model. Kit$^{W\text{-}sh/W\text{-}sh}$ is a spontaneous mutation in the promoter region of the c-kit gene which is critical for mast cell survival.[10] As the c-kit gene itself is intact, c-kit expression is preserved to some extent in the early life of these mice. Consequently, systemic mast cell deficiency does not develop until 4 weeks old. Jackson Laboratories (USA) was the original supplier of both the Kit$^{W\text{-}sh/W\text{-}sh}$ and apoE$^{-/-}$ mice and these were subsequently bred in-house. Both strains were on a C57BL/6J background. In order to generate a hyperlipidaemic mouse line lacking mast cells, apoE$^{-/-}$ and Kit$^{W\text{-}sh/W\text{-}sh}$ mice were cross-bred. For the normolipidaemic Kit$^{W\text{-}sh/W\text{-}sh}$ mice, the congenic control was C57BL/6J while for the hyperlipidaemic apoE$^{-/-}$Kit$^{W\text{-}sh/W\text{-}sh}$ mice, the apoE$^{-/-}$ was the congenic control. All mice were maintained on a cycle of 12-h periods of light and dark and allowed access to normal chow diet and water *ad libitum*. Male mice (about 10 to 20 weeks old) were used in this study.

Surgery

The mouse vein graft model is a well-established model for studying neointimal hyperplasia.[13,14] Briefly, i.p. injection of sodium pentobarbital (60 mg/kg) was used as an anaesthetic agent with top-up doses administered as appropriate and depending on the depth of anaesthesia demonstrated by the pedal withdrawal reflex. All animals received perioperative analgesic cover (buprenorphine; 0.05 mg/kg body weight, s.c.). From a donor mouse, the thoracic inferior vena cava was carefully harvested. In all experiments

the donor mouse was a male mouse of the same genotype. In the recipient mouse, the right common carotid artery was prepared by isolating it, tying two sutures around the middle and cutting. A nylon cuff was then sleeved onto the distal arterial end. In order to graft the vein onto artery rather than nylon cuff, the artery was everted back over the cuff and ligated using 8/0 silk suture (Ethicon, Livingston, UK). The proximal end of the carotid artery was prepared in an identical fashion. The vena cava to be grafted was then sleeved onto each arterial end in turn and tied into position with 8/0 suture. Twenty-eight days after grafting, mice were euthanised by a rising concentration of CO_2. The neck was opened and the vein graft removed and placed in physiological bathing solution.

Perivascular mast cell reconstitution

In a previous study from our laboratory, we established a reliable method for reconstituting mast cells locally to the perivascular region. The advantage of this method is that it re-establishes a local mast cell reconstitution in the $Kit^{W\text{-}sh/W\text{-}sh}$ mice without mast cells being present elsewhere.[14] Thus, this method allows the role of perivascular mast cells to be investigated without the consequences of systemic mast cell reconstitution, which can include ectopic mast cell accumulation and abnormal distribution of mast cells in target organs/tissues.[10,14] Briefly, C57BL/6J mice were used as the source of bone marrow cells and these were cultured with mast cell-differentiating media (containing murine IL-3 and stem cell factor; PeproTech, New Jersey, USA) for 4 weeks as we have described previously.[14] To confirm that the cultured cells were in fact differentiated into mast cells, flow cytometry with anti-c-Kit and anti-FcεRI antibodies (eBioscience, Hatfield, UK) was used.[14] To investigate the impact of local reconstitution of mast cells on neointima formation within the vein graft, the perivascular area of the right common carotid artery was injected with the bone marrow-derived mast cells (BMMCs) as follows. Briefly, the recipient $Kit^{W\text{-}sh/W\text{-}sh}$ mouse was anaesthetised with sodium pentobarbital as previously described and the common right carotid artery was exposed. BMMCs were injected around the artery (1 million cells per mouse). A suture of size 6/0 was used to close the wound and the animal was kept for 4 weeks to allow repopulation of the perivascular area with a mast cell population similar to that seen in wild type (C57BL/6) mice.[14] Vein graft surgery was performed 4 weeks after mast cell reconstitution.

Histology and immunostaining

Vein grafts were perfusion-fixed to maintain graft patency and then stored overnight in 10% formalin before being embedded in paraffin. For each vein graft, serial sections of 5 μm thickness were cut from 5 evenly divided regions from the proximal to the distal end. In all cases, 5 slides (1 slide from each region) were stained as outlined below and the values averaged for each vein graft. The advantage of this method is that the average value gives an indication of the value from the whole of the graft rather than one discrete area which may not be representative of the graft as a whole. For general and gross morphology and for planimetry studies, haematoxylin and eosin staining was used and the slides were photographed and analysed using Image pro plus software (Media Cybernetics, Marlow, UK). Collagen was stained by picrosirius red and visualised under polarised light. As with other stains used, at least 5 slides were used from the length of the graft to give a mean intensity of the total fluorescent signal and this value was used for comparison of collagen content between groups. Elastin was identified by Verhoeff Van Gieson staining and mean intensity of light absorption of the bluish dark stain within the neointima was quantified. To avoid batch-to-batch variation, for each staining protocol, all the vein graft samples were stained at one time. For immunostaining, slides were de-waxed and pressure-cooked in citrate buffer to retrieve antigens of interest. To study proliferation in vein grafts, one slide per vein graft was stained using an antibody to the marker Ki67 (rabbit anti-Ki67 antibody, Abcam). The percentage cell proliferation was presented as the ratio of Ki67 positive nuclei over total nuclei in the wall of the vein graft.

Statistics

In all experiments, data are presented as mean ± standard error of the mean where *n* refers to the number of mice. To make comparisons between two groups an unpaired Student's *t*-test was used as long as the data had a normal distribution and, for data not normally distributed, the non-parametric Mann Whitney test was used. To compare data from multiple groups a one-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used as appropriate. Statistical analyses were performed using Graph Pad Prism v6.04. In all comparisons, a significant difference was assumed when $P < 0.05$.

RESULTS

Mast cells promote elastin but not collagen deposition in vein grafts by 4 weeks

Significant collagen deposition was identified in all vein grafts at 4 weeks. The majority of collagen was present in the neo-adventitia and perivascular area [Figure 1A]. In contrast, elastin was only detected in the neointima [Figure 1B and C]. Mast cell deficiency ($Kit^{W\text{-}sh/W\text{-}sh}$) had no impact on collagen deposition,

but suppressed elastin deposition by 6.5 fold. Local mast cell reconstitution (4 weeks before vein graft implantation) rescued the impaired elastin deposition in $Kit^{W\text{-}sh/W\text{-}sh}$ vein grafts (23.5 ± 4.8 *vs.* 3.6 ± 1.1 *vs.* 26.4 ± 4.4 arbitrary units/mm^2; C57BL/6J *vs.* $Kit^{W\text{-}sh/W\text{-}sh}$ *vs.* $Kit^{W\text{-}sh/W\text{-}sh}$ + lcMC; $P < 0.01$ by one-way ANOVA). Mast cell deficiency in hyperlipidaemic mice induced a similar suppression of neointimal elastin (37.6 ± 4.0 *vs.* 13.4 ± 2.9 arbitrary units/mm^2; $apoE^{-/-}$ *vs.* $apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$; $P < 0.01$ by Student's *t*-test).

Mast cell deficiency increased chronic neointimal proliferation and thickening in $apoE^{-/-}$ mice but not in C57BL/6J mice

Mast cell deficiency moderately suppressed neointima formation in normolipidaemic mice 4 weeks after grafting ($1.7 \times 10^5 \pm 0.2 \times 10^5$ *vs.* $1.1 \times 10^5 \pm 0.1 \times 10^5$ μm^2; C57BL/6J *vs.* $Kit^{W\text{-}sh/W\text{-}sh}$; $P < 0.01$ by Student's *t*-test), but induced a 2-fold increase in the hyperlipidaemic mice ($1.9 \times 10^5 \pm 0.2 \times 10^5$ *vs.* $4.2 \times 10^5 \pm 0.8 \times 10^5$ μm^2; $apoE^{-/-}$ *vs.* $apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$; $P < 0.01$ by Student's *t*-test; Figure 2A and B). Cell proliferation within the vein graft at 4 weeks was very low in both C57BL/6J and $Kit^{W\text{-}sh/W\text{-}sh}$ (2.1 ± 0.5% *vs.* 1.8 ± 0.3%; C57BL/6J *vs.* $Kit^{W\text{-}sh/W\text{-}sh}$), but significantly elevated in $apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$ compared to $apoE^{-/-}$ (1.9 ± 0.5% *vs.* 5.3 ± 1.4%; $apoE^{-/-}$ *vs.* $apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$; $P < 0.05$ by Mann Whitney test; Figure 2A and C).

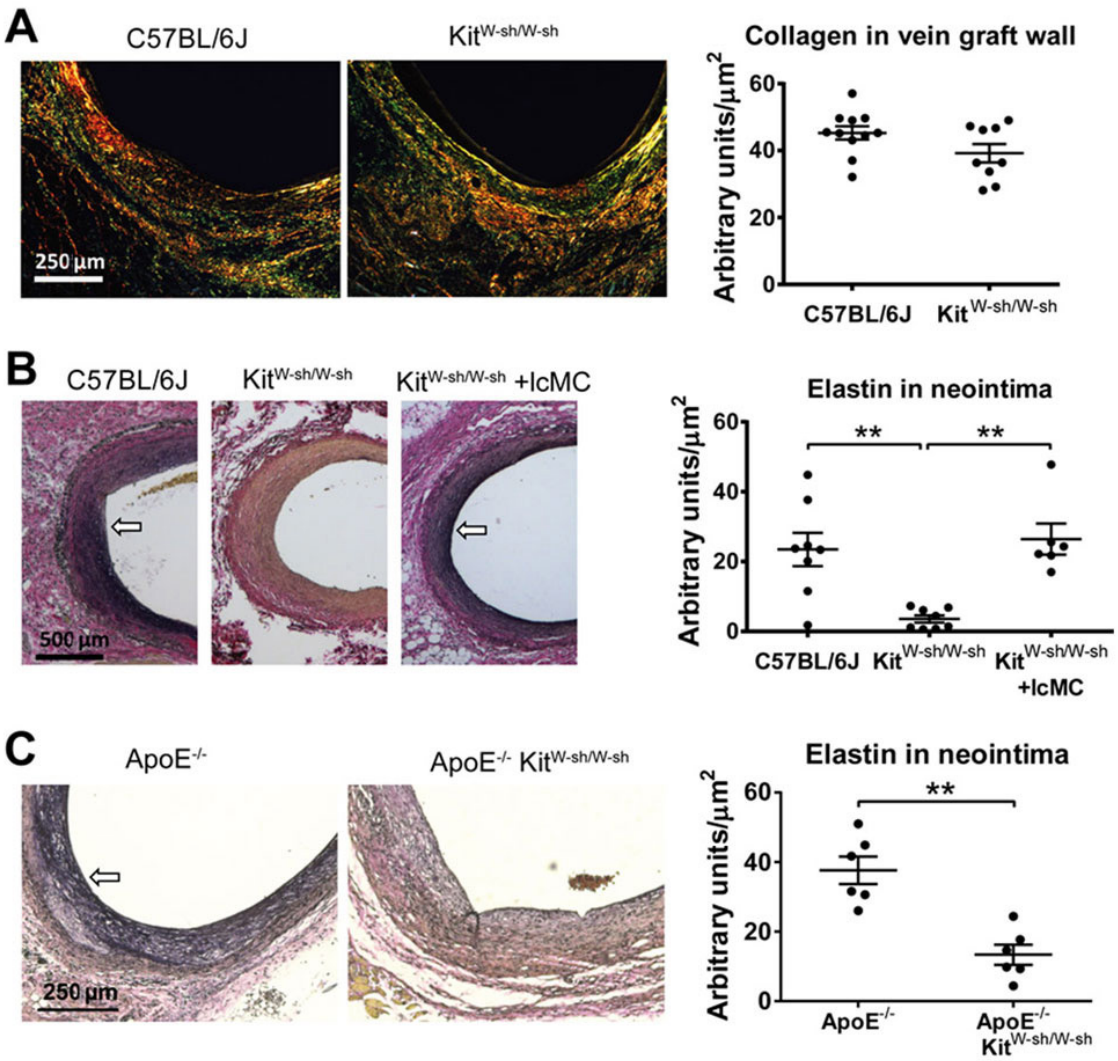

Figure 1: Mast cell deficiency suppressed elastin deposition in vein graft. (A) Picrosirus red staining demonstrated that both C57BL/6J and $Kit^{W\text{-}sh/W\text{-}sh}$ vein grafts synthesised large amount of collagen (in green, yellow and red colour) in the adventitia and perivascular connective tissue by 4 weeks. No statistical difference was detected in collagen content between groups; (B and C) Elastin deposition was restricted to the vein graft neointima (visualised by Verhoeff Van Gieson staining, indicated by arrows). Mast cell deficient $Kit^{W\text{-}sh/W\text{-}sh}$ vein grafts contained a significantly lower level of elastin versus congenic C57BL/6J vein grafts, which was rescued by local mast cell reconstitution. Mast cell deficiency in hyperlipidaemic mice ($apoE^{-/-}$) also significantly suppressed elastin deposition in the vein graft by 4 weeks. $n = 6$; $^{**}P < 0.01$. One-way analysis of variance plus Tukey *post hoc* test was used for multiple group comparison and unpaired student's *t*-test for two group comparison

DISCUSSION

This study demonstrates firstly that perivascular mast cells elevate neointimal elastin deposition under both normolipidaemic and hyperlipidaemic conditions, and secondly that they suppress neointimal thickening in hyperlipidaemic mice possibly via down regulation of cell proliferation within the vein graft.

Elastin is one of the fundamental structural proteins of the arterial wall that regulates vascular elasticity and stabilises smooth muscle cells.[18] The present study demonstrates that mast cells play a previously unrecognised role in promotion of elastin deposition during vein graft remodelling. The causality is demonstrated by the data showing that mast cell deficiency reduced, and mast cell reconstitution rescued, elastin deposition in the vein graft. Although the exact mechanism of how mast cells regulate elastin deposition is not yet clear, there is a consensus within the literature that heparin is the potential mediator. The evidence for the involvement of heparin is three-fold. Firstly, mast cells are the only cell type that produces heparin *in vivo*.[19] Secondly, heparin is known to promote elastogenesis and supress neointima hyperplasia in injured arteries and vascular grafts.[20,21] Being the most negatively charged molecule in biological systems, heparin covalently binds to the positively charged tropoelastin to accelerate tropoelastin coacervation and elastic fibre formation.[22-24] However, the very short half-life of heparin limits its pharmacological potency for therapeutic purposes.[25] Only continuous intravenous delivery,[26-28] but not short term treatment,[29] inhibited neointima thickening. Interestingly, perivascular delivery of heparin required a much smaller dose and was more effective in suppression of neointima hyperplasia,[20,25] which matches the source of endogenous heparin from perivascular mast cells. Thirdly, mast cell granules are enriched with heparin and the heparin-based particles are capable of long distance travel within tissue[30] which makes perivascular mast cells an ideal and indeed the only source for continuous heparin supply to assist vascular elastogenesis.

It is intriguing that mast cell-dependent elastin deposition had a divergent impact on neointimal hyperplasia in normolipidaemic and hyperlipidaemic vein grafts. This could be a consequence of the different dynamics of neointima formation and vascular matrix remodelling. In normolipidaemic mice, the neointima formation is driven by acute inflammation and proliferation which peak within one week and are complete by 2 weeks.[13,31] During the 3rd and 4th weeks, the neointimal proliferation decreases

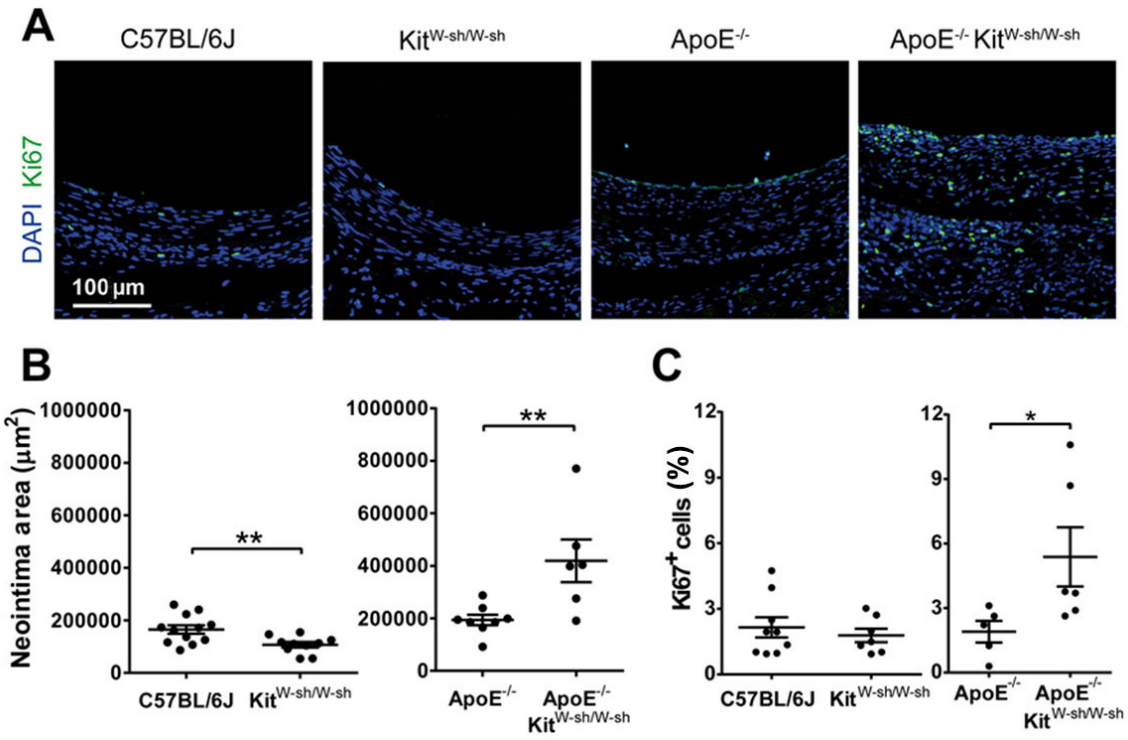

Figure 2: Mast cell deficiency in hyperlipidaemic mice increased neointima area with elevated chronic cell proliferation. (A) Representative images of cell proliferation in 4-week-old vein grafts. Proliferating cells were labelled using an antibody against Ki67 (green) and cell nuclei by DAPI (blue). The neointimal area in apoE$^{-/-}$ Kit$^{W\text{-}sh/W\text{-}sh}$ vein grafts was significantly higher than all other groups (B), associated with elevated cell proliferation by 4 weeks (C). $n > 6$ for all groups; $^{**}P < 0.01$ by one-way analysis of variance plus Tukey *post hoc* test

over 10-fold and the majority of the neointimal cells transdifferentiate into smooth muscle-like cells that initiate vascular matrix remodelling including elastin deposition.[13,14,18,31] Therefore, elastin is unlikely to regulate the early events of neointima formation in the normolipidaemic vein graft. The modest reduction in neointimal area in the $Kit^{W\text{-}sh/W\text{-}sh}$ vein graft is attributed to the damped cell proliferation during the first week rather than altered elastin deposition.[14]

Both our data and the literature[32] demonstrated that $apoE^{-/-}$ mice, when fed on normal chow diet, do not demonstrate any enhancement of neointimal hyperplasia. The low cell proliferation level in $apoE^{-/-}$ vein grafts at 4 weeks in the current study suggests that the neointima was stabilised, similar to that in C57BL/6J. These observations are in contrast with *in vitro* evidence that $apoE^{-/-}$ smooth muscle cells are predisposed to a higher rate of proliferation.[17] One clear difference between data from cell cultures and neointima formation *in vivo* is the absence of the rich extracellular matrix environment. $ApoE^{-/-}$ vein grafts at 4 weeks contained large amounts of elastin that can stabilise neointimal smooth muscle cells.[18] The reduction in neointimal elastin in $apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$ grafts was correlated with a dramatic increase in cell proliferation within the vein graft wall, suggesting that the presence of elastin is an important protective mechanism against chronic smooth muscle cell proliferation under pro-atherogenic conditions.

Mast cells, as innate immune cells, are well-documented to have pro-inflammatory effects in many pre-clinical vascular studies.[6] Induction of mast cell activation in a pro-inflammatory environment, either using pharmacological activators or long term western diet in pro-atherosclerotic mouse models ($apoE^{-/-}$ or $LDLr^{-/-}$), was a common feature of these studies. The widely accepted paradigm is that high levels of pro-inflammatory factors and proteinases released by activated mast cells promote vascular inflammation and development of vascular lesions. Indeed activated mast cells may be the explanation for the clinical correlation between mast cell-mediated allergic disease and atherosclerosis.[33] However, the role of unstimulated mast cells in normal vasculature or in stable vascular disease is not fully understood. The current study provides the first evidence that mast cells could exert a positive effect in vascular remodelling via regulation of elastin deposition. Our unpublished data suggests that the expression of inflammatory cytokines in vein grafts during the remodelling stage (4 weeks after implantation) is negligible. We therefore speculate that the mast cell-dependent regulation of elastin is independent of immunological/inflammatory mechanisms, but mediated by heparin (as discussed above) or some unknown mechanisms.

The mast cell-elastin-axis could potentially be extrapolated to vascular development as the first appearance of mast cells around the foetal aorta is before the formation of aortic elastic laminae.[34] This suggests that mast cells may also contribute to the foetal arteriogenesis by assisting formation of elastic fibres. In addition to the perivascular area, the mast cell population is clearly enriched in all the elastic tissue/organs including arteries, skin and lung, indicating a universal link between mast cells and elastin. Under pathological conditions, an increase of mast cell number is often associated with elastin-related tissue fibrosis.[35,36] In a skin photodamage model, chronic ultraviolet light exposure increases skin mast cell number accompanied by a 3.6 fold elevation in elastin content, which was not observed in the mast cell-deficient $Kit^{W/W\text{-}v}$ mice.[37] Similar correlation was also present in human skin.[38] Non-elastic organs, such as the liver usually hosts only a very small population of mast cells.[39] In liver disease, an increase of hepatic elastin is one of the most distinct features of hepatic fibrosis and cirrhosis,[36] correlated with a significant increase in hepatic mast cells.[39] In contrast to other leukocyte recruitment, the increase of mast cell number persisted with the chronic progress of hepatic cirrhosis,[40] suggesting the mast cell-mediated pathological elastin accumulation was beyond inflammatory mechanisms.

Collectively, accumulating evidence suggests that the mast cell-elastin axis may be a universal mechanism that regulates physiological and pathological elastin metabolism. Our findings, in line with other literature, raise the question of whether mast cells regulate vascular elastin metabolism via mechanisms independent from their immunological function. More comprehensive investigation is required to clarify the complex role of mast cells in vascular development and remodelling.

The current study used a mast cell-deficient mouse model ($Kit^{W\text{-}sh/W\text{-}sh}$) and local mast cell reconstitution to demonstrate that perivascular mast cells promoted neointimal elastin remodelling in vein grafts. Limited by the experimental period, it was not clear whether perivascular mast cells merely accelerated the elastin deposition in the 4-week time window, or permanently increased the elastin level in the neointima. Unlike the elastic lamina in healthy arteries, the elastic fibres in the neointima were diffuse. The presence of mast cells only increased the amount of elastic fibres but did not improve the structure of the matrix. Further

experiments are required to clarify whether the quantitative increase in elastin content has a positive impact on vascular compliance and vasomotor function, which may also influence the neointima hyperplasia.

In conclusion, this is the first study to demonstrate the mast cell-dependent regulation of elastin deposition in vein grafts, and subsequent suppression of chronic vein graft hyperplasia in hyperlipidaemic mice. This study sheds new light on the function of perivascular mast cells on vascular lesion formation and remodelling, and provides new targets for therapeutic intervention for vein graft failure and other stenotic vascular disease.

Authors' contributions

Performing all the experiments, acquiring and analysing the data and writing the first draft of the paper: J. Wu
Designing the experiments, manuscript editing and reviewing: C. Lawrence, R.M. Wadsworth, S. Kennedy

Financial support and sponsorship

This work was supported by a British Heart Foundation project grant (PG/09/095). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

The study had been granted by the University of Strathclyde ethical review committee.

REFERENCES

1. St John AL, Abraham SN. Innate immunity and its regulation by mast cells. *J Immunol* 2013;190:4458-63.
2. Kennedy S, Wu J, Wadsworth RM, Lawrence CE, Maffia P. Mast cells and vascular diseases. *Pharmacol Ther* 2013;138:53-65.
3. Sun J, Sukhova GK, Wolters PJ, Yang M, Kitamoto S, Libby P, MacFarlane LA, Mallen-St Clair J, Shi GP. Mast cells promote atherosclerosis by releasing proinflammatory cytokines. *Nat Med* 2007;13:719-24.
4. Zhang J, Sun J, Lindholt JS, Sukhova GK, Sinnamon M, Stevens RL, Adachi R, Libby P, Thompson RW, Shi GP. Mast cell tryptase deficiency attenuates mouse abdominal aortic aneurysm formation. *Circ Res* 2011;108:1316-27.
5. de Vries MR, Wezel A, Schepers A, van Santbrink PJ, Woodruff TM, Niessen HW, Hamming JF, Kuiper J, Bot I, Quax PH. Complement factor C5a as mast cell activator mediates vascular remodelling in vein graft disease. *Cardiovasc Res* 2013;97:311-20.
6. Bot I, Shi GP, Kovanen PT. Mast cells as effectors in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2015;35:265-71.
7. Bot I, de Jager SC, Zernecke A, Lindstedt KA, van Berkel TJ, Weber C, Biessen EA. Perivascular mast cells promote atherogenesis and induce plaque destabilization in apolipoprotein E-deficient mice. *Circulation* 2007;115:2516-25.
8. Wang J, Sjöberg S, Tia V, Secco B, Chen H, Yang M, Sukhova GK, Shi GP. Pharmaceutical stabilization of mast cells attenuates experimental atherogenesis in low-density lipoprotein receptor-deficient mice. *Atherosclerosis* 2013;229:304-9.
9. Tang Y, Yang Y, Wang S, Huang T, Tang C, Xu Z, Sun YH. Mast cell degranulator compound 48-80 promotes atherosclerotic plaque in apolipoprotein E knockout mice with perivascular common carotid collar placement. *Chin Med J (Engl)* 2009;122:319-25.
10. Grimbaldeston MA, Chen CC, Piliponsky AM, Tsai M, Tam SY, Galli SJ. Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. *Am J Pathol* 2005;167:835-48.
11. Wu J, Zhang C. Neointimal hyperplasia, vein graft remodeling, and long-term patency. *Am J Physiol Heart Circ Physiol* 2009;297:H1194-5.
12. Owens CD, Gasper WJ, Rahman AS, Conte MS. Vein graft failure. *J Vasc Surg* 2015;61:203-16.
13. Wu J, Wadsworth RM, Kennedy S. Inhibition of inducible nitric oxide synthase promotes vein graft neoadventitial inflammation and remodelling. *J Vasc Res* 2011;48:141-9.
14. Wu J, Grassia G, Cambrook H, Ialenti A, MacRitchie N, Carberry J, Wadsworth RM, Lawrence C, Kennedy S, Maffia P. Perivascular mast cells regulate vein graft neointimal formation and remodeling. *Peer J* 2015;3:e1192.
15. de Vries MR, Simons KH, Jukema JW, Braun J, Quax PH. Vein graft failure: from pathophysiology to clinical outcomes. *Nat Rev Cardiol* 2016;13:451-70.
16. de Vries MR, Wezel A, Schepers A, van Santbrink PJ, Woodruff TM, Niessen HW, Hamming JF, Kuiper J, Bot I, Quax PH. Complement factor C5a as mast cell activator mediates vascular remodelling in vein graft disease. *Cardiovasc Res* 2013;97:311-20.
17. Kothapalli D, Castagnino P, Rader DJ, Phillips MC, Lund-Katz S, Assoian RK. Apolipoprotein E-mediated cell cycle arrest linked to p27 and the Cox2-dependent repression of miR221/222. *Atherosclerosis* 2013;227:65-71.
18. Lannoy M, Slove S, Jacob MP. The function of elastic fibers in the arteries: beyond elasticity. *Pathol Biol* 2014;62:79-83.
19. Carlsson P, Kjellén L. Heparin biosynthesis. In: Lever R, Mulloy B, Page CP, editors. Handbook of experimental pharmacology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 23-41.
20. Teomim D, Fishbien I, Golomb G, Orloff L, Mayberg M, Domb AJ. Perivascular delivery of heparin for the reduction of smooth muscle cell proliferation after endothelial injury. *J Control Release* 1999;60:129-42.
21. Saitow C, Kaplan DL, Castellot JJ. Heparin stimulates elastogenesis: application to silk-based vascular grafts. *Matrix Biol* 2011;30:346-55.
22. Tu Y, Weiss AS. Transient tropoelastin nanoparticles are early-stage intermediates in the coacervation of human tropoelastin whose aggregation is facilitated by heparan sulfate and heparin decasaccharides. *Matrix Biol* 2010;29:152-9.
23. Cain SA, Baldock C, Gallagher J, Morgan A, Bax DV, Weiss AS, Shuttleworth CA, Kielty CM. Fibrillin-1 interactions with heparin: implications for microfibril and elastic fiber assembly. *J Biol Chem* 2005;280:30526-37.
24. Tu Y, Weiss AS. Glycosaminoglycan-mediated coacervation of tropoelastin abolishes the critical concentration, accelerates coacervate formation, and facilitates spherule fusion: implications for tropoelastin microassembly. *Biomacromolecules* 2008;9:1739-44.

25. Edelman ER, Adams DH, Karnovsky MJ. Effect of controlled adventitial heparin delivery on smooth muscle cell proliferation following endothelial injury. *Proc Natl Acad Sci U S A* 1990;87:3773-7.
26. Capron L, Jarnet J, Heudes D, Joseph-Monrose D, Bruneval P. Repeated balloon injury of rat aorta: a model of neointima with attenuated inhibition by heparin. *Arterioscler Thromb Vasc Biol* 1997;17:1649-56.
27. Chajara A, Heudes D, Peronneau I, Jarnet J, Basset A, Capron L. Effects of heparinoids on the sclerotic reaction of rat thoracic aorta to injury: comparison between standard and low-molecular-weight heparins in vitro and in vivo. *J Cardiovasc Pharmacol* 1994;23:995-1003.
28. Guyton JR, Rosenberg RD, Clowes AW, Karnovsky MJ. Inhibition of rat arterial smooth muscle cell proliferation by heparin. In vivo studies with anticoagulant and nonanticoagulant heparin. *Circ Res* 1980;46:625-34.
29. Ellis SG, Roubin GS, Wilentz J, Douglas JS, King SB. Effect of 18- to 24-hour heparin administration for prevention of restenosis after uncomplicated coronary angioplasty. *Am Heart J* 1989;117:777-82.
30. Kunder CA, St John AL, Li G, Leong KW, Berwin B, Staats HF, Abraham SN. Mast cell-derived particles deliver peripheral signals to remote lymph nodes. *J Exp Med* 2009;206:2455-67.
31. Marx SO, Totary-Jain H, Marks AR. Vascular smooth muscle cell proliferation in restenosis. *Circ Cardiovasc Interv* 2011;4:104-11.
32. Matter CM, Ma L, von Lukowicz T, Meier P, Lohmann C, Zhang D, Kilic U, Hofmann E, Ha SW, Hersberger M, Hermann DM, Lüscher TF. Increased balloon-induced inflammation, proliferation, and neointima formation in apolipoprotein E (ApoE) knockout mice. *Stroke* 2006;37:2625-32.
33. Liu CL, Zhang JY, Shi GP. Interaction between allergic asthma and atherosclerosis. *Transl Res* 2016;174:5-22.
34. Wulff BC, Parent AE, Meleski MA, DiPietro LA, Schrementi ME, Wilgus TA. Mast cells contribute to scar formation during fetal wound healing. *J Invest Dermatol* 2012;132:458-65.
35. Wilgus TA, Wulff BC. The importance of mast cells in dermal scarring. *Adv Wound Care (New Rochelle)* 2014;3:356-65.
36. Pellicoro A, Aucott RL, Ramachandran P, Robson AJ, Fallowfield JA, Snowdon VK, Hartland SN, Vernon M, Duffield JS, Benyon RC, Forbes SJ, Iredale JP. Elastin accumulation is regulated at the level of degradation by macrophage metalloelastase (MMP-12) during experimental liver fibrosis. *Hepatology* 2012;55:1965-75.
37. Gonzalez S, Moran M, Kochevar IE. Chronic photodamage in skin of mast cell-deficient mice. *Photochem Photobiol* 1999;70:248-53.
38. Grimbaldeston MA, Simpson A, Finlay-Jones JJ, Hart PH. The effect of ultraviolet radiation exposure on the prevalence of mast cells in human skin. *Br J Dermatol* 2003;148:300-6.
39. Farrell DJ, Hines JE, Walls AF, Kelly PJ, Bennett MK, Burt AD. Intrahepatic mast cells in chronic liver diseases. *Hepatology* 1995;22:1175-81.
40. Jeong WI, Lee CS, Park SJ, Chung JY, Jeong KS. Kinetics of macrophages, myofibroblasts and mast cells in carbon tetrachloride-induced rat liver cirrhosis. *Anticancer Res* 2002;22:869-77.

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DOI: 10.20517/2574-1209.2016.04

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Potential use of buccal epithelium for genetic diagnosis of atherosclerosis using mtDNA mutations

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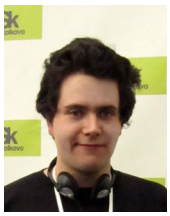
How to cite this article: Sinyov VV, Sazonova MA, Ryzhkova AI, Galitsyna EV, Melnichenko AA, Postnov AY, Orekhov AN, Grechko AV, Sobenin IA. Potential use of buccal epithelium for genetic diagnosis of atherosclerosis using mtDNA mutations. *Vessel Plus* 2017;1:145-50.



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Article history:
Received: 10 Oct 2016
Accepted: 6 Jan 2017
Published: 26 Sep 2017

Key words:
Buccal cell,
mutation,
mtDNA,
threshold heteroplasmy level,
mitochondrial genome,
atherosclerosis

ABSTRACT

Aim: The aim of this pilot study was to compare the heteroplasmy levels of specific mitochondrial (mt)DNA mutations in human buccal epithelial and whole blood cells in participants with different degrees of predisposition to atherosclerosis. The potential for buccal epithelium to be used for the genetic diagnosis of atherosclerosis using mtDNA mutations was assessed. **Methods:** Samples of buccal epithelial and whole blood cells were obtained from 134 donors. DNA was extracted from the samples and subjected to polymerase chain reaction and pyrosequencing. The threshold heteroplasmy levels of the mutations m.12315G>A, m.3336T>C, m.1555A>G, m.13513G>A, and m.3256C>T were analyzed in order to assess the potential for using buccal epithelium and whole blood for the genetic diagnosis of atherosclerosis. **Results:** The threshold heteroplasmy levels of the assessed mitochondrial mutations did not significantly differ between buccal epithelial and whole blood cells. **Conclusion:** Buccal epithelial cells may be preferable to whole blood cells for analyzing the association of mitochondrial genome mutations with atherosclerosis.

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INTRODUCTION

Investigations of the human genome make the early, presymptomatic diagnosis of different pathologies possible. According to the literature,[1-4] various pathologies, including atherosclerosis, may be associated with mitochondrial mutations. The phenomenon of heteroplasmy is typical for the mitochondrial genome, meaning that a quantitative assessment of mutant alleles in the mitochondrial genome is necessary when investigating the association of mitochondrial mutations with diseases, in particular, the heteroplasmy level of mutations associated with pathologies should be evaluated.

Because of the instability of the mitochondrial genome, both somatic and hereditary mutations often occur. These can accumulate, influencing the phenotype of the carrier. According to the literature, heteroplasmy of the mitochondrial genome is common in normal human cells.[5] During human embryogenesis, in the process of determining cells and tissues, morphologic and chemical differences occur between cells. As a result of cell division, mitochondria are randomly distributed between cells. This determines the difference between cells in the ratio of normal and mutant molecules of mitochondrial (mt)DNA. It has previously been established that single nucleotide polymorphisms of the mitochondrial genome are unevenly distributed in the tissues and organs of adults.[6-8] This uneven distribution impedes the use of biomarkers in diagnosing different diseases. Therefore, the identification of biomarkers for the non-invasive genetic diagnosis of pathologies seems an interesting prospect.

The aim of the present pilot study was to compare heteroplasmy levels in mtDNA m.12315G>A, m.3336T>C, m.1555A>G, m.13513G>A, and m.3256C>T between human buccal epithelial cells and whole blood cells in individuals with different degrees of predisposition to atherosclerosis. The potential use of buccal epithelium for the genetic diagnosis of atherosclerosis using mutations in mtDNA was assessed.

METHODS

This study was carried out according to the Declaration of Helsinki and with permission of an ethics committee (Russian Cardiology Research and Production Complex, Moscow, Russia). Samples were taken from 134 donors, all of whom provided a written informed consent.

Samples of buccal epithelial and whole blood cells were obtained from the donors.

High-resolution B-mode ultrasonography using a linear vascular transducer at 7.5 MHz with the SonoScape SSI-1000 ultrasonic scanner (SonoScape Medical Corp., Shenzhen, China) was used to determine the degree of arterial stenosis of each participant. Intima-medial thickness (IMT) was measured using the program ProSound (R. Selzer, California Institute of Technology, Pasadena, USA). Ultrasonographic examination of the carotid arteries included scanning the left and right carotid arteries with a focus on the back wall of the artery in three fixed projections: anterolateral, lateral, and posterolateral.[9]

DNA phenol-chloroform extraction

Total DNA for this study was isolated from whole blood using phenol-chloroform extraction.[10,11] This DNA isolation method included the following steps: (1) cell lysis (using sodium dodecyl sulfate); (2) enzymatic degradation of proteins with proteinase K; (3) cell lysate deproteinization with phenol and chloroform.

The above-mentioned stages of pure DNA product isolation came amid centrifugation to remove denatured proteins and fragments of cell organelles.

Next, DNA was precipitated from the solution using ethanol and, following centrifugation, the precipitate was dissolved in a buffer solution.

Polymerase chain reaction

Polymerase chain reaction (PCR) was conducted with the aim of amplifying the short-chain mtDNA fragments to achieve a sufficient number of amplicon copies for further analysis of these fragments. The conditions for PCR were mentioned in previous publications.[4,10-12]

Pyrosequencing

Short-chain amplicons obtained during PCR were subjected to pyrosequencing in order to determine the nucleotide sequences of these fragments and assess heteroplasmy levels in the investigated mtDNA positions. This work was performed using the PSQ96MA pyrosequencer (Biotage AB, Uppsala, Sweden), as previously described.[13]

Analysis of heteroplasmy levels in mtDNA mutations

Quantitative assessment of the mutant allele involved estimating the heteroplasmy levels of mtDNA mutations, using the pyrogram peak height of each sample in the studied region of a single-stranded PCR fragment of mitochondrial genome. The general formula for calculating the percentage of heteroplasmy was as follows:[10,11]

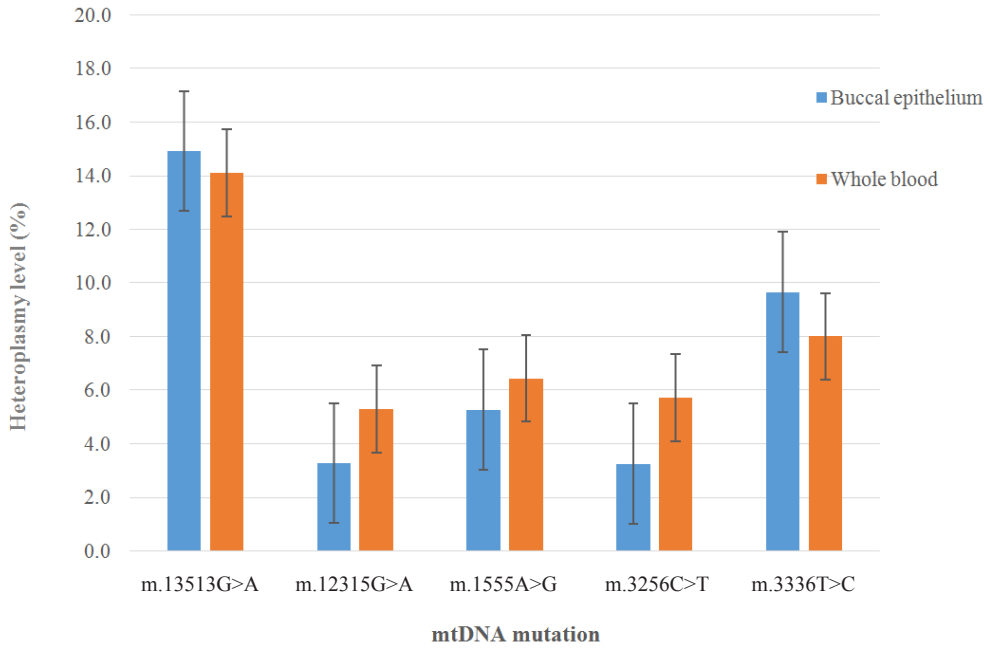

Figure 1: The heteroplasmy level of mitochondrial genome mutations in whole blood and buccal epithelium from 134 donors. Error bars show the standard error. mtDNA: mitochondrial DNA

$P = [(h - N)/(M - N)]*100\%,$

Where: *P* is the percentage heteroplasmy; *h* is the peak height of the investigated nucleotide; *N* is the peak height of the investigated nucleotide, relevant to the presence of 100% of normal alleles in a sample; *M* is the peak height of the investigated nucleotide, relevant to the presence of 100% of mutant alleles in a sample.

Statistical processing of the data was carried out using the software package SPSS version 22.0 (SPSS Inc., USA).[14] Independent samples were assessed using *t*-tests and Mann-Whitney *U*-tests, and correlation analyses were also conducted. Differences were considered significant at $P < 0.05$.

Next, the possibility that buccal epithelium might be equivalent to whole blood for the genetic diagnosis of atherosclerosis was assessed. For this purpose the threshold heteroplasmy level of mutations was used, after which in individuals atherosclerotic plaques were detected or antiatherogenic effect of these single nucleotide substitutions manifests.[14] According to the data, previously obtained by the members of our laboratory,[11] the threshold heteroplasmy level of mtDNA mutations for atherosclerotic plaques in human carotid arteries for atherogenic mutations is: 17.5% for m.1555A>G; 6.5% for m.3336T>C; 7.5% for m.12315G>A; and 15.5% for m.3256C>T. For the anti-atherogenic mutation m.13513G>A, the threshold level of heteroplasmy is 32.5%.

RESULTS

The investigated sample of 134 participants was divided by gender and age. All the examined individuals had their IMT measured. A total of 35 of the donors were men, with a mean ± standard deviation (SD) age of 61 ± 10 years and an IMT of 0.84 ± 0.21 mm. The remaining 97 donors were women, with a mean ± SD age of 61 ± 11 years and an IMT of 0.79 ± 0.41 mm.

During the quantitative assessment of the mutant allele of mitochondrial genome, data on the heteroplasmy levels in buccal epithelial and whole blood cells were obtained [Figure 1]. The obtained data were analyzed using the *t*-test for independent samples.

Significant differences in the heteroplasmy level of the mitochondrial genome mutation m.3256C>T were found between buccal epithelial and whole blood cells ($P = 0.01$). However, there were no differences in the mutations m.13513G>A ($P = 0.48$), m.3336T>C ($P = 0.65$), m.12315G>A ($P = 0.13$), and m.1555A>G ($P = 0.23$).

The difference between average heteroplasmy levels of the investigated mutations did not exceed 5% and the standard error in a range of cases was higher than the mean level. This is probably connected to the fact that each individual has a different heteroplasmy level of mtDNA mutations, and that heteroplasmy levels significantly differ within an investigated sample. Therefore, it is unlikely that the impact of the

Table 1: Significance of rank differences in heteroplasmy levels between human buccal epithelium and whole blood

	m.12315G>A	m.13513G>A	m.1555A>G	m.3256C>T	m.3336T>C
Mann-Whitney *U*-test	8,167	7,761	7,625	6,554	595
P value	0.20	0.18	0.36	0.08	0.80

Table 2: Ranking of atherosclerotic plaques in the lumen of blood vessels of the brachiocephalic bed, depending on the degree of stenosis

Atherosclerotic plaque size (points)	Degree of stenosis (%)
0	0
1	< 20
2	20-40
3	> 40

heteroplasmy level of mitochondrial genome mutations on different human diseases can be evaluated using the percentage expression of mutational burden. To determine the difference in heteroplasmy levels among samples, it is necessary to convert heteroplasmy levels into rank values using the threshold value of heteroplasmy percentage. It should be noted that, despite the fact that the average heteroplasmy level for a number of mutations was much lower than the threshold level (e.g. for mutation m.13513G>A, the threshold heteroplasmy level was 32.5% while the average value of this parameter for the buccal epithelium and whole blood was 15% and 14%, respectively), some individuals had heteroplasmy levels above the threshold level.

After ranking the heteroplasmy levels, differences in the mutational burden distribution in buccal epithelium and whole blood were determined using the Mann-Whitney *U*-test [Table 1]. As can be seen in Table 1, the threshold heteroplasmy level of the mutations m.12315G>A, m.13513G>A, m.1555A>G, m.3256C>T, and m.3336T>C, which are associated with atherosclerotic plaques, did not significantly differ between the buccal epithelium and whole blood.

Consequently, DNA samples from both the buccal epithelium and the whole blood may be of use for the molecular genetic diagnosis of atherosclerosis using mitochondrial genome mutations associated with this disease.

To check the distribution of heteroplasmy values in different cell types and their association with gender, age, the degree of angiostenosis, and IMT, a correlation analysis of heteroplasmy levels in the buccal epithelium and whole blood was carried out. The parametric variables of age and IMT were compared with the average heteroplasmy levels of the mitochondrial genome mutations m.12315G>A, m.13513G>A, m.1555A>G, m.3256C>T, and m.3336T>C, while the non-parametric variables of atherosclerotic plaques and gender were compared with threshold heteroplasmy level distributions. Variability was assessed according to the available data on the size of an atherosclerotic plaque (in points) [Table 2].

A correlation analysis was performed between the threshold levels of heteroplasmy in the mitochondrial genome mutations m.12315G>A, m.13513G>A, m.1555A>G, m.3256C>T, and m.3336T>C in the buccal epithelium and whole blood, and the atherosclerotic plaque size, IMT, age, and gender of individuals from the investigated sample [Tables 3 and 4]. The differences were considered significant at 95% probability of faultless prognosis. These analyses found that the heteroplasmy level of mutation m.1555A>G in the buccal epithelium was significantly positively correlated with IMT and negatively correlated with age [Table 3]. In whole blood, the heteroplasmy level of mutation m.1555A>G was significantly positively correlated with IMT, similar to the correlation in the buccal epithelium.

DISCUSSION

A range of scientific publications have indicated that mitochondrial genome mutations, which are associated

Table 3: Correlation of heteroplasmy level of mtDNA mutations in human buccal epithelium with plaques, IMT, gender, and age

Variable		m.13513G>A	m.12315G>A	m.1555A>G	m.3256C>T	m.3336T>C
Plaque	Spearman correlation coefficient	0.09	0.03	-0.11	-0.12	-0.02
	P value	0.31	0.78	0.22	0.19	0.81
Gender	Spearman correlation coefficient	0.02	-0.02	0.15	-0.16	-0.10
	P value	0.87	0.79	0.10	0.10	0.33
Age	Pearson correlation coefficient	-0.07	-0.13	-0.33*	-0.21	-0.06
	P value	0.40	0.16	0.01	0.20	0.56
IMT	Pearson correlation coefficient	-0.07	0.14	0.53*	0.04	-0.05
	P value	0.40	0.12	0.01	0.67	0.61

*Highly significant. mtDNA: mitochondrial DNA; IMT: intima-medial thickness

Table 4: Correlation of heteroplasmy level of mtDNA mutations in human whole blood with plaques, IMT, gender, and age

Variable		m.13513G>A	m.12315G>A	m.1555A>G	m.3256C>T	m.3336T>C
Plaque	Spearman correlation coefficient	-0.01	-0.08	0.04	-0.02	-0.08
	P value	0.89	0.37	0.66	0.82	0.42
Gender	Spearman correlation coefficient	0.10	-0.11	0.11	0.10	-0.10
	P value	0.29	0.21	0.22	0.30	0.32
Age	Pearson correlation coefficient	0.04	0.08	-0.07	-0.11	0.03
	P value	0.68	0.34	0.43	0.25	0.79
IMT	Pearson correlation coefficient	0.35	0.05	0.26*	0.07	-0.01
	P value	0.10	0.56	0.01	0.49	0.91

*Highly significant. mtDNA: mitochondrial DNA; IMT: intima-medial thickness

with several human diseases, can be unequally distributed in tissues and organs.[15,16] This has been shown in research with both healthy participants and those affected by disease.[17,18] Therefore, we studied the heteroplasmy levels of the mutations m.12315G>A, m.13513G>A, m.1555A>G, m.3256C>T, and m.3336T>C, which have been associated with atherosclerosis, and the distribution of these mutations in different tissues and organs. To compare different cells and tissues, we used the parameter of the threshold heteroplasmy level of mutations. This enabled us to calculate average differences in heteroplasmy levels among samples from various participants. This parameter was calculated on the basis of the correlation between mtDNA mutations and the degree of atherosclerosis of the arterial vessel wall.[9] The results indicate the absence of significant differences in threshold heteroplasmy levels of the mitochondrial genome mutations m.12315G>A, m.13513G>A, m.1555A>G, m.3256C>T, and m.3336T>C between human buccal epithelium and whole blood. In addition, we studied the variability of heteroplasmy levels in the same mutations in participants with different IMTs and different degrees of luminal occlusion. No correlation between the heteroplasmy level of mtDNA mutations and atherosclerotic plaque size was found. This may indicate an insufficient sample size to identify such a relationship. However, a significant direct correlation of mutation m.1555A>G with IMT was detected for DNA samples from both the buccal epithelium and whole blood. In line with previous research,[16,19-21] we found no correlation between the level of mutational burden and either gender or age, apart from an inverse correlation of mutation m.1555A>G with age in buccal epithelial samples.

It is possible that the data on mutation m.1555A>G indicate that individuals with a high heteroplasmy level of this mutation may have a higher IMT than their peers with lower heteroplasmy levels. It is important to note that mutation m.1555A>G is localized in the gene of subunit 12S of the mitochondrial genome. The association of this mutation with increased IMT may suggest that defects in mitochondrial ribosomes can lead to a decrease in the protein chains of respiratory enzymes. This might result in decreased ATP synthesis in cells, leading to oxidative stress and the unlimited proliferation of mutant cells. Such a process might culminate in increased IMT in the carotid arteries and the formation of atherosclerotic plaques (i.e. atherosclerosis).

In conclusion, the buccal epithelium and whole blood are quite common subjects of investigation in medicine, in particular in the genetic diagnosis of various human diseases. The data obtained during the present study suggest that DNA samples from either the buccal epithelium or whole blood can be used to determine the heteroplasmy levels of the mitochondrial genome mutations m.12315G>A, m.13513G>A, m.1555A>G, m.3256C>T, and m.3336T>C, which have been associated with atherosclerosis. It should be emphasized that buccal epithelial samples are far easier to collect, making this a preferable tissue for studying the association of mitochondrial genome mutations with atherosclerosis and the search for new biomarkers.

These findings will be of particular interest to specialists in medical genetics and medical practitioners.

Authors' contributions

Manuscript's conception and writing: V.V. Sinyov, M.A. Sazonova, A.Y. Postnov, A.N. Orekhov
Data base fulfillment: V.V. Sinyov, M.A. Sazonova, A.I. Ryzhkova, E.V. Galitsyna, A.A. Melnichenko
Manuscript's revision: A.V. Grechko, I.A. Sobenin

Financial support and sponsorship

This study was supported by the Russian Science Foundation (grant #14-14-01038).

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial

relationships that could be construed as a potential conflict of interest.

Patient consent

All of the participants completed signed consent forms.

Ethics approval

This study was carried out according to the Declaration of Helsinki and with permission of an ethics committee (Russian Cardiology Research and Production Complex, Moscow, Russia).

REFERENCES

1. Sazonova MA, Budnikov YY, Khasanova ZB, Anton P, Sobenin IA, Orekhov A. Direct quantitative assessment of mutant allele in mitochondrial genome in atherosclerotic lesion of human aorta. *Atheroscler Suppl* 2007;8:45-6.
2. Postnov AY, Sazonova MA, Budnikov YY, Khazanova ZB, Sobenin IA, Orekhov AN. PO5-116 Association of somatic mitochondrial mutations with atherosclerosis. *Atheroscler Suppl* 2007;8:46.
3. Sobenin IA, Sazonova MA, Ivanova MM, Zhelankin AV, Myasoedova VA, Postnov AY, Nurbaev SD, Bobryshev YV, Orekhov AN. Mutation C3256T of mitochondrial genome in white blood cells: novel genetic marker of atherosclerosis and coronary heart disease. *PLoS One* 2012;7:e46573.
4. Sobenin IA, Sazonova MA, Postnov AY, Bobryshev YV, Orekhov AN. Mitochondrial mutations are associated with atherosclerotic lesions in the human aorta. *Clin Dev Immunol* 2012;2012:832464.
5. Li H, Liu D, Lu J, Bai Y. Physiology and pathophysiology of mitochondrial DNA. *Adv Exp Med Biol* 2012;942:39-51.
6. de Laat P, Koene S, van den Heuvel LP, Rodenburg RJ, Janssen MC, Smeitink JA. Clinical features and heteroplasmy in blood, urine and saliva in 34 Dutch families carrying the m.3243A>G mutation. *J Inherit Metab Dis* 2012;35:1059-69.
7. Naue J, Hörer S, Sänger T, Strobl C, Hatzer-Grubwieser P, Parson W, Lutz-Bonengel S. Evidence for frequent and tissue-specific sequence heteroplasmy in human mitochondrial DNA. *Mitochondrion* 2015;20:82-94.
8. Chinnery PF, Zwijnenburg PJ, Walker M, Howell N, Taylor RW, Lightowlers RN, Bindoff L, Turnbull DM. Nonrandom tissue distribution of mutant mtDNA. *Am J Med Genet* 1999;85:498-501.
9. Miasoedova VA, Kirichenko TV, Orekhova VA, Sobenin IA, Mukhamedova NM, Martirosian DM, Karagodin VP, Orekhov AN. Study of intima-medial thickness (IMT) of the carotid arteries as an indicator of natural atherosclerosis progress in Moscow population. *Patol Fiziol Eksp Ter* 2012;(3):104-8. (in Russian)
10. Sazonova MA, Orekhov AN, Sobenin IA. Mitochondrial genome defects andatherosclerosis. Role of mitochondrial genome pathologies in atherosclerotic lesionsformation of an arterial wall. Palmarium Academic Publishing; 2014. (in Russian)
11. Sazonova M, Budnikov E, Khasanova Z, Sobenin I, Postnov A, Orekhov A. Studies of the human aortic intima by a direct quantitative assay of mutant alleles in the mitochondrial genome. *Atherosclerosis* 2009;204:184-90.
12. Sazonova MA, Sinyov VV, Barinova VA, Ryzhkova AI, Zhelankin AV, Postnov AY, Sobenin IA, Bobryshev YV, Orekhov AN. Mosaicism of mitochondrial genetic variation in atherosclerotic lesions of the human aorta. *Biomed Res Int* 2015;2015:825468.
13. Alderborn A, Kristofferson A, Hammerling U. Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing. *Genome Res* 2000;10:1249-58.
14. IBM Analytics. IBM SPSS. Available from: http://www.ibm.com/analytics/us/en/technology/spss/spss-trials.html#spss-trials%20n.d. [Last accessed on Jan 17, 2017]
15. Wallace DC, Brown MD, Lott MT. Mitochondrial DNA variation in human evolution and disease. *Gene* 1999;238:211-30.
16. Frederiksen AL, Andersen PH, Kyvik KO, Jeppesen TD, Vissing J, Schwartz M. Tissue specific distribution of the 3243A->G mtDNA mutation. *J Med Genet* 2006;43:671-7.
17. Litvinova NA, Voronkova AS, Nikolaeva EA, Sukhorukov VS. Tissue-specific features of mitochondrial DNA polymorphisms. *Russian Bull Perinatol Pediatr* 2015;60:76-8. (in Russian)
18. Pyle A, Taylor RW, Durham SE, Deschauer M, Schaefer AM, Samuels DC, Chinnery PF. Depletion of mitochondrial DNA in leucocytes harbouring the 3243A->G mtDNA mutation. *J Med Genet* 2007;44:69-74.
19. Spyropoulos A, Manford M, Horvath R, Alston CL, Yu-Wai-Man P, He L, Taylor RW, Chinnery PF. Near-identical segregation of mtDNA heteroplasmy in blood, muscle, urinary epithelium, and hair follicles in twins with optic atrophy, ptosis, and intractable epilepsy. *JAMA Neurol* 2013;70:1552-5.
20. Lee HY, Chung U, Park MJ, Yoo JE, Han GR, Shin KJ. Differential distribution of human mitochondrial DNA in somatic tissues and hairs. *Ann Hum Genet* 2006;70:59-65.
21. Roberts KA, Calloway C. Characterization of mitochondrial DNA sequence heteroplasmy in blood tissue and hair as a function of hair morphology. *J Forensic Sci* 2011;56:46-60.

Shaikhrezai *et al. Vessel Plus* 2017;1:151-4
DOI: 10.20517/2574-1209.2017.06

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Case Report **Open Access**

Bjork-Shiley tricuspid valve endocarditis thirty years after implantation

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How to cite this article: Shaikhrezai K, Singh SSA, Spadaccio C, Hunter S. Bjork-Shiley tricuspid valve endocarditis thirty years after implantation. *Vessel Plus* 2017;1:151-4.

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Article history:
Received: 4 Mar 2017
Accepted: 22 Aug 2017
Published: 26 Sep 2017

Key words:
Mechanical valve, tricuspid valve stenosis, endocarditis

ABSTRACT

The Bjork-Shiley valve has been in use since 1971. Its longevity has been well reported in the literature. The study report a case of the Bjork-Shiley valve implanted in a young man, who presents with bacterial endocarditis 30 years after implantation of the valve. Transthoracic echocardiography showed vegetations in the mechanical tricuspid valve, with positive blood cultures csonfirming the diagnosis. Artificial valves increase the risk of contracting endocarditis but this is the first report of a case of bacterial endocarditis occurring in a mechanical tricuspid valve replacement 30 years after the initial implantation.

INTRODUCTION

The choice of valve implantation is a complex decision that is usually made after consultation with the family and the patient. In the era of transcatheter valve insertions, the decision may start favouring the bioprosthetic valves. Recent updates in pericardial valves and anti-calcification techniques have also resulted in more durable bioprosthetic valves with outcomes surpassing 20 years expected, although this is yet to be seen in younger patients' due to the preference for a more durable mechanical valve given the potential longevity offered. Mechanical valves are also likelier to be used in the setting of mitral stenosis which almost invariably

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occurs alongside atrial fibrillation which is an indication for anticoagulation in itself. Point of care testing for anticoagulation efficiency with allied health professional led international normalised ratio clinics have been in favour of mechanical valves but the introduction of direct oral anti-coagulants (DOACs) for first line atrial fibrillation treatment has brought a paradigm shift favouring bioprosthetic valves. Tricuspid valve replacements however, are less common compared to the mitral valve, thereby lacking a consensus on the optimal choice of valve. As the right heart is a lower pressure system, the stasis of blood causing increase thrombogenicity may favour the use of tissue based valves. Artificial valves do predispose patients to an increased risk of bacterial endocarditis. This can be attributed to adherent surfaces of suture lines, turbulent flows and nidus for infections in microthrombi produced especially in mechanical valves.

We present a case report of a patient who presented 30 years after his first operation with bacterial endocarditis.

CASE REPORT

A 48-year-old Caucasian male presented to our institution with fever, shortness of breath and increasing fatigability. His past medical history included a previous tricuspid valve replacement with a Bjork-Shiley tilting disc valve in 1986 due to endocarditis from an unknown origin and severe tricuspid valve regurgitation.

As part of his follow-up, he attended annual surveillance echocardiography clinics. A possible pannus forming around the mechanical valve prosthesis was noted on his latest scan. This extensively reduced the effective orifice area. As he was well and asymptomatic, a plan was made to repeat the echocardiogram in 6 months' time. However, he became severely unwell following a urinary tract infection and required intravenous antibiotic therapy.

He was found collapsed and presented to the intensive care unit in our institute requiring mechanical ventilation. He had no pathognomonic signs of bacterial endocarditis such as splinter haemorrhages, Roth's spots, Osler's nodes and Janeway lesions. An urgent echocardiogram highlighted large prominent vegetations with severe tricuspid valve stenosis [Video 1]. Blood cultures were positive for *Staphylococcus Aureus*.

He had multiple episodes of non-sustained ventricular tachycardia. He later developed an acute kidney injury and the following a multidisciplinary team decision, he underwent an urgent redo-operation.

After right atriotomy, pannus and vegetation was observed on the mechanical valve were clearly visible [Figures 1 and 2]. The mechanical valve was explanted and replaced with a 31-mm bioprosthetic valve. His post-operative period was uneventful but for an episode of acute kidney injury which settled with intravenous fluids. He made a good recovery and was discharged on the 20th post-operative day. He was seen in the post-operative clinic after discharge and reports being well with no further complications.

DISCUSSION

Tricuspid valve endocarditis is a well recognised disease with a wide spectrum of pathologies which is not limited to intravenous drug abuse and can be caused even by monitoring lines.[1] Up to 40% of prosthetic valve endocarditis is cause by *Staphylococci* infections.[2] Artificial valve endocarditis is also more common in the atrioventricular valve owing to reduced flow velocities across the valves particularly in the right side of the heart. Pannus growth tends to occur in the tissue valve interface and tracks along the suture lines as seen in our patient. However, encroachment into

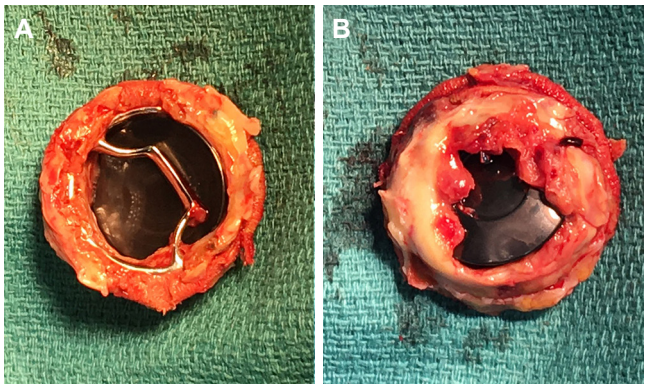

Figure 1: Pannus formation on atrial (A) and ventricular (B) surface of the valve; the patient remained asymptomatic and compliant with warfarinisation with no thromboemboli event during the 30-year lifespan of the valve

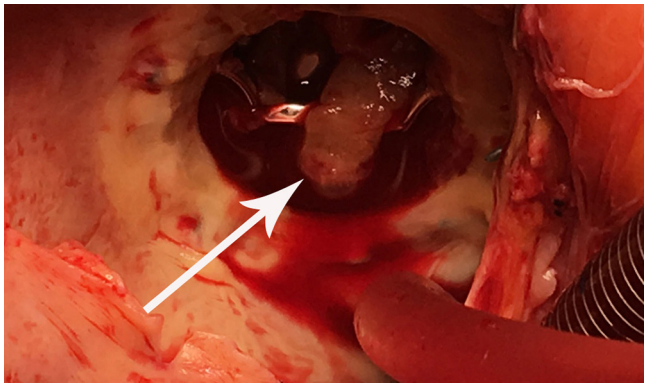

Figure 2: Vegetation (arrow) on atrial surface of the valve from surgeon's view; the endocarditis was caused by pyelonephritis with *Staphylococcus Aureus*

the valve orifice is uncommon. Pannus formation was the causes mechanical valve obstruction in about 10% of valves.[3]

Implantation of prosthetic valve in tricuspid position has been debated frequently in the literature with a consensus on bioprosthesis favouring mechanical valve due to the increased occurrence of valve-related events, especially the composite of thrombosis, embolism, and bleeding high risk of thrombosis when a mechanical valve is implanted in tricuspid position.[4,5] The management of thrombosis can be challenging and depending on patient clinical manifestation or degree of valvular obstruction the management can vary from observation to emergency operation.[6]

With the emergence of transcatheter technology, bioprosthetic valve implantation in the tricuspid position is an ideal alternative while future valve-in-valve implantation feasible in the event of valve failure.[7] To our knowledge there is no report of mechanical valve implantation lasting more than 30 years in the tricuspid position. The abovementioned patient had excellent compliance with anticoagulation therapy, thus explaining his mechanical valve longevity without any episodes of thrombosis. It has been observed that non-compliance with coumadin plays a pivotal role in thrombosis and valve malfunction.[4]

Bjork-Shiley tilting disc valves had a good clinical profile following decades of implantation. However, they became obsolete following the invention of more innovative mechanical valves with better profiles facilitated by double tilting discs. In 1979, the convexo-concave design of the Bjork-Shiley valve came under scrutiny as it was prone to fractures in the outflow strut. This resulted in the food and drug administration withdrawing approval of the 1986, around the time our patient had his valve implanted.[8]

Despite advancements in valve design and refinement in surgical techniques, the type of valve implantation (mechanical versus bioprosthesis) remains a dilemma among surgeons in all 4 valves positions. Although, patient autonomy dictates that they be given priority to choose the type of valve, a surgeons input is of paramount importance. The evolving technology has influenced the guidelines with rapid amendments and new published editions. Newer generations of mechanical valves with better performance with regards to amplitude and duration of regional backflow velocities across the mechanical valve as the main culprit for thrombosis are currently available.[9] DOACs may have a role in the mechanical valves of the future despite the findings of the recently published RE-ALIGN trial.[10] It may be a reality to have alternatives to coumadin therapy for mechanical valves in the near future.

DECLARATIONS

Authors' contributions

Writing manuscript, operating surgeon: K. Shaikhrezai
Writing and reviewing manuscript: S.S.A. Singh
Reviewing manuscript: C. Spadaccio
Supervision and operating surgeon: S. Hunter

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Written consent was obtained from the patient preoperatively.

Ethics approval

Medical Photography was obtained with permission from the patient and the Local Clinical Governance Committee.

REFERENCES

1. Sundhu M, Mohapatra S, Arobelidze S, Gundelly P, Changarath Vijayan AK. Infective endocarditis in a patient with celiac disease after censtral venous catheter insertion. *Cureus* 2017;9:e1027.
2. Selton-Suty C, Célard M, Le Moing V, Doco-Lecompte T, Chirouze C, Iung B, Strady C, Revest M, Vandenesch F, Bouvet A, Delahaye F, Alla F, Duval X, Hoen B; AEPEI Study Group. Preeminence of staphylococcus aureus in infective endocarditis: a 1-year population-based survey. *Clin Infect Dis* 2012;54:1230-9.
3. Deviri E, Sareli P, Wisenbaugh T, Cronje SL. Obstruction of mechanical heart valve prostheses: clinical aspects and surgical management. *J Am Coll Cardiol* 1991;17:646-50.
4. Yaminisharif A, Alemzadeh-Ansari MJ, Ahmadi SH. Prosthetic tricuspid valve thrombosis: three case reports and literature review. *J Tehran Heart Cent* 2012;7:147-55.
5. Betancur-Gutiérrez A, Bayón-Fernández J, García-Rodríguez MÁ, García-Egido A, Garrote-Coloma C, Fernández-Vázquez F. Thrombosis of a mechanical tricuspid valve prosthesis resolved with fibrinolysis. *Rev Esp Cardiol (Engl Ed)* 2014;67:1064-6.
6. Joint Task Force on the Management of Valvular Heart Disease of the European Society of Cardiology (ESC); European Association for Cardio-Thoracic Surgery (EACTS); Vahanian A, Alfieri O, Andreotti F, Antunes MJ, Barón-Esquivias G, Baumgartner H, Borger MA, Carrel TP, De Bonis M, Evangelista A, Falk V, Iung B, Lancellotti P, Pierard L, Price S, Schäfers HJ, Schuler G, Stepinska J, Swedberg K, Takkenberg J, Von Oppell UO, Windecker S, Zamorano JL, Zembala M. Guidelines on the management of valvular heart disease (version

2012). *Eur Heart J* 2012;33:2451-96.
7. Panaich SS, Eleid MF. Tricuspid valve-in-valve implantation for failing bioprosthetic valves: an evolving standard of care. *Ann Transl Med* 2016;4:410.
8. Blackstone EH. Could it happen again? the björk-shiley convexo-concave heart valve story. *Circulation* 2005;111:2717-9.
9. Chaux A, Gray RJ, Stupka JC, Emken MR, Scotten LN, Siegel R. Anticoagulant independent mechanical heart valves: viable now or still a distant holy grail. *Ann Transl Med* 2016;4:525.
10. Shazly A, Afifi A. RE-ALIGN: first trial of novel oral anticoagulant in patients with mechanical heart valves - the search continues. *Glob Cardiol Sci Pract* 2014;2014:88-9.

Tummala *et al. Vessel Plus* 2017;1:155-8
DOI: 10.20517/2574-1209.2016.14

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Case Report **Open Access**

Leriche's syndrome: a rare complication following anterior approach lumbar spinal surgery

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How to cite this article: Tummala R, Ravakhah K, Gupta A. Leriche's syndrome: a rare complication following anterior approach lumbar spinal surgery. *Vessel Plus* 2017;1:155-8.

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Article history:
Received: 3 Dec 2016
Accepted: 9 Jan 2017
Published: 26 Sep 2017

Key words:
Leriche's syndrome,
anterior lumbar interbody fusion,
aortoiliac atherosclerotic disease,
thrombus,
aortoiliac endarterectomy,
aortobifemoral bypass

ABSTRACT

Leriche's syndrome is an aortic occlusive disease, which is due to obliteration of distal aorta above the site of bifurcation of common iliac arteries. The classic triad of symptoms include claudication, impotence, and absent or decreased femoral pulses. It may be acute or chronic in onset. Most of the cases are chronic in nature due to baseline pathophysiology involving atherosclerotic changes in the aorta. There are many causes of acute leriche's syndrome like surgical manipulation, trauma, thromboembolic disease, hypercoagulability, atrial fibrillation, neoplasm, intraplaque hemorrhage in an aneurysm. Post-surgical Leriche's syndrome is rare and needs a strong index of suspicion to diagnose. The authors highlight a case of Leriche's syndrome, as a post-surgical complication and its clinical importance.

INTRODUCTION

Leriche's syndrome is an aortic occlusive disease, which is due to obliteration of distal aorta above the site of bifurcation of common iliac arteries.[1] The classic triad of symptoms include claudication, impotence, and absent or decreased femoral pulses.[2] It may be acute or chronic in onset. Most of the cases

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are chronic in nature due to baseline pathophysiology involving atherosclerotic changes in the aorta. Risk factors like hyperlipidemia, hypertension, diabetes would aggravate the underlying chronic condition.[1] Acute cases are rare and are usually related to acute thrombus occlusion.

Injuries of the thoracic and abdominal aorta after spine surgery are rare but may result in severe life-threatening complications. Acute and chronic vascular injuries such as perforations leading to major bleeding or hematoma formation, erosions or pseudoaneurysm formation are some of the vascular complications of lower spinal surgeries.[2-4] However, most injuries are delayed due to chronic irritation of the aortic wall.

The majority of spinal surgery complications highlight neurological sequelae, while vascular issues are less frequent.[5] Post spinal surgery Leriche's syndrome often misdiagnosed because of overlapping symptoms of pseudo-claudication from spinal canal stenosis. We highlight a case of acute Leriche's syndrome after anterior lumbar interbody fusion (ALIF) surgery, and its presentation.

CASE REPORT

A 58-year-old male patient presented to the hospital 3 weeks after ALIF surgery at L2-S1, performed due to lumbar spinal stenosis. He reported sudden numbness, tingling and weakness of both lower extremities from the waist down. He had none of these lower extremity symptoms before surgery. Prior to his visit to our Emergency Department (ED), he was discharged from two EDs in last 48 h with the diagnosis of diskitis. Review of the system included new onset leg claudication but no rest pain. The patient had the blood pressure of 140/95 mmHg, a heart rate of 80 beats/min and a respiratory rate of 16 breaths/min, whereas laboratory tests were inconspicuous. His past medical history was significant for osteoarthritis, chronic back pain, and hypertension. There was no prior history of peripheral vascular disease, coronary artery disease, hypercoagulable state or prior thrombosis formation. Only past surgical history included an operative history of ALIF of L2-S1, 3 weeks prior to presentation for lumbar spinal stenosis. He was a chronic smoker with 30 pack year's history. There was no associated fever, abdominal pain, nausea, vomiting, bladder or bowel incontinence. Initially, all his symptoms were attributed to post-surgical changes leading to pseudo-claudication and other symptoms. At this point, our differentials for sudden sensory and motor deficit in lower extremities were spinal cord compression, spinal cord abscess, retroperitoneal hematoma, and myelopathy.

On physical examination, he was alert and oriented. There was no point tenderness noted on palpation of the back. Abdomen was soft on palpation and normal bowel sounds heard without any tenderness. In the neurological examination, there was a loss of sensation to fine and crude touch in both lower extremities up to the mid thighs (L2-S1) and 4/5 power with +2 reflexes (patellar and ankle). The motor and sensory system of L1 distribution were normal. Babinski's sign was present bilaterally. Rectal sphincter tone was normal. The patient had no sensory or motor deficits in the upper extremities. I-XII cranial nerves intact. Vitals were stable.

Review of operative note revealed, that anterior aspect of lumbosacral spines was reached by the surgeon through retroperitoneal approach, following left paramedian incision. Exposure of the disks was accomplished after mobilization of left iliac vessels to the right side and retraction by a malleable retractor. During disk removal and positioning the cages, the major vessels were mobilized to right side and protected by the retractor. No direct injury to any vessels or excessive bleeding leading to hematoma was noted intraoperatively. The operative time was approximately 4 h. Routine blood works including complete blood count with differentiation, complete metabolic profile, erythrocyte sedimentation rate, C-reactive protein, creatinine kinase were normal.

Due to a strong suspicion of complications from recent spinal surgery computed tomography (CT) of the thoracolumbar spine was ordered which showed anterior interbody fusion changes at L2-S1 with intact hardware. Mild to moderate multilevel central canal narrowing was noted in CT scan, which was secondary to scar tissue in the anterior epidural recesses, consistent with recent surgical history. The imaging study ruled out any critical central canal stenosis, acute lumbar osseous injury or paraspinal abscess. There was not enough evidence of myelopathy and radiculopathy from the CT and blood works, explaining the symptoms. His condition did not improve despite steroid treatment as well. Then we started looking for vascular causes. CT angiography of abdomen and pelvis was performed, which ruled out intramural hematoma or aortic dissection. However, there was an extensive aortoiliac atherosclerotic disease with long segment occlusive thrombosis of the infrarenal abdominal aorta by a crescentic mural thrombus [Figures 1 and 2].

As part of acute thrombosis work up, hypercoagulable

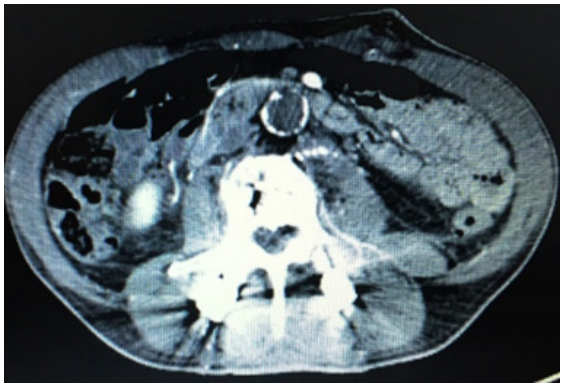

Figure 1: Enhanced computed tomography of thorax and abdomen imaging (sagittal reformation) shows a complete thrombosis of the distal aorta below the left renal artery, extending to the common iliac bifurcations

studies were normal. No abnormal cardiac rhythm including atrial fibrillation was detected in the telemetry. Transesophageal echocardiography ruled out any left atrial thrombus. There were no compressive lymph nodes or any other structure compressing on the aorta. Diagnosis of acute Leriche's syndrome was established which was attributed to acute vascular injury following ALIF. The patient underwent emergent aortoiliac endarterectomy and aorta bifemoral bypass. During the surgery, acute thrombus was found in the more proximal infrarenal aorta which was retrieved and then dacron graft was placed. His postoperative period was uneventful with complete resolution of symptoms within next 48 h. Postoperative, the patient was started on antithrombotic therapy. The symptoms completely resolved post vascular surgery interventions and he was followed up as an outpatient after a week. He resumed his regular lifestyle without any residual symptoms. Although the patient had risk factors for Leriche's syndrome, the acuity of the presentation was attributed to his recent surgical intervention where abdominal aorta was likely traumatized. The injury to aortic endothelium resulted in acute thrombus formation.

DISCUSSION

Leriche's syndrome is an occlusive disease of the aorta which is characterized by a triad of symptoms like erectile dysfunction, claudication of thighs and legs and diminished or absent femoral pulse.[6]

There are many causes of acute Leriche's syndrome like surgical manipulation, trauma, thromboembolic disease, hypercoagulability, atrial fibrillation, neoplasm, intraplaque hemorrhage in an aneurysm. Post-surgical Leriche's syndrome is rare and needs a strong index of suspicion to diagnose. Surgical treatment of adult lumbar spinal disorders is associated with substantial

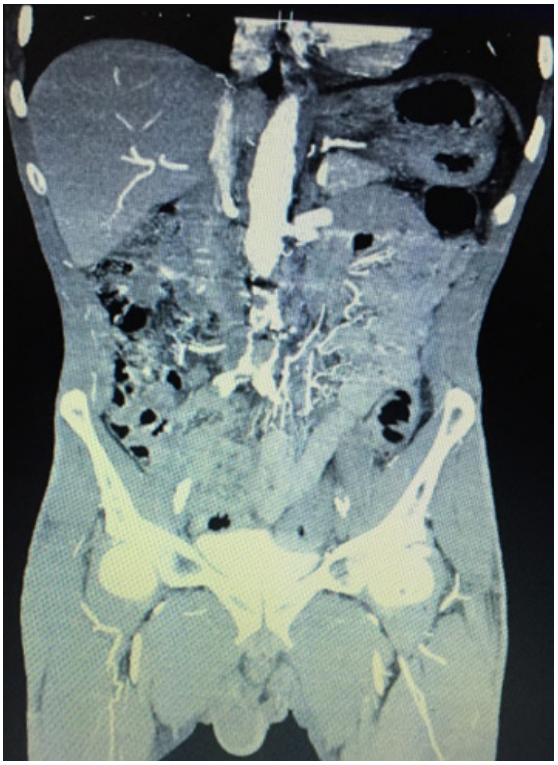

Figure 2: Enhanced computed tomography imaging (axial) demonstrates an acute thrombus in the abdominal aorta causing complete occlusion

risk of intra, peri, and postoperative complications.[7] A systematic review conducted by Wood *et al*.[8] showed that vascular injury in elective anterior lumbosacral surgery is less than 5% and complications being thrombosis, pulmonary embolism, and prolonged hospitalization. It is shown that vascular complications after ALIF range from 1.9% to 5.6% in the general population.[5,9,10] The vascular complications can be in the form of acute thrombosis, retroperitoneal hemorrhage, and injury to the major blood vessels.

We highlight the rare case of acute Leriche's syndrome following ALIF surgery which was initially misdiagnosed. Firstly, the patient's presentation of sensory and motor impairment misled physicians to have an impression of neuropathy. Secondly, ischemic neuropathy is rarely caused by Leriche's syndrome.[11,12] Another reason for misdiagnosis is his recent spinal surgery. Though the patient was at risk for thrombus formation due to risk factors and atherosclerosis, the interesting part was the acute presentation of symptoms. There was a case study in 2003 which reported 8 cases arterial complication following ALIF in which possible risk factors were analyzed. The authors encountered 6 cases of common iliac artery occlusion and two cases of acute vasospasm as a complication of ALIF.[5] Another article in 2010 documented the incidence of vascular complications during ALIF in 212 consecutive patients,

out of which 13 (6.1%) vascular injuries occurred of which 5 were major (38.5%). One major arterial injury (0.5%) occurred and required arterial thrombectomy and stent placement.[9] A case report of an acute Leriche-like syndrome after posterior instrumentation of the spine highlighting iatrogenic trauma to the aorta during spinal surgeries was described by in a 47-year-old female who developed an acute occlusion of the infrarenal aorta after posterior transpedicular instrumentation of an L1 burst-fracture.[13]

In the literature review, it was found that ALIF surgery at L2-L5 levels is most likely prone to cause vascular injury,[3-5] just as the procedure in our case. The explained reason is that left common iliac vessels normally transverse the prevertebral space the L4-5 interspace, and thus prevents direct access to the spine. These vessels must be mobilized with the help of retractors from left to right to expose the bony midline.[8] And there might be a progression of the injury from iliac artery upwards to the aorta. We believe the retraction of vessels can promote thrombus formation, through a mechanical injury to the intima of the artery. The mechanism of thrombus formation is detailed below. The retraction of the artery is considered as a non-penetrating arterial injury which has been shown that stretching of the artery can cause a rupture of the intimal layer.[14] The second reasoning is vascular retraction causes turbulent flow near the retraction site. The disturbance in normal flow cause platelets to adhere to the injured endothelium and lead to the process of primary hemostasis.[15] The turbulent flow also prevents dilution by fresh flowing blood with activated clotting factors, which promote secondary hemostasis and eventual thrombus formation.[15] The aortic occlusion of Leriche's syndrome is usually caused by diffuse atherosclerotic changes, exacerbated by smoking, diabetes, hypertension, and hypercholesterolemia.[1] However, iatrogenic trauma to the blood vessels as a cause for thrombosis has been documented.[16]

The retraction of iliac vessels in our case during ALIF could have promoted endothelial injury leading to thrombus formation. In addition, smoking itself can promote hypercoagulability via unknown mechanisms.[15]

Authors' contributions

Patient's treatment and manuscript's preparation: R. Tummala
Manuscript review: K. Ravakhah and A. Gupta

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Appropriate consent was obtained from the patient regarding the usage of details for educational purposes.

Ethics approval

This case report is waived for ethical approval.

REFERENCES

1. Lee WJ, Cheng YZ, Lin HJ. Leriche's syndrome. *Int J Emerg Med* 2008;1:223.
2. Kakkos SK, Shepard AD. Delayed presentation of aortic injury by pedicle screws: report of two cases and review of the literature. *J Vasc Surg* 2008;47:1074-82.
3. Baker JK, Reardon PR, Reardon MJ, Heggeness MH. Vascular injury in anterior lumbar surgery. *Spine (Phila Pa 1976)* 1993;18:2227-30.
4. Brau SA, Delamarter RB, Schiffman ML, Williams LA, Watkins RG. Vascular injury during anterior lumbar surgery. *Spine J* 2004;4:409-12.
5. Kulkarni SS, Lowery GL, Ross RE, Ravi Sankar K, Lykomitros V. Arterial complications following anterior lumbar interbody fusion: report of eight cases. *Eur Spine J* 2003;12:48-54.
6. Jawor WJ, Plice SG. Thrombotic obliteration of the abdominal aorta; report of a case. *J Am Med Assoc* 1952;149:142-3.
7. Faciszewski T, Winter RB, Lonstein JE, Denis F, Johnson L. The surgical and medical perioperative complications of anterior spinal fusion surgery in the thoracic and lumbar spine in adults. A review of 1223 procedures. *Spine (Phila Pa 1976)* 1995;20:1592-9.
8. Wood KB, Devine J, Fischer D, Dettori JR, Janssen M. Vascular injury in elective anterior lumbosacral surgery. *Spine (Phila Pa 1976)* 2010;35:S66-75.
9. Heary RF, Benzel EC, Vaicys C. Anterior lumbar interbody fusion. In: Benzel's Spine surgery: Techniques, Complication, Avoidance, and Management, 2nd ed. Philadelphia: Elsevier; 2005. p. 474-85.
10. Garg J, Woo K, Hirsch J, Bruffey JD, Dilley RB. Vascular complications of exposure for anterior lumbar interbody fusion. *J Vasc Surg* 2010;51:946-50.
11. McCoy CE, Patierno S, Lotfipour S. Leriche syndrome presenting with multisystem vaso-occlusive catastrophe. *West J Emerg Med* 2015;16:583-6.
12. Yoon DH, Cho H, Seol SJ, Kim T. Right calf claudication revealing leriche syndrome presenting as right sciatic neuropathy. *Ann Rehabil Med* 2014;38:132-7.
13. Blocher M, Mayer M, Resch H, Ortmaier R. Leriche-like syndrome as a delayed complication following posterior instrumentation of a traumatic L1 fracture: a case report and literature review. *Spine (Phila Pa 1976)* 2015;40:1195-7.
14. Sonmez B, Yorukoglu Y, Williams BT. Traction injury in the internal mammary artery. Report of a case and review the literature. *J Cardiovasc Surg (Torino)* 1990;31:592-4.
15. Mitchell RN, Cotran RS. Hemodynamic disorders, thrombosis, and shock. In: Cotran RS, Kumar V, Collins T, eds. Robbins pathological basis of disease, 6th ed. Philadelphia: WB Saunders; 1999. p. 124-9.
16. Huang JH, Lee CH, Tsai TC, Peng SY. Perioperative thrombotic occlusion of left external iliac artery during anterior lumbar interbody fusion. *Arch Orthop Trauma Surg* 2008;128:1107-10.

Bertellotti *et al. Vessel Plus* 2017;1:159-62
DOI: 10.20517/2574-1209.2017.10

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Case Report **Open Access**

Stab wound with transection of left vertebral artery at V3

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How to cite this article: Bertellotti R, Ogun OA, Mironov A, Asensio JA. Stab wound with transection of left vertebral artery at V3. *Vessel Plus* 2017;1:159-62.

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Article history:
Received: 3 Apr 2017
Accepted: 15 Jun 2017
Published: 26 Sep 2017

Key words:
Vertebral artery,
stab wound,
vertebral artery injuries

ABSTRACT

While vertebral artery injuries are uncommon, they can have significant morbidity if not identified and treated in a timely fashion. While the majority of vertebral artery injuries are the result of blunt injury and typically have favorable outcomes, a substantial percentage of patients with penetrating injury to the neck may also have vertebral artery injury necessitating angiographic or operative intervention. A 45-year-old male sustained a single stab wound to the apex of the posterior triangle of the neck, below the left mastoid process. At the scene, Emergency Medical Services personnel reported large blood loss and upon arrival, his initial vital signs were consistent with Class II/III hemorrhagic shock. Physical examination revealed a 9 cm longitudinal and deep laceration which began to bleed rapidly and profusely during his inital evaluation. The patient was intubated and rapidly transported to the operating room for exploration of the wound with direct control of the suspected vascular injury via suture ligation and application of vascular clips and to interventional radiology suite for embolization. Operative control was necessary however, immediate post-operative angiography allowed confirmation of collateral cerebral perfusion. The patient had an uneventful recovery and was evaluated in the Trauma Clinic during his 7, 14, 30 and 60-day follow-up.

INTRODUCTION

The vertebral artery is located deep within the neck therefore, it is rarely injured. An epidemiology study by Hsu *et al.*[1] identified 14 cases, 0.08% of vertebral artery injuries in a multi-institutional database of

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16,582 patients, a prevalence rate of 8.4 per 10,000. When examining penetrating trauma to the neck, the incidence has been reported as between 1.0-7.4%, with some variation attributed to mechanism (gunshot wound versus stab wound).[2] Some believe however, that the low incidence of vertebral artery injuries previously reported is due to inefficient diagnostic tools, before the advent of routine angiography in penetrating neck trauma.[3,4] Even with angiography, there are variations in vertebral artery anatomy that should be taken into consideration. Preoperative imaging revealed anomalous variations in 50% of patients with vertebral artery injuries, in a multicenter study.[1] With increasing incidence of vertebral artery injury in patients with cervical spine trauma,[5] vertebral artery injuries should be ruled out in patients presenting with neck and cervical spine trauma.[6] Early diagnosis and intervention is critical to successful management of vertebral artery injuries. Multiple complications result from these types of injuries, and the point of entry and exit of the foreign object in penetrating injuries to the neck could predict susceptibility to injury and outcome.[5,7]

In a prospective study by Jang *et al*.,[6] none of the patients experienced secondary neurologic deterioration from vertebrobasilar ischemia, similarly the patient in this case report also did not develop neurologic sequelae. Other studies have also shown that with proper management, patients experience uneventful recovery without residual effects.[1] While neurologic deficits are rare complications of vertebral artery injury, when neurologic deficit occurs, they could be devastating and permanent.[1] Other severe outcomes are complications of stroke, pseudoaneurysm, late-onset hemorrhage, brain stem and cerebellar infarcts, and death;[1,5,8,9] early diagnosis and management are therefore critical to positive outcome.

This case report aims to describe the roles of surgical procedures and interventional radiology in the successful management of emergent vertebral artery injuries.

CASE REPORT

A 45-year-old male who sustained a single stab wound at the apex of the posterior triangle of the neck below the left mastoid process was transported to the level 1 trauma center by Emergency Medical Services personnel, who reported large blood loss at the scene. Upon arrival, the patient's initial vital signs were: blood pressure (BP) 106/51 mmHg, pulse 139 bpm. Physical examination revealed a 9-cm longitudinal deep laceration with no active bleeding and the interventional radiologist was consulted. Subsequently, the wound began to bleed rapidly and profusely. Pressure was applied. The patient was intubated; massive transfusion protocol was activated and the patient was rapidly transported to the operating room (OR).

In the OR, the wound was explored by enlarging the incision longitudinally, and interventional radiology (IR) was contacted. Ideally, the patient should have gone directly to IR suite upon his admission. Unfortunately, the patient began to bleed significantly, thus precluding this option. IR however, had already been consulted. In the OR, the goal was to obtain hemostasis through direct control of the vessel; however, the location of the injury at the skull base precluded this. Temporary control in the OR was therefore obtained.

In the OR, the sternocleidomastoid was partially transected. Bleeding was controlled from muscular arterial and venous branches. A deeper wound track in the anteromedial aspect of wound was partially explored and bleeding was controlled. The sternocleidomastoid was re-approximated and the wound was closed. Estimated blood loss (EBL) was 1,100 mL. Total fluid replacement was 3,900 mL: crystalloids 2,700 mL and 4 units packed red blood cells (PRBCs) 1,200 mL.

The patient was transported to the intensive care unit (ICU) pending IR arrival. Immediately upon arrival, the patient became hypotensive with a systolic BP - 55 mmHg. He bled massively. Digital control was established and he was returned to the OR.

Senior trauma surgery staff was paged signal transducer and activator of transcription to the OR. The patient was then re-explored. The longitudinal incision was extended and the sternocleidomastoid muscle was completely transected along with the splenius capitis muscle. Transverse processes of C1-C2 were palpated and the pre-vertebral fascia was entered. The vertebral artery at V3 (third portion) was noted to be partially transected extracranially; it was controlled with vascular clips. Paired vertebral veins were controlled in the same fashion. Hemorrhage was thus controlled. The accessory spinal nerve was not visualized. Bone wax was applied and the wound closed. EBL was 3,000 mL. Total volume replacement was 6,400 mL: crystalloids 4,000 mL, 6 units PRBCs 1,800 mL, 2 units fresh frozen plasma 600 mL.

During the second intervention, a more complete exploration identified the injury thus allowing for definitive control.

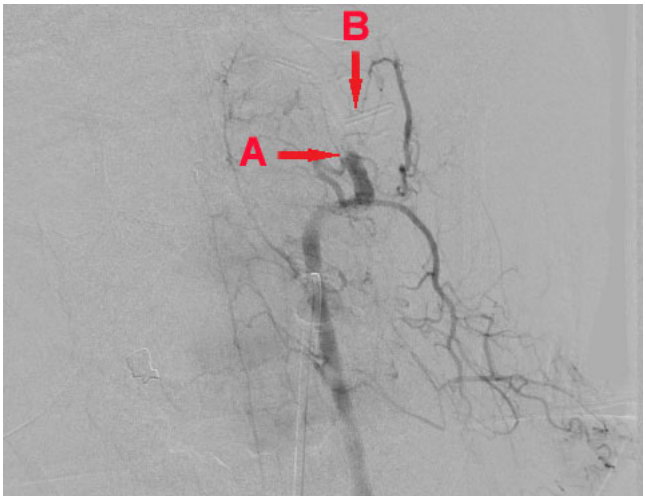

Figure 1: Vertebral artery surgically controlled prior to embolization (A) and digital subtraction shadow of surgically applied clips (B)

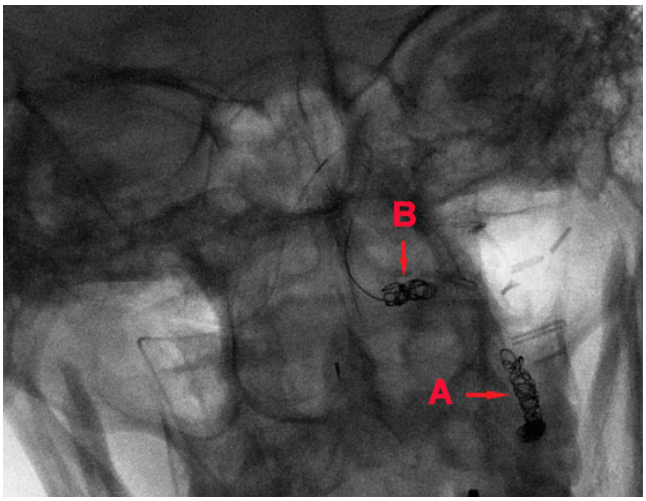

Figure 3: The micro catheter was placed in retrograde fashion in the left distal vertebral artery immediately above the extradural pseudoaneurysm during deployment. Endovascular approach obtained complete proximal (A) and distal (B) trapping of the extra dural left vertebral artery pseudoaneurysm above the surgical clips

Angiographic visualization of the vasculature was performed by catheter angiography, which revealed that complete acute occlusion of the left vertebral artery at the C1-C2 level was successfully achieved during the second surgical intervention [Figure 1]. The catheterization of the right vertebral artery showed normal perfusion with opacification of the very distal segment of the left vertebral artery. The late phase of angiography depicted a pattern consistent with an extra-dural pseudoaneurysm noted above the surgically placed clips.

The first step during angiography included endovascular embolization by coils of the left vertebral artery proximal to the visualized surgical occlusion. The second stage proceeded by navigation of the

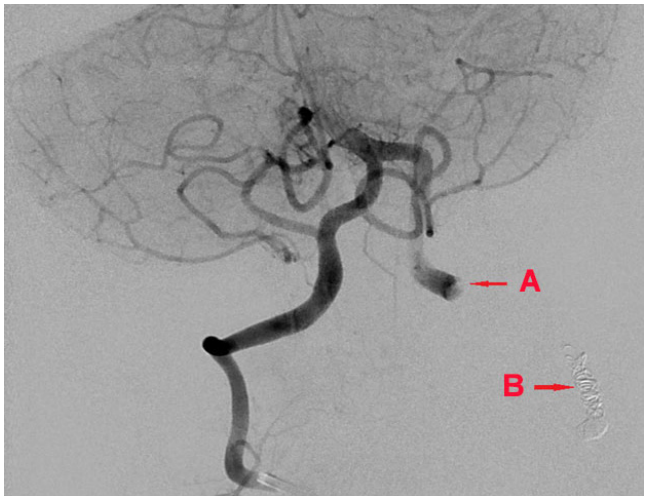

Figure 2: Right vertebral artery angiography after embolization by coils of the very proximal part of left vertebral artery (arrow showing coils, B). Retrograde opacity of the distal left vertebral artery shows persistent extravasation of contrast with an extradural pseudoaneurysm above the surgically placed clips which had controlled all bleeding operatively (A) secondary to vessel transection from the stab wound

micro-catheter through the right vertebral artery in retrograde fashion into the very distal part of the left vertebral artery with navigation of the catheter tip above the level of the surgically placed clips [Figure 2]. Embolization in this location was performed again by lumen occlusion with coils with meticulous preservation of the anterior spinal artery, which had its origin from the distal left vertebral artery. The territory of the left posterior inferior cerebellar artery was satisfactory collateralized by the left anterior inferior cerebellar artery. Thus, this endovascular approach obtained complete proximal and distal trapping of the left vertebral artery pseudoaneurysm above the surgically placed clips [Figure 3].

The patient was transferred to the ICU, extubated on the second post-operative day and transferred to the floor. He was observed for 2 days and discharged on post-operative day 4. No problems were noted with balance when ambulating. He had an uneventful recovery. He was evaluated in the Trauma Clinic during his 7, 14, 30 and 60-day follow-up with no sequelae noted from this rare and complex vascular injury.

DISCUSSION

Penetrating vertebral artery injuries are rare and their injuries were previously missed prior to the routine use of angiography in diagnosing penetrating neck injuries.

This patient specifically, sustained a penetrating injury to the vertebral artery and had hard signs of vascular injury with profuse bleeding at the scene and upon

examination of the wound during his initial evaluation. This is the most common presentation in penetrating vertebral artery injury at 50%, while 30% present only with soft signs, and 20% with no overt signs.[2] This frank hemorrhage was the impetus for the decision for operative exploration at the onset. This decision also corresponds to the algorithm suggested by Greer *et al*.[10] in their series of penetrating vertebral artery injuries managed during the military campaigns in Iraq and Afghanistan. Early operative intervention was also recommended by Reid and Weigelt[11] in their series of 43 patients from 1976-1986; both series agree that patients without acute life-threatening injuries can be safely evaluated without imaging studies (computed tomography angiography, digital subtraction angiography) with minimal risk to the patient.

Vertebral artery injuries though uncommon, could be fatal. Operative management is difficult and requires significant experience, in some cases, definitive control can be achieved through endovascular embolization by interventional radiology. While these approaches are described in the literature, there is little data on the multimodal therapy provided in this patient's care. Operative control was necessary however, immediate post-operative angiography allowed confirmation of collateral cerebral perfusion. The use of coil embolization in addition to ligation may also decrease the risk of post-operative morbidity such as fistula formation, re-bleeding, and pseudoaneurysm formation. Additional investigation of this approach is therefore warranted.

Authors' contributions

Study conception and design: J.A. Asensio
Analysis and interpretation of data: R. Bertellotti, O.A. Ogun, A. Mironov, J.A. Asensio
Drafting of manuscript: R. Bertellotti, O.A. Ogun
Critical revision: R. Bertellotti, O.A. Ogun, A. Mironov, J.A. Asensio

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable as there is no identifying data.

Ethics approval

Not required for this type of publication in our institution.

REFERENCES

1. Hsu WK, Kannan A, Mai HT, Fehlings MG, Smith ZA, Traynelis VC, Gokaslan ZL, Hilibrand AS, Nassr A, Arnold PM, Mroz TE, Bydon M, Massicotte EM, Ray WZ, Steinmetz MP, Smith GA, Pace J, Corriveau M, Lee S, Isaacs RE, Wang JC, Lord EL, Buser Z, Riew KD. Epidemiology and outcomes of vertebral artery injury in 16,582 cervical spine surgery patients: an AOSpine North America multicenter study. *Global Spine J* 2017;7:S21-7.
2. Asensio JA, Trunkey DD. Current Therapy of Trauma and Surgical Critical Care. Philadelphia, PA: Mosby, Elsevier; 2008.
3. Roper PR, Guinto FC Jr, Wolma FJ. Posttraumatic vertebral artery aneurysm and arteriovenous fistula: a case report. *Surgery* 1984;96:556-9.
4. Golueke P, Sclafani S, Phillips T, Goldstein A, Scalea T, Duncan A. Vertebral artery injury -- diagnosis and management. *J Trauma* 1987;27:856-65.
5. Tannoury C, Degiacomo A. Fatal vertebral artery injury in penetrating cervical spine trauma. *Case Rep Neurol Med* 2015;2015:571656.
6. Jang JW, Lee JK, Hur H, Seo BR, Lee JH, Kim SH. Vertebral artery injury after cervical spine trauma: a prospective study using computed tomographic angiography. *Surg Neurol Int* 2011;2:39.
7. Elkin DC, Harris MH. Arteriovenous aneurysm of the vertebral vessels. *Ann Surg* 1946;124:934-51.
8. Ohya J, Miyoshi K, Oka H, Matsudaira K, Fukushima M, Nagata K. Optimal measurement for "posterolateral protrusion" of the vertebral artery at the craniovertebral junction using computed tomography angiography. *J Craniovertebr Junction Spine* 2014;5:151-6.
9. Wang M. Death due to extensive cervicomedullary infarction following iatrogenic vertebral artery occlusion. *Forensic Sci Med Pathol* 2012;8:334-7.
10. Greer LT, Kuehn RB, Gillespie DL, White PW, Bell RS, Armonda RA, Fox CJ. Contemporary management of combat-related vertebral artery injuries. *J Trauma Acute Care Surg* 2013;74:818-24.
11. Reid JD, Weigelt JA. Forty-three cases of vertebral artery trauma. *J Trauma* 1988;28:1007-12.

Orekhov *et al. Vessel Plus* 2017;1:163-5
DOI: 10.20517/2574-1209.2017.33

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Topic: Atherosclerosis and Related Diseases **Open Access**

Introduction of the special issue "Atherosclerosis and Related Diseases"

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How to cite this article: Orekhov AN, Ivanova EA. Introduction of the special issue "Atherosclerosis and Related Diseases". *Vessel Plus* 2017;1:163-5.

Article history: Received: 19 Sep 2017 Accepted: 20 Sep 2017 Published: 28 Dec 2017

Atherosclerosis and atherosclerotic diseases remain the problem number one of current medicine and health care being the cause of myocardial infarction, stroke, sudden death, and other common causes of mortality and disability. Atherosclerotic diseases account for more than 50% of total mortality in industrialized societies. Atherosclerotic lesion development has a long asymptomatic phase. Therefore, in many cases, the first clinical manifestations of atherosclerosis appear when the lesion is already well developed causing significant narrowing of the vascular lumen. Current treatment of atherosclerosis is mainly symptomatic and does not affect the atherosclerotic lesion *per se*. Frequently, symptomatic therapy that improves the state of the patient even provokes further development of atherosclerosis. Unfortunately, direct anti-atherosclerotic therapy aimed at regression of atherosclerotic plaques remains to be developed. Such development should become a major goal of modern medicine and pharmaceutical industry, taking into account the burden and clinical significance of the disease.

The development of novel anti-atherosclerotic therapies is hindered by the lack of knowledge of the disease mechanisms and the absence of comprehensive concepts of the disease pathogenesis. Detailed studying of the disease mechanisms at molecular and cellular level using modern methods of analysis should attempt to solve this problem. Among the mechanisms to be studied at the first place, are lipid metabolism, innate immunity, chronic inflammation and cell differentiation. In addition to the traditional methods of morphology and biochemistry, the most advanced techniques of cellular and molecular biology should be applied. The results of these studies should contribute to the development of novel comprehensive concepts of the pathogenesis of atherosclerosis and to identification of novel pharmacological tagets for direct anti-atherosclerotic therapy. During the recent years, in addition to the widely-accepted lipid concept of atherogenesis, new targets for anti-atherosclerotic therapy associated with innate immunity and inflammation were proposed.

Three articles of this special issue are discussing the mechanisms of atherogenesis and the diseases associated with atherosclerosis. It is commonly accepted that modified low density lipoprotein (LDL) plays a key role in the initiation and development of

atherosclerotic lesions. Several types of atherogenic LDL modifications have been discovered. Alipov *et al.*[1] from Russia and France investigated modified LDL with reduced sialic acid content (desialylated LDL). Desialylation is one of the atherogenic modifications observed in circulating atherogenic LDL *in vivo*. The authors reviewed the available data on differences between native and desialylated LDL circulating in the blood of patients[1]. Desialylated LDL is small, dense and highly susceptible to oxidation. This atherogenic modification leads to increased cholesterol intake by macrophages and smooth muscle cells. Thus, it can be argued that the circulating modified LDL is susceptible to multiple modifications and is a trigger for atherogenesis.

Insulin-dependent (type 1) diabetes mellitus is known to be associated with accelerated atherosclerosis development, although the reasons for premature atherogenesis in diabetic patients remain obscure. Several hypotheses exist that attempt to reveal the molecular, cellular and biochemical mechanisms of premature atherogenesis in diabetic patients, but all of them possess insufficient explanatory properties. Sobenin *et al.*[2] from Russia tested a non-obvious hypothesis that insulin treatment may have a pro-atherogenic side effect on major atherosclerotic manifestations at the cellular level, namely, on proliferative activity and intracellular cholesterol accumulation. The obtained results suggest that insulin does not exert a direct atherogenic action at the level of arterial cells, with respect to proliferative activity and cholesterol content.

Diabetes mellitus type 2 is characterized by rapid progression of atherosclerosis. The development of atherosclerosis is largely determined by immune and inflammatory cells, primarily monocyte-derived macrophages. Recent studies demonstrated a relationship between the progression of atherosclerotic plaque and the ratio of pro-inflammatory and anti-inflammatory activated macrophages. Nikiforov *et al.*[3] from Russia and Belgium studied the ability of circulating monocytes from patients with diabetes, coronary heart disease and healthy subjects to activate into pro-inflammatory phenotype. Unexpectedly, they found that in patients with diabetes, monocytes were prone to pro-inflammatory stimulation to a higher degree than monocytes from healthy individuals. At the same time, monocytes from patients with coronary heart disease did not respond to stimulation. The mechanisms of this interesting difference should be investigated.

Genetic diagnostics is a very promising direction for clinical use in various diseases including atherosclerosis. Sazonova *et al.*[4] from Russia and Italy aimed to determine the threshold of heteroplasmy levels of mitochondrial DNA (mtDNA) mutations for diagnosis and prognosis of atherosclerotic lesions' appearance and development. The threshold heteroplasmy levels of 11 mitochondrial genome mutations associated with atherosclerosis were detected. The authors suggested that these markers may be used for evaluation of predisposition to atherosclerotic lesions development in humans.

Plasma D-dimer, a product of plasmin fibrinolysis, is a known biomarker of coagulation. In the Letter to Editor, Myasoedova *et al.*[5] from Italy suggested that D-dimer levels can be helpful for diagnostic and risk stratification of patients with both acute cardiac states and atherosclerosis. They focused on the relationship between inflammation and hemostasis to identify D-dimer biological mechanisms and its effects.

Five articles of the special issue are focused on clinical and diagnostic questions. In dyslipidemia, two main players are identified: LDL and high-density lipoprotein (HDL). The protective role of HDL against atherosclerosis is currently well known. However, the protective efficacy of HDL may be affected by structural and functional alterations of lipoprotein particles. Harangi *et al.*[6] from Hungary aimed to evaluate qualitative and quantitative markers of HDL in dyslipidemic patients and healthy control subjects. Their findings highlight the importance of HDL-associated pro- and antioxidant enzymes, suggesting the possible clinical benefit of these markers in dyslipidemia.

High blood lipid level remains a major risk factor for many diseases atherosclerotic disease including coronary artery disease. Samaha *et al.*[7] from Lebanon and Qatar have carried out cross-sectional survey to evaluate the effects of dietary and lifestyle habits on several blood lipid parameters in the Lebanese population. They revealed that hyperlipidemia affects more than half of the Lebanese population. Prevalence of hypercholesterolemia, hypertriglyceridemia and high levels LDL cholesterol was higher in smokers, physically inactive individuals or those who consume fatty meat or eggs. The authors emphasize that the majority of the individuals were unaware of their lipid profile, which mandates concerted efforts for both patient and public education.

Abdominal obesity and excessive body weight are associated with the development of atherosclerotic cardiovascular disease and with increased risk of sudden cardiac death, atrium fibrillation and other forms of arrhythmias. Bilovol *et al.*[8] from Ukraine

aimed to evaluate the probability of developing atrial fibrillation depending on the body mass index and adipokines levels in the general population. The obtained results indicate that while obesity was associated with different metabolic, hormonal and hemodynamic changes that affect heart muscle causing its structural and functional changes, the same changes were present in patients with body weight deficit, associated with similar pathogenic changes, in particular, with atrial fibrillation development.

We express our gratitude to all authors and hope that the readers will find our special issue interesting and useful.

DECLARATIONS

Authors' contributions
Wrote the manuscript: A.N. Orekhov
Edited the text and formatted the manuscript: E.A. Ivanova

Financial support and sponsorship
None.

Conflicts of interest
There are no conflicts of interest.

Patient consent
Not applicable.

Ethics approval
Not applicable.

REFERENCES

1. Alipov VI, Sukhorukov VN, Karagodin VP, Grechko AV, Orekhov AN. Chemical composition of circulating native and desialylated low density lipoprotein: what is the difference? *Vessel Plus* 2017;1:107-15.
2. Sobenin IA, Orekhova VA, Grechko AV, Orekhov AN. Is insulin pro-atherogenic at the cellular level? *Vessel Plus* 2017; doi: 10.20517/2574-1209.2017.19
3. Nikiforov NG, Galstyan KO, Nedosugova LV, Elizova NV, Kolmychkova KI, Ivanova EA. Proinflammatory monocyte polarization in type 2 diabetes mellitus and coronary heart disease. *Vessel Plus* 2017; doi: 10.20517/2574-1209.2017.21
4. Sazonova MA, Ryzhkova AI, Sinyov VV, Galitsyna EV, Orekhova VA, Melnichenko AA, Orekhov AN, Ravani AL, Sobenin IA. New markers of atherosclerosis: a threshold level of heteroplasmy in mtDNA mutations. *Vessel Plus* 2017; doi: 10.20517/2574-1209.2017.16
5. Myasoedova VA, Poggio P, Parolari A. A prominent role of D-dimer in inflammation and atherosclerosis. *Vessel Plus* 2017;1:96-7.
6. Harangi M, Szentpéteri A, Nádró B, Lőrincz H, Seres I, Páll D, Paragh G. HDL subfraction distribution and HDL function in untreated dyslipidemic patients. *Vessel Plus* 2017; doi: 10.20517/2574-1209.2017.27
7. Samaha AA, Zouein F, Gebbawi M, Fawaz M, Houjayri R, Samaha R, Baydoun S, Eid AH. Associations of lifestyle and dietary habits with hyperlipidemia in Lebanon. *Vessel Plus* 2017;1:98-106.
8. Bilovol O, Shaposhnikova Y, Ilchenko I, Shalimova A. Relationship between peculiarities of atrial fibrillation, body mass index and adipokines levels. *Vessel Plus* 2017; doi: 10.20517/2574-1209.2017.17

Harangi *et al. Vessel Plus* 2017;1:166-73
DOI: 10.20517/2574-1209.2017.27

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Topic: Atherosclerosis and Related Diseases **Open Access**

HDL subfraction distribution and HDL function in untreated dyslipidemic patients

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How to cite this article: Harangi M, Szentpéteri A, Nádró B, Lőrincz H, Seres I, Páll D, Paragh G. HDL subfraction distribution and HDL function in untreated dyslipidemic patients. *Vessel Plus* 2017;1:166-73.

Article history:
Received: 31 Jul 2017
Accepted: 18 Sep 2017
Published: 28 Dec 2017

Key words:
High-density lipoprotein, subfraction, paraoxonase, myeloperoxidase, dyslipidemia

ABSTRACT

Aim: The protective role of high-density lipoprotein (HDL) against atherosclerosis is well known. However, both structural and functional changes of the HDL particles may affect its protective efficacy. Increased levels of HDL-associated myeloperoxidase (MPO) and decreased HDL-linked paraoxonase-1 (PON1) activity have been reported in dyslipidemic patients. Some changes in HDL subfraction distributions were also studied previously, but data on structural and functional changes in dyslipidemia are not complete. Therefore, the authors aimed to evaluate these qualitative and quantitative markers of HDL in dyslipidemic patients and healthy control subjects. **Methods:** Anthropometric parameters, serum levels of lipoproteins and MPO, as well as PON1 activities were investigated in 81 untreated dyslipidemic patients and in 32 healthy gender-matched controls. Additionally, HDL subfractions were detected by an electrophoretic method on polyacrylamide gel (Lipoprint). **Results:** Significantly higher glucose, hemoglobin A1c, total cholesterol, low-density lipoprotein-cholesterol, triglyceride, lipoprotein(a), apolipoprotein B, C-reactive protein, and MPO levels were found in patients compared to the healthy subjects. There were no significant differences in PON1 paraoxonase and arylesterase activities between the two study groups, but MPO/PON1 ratio was significantly higher in patients. There was a shift towards the smaller HDL subfractions, but only the intermediate HDL ratio was significantly lower in patients compared to controls. **Conclusion:** The results highlight the importance of HDL-associated pro- and antioxidant enzymes suggesting the possible clinical benefit of MPO/PON1 calculation and confirm that quantification of HDL-C level alone provides limited data regarding HDL's cardioprotective effect. Calculation of MPO/PON1 ratio may be a useful cardiovascular marker in dyslipidemia.

INTRODUCTION

High-density lipoprotein (HDL) is a fraction of small, dense, protein-rich lipoprotein that is highly heterogeneous in their structural, chemical and biological properties. Using different analytical methods HDL can be separated to subclasses differing in size, density, shape and lipid and protein

composition[1]. Therefore, the subfraction distribution of HDL has implications for their functions. Although the compositional and functional heterogeneity of HDL particles is well known, HDL is often regarded as a single entity characterized by measurement of HDL-cholesterol (HDL-C) levels. Previous epidemiological studies have clearly shown that levels of HDL-C are inversely associated with the risk of coronary artery disease and its thrombotic complications[2,3]. Over the last few decades, substantial progress has been made in understanding how the HDL particle exerts protective effects on the vessel wall. HDL and its main protein constituent, apolipoprotein A1 (ApoA1) is protective in processes involved in atherogenesis, including mediation of reverse cholesterol transport, protection against oxidative stress, and inhibition of cytokine-induced expression of cellular adhesion molecules on endothelial cells. However, under particular circumstances, HDL particles may become dysfunctional caused by the loss of antioxidant and anti-inflammatory proteins in combination with a gain of acute phase proteins and some further pro-inflammatory components. This pro-inflammatory, dysfunctional HDL is unable to protect low-density lipoprotein (LDL) from oxidation and to prevent monocyte migration into the vessel wall induced by oxidized LDL particles[4]. Myeloperoxidase (MPO) mediates the oxidation of ApoA1 that creates a dysfunctional HDL particle, which activates nuclear factor kappa-B and promotes inflammation in the vessel wall[5]. MPO level and its role in oxidative stress and inflammation has been implicated in the pathophysiology of cardiovascular diseases such as coronary artery disease[6]. Human paraoxonase-1 (PON1) is a calcium-dependent lactonase that is produced by the liver and almost exclusively associated with HDL[7]. Although hydrolysis of homocysteine thiolactone might represent a major physiologic function of PON1[8], several further substrates have been described including other lactones[9], organophosphates and lipid peroxides[7]. Interestingly, some previous studies proved that PON1 is predominantly associated to the smaller and denser HDL3 subfractions, and PON1 activity of HDL2 was only 4% of that in HDL3[10]. Furthermore, previous data indicate that MPO, PON1 and HDL from a functional ternary complex in which MPO and PON1 inhibit each other's activity demonstrating the dysfunction of the HDL particle[11].

Dyslipidemia characterized by high levels of triglyceride, total and LDL cholesterol is an established risk factor for coronary heart disease. Therefore, to reduce the risk of cardiovascular complications lipid lowering treatment is widely used in this patient population. Additionally, combined forms of hyperlipidemia often require combined drug therapy. At the same time, it must be noted that lipid lowering agents, such as statins[12,13], fibrates[14] and ezetimibe[15] have a significant effect on HDL composition and function. Consequently, evaluating the effect of various lipid abnormalities on HDL properties in patients on lipid lowering therapy can be misleading[16].

Therefore, we investigated the structural and some functional HDL properties in newly diagnosed, untreated dyslipidemic patients and in healthy controls to evaluate the effect of dyslipidemia on the structural and functional properties of HDL characterized by the serum levels of HDL subfractions and MPO, PON1 paraoxonase and arylesterase activities and PON1 phenotyping. We hypothesized that the level and ratio of large HDL subfractions are higher, and the level and ratio smaller HDL subfractions are lower in dyslipidemic subjects compared to healthy controls. A lower number of smaller HDL subfractions may result in lower PON activities and higher MPO levels.

METHODS

Study population

The study protocol was approved by local and regional ethics committees and carried out in accordance with the Declaration of Helsinki of World Medical Association. All investigated subjects gave their written informed consent to participate in the study. We enrolled eighty-one newly diagnosed, untreated patient with Fredrickson type IIa and IIb hyperlipidemia that were referred to our lipid outpatient clinic at Department of Internal Medicine, University of Debrecen. Physical examination and carotid ultrasound were performed regularly. Further vascular imaging techniques (Doppler ultrasound and computer tomography) were performed in case of complaints or abnormal physical and electrocardiography examinations.

We excluded patients with pre-existing vascular complications from the study. Vascular complications were defined as ischemic heart disease (myocardial infarction or coronary sclerosis), ischemic cerebrovascular disease (ischemic stroke, transient ischemic attack, carotid artery stenosis/occlusion) and peripheral arterial disease. Vascular complications were established by patient history or upon the results of imaging techniques. Any lesions with measurable intravascular stenosis were defined as clinically significant. Further exclusion criteria included previous and ongoing lipid lowering therapy, chronic inflammatory conditions, autoimmune disease, and

endocrine or active liver disease including type 1 and 2 diabetes mellitus, chronic renal disease and malignancy.

Furthermore, thirty-two individuals were enrolled as a control population confirmed to be healthy by clinical and laboratory examinations.

Biochemical assays

After an overnight fasting period venous blood samples were taken into evacuated tubes and sera were prepared immediately by centrifugation at 1,500 × *g* for 10 min at 4 °C. Multiple aliquots of each samples were separated and stored at -70 °C. Routine laboratory analyses: total cholesterol, HDL-C, LDL-cholesterol (LDL-C), C-reactive protein (CRP), triglyceride, ApoA1, apolipoprotein B (ApoB), lipoprotein(a), hemoglobin A1c (HbA1c), and super-sensitive thyroid stimulating hormone were performed from fresh sera with Cobas c501 autoanalyzer (Roche Ltd., Mannheim, Germany) in the Department of Laboratory Medicine of University of Debrecen. Tests were performed according to the recommendation of the manufacturer. All the reagents were purchased from the same vendor.

Paraoxonase-1 activities and phenotype

PON1 paraoxonase activity was measured by a kinetic, semi-automated method. Briefly, paraoxon (O,O-diethyl-O-p-nitrophenyl-phosphate, Sigma, Hungary) was used as a substrate, and the generation of 4-nitrophenol was measured on a microtiter plate (Greiner Bio-One GmbH, Germany). The total of 15 µL serum was mixed with 285 µL Tris-HCl buffer (100 mmol/L, pH = 8.0) containing 2 mmol/L $CaCl_2$ and 5.5 mmol/L paraoxon. The absorbance was monitored at 405 nm (25 °C), in every minute for 6 min by a Beckman Coulter DTX880 Plate Reader (Beckman Coulter, California, USA) equipped with multimode detector. Enzyme activity was calculated using the molar extinction coefficient 17,600 $M^{-1}cm^{-1}$. PON1 paraoxonase activity is expressed as units per liter of serum, where 1 unit equals 1 µmol of substrate hydrolyzed per minute.

PON1 arylesterase activity was assayed by a standard containing 1 mmol/L phenylacetate substrate (Sigma, Hungary) in 20 mmol/L Tris-HCl, pH = 8.0. The reaction was started by adding the serum and the absorbance was monitored at 270 nm. Blanks were included to correct for the spontaneous hydrolysis of phenylacetate. We calculated the enzyme activity using the molar extinction coefficient 1,310 $M^{-1}cm^{-1}$. PON1 arylesterase activity is expressed in U/mL; 1 U is defined as 1 µmol phenylacetate hydrolyzed per minute.

To calculate PON1 phenotype the dual substrate method was used[17]. The genetic polymorphism at codon 192 Q→R (Arg/Gln at position 192) has the most significant impact on the enzyme activity as hydrolysis of paraoxon is faster by the *R* allele than by the *Q* allele. The allozyme determined by the *R* allele was designated type B, while the allozyme identified by the *Q* allele was nominated type A. In contrast, both *R* and *Q* alleles had similar arylesterase activity. The ratio of the hydrolysis of paraoxon in the presence of 1 mol/L NaCl (salt-stimulated paraoxonase) to the hydrolysis of phenylacetate was used to assign individuals to one of the three possible PON1 phenotypes: AA (low activity), AB (intermediate activity) and BB (high activity). Cut-off values between phenotypes were as follows: ratio below 3.0 for AA, ratio between 3.0 and 7.0 for AB and ratio over 7.0 for BB phenotype.

Serum myeloperoxidase concentration measurement

Myeloperoxidase serum concentrations were measured by commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Europe Ltd., Abington, England). The ELISA assay was performed according to the manufacturer's instructions. The intra- and inter-assay coefficient of variations was 6.5-9.4%.

HDL subfraction analysis

HDL subfractions were determined using an electrophoretic method on polyacrylamide gel with the Lipoprint System (Quantimetrix Corp., CA, USA) according to manufacturer's instructions. This commercially available system separates HDL subfractions from human serum on the basis of their size applying preloaded gel tubes for HDL determinations.

Concisely, 25 µL serum was added to the polyacrylamide gel tubes along with 300 µL loading gel solution. The tubes contained Sudan Black as a lipophilic dye and were photopolimerized at room temperature for 30 min. Electrophoresis with tubes containing sera samples and the manufacturer's quality controls were performed at a constant of 3 mA/tube for 50 min. Subfraction bands were scanned with an ArtixScan M1 digital scanner (Microtek International Inc., CA, USA) and were identified by their mobility (Rf) using very-LDL (VLDL) + LDL as the starting (Rf 0.0) and albumin as the ending (Rf 1.0) reference point.

Ten HDL subfractions were differentiated between

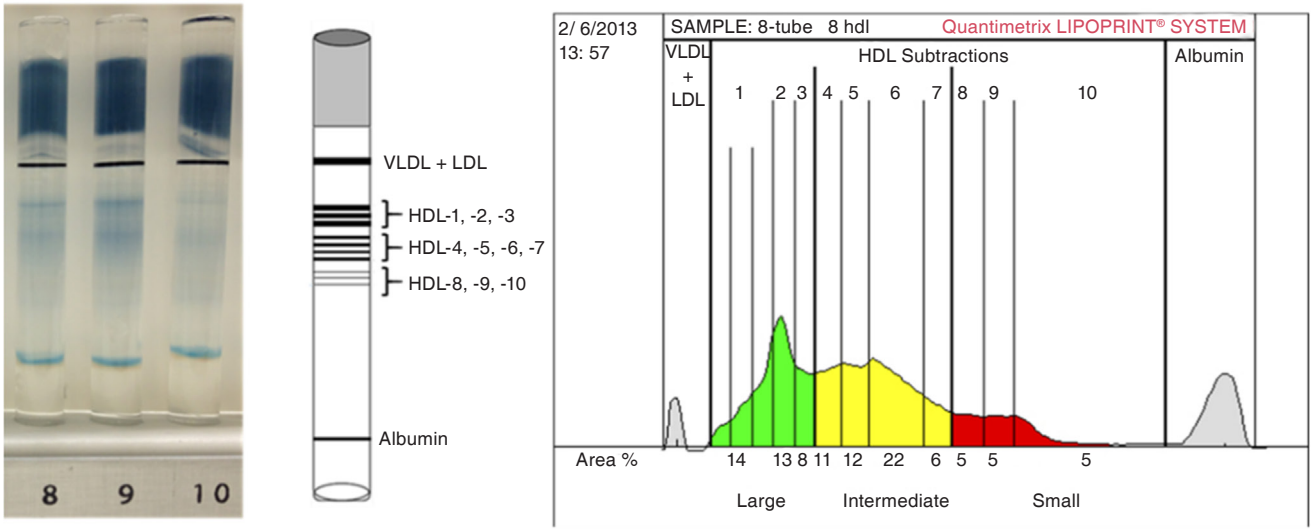

Figure 1: HDL subfraction profile in a healthy subject determined using an electrophoretic method on polyacrylamide gel with the Lipoprint System. Ten HDL subfractions were differentiated between VLDL + LDL and albumin peaks, and were grouped into three major classes: large (from HDL1 to HDL3), intermediate (from HDL4 to HDL7) and small (HDL8 to HDL10) HDL subfractions. HDL: high-density lipoprotein; VLDL: very-low-density lipoprotein

VLDL + LDL and albumin peaks, and were grouped into three major classes: large (from HDL1 to HDL3), intermediate (from HDL4 to HDL7) and small (HDL8 to HDL10) HDL subfractions. Cholesterol concentrations of the HDL particle subsets were calculated by multiplying the total cholesterol concentration of the samples by the relative area under the curve of the subfraction bands [Figure 1].

Statistical methods

Statistical analysis was performed by STATISTICA (ver 8.0; StatSoft Inc., Tulsa, OK, USA). We tested the normality of data distribution by Kolmogorov-Smirnov test. Data are presented by descriptive analysis [mean ± SD in case of normal distribution, or median (lower quartile - upper quartile) in the case of non-normal distribution]. Comparisons between groups were performed by Student's unpaired *t*-test in case of normally distributed variables and by Mann-Whitney *U*-test in case of variables with non-normal distribution. Correlations between continuous variables were assessed by linear regression analysis using Pearson's test. Differences with $P < 0.05$ were considered to be statistically significant.

RESULTS

Significantly higher total cholesterol, LDL-C, triglyceride, lipoprotein(a), apo B and CRP levels were found in the untreated dyslipidemic patients compared to healthy controls. A few patients had higher CRP levels, however, we could not find significant correlations between the studied parameters and the serum level of CRP. Serum glucose and HbA1c levels were also significantly higher in patients, although they remained in the normal range. Mean age and body mass index of patients were also significantly higher compared to controls. Concentration of serum MPO was significantly higher in patients [Figure 2A]. We could not find significant differences in PON1 paraoxonase and arylesterase activities between the two study groups. Large inter-individual variations in PON1 paraoxonase and arylesterase enzyme activities were found both in dyslipidemic patients and controls [Figure 2C and D]. The MPO/PON1 ratio was significantly higher in dyslipidemic patients compared to controls [Table 1 and Figure 2B].

Significant negative correlation was detected between PON1 arylesterase activity and the concentration of MPO ($r = -0.38$; $P < 0.001$) in the whole study group (data not shown). Analyzing these associations in the two groups separately, correlation remained significant only in patients ($r = -0.38$; $P < 0.001$). The PON1 phenotype distribution was as follows: in the patient group 80.2 % ($n = 65$) were AA, 19.8 % ($n = 16$) were AB phenotype, and there were no patients with BB phenotype. The phenotype distribution (AA-AB) was 87.5% ($n = 28$), 12.5% ($n = 4$) in controls, respectively. The allelic frequencies followed the Hardy-Weinberg equilibrium and no significant differences were found between the subgroups.

The absolute amounts and ratios of lipoprotein subfractions are shown on Table 2. Although HDL-C levels fell into the normal range in both studied groups,

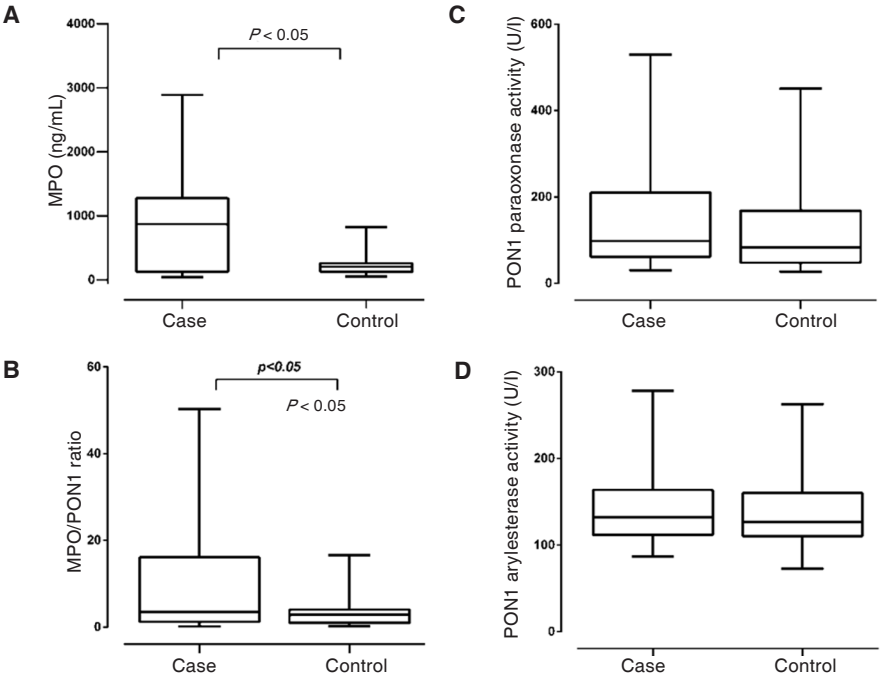

Figure 2: Levels of serum MPO (A), MPO/PON1 ratio (B), activities of serum paraoxonase (C), and arylesterase (D) in dyslipidemic patients and controls. MPO: myeloperoxidase; PON1: paraoxonase-1

Table 1: Laboratory parameters and anthropometric data of the patients and healthy controls

	Dyslipidemic patients	Healthy controls	*P*
Number of patients	81	32	
Age (years)	48.9 ± 14.3	41.8 ± 5.9	< 0.01
Female/male	49/32	19/13	NS
BMI (kg/m^2)	28.1 ± 5.0	24.5 ± 2.5	< 0.01
Waist circumference (cm)			
Females	89.4 ± 13.2	85.7 ± 6.6	< 0.05
Males	101.7 ± 11.2	92.75 ± 7.6	< 0.05
Glucose (mmol/L)	5.1 (4.8-5.5)	4.8 (4.6-5.1)	< 0.01
HbA1C (%)	5.4 (5.2-5.9)	5.1 (4.8-5.3)	< 0.01
Total cholesterol (mmol/L)	7.07 ± 1.69	5.07 ± 0.78	< 0.01
LDL-C (mmol/L)	4.52 ± 1.43	2.92 ± 0.51	< 0.01
HDL-C (mmol/L)	1.56 ± 0.54	1.55 ± 0.46	NS
Triglyceride (mmol/L)	1.6 (1.1-3.0)	1.2 (0.8-1.4)	< 0.01
Lipoprotein(a) (mg/L)	191 (77-587)	70 (30-214)	< 0.01
Apolipoprotein A1 (g/L)	1.71 ± 0.59	1.68 ± 0.31	NS
Apolipoprotein B (g/L)	1.33 ± 0.37	0.94 ± 0.18	< 0.01
hsCRP (mg/L)	2.4 (1.0-45.3)	2.0 (0.6-2.9)	< 0.05
Thyroid stimulating hormone (mU/L)	2.1 ± 1.29	1.94 ± 1.1	NS
Myeloperoxidase (ng/mL)	869 (131-1,272)	205 (125-257)	< 0.05
Paraoxonase activity (U/L)	98 (62-210)	83 (48-167)	NS
Arylesterase activity (U/L)	132 (112-162)	127 (110-160)	NS
Myeloperoxidase/paraoxonase (ug/U)	3.56 (1.28-16.03)	2.81 (0.89-4.03)	< 0.05

Data are presented as mean ± standard deviation or median (lower-upper quartiles). NS: non-significant; BMI: body mass index; HbA1c: hemoglobin A1c; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; hsCRP: high sensitivity C-reactive protein

there was a shift toward the small-sized HDL particles in dyslipidemic patients compared to controls. The ratio of intermediate HDL subfraction was found to be significantly lower in dyslipidemic patients compared to controls, but there were no further significant differences between patients and controls [Table 2].

DISCUSSION

The measurement of serum levels of HDL-C has

Table 2: Concentration and ratio of high-density lipoprotein subfractions in dyslipidemic patients and healthy controls

	Dyslipidemic patients	Healthy controls	*P*
HDL subfraction concentrations (mmol/L)			
Large HDL	0.42 (0.27-0.67)	0.45 (0.31-0.61)	NS
Intermediate HDL	0.73 (0.59-0.87)	0.75 (0.66-0.85)	NS
Small HDL	0.34 (0.23-0.43)	0.28 (0.25-0.34)	NS
HDL subfraction ratios (%)			
Large HDL	29.7 (21.5-35.4)	31.6 (24.4-34.6)	NS
Intermediate HDL	48.3 (45-51.4)	51.5 (48.3-54.3)	< 0.05
Small HDL	21.6 (16.4-26.6)	18.4 (15.5-22.5)	NS

Values are presented as mean ± standard deviation or median (lower-upper quartiles). NS: non-significant; HDL: high-density lipoprotein

been standardized and accepted in everyday clinical practice, but it fails to capture the complexity of HDL structure and function. Previous proteomic studies have revealed more than 100 proteins on HDL particles including structure proteins, enzymes, complement components and proteinase inhibitors. It became clear that the protective role of HDL against atherogenesis may relate to its composition as much as to its concentration in the plasma[18]. By contrast, there is no accepted gold standard to measure the physical and functional properties of HDL particles, although several tests and assays have been reported in the last few decades[1]. Consequently, results of the various studies are often not comparable, confusing and not applicable in clinical practice. Indeed, the assessment of HDL structure and functions has become a high priority novel target to investigate the association between HDL and cardiovascular risk. Therefore, any further data on parallel investigation of HDL subfractions and functional markers may improve our knowledge on this field.

Because of the various techniques used for HDL subfraction detection, it is not surprising that considerable controversy exists as to the clinical usefulness of HDL subfractions for the prediction of cardiovascular risk. Basically, HDL-C includes two major subfractions: lipid-enriched, larger HDL2, which has a major role in reverse cholesterol transport, and protein-enriched, smaller HDL3, whose anti-atherogenic role is less clear, but is able to bind several antioxidant enzymes including PON1. Although the results of previous studies are not concordant, a higher ratio of the larger HDL2 particles may protective against atherogenesis, while the smaller subclasses are positively correlated with the risk of cardiovascular disease[19]. We found a shift towards the smaller HDL subfractions, which may be unfavorable for our dyslipidemic patients. On the other hand, most of these changes were not significant, therefore the importance of alterations in HDL subfractions might not be crucial, at least in dyslipidemic patients without manifest vascular complications. It must be noted that there can be notable differences in data produced by different analytical techniques applied for HDL subclass analysis. Still, these data may shift the focus from HDL subfractions to HDL function.

Measurement of PON1 activity is an accepted indicator of the HDL antioxidant property and a promising biomarker of HDL function independently of HDL-C levels[20]. PON1 inhibits lipoprotein oxidation and macrophage foam cell formation. Moreover, it possesses homocysteine-thiolactonase activity and stimulates macrophage cholesterol efflux that may also be responsible for its anti-atherogenic properties[7]. A previous meta-analysis comprising 47 studies reported markedly lower PON1 activities in patients with coronary heart disease than in unaffected controls[21]. Decreased paraoxonase activities were found in a number of medical conditions including familial hypercholesterolemia and diabetes mellitus[7]. However, it is also well known that paraoxonase activities can vary by over 40-fold between individuals, in part because of its genetic polymorphisms[22,23]. Although many environmental and pharmaceutical modulators of PON1 are known, by far the biggest effect on PON1 activity levels is through these polymorphisms. The coding region PON1-Q192R polymorphism determines a substrate dependent effect on activity. Some substrates e.g. paraoxon are hydrolyzed faster by the R- isoform while others such as phenylacetate and diazoxon are hydrolyzed more rapidly by the Q-isoform[22]. Therefore using the dual substrate method the PON1-Q192R polymorphism can be evaluated. In the present study we could not find significant differences in PON1 paraoxonase and arylesterase activities between patients and controls, but we also observed the large inter-individual variations in enzyme activities. Therefore, we evaluated the PON1 Q192R phenotype distribution and allelic frequencies. The allelic frequencies followed the Hardy-Weinberg

equilibrium and no significant differences were found between the subgroups. According to the results, because of the large inter-individual variability, PON1 activity measurement alone might not became a useful biomarker for cardiovascular risk prediction in dyslipidemic patients without vascular complications.

MPO is a leukocyte-derived heme protein that binds to HDL. As a part of the innate immune host defense system, MPO uses hydrogen peroxide to generate an array of reactive oxidant and free radical species such as hypochlorous acid possessing antimicrobial effect. However, these reactive species can also foster oxidative injury to host molecules as well. Indeed, MPO catalyzes generation of nitrating oxidants and promotes both protein modifications and initiates lipid peroxidation leading to enhanced atherosclerosis[24]. Plasma, serum, and leukocyte MPO levels have been associated with coronary artery disease[25]; incident risk of myocardial infarction, death, and need for revascularization[26,27]. In a previous study we found significantly elevated MPO levels in overweight hyperlipidemic patients with or without cardiovascular complications[28]. In the present study we also found significantly higher MPO levels in dyslipidemic patients without any vascular complications compared to healthy subjects.

Interestingly, both PON1 and MPO interact at the same site on HDL, reciprocally modulate each other's function, influencing the antioxidant and anti-inflammatory function of HDL[11]. Our previous study on a similar patient population also highlighted the importance of this reciprocal inhibition[29]. Furthermore, a recent study showed that serum MPO/PON1 ratio may be a potential indicator of dysfunctional high-density lipoprotein and risk stratification in coronary artery disease[30]. Therefore, MPO/PON1 ratio was also calculated and we found significantly higher MPO/PON1 ratio in our dyslipidemic patients compared to controls indicating an increased risk for cardiovascular complications. Moreover, in line with the results of previous studies, significant negative correlation was found between myeloperoxidase levels and PON1 arylesterase activities that demonstrates the reciprocal inhibition between these two HDL-associated enzymes. Hence, calculation of MPO/PON1 ratio may give information about the function of the enzyme complex and characterize HDL function.

Some limitations of the study must be noted. The power of the study may be reduced because of the relatively small size of the study population. Age was significantly different between the patients and controls, however, we could not find significant correlations between the studied parameters and the age. Therefore, we concluded that age may not influence our results in this study. It must be noted that HDL subfraction ratios are derived secondary parameters that may include additional error based on method of calculation. Data on further HDL functional assays such as HDL cholesterol efflux assay, measurement of lecithin: cholesterol acyltransferase or platelet-activating factor-acetylhydrolase activity would add further information on HDL property.

In summary, we found altered HDL function in dyslipidemic patients, characterized by increased level of MPO and MPO/PON1, even in patients without clinically detectable symptoms of vascular complications. However, PON1 paraoxonase and arylesterase activities were unaltered. There was a shift towards the smaller HDL subfractions, but these changes were not significant; indicating that the importance of alterations in HDL subfractions might not be crucial in patients with dyslipidemia. Our results highlights the importance of HDL-associated pro- and antioxidant enzymes suggesting the possible clinical benefit of MPO/PON1 calculation and confirms that quantification of HDL-C level alone provides limited data regarding HDL's cardioprotective effect. Further studies on larger patient populations are needed to identify and characterize the best markers of HDL functions.

Data on HDL structural and functional properties may improve the efficacy of cardiovascular risk prediction and development of novel anti-atherogenic treatment strategies in dyslipidemia.

DECLARATIONS

Authors' contributions

Study design: M. Harangi, G Paragh
Development of methodology: I. Seres
Collection of data: B. Nádró, M. Harangi
Analysis and/or interpretation of data: A. Szentpéteri, H. Lőrincz
Writing (not revising) all or sections of the manuscript: M. Harangi
Manuscript review: G. Paragh, D. Páll
Supervision: G. Paragh

Financial support and sponsorship

This research was supported by a grant from the National Research, Development and Innovation (NFKI) (OTKA 115723) and by the GINOP-2.3.2-15-2016-00062 project. The project is co-financed by the European Union under the European Regional Development Fund.

Conflicts of interest
There are no conflicts of interest.

Patient consent
Informed consent was obtained from all patients after approval of the local ethics committee.

Ethics approval
The study was approved by local and regional ethics committees.

REFERENCES

1. Hafiane A, Genest J. High density lipoproteins: Measurement techniques and potential biomarkers of cardiovascular risk. *BBA Clin* 2015;3:175-88.
2. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L; INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004;364:937-52.
3. Ramirez A, Hu PP. Low high-density lipoprotein and risk of myocardial infarction. *Clin Med Insights Cardiol* 2015;9:113-7.
4. Annema W, von Eckardstein A. Dysfunctional high-density lipoproteins in coronary heart disease: implications for diagnostics and therapy. *Transl Res* 2016;173:30-57.
5. Huang Y, DiDonato JA, Levison BS, Schmitt D, Li L, Wu Y, Buffa J, Kim T, Gerstenecker GS, Gu X, Kadiyala CS, Wang Z, Culley MK, Hazen JE, Didonato AJ, Fu X, Berisha SZ, Peng D, Nguyen TT, Liang S, Chuang CC, Cho L, Plow EF, Fox PL, Gogonea V, Tang WH, Parks JS, Fisher EA, Smith JD, Hazen SL. An abundant dysfunctional apolipoprotein A1 in human atheroma. *Nat Med* 2014;20:193-203.
6. Khine HW, Teiber JF, Haley RW, Khera A, Ayers CR, Rohatgi A. Association of the serum myeloperoxidase/high-density lipoprotein particle ratio and incident cardiovascular events in a multi-ethnic population: observations from the dallas heart study. *Atherosclerosis* 2017;263:156-62.
7. Mackness M, Mackness B. Human paraoxonase-1 (PON1): Gene structure and expression, promiscuous activities and multiple physiological roles. *Gene* 2015;567:12-21.
8. Perła-Kaján J, Jakubowski H. Paraoxonase 1 and homocysteine metabolism. *Amino Acids* 2012;43:1405-17.
9. Ben-David M, Elias M, Filippi JJ, Duñach E, Silman I, Sussman JL, Tawfik DS. Catalytic versatility and backups in enzyme active sites: the case of serum paraoxonase 1. *J Mol Biol* 2012;418:181-96.
10. Gugliucci A, Menini T. Paraoxonase 1 and HDL maturation. *Clin Chim Acta* 2015;439:5-13.
11. Huang Y, Wu Z, Riwanto M, Gao S, Levison BS, Gu X, Fu X, Wagner MA, Besler C, Gerstenecker G, Zhang R, Li XM, DiDonato AJ, Gogonea V, Tang WH, Smith JD, Plow EF, Fox PL, Shih DM, Lusis AJ, Fisher EA, DiDonato JA, Landmesser U, Hazen SL. Myeloperoxidase, paraoxonase-1, and HDL form a functional ternary complex. *J Clin Invest* 2013;123:3815-28.
12. Harangi M, Mirdamadi HZ, Seres I, Sztanek F, Molnár M, Kassai A, Derdák Z, Illyés L, Paragh G. Atorvastatin effect on the distribution of high-density lipoprotein subfractions and human paraoxonase activity. *Transl Res* 2009;153:190-8.
13. Ferretti G, Bacchetti T, Sahebkar A. Effect of statin therapy on paraoxonase-1 status: A systematic review and meta-analysis of 25 clinical trials. *Prog Lipid Res* 2015;60:50-73.
14. Balogh Z, Seres I, Harangi M, Kovács P, Kakuk G, Paragh G. Gemfibrozil increases paraoxonase activity in type 2 diabetic patients. A new hypothesis of the beneficial action of fibrates? *Diabetes Metab* 2001;27:604-10.
15. Nakou ES, Filippatos TD, Kiortsis DN, Derdemezis CS, Tselepis AD, Mikhailidis DP, Elisaf MS. The effects of ezetimibe and orlistat, alone or in combination, on high-density lipoprotein (HDL) subclasses and HDL-associated enzyme activities in overweight and obese patients with hyperlipidaemia. *Expert Opin Pharmacother* 2008;9:3151-8.
16. Harangi M, Seres I, Harangi J, Paragh G. Benefits and difficulties in measuring HDL subfractions and human paraoxonase-1 activity during statin treatment. *Cardiovasc Drugs Ther* 2009;23:501-10.
17. Smolen A, Eckerson HW, Gan KN, Hailat N, La Du BN. Characteristics of the genetically determined allozymic forms of human serum paraoxonase/arylesterase. *Drug Metab Dispos* 1991;19:107-12.
18. Schaefer EJ, Anthanont P, Asztalos BF. High-density lipoprotein metabolism, composition, function, and deficiency. *Curr Opin Lipidol* 2014;25:194-9.
19. Li JJ, Zhang Y, Li S, Cui CJ, Zhu CG, Guo YL, Wu NQ, Xu RX, Liu G, Dong Q, Sun J. Large HDL subfraction but not HDL-C is closely linked with risk factors, coronary severity and outcomes in a cohort of nontreated patients with stable coronary artery disease: a prospective observational study. *Medicine (Baltimore)* 2016;95:e2600.
20. Soran H, Schofield JD, Durrington PN. Antioxidant properties of HDL. *Front Pharmacol* 2015;6:222.
21. Wang M, Lang X, Cui S, Zou L, Cao J, Wang S, Wu X. Quantitative assessment of the influence of paraoxonase 1 activity and coronary heart disease risk. *DNA Cell Biol* 2012;31:975-82.
22. Mackness B, Turkie W, Mackness M. Paraoxonase-1 (PON1) promoter region polymorphisms, serum PON1 status and coronary heart disease. *Arch Med Sci* 2013;9:8-13.
23. Hernández-Díaz Y, Tovilla-Zárate CA, Juárez-Rojop IE, González-Castro TB, Rodríguez-Pérez C, López-Narváez ML, Rodríguez-Pérez JM, Cámara-Álvarez JF. Effects of paraoxonase 1 gene polymorphisms on heart diseases: systematic review and meta-analysis of 64 case-control studies. *Medicine (Baltimore)* 2016;95:e5298.
24. Zhou B, Zu L, Chen Y, Zheng X, Wang Y, Pan B, Dong M, Zhou E, Zhao M, Zhang Y, Zheng L, Gao W. Myeloperoxidase-oxidized high density lipoprotein impairs atherosclerotic plaque stability by inhibiting smooth muscle cell migration. *Lipids Health Dis* 2017;16:3.
25. Teng N, Maghzal GJ, Talib J, Rashid I, Lau AK, Stocker R. The roles of myeloperoxidase in coronary artery disease and its potential implication in plaque rupture. *Redox Rep* 2017;22:51-73.
26. Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Münzel T, Simoons ML, Hamm CW; CAPTURE Investigators. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation* 2003;108:1440-5.
27. Brennan ML, Penn MS, Van Lente F, Nambi V, Shishehbor MH, Aviles RJ, Goormastic M, Pepoy ML, McErlean ES, Topol EJ, Nissen SE, Hazen SL. Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med* 2003;349:1595-604.
28. Szentpéteri A, Zsíros N, Varga VE, Lőrincz H, Katkó M, Seres I, Fülöp P, Paragh G, Harangi M. Paraoxonase-1 and myeloperoxidase correlate with vascular biomarkers in overweight patients with newly diagnosed untreated hyperlipidaemia. *Vasa* 2017;46:370-6.
29. Zsíros N, Koncsos P, Lőrincz H, Seres I, Katkó M, Szentpéteri A, Varga VE, Fülöp P, Paragh G, Harangi M. Paraoxonase-1 arylesterase activity is an independent predictor of myeloperoxidase levels in overweight patients with or without cardiovascular complications. *Clin Biochem* 2016;49:862-7.
30. Haraguchi Y, Toh R, Hasokawa M, Nakajima H, Honjo T, Otsui K, Mori K, Miyamoto-Sasaki M, Shinohara M, Nishimura K, Ishida T, Hirata K. Serum myeloperoxidase/paraoxonase 1 ratio as potential indicator of dysfunctional high-density lipoprotein and risk stratification in coronary artery disease. *Atherosclerosis* 2014;234:288-94.

Sobenin *et al. Vessel Plus* 2017;1:174-81
DOI: 10.20517/2574-1209.2017.19

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Topic: Atherosclerosis and Related Diseases **Open Access**

Is insulin pro-atherogenic at the cellular level?

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How to cite this article: Sobenin IA, Orekhova VA, Grechko AV, Orekhov AN. Is insulin pro-atherogenic at the cellular level? *Vessel Plus* 2017;1:174-81.

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Article history:
Received: 9 Jun 2017
Accepted: 13 Sep 2017
Published: 28 Dec 2017

Key words:
Diabetes mellitus,
atherosclerosis,
insulin,
cultured subendothelial cells,
cholesterol accumulation,
cellular proliferation

ABSTRACT

Aim: This study was undertaken to test the hypothesis that insulin treatment has unexpected pro-atherogenic effects at the cellular level, namely, proliferative activity and intracellular cholesterol content. **Methods**: Primary cultures of subendothelial cells derived from non-atherosclerotic human aorta and mouse peritoneal macrophages were used to investigate the *in vitro* effect of insulin on atherosclerosis-related parameters, such as cellular cholesterol content and proliferation rate. Additionally, the effect of insulin treatment in 33 type 1 diabetic patients on serum atherogenicity (i.e. its ability to induce cholesterol accumulation in cultured cells) was investigated. **Results**: Insulin (1-1,000 μU/mL) did not affect [^{3}H]-thymidine incorporation or cholesterol content in either type of cultured cell. Most blood sera obtained from type 1 diabetic patients induced a 1.5- to 1.7-fold increase in cholesterol content of cultured cells, but this effect did not correlate with serum insulin levels. Exogenous insulin added to cultured cells did not modify the effect of patient's sera on cholesterol level and proliferation of cultured cells. **Conclusion**: The results suggest that insulin does not exert direct atherogenic actions at the level of arterial cells, with the respect to proliferative activity and cholesterol content.

Quick Response Code:

INTRODUCTION

Insulin-dependent (type 1) diabetes mellitus is known to favor an accelerated development of atherosclerosis, although the reasons for premature atherogenesis in diabetic patients remain obscure. Several hypotheses exist, which try to describe the molecular, cellular and biochemical mechanisms of premature atherogenesis in diabetic patients, but all have failed to sufficiently explain this phenomenon. Proposed mechanisms include the harmful effects of advanced glycosylation end products, modification of low density lipoproteins by non-enzymatic glycosylation, lipoprotein retention in the arterial wall, enhanced oxidative stress, inflammation, endothelial dysfunction, and alterations in metabolism of vitamins and minerals[1-5]. In addition to these potential factors, it is necessary to consider the possible role of insulin in the process of atherosclerosis development. Since hyperinsulinemia is associated with increased cardiovascular risk, hyperinsulinemia and hepatic portal hypoinsulinemia due to injection of insulin preparations widely used in the treatment of type 1 diabetic patients could be responsible, at least in part, for cardiovascular consequences of the disease. The results of epidemiological studies and experimental studies in animal models supported systemic hyperinsulinemia as a major plausible factor in the development of atherosclerosis in diabetic patients[6-10]. Insulin resistance is strongly associated with hyperinsulinemia, and is considered as the major pathologic mechanism for susceptibility to premature atherosclerosis and cardiovascular disease[11,12]. However, the effects of exogenous insulin on atherosclerosis progression have not been sufficiently studied. Experimental studies demonstrate direct *in vitro* effects of insulin on angiogenesis, as well as on endothelial and arterial smooth muscle cell proliferation[13,14]. Nevertheless, this contention remains controversial, and there is no definitive settlement on the role of insulin in atherogenesis[15-17]. To elucidate this matter, the present study tested the influence of insulin on cellular proliferation and total cholesterol content in cultured subendothelial cells derived from human aorta and mouse peritoneal macrophages. Additionally, the effect of exogenous insulin on atherogenicity of serum from diabetic patients was investigated.

METHODS

Patients

This study was conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983. It was approved by the local ethics committee of the Institute for Atherosclerosis Research, Skolkovo Innovation Center, Moscow, Russia. All participants gave their written informed consent prior to their inclusion in the study.

The group of study participants consisted of 33 type 1 diabetic patients aged 20 to 44 (17 men, 16 women) with diabetes duration ranging from 0 to 16 years. They were characterized by rather low level of antibodies to insulin (less than 50 nU/mL), therefore, the possibility of insulin resistance attributable to antibodies was excluded[18]. The type of diabetes was verified according to recommendations from the American Diabetes Association[19]. None of the patients had clinical manifestations of coronary heart disease. All patients received intensive insulin therapy with glargine ("Lantus", Sanofi-Aventis Deutschland GmbH, Germany) as basal insulin, and glulisine ("Apidra", Sanofi-Aventis Deutschland GmbH, Germany) as prandial insulin. None of the patients had coronary heart disease or other clinical manifestations of atherosclerosis, arterial hypertension or kidney disease. The selection and recruitment of study participants was designed to avoid any significant comorbidity or drug administration, which could potentially affect the results of experiments. Venous blood (5 mL) was drawn once in the morning before meals at the first day at the hospital. Fasting blood glucose was 8.2 ± 0.4 mmol/L, HbA1c was 9.2 ± 0.3%, serum cholesterol level was 5.2 ± 0.2 mmol/L, and triglyceride level was 1.4 ± 0.2 mmol/L.

Additionally, the second group of study participants consisted of 3 patients with newly diagnosed type 1 diabetes mellitus (1 man and 2 women aged 18, 19 and 23, respectively), who were characterized by low levels of immunoreactive insulin (IRI) and residual C-peptide secretion (1.6 ± 0.5 mU/L and 0.8 ± 0.2 ng/mL, respectively), and significantly elevated blood glucose levels (20.0 ± 0.2 mmol/L) without severe ketoacidosis on admission to hospital. Intensive therapy with insulin glulisine and insulin glargine was started immediately. For monitoring of glucose level, IRI level and blood serum atherogenicity, venous blood was taken several times throughout the duration of hospitalization immediately before the start of intensive insulin therapy, then at 30 min, 2 h, 6 h, 12 h, 24 h and 7 days after the first insulin injection. On the 10th day, the patients were discharged from the hospital and were examined at outpatient clinics 21 days after the start of intensive insulin therapy.

Blood collected from patients was incubated for 1 h at 37 ºC and centrifuged for 30 min at 1,000 *g*. The resultant serum samples were stored frozen (-20 ºC) for 4-7 days before examination.

Reagents

Medium 199, fetal calf serum (FCS), penicillin, streptomycin, Fungizone and L-glutamine were purchased from GIBCO Europe (Paisley, UK). Collagenase type II was obtained from Worthington Diagnostic System (Freehold, UK). [6-^{3}H]-thymidine (21 Ci/mmol) was purchased from Amersham International (Amersham, UK). Assay kits for total cholesterol determination were from Cell Biolabs, Inc. (San Diego, CA, USA). Other reagents were purchased from Sigma-Aldrich Co., LLC (St. Louis, MO, USA).

Cell culture

To study the direct effects of insulin on intracellular cholesterol accumulation and cellular proliferation, cells were isolated from the non-atherosclerotic sub-endothelial (elastico-hyperplastic) sublayer of the intima by digestion of human aortic tissue with 0.15% collagenase under sterile conditions, as described elsewhere[20]. The autopsy material was taken from subjects aged 50 to 58 who had died suddenly by accident. After digestion, enzyme-isolated cells were separated from the remaining tissue by filtering through nylon mesh, and subsequently resuspended in Medium 199 containing standard additives: 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL Fungizone and 10% of FCS. Cells were seeded into 48-well or 24-well tissue culture plates at a density of (3-4) × 10^4 cells/cm^2 of growth area. The cells were cultured at 37 ºC in a humidified CO_2-incubator (95% air and 5% CO_2). The primary cultures contained a mixed cell population made up primarily (95%) of typical and modified smooth muscle cells as it was earlier defined by the ultrastructural features and immunofluorescent markers. The medium was changed each day. On the 7th day in culture, Medium 199 containing 40% blood serum from type 1 diabetic patients, 1 μCi/mL [^{3}H]-thymidine and the indicated concentrations of insulin (0.001-1,000 mU/mL), or Medium 199 containing 10% of FCS, 1 μCi/mL [^{3}H]-thymidine and insulin (0.001-1,000 mU/mL) were applied to cell cultures. After 24 h incubation, cells were rinsed 3 times with phosphate buffered saline (PBS), 3 times with PBS containing 0.2% bovine serum albumin, and again 3 times with PBS. Cellular lipids were extracted with the mixture of n-hexane and isopropanol (3:2, vol:vol) as described elsewhere[20]. Total cholesterol content was determined enzymatically using commercial assay kits. After delipidation, the cells were rinsed 3 times with 5% trichloroacetic acid and then were dissolved in 0.1 mol/L NaOH. Aliquots were used for cellular protein determination and [^{3}H]-thymidine incorporation as described by Tertov *et al.*[21].

To measure serum atherogenicity (i.e. its ability to induce cholesterol accumulation in cultured cells), BALB/c mouse resident peritoneal macrophages were taken from non-stimulated animals as described by Adams[22]. Afterward, 2 × 10^5 cells were seeded into each well of 24-well plates in Medium 199 containing antibiotics and 10% of FCS. After a 4-h incubation, the cultures were rinsed with Medium 199 to remove unattached cells. Then cells were incubated for additional 4 h in Medium 199 containing 10% of examined patient's serum. Lipid extraction, cellular protein and cholesterol measurements were performed as described above.

Statistical analysis

Results are reported as mean ± SEM, based on triplicate measurements in each of 4 independent experiments. Significance of differences was evaluated using the IBM SPSS 20.0 statistical program package and was assumed for P values < 0.05.

RESULTS

Twenty-five, or 70% of 33 type 1 diabetic patient's sera exhibited an atherogenic potential, i.e. increased total cholesterol content of cultured mouse peritoneal macrophages by 50-70% as compared to control. This effect did not depend on patient's age, gender, plasma lipid levels, or diabetes duration. Moreover, serum-induced cholesterol accumulation did not correlate with IRI level in tested serum samples (r = -0.09, P > 0.6).

In those 3 patients with newly diagnosed type 1 diabetes mellitus, blood serum obtained immediately before the beginning of intensive insulin therapy induced significant cholesterol accumulation in cultured mouse peritoneal macrophages [Table 1]. As it was expected, after the start of intensive insulin therapy, IRI level increased rapidly, whereas C-peptide level remained low (the data not shown). By the end of the 1st day of intensive insulin therapy, blood glucose decreased to 10.5 ± 0.8 mmol/L, and IRI level accounted for 28 ± 3 mU/L. Further treatment resulted in the achievement of satisfactory glycemic control (data not shown). Along with these changes, the atherogenic effect of serum stayed practically invariable up to the 21st day of intensive insulin therapy, despite the steady decrease in blood glucose and elevation in IRI level [Table 1]. Therefore, the ability of diabetic blood serum to induce intracellular cholesterol accumulation was not directly associated with either plasma insulin concentration or the state of glycemic control.

We have also studied the direct effect of insulin on cholesterol content and proliferative activity in cultured

Table 1: The effect of insulin therapy in newly diagnosed type 1 diabetic patients on plasma IRI level and blood serum atherogenicity

Time	IRI level (mU/L)	Intracellular cholesterol accumulation, % of control (*)
Patient 1		
Before insulin therapy	1.7	200 ± 8
30 min	8	205 ± 6
2 h	10	195 ± 7
6 h	13	202 ± 5
12 h	27	186 ± 6
24 h	23	180 ± 9
7 days	29	195 ± 8
21 days	35	180 ± 9
Patient 2		
Before insulin therapy	0.8	160 ± 7
30 min	6	162 ± 8
2 h	11	168 ± 3
6 h	15	160 ± 7
12 h	30	164 ± 5
24 h	34	170 ± 6
7 days	20	164 ± 7
21 days	27	169 ± 6
Patient 3		
Before insulin therapy	2.4	220 ± 9
30 min	7.8	219 ± 6
2 h	10	211 ± 4
6 h	15	208 ± 6
12 h	25	219 ± 4
24 h	28	209 ± 5
7 days	50	199 ± 5
21 days	52	214 ± 5

Total cholesterol content of cultured mouse peritoneal macrophages incubated in the presence of 10% tested patients' serum was determined as described in methods section. Cholesterol content of control cells incubated with Medium 199 containing 10% of FCS was taken for 100%. *: significant intracellular cholesterol accumulation, $P < 0.05$; IRI: immunoreactive insulin; FCS: fetal calf serum

human aortic intima cells [Table 2] and mouse peritoneal macrophages. At various concentrations (1-1,000 mU/mL), insulin had no effect on intracellular cholesterol content. Moreover, insulin did not stimulate proliferative activity of cultured intimal cells; slight deviations seemed to be random and non-systematic [Table 2]. Additionally, the lack of insulin effect on intracellular cholesterol content and [^{3}H]-thymidine incorporation was registered when extremely low (0.001, 0.01 and 0.1 mU/mL) insulin concentrations were used (data not shown). Similar results were obtained in cultured mouse peritoneal macrophages (data not shown).

The atherogenic effects of sera randomly taken from four type 1 diabetic patients were further studied using the primary culture of human intimal aortic cells. These

Table 2: The effect of insulin on [^{3}H]-thymidine incorporation and total cholesterol content of cultured intimal human cells

Insulin concentration (mU/mL)	Intracellular total cholesterol content (μg/mg cell protein)	[^{3}H]-thymidine incorporation (dpm/μg cell protein)
Control	39 ± 2	36 ± 2
1	40 ± 1	40 ± 2
10	40 ± 2	38 ± 1
100	40 ± 1	34 ± 1
1,000	38 ± 2	39 ± 3

Total cholesterol content of subendothelial intimal cells and [^{3}H]-thymidine incorporation was determined as described in methods section

studies revealed that the sera taken from 2 patients induced a significant increase in total cholesterol content of cultured human intimal cells, while 2 other serum samples proved to be non-atherogenic, i.e. did not produce the changes in intracellular cholesterol as compared to control cells incubated in the absence of human serum [Table 3]. Insulin addition at concentrations of 1-1,000 mU/mL to the cultured cells did not affect serum atherogenicity [Table 3]. One of the sera induced enhanced [^{3}H]-thymidine incorporation by cultured cells along with pronounced cholesterol accumulation. Insulin addition did not modify this effect. Similarly, no significant changes in [^{3}H]-thymidine incorporation were observed in cells incubated with 3 other serum samples, even when insulin was used in high concentrations [Table 3]. When these serum samples were tested on cultured mouse peritoneal macrophages, similar results were obtained with respect to either cellular cholesterol content or [^{3}H]-thymidine incorporation (data not shown).

DISCUSSION

Enhanced cellular proliferative activity and deposition of intracellular lipids in the vessel wall, mainly free and esterified cholesterol are typical features of early atherosclerotic lesions. Previous studies demonstrated that blood sera or low-density lipoprotein from most of type 1 diabetic patients, unlike healthy subjects' sera, are able to induce cholesterol deposition in cultured macrophages and human intimal cells[20,23-27]. However, one cannot rule out the possibility that some other factors may play a role. Hyperinsulinemia is thought to be an independent risk factor for atherosclerosis, as was demonstrated earlier in numerous epidemiologic studies[10,28-31]. On the other hand, most of the data were obtained on groups of non-diabetic individuals or type 2 diabetic patients. At the same time, type 1 diabetes is characterized by elevated insulin levels

Table 3: The effect of exogenous insulin addition on atherogenic properties of diabetic patients' serum

	Intracellular cholesterol content, μg/mg cell protein at insulinconcentration (mU/mL)					[^{3}H]-thymidine incorporation, dpm/mg cell protein at insulinconcentration (mU/mL)				
	0	1	10	100	1,000	0	1	10	100	1,000
Control	43 ± 1	45 ± 1	42 ± 2	45 ± 1	44 ± 2	16 ± 3	19 ± 2	17 ± 1	19 ± 2	20 ± 2
Patient 4	43 ± 3	45 ± 2	46 ± 2	44 ± 2	47 ± 3	16 ± 3	12 ± 4	16 ± 5	19 ± 6	20 ± 2
Patient 5	44 ± 3	45 ± 2	47 ± 2	47 ± 2	46 ± 3	18 ± 3	15 ± 2	16 ± 1	13 ± 3	14 ± 3
Patient 6	69 ± 3*	69 ± 5*	71 ± 4*	74 ± 3*	74 ± 4*	12 ± 2	15 ± 2	15 ± 4	17 ± 2	18 ± 5
Patient 7	82 ± 4*	81 ± 2*	86 ± 3*	86 ± 3*	81 ± 5*	27 ± 3#	28 ± 3#	35 ± 4#	34 ± 4#	30 ± 3#

Subendothelial intimal cells were incubated with 40% patient's serum with or without exogenous insulin addition, as described in Methods section.*: significant intracellular cholesterol accumulation, $P < 0.05$; #: significant increase in [^{3}H]-thymidine incorporation, $P < 0.05$

due to exogenous insulin administration, but the role of insulin in atherogenesis in this category of patients remains unknown.

Theoretically, there are many reasons why insulin might affect the vessel wall in diabetic patients. Insulin is a widely recognized and important growth factor. Initially, the hypothesis on insulin atherogenicity derived from old studies in non-diabetic animals given large doses of insulin, which showed enhanced cholesterol synthesis in the aorta[32-34]. Later studies showed that atherosclerosis-like biochemical and histological changes in aortas of Wistar rats could be induced by long-term insulin treatment and hyperinsulinemia[35,36]. However, these findings were not supported in other studies in hyperinsulinemic or insulin-treated animals[37,38]. Insulin did not produce any effect on the transfer of cholesterol from circulation into arterial wall[39]. It was also demonstrated that insulin did not modify lipogenesis by the rat intima-media even at very high concentrations[40].

It is suggested that insulin may cause an increased cellular proliferation in tissue culture[13,41]. However, to demonstrate such an effect of insulin, the cells needed to be starved in serum-deficient medium for several days, the conditions being far from physiological, and then only at high insulin concentrations[42]. However, Ledet had not been able to demonstrate the growth-stimulating effect of insulin; moreover, there was a growth-promoting effect of serum from recent-onset type 1 diabetic patients, although serum insulin levels were extremely low[43]. Also, against the "insulin hypothesis" stand the observations of beneficial effects of insulin treatment on the development of atherosclerosis-like lesions in streptozotocin-diabetic rats. In well-controlled rats, tunica media of coronary arteries contained the normal relative amount of connective tissue and number of cells, as compared to the poorly controlled diabetic animals[44]. Additionally, hyperinsulinemia did not stimulate the early stages of arterial smooth muscle cells proliferation in the rat after aortic injury[45]. So, the speculated pro-atherogenic effects of insulin were intensively discussed for a long time, but then left aside for decades, without reaching consensus.

All further studies focused on clinical effectiveness of insulin therapy, with a partial respect to cardiovascular outcomes, which could serve as the indirect markers of proatherogenic or, vice versa, beneficial effects of insulin on atherosclerosis progression. Large observational studies performed in diabetic patients did not solve the controversy on the effects of insulin treatment on atherosclerosis progression and cardiovascular risk, although they have demonstrated some reduction in cardiovascular events under improved glycemic control during the long-term follow-up[46]. The Epidemiology of Diabetes Interventions and Complications (EDIC) Study, which was designed as the follow-up long-term study of the Diabetes Control and Complications Trial (DCCT), was aimed to estimate the effects of glycemic control on major cardiovascular endpoints (all fatal and nonfatal cardiovascular events, angina, revascularization, and also fatal and nonfatal myocardial infarction, and stroke as secondary endpoints) in type 1 diabetes mellitus patients[47,48]. The EDIC study participants were followed annually, and after a mean of 18 years from the start of the DCCT, a 42% reduction in cardiovascular disease outcomes was observed along with a 57% reduction in fatal and nonfatal myocardial infarction, and stroke[48]. Thus, prospective studies have provided indirect evidence supporting the absence of harmful effects of intensive insulin therapy on atherosclerosis progression in type 1 diabetic patients. Moreover, intensive insulin therapy was shown to be beneficial with respect to instrumental measures of atherosclerosis, namely, coronary artery calcification and carotid intima media thickness[49,50].

In contrast, studies in type 2 diabetic patients who were appointed to intensive insulin therapy provided rather controversial results. The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated a non-significant 16% decrease in fatal and nonfatal myocardial infarction along with a non-significant 6%

decrease in all-cause mortality in newly diagnosed type 2 diabetic patients followed-up for 10 years[51]. Post-trial follow-up for another 10 years demonstrated 15% reduction in myocardial infarction, and 13% reduction in all-cause mortality[52]. However, it is difficult to interpret the UKPDS results, since intensive glycemic control was achieved by sulfonylurea derivatives and/or insulin. Later studies mainly aimed to assess cardiovascular disease risk in intensively controlled type 2 diabetic patients [Action to Control Cardiovascular Risk in Diabetes (ACCORD); Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified-Release Controlled Evaluation (ADVANCE); Veterans Affairs Diabetes Trial (VADT)] failed to reproduce the beneficial effects observed in UKPDS[53-55]. It should be noted that insulin use ranged from 41-90% among intensively controlled subjects in these trials. Within 3.5-5.6 years of follow-up, no significant reductions in cardiovascular disease were demonstrated. Moreover, the ACCORD trial was prematurely stopped after a median follow-up of 3.4 years due to a statistically significant 22% higher all-cause mortality just in a subgroup with intensive glucose control[56]. Thus, it could be proposed that intensive glucose lowering in treatment of type 2 diabetic patients is associated with a higher incidence of mortality, although the role of insulin treatment was never elucidated. However, the most recent meta-analysis of the benefits and harms of intensive glucose lowering therapy performed on the data from 58,160 type 2 diabetic patients in 13 randomized controlled trials has provided the evidence that intensive glucose lowering therapy compared to conventional glucose control therapy is associated with a reduced risk of major cardiovascular events and myocardial infarction. At the same time, intensive glucose lowering therapy does not affect significantly the risks of cardiac death, stroke, congestive heart failure, and total mortality[57].

The results of our study show that insulin does not exert direct atherogenic actions, at least on such phenotypic characteristics as intracellular cholesterol accumulation and cell proliferation. An effect on cholesterol content or proliferative activity was not observed in cultured cells, a result that could possibly be attributed to the high concentration of serum used. Certainly, these results do not indicate that insulin has no proatherogenic effect at all, and numerous possibilities of atherogenic action remain, which could be detected by other parameters. On the other hand, blood serum atherogenicity in type 1 diabetic patients did not correlate with insulin level in serum samples. The addition of exogenous insulin to the cultural medium did not modify atherogenic effects of diabetic patients' sera. The obtained results are in good agreement with the data on the absence of atherogenicity of insulin from studies performed on experimental models different from ours[37-40,44,45].

It must be noted that this study has certain limitations. First, the power of the study may be limited because of the small sample size. Second, the second group of patients with newly diagnosed type 1 diabetes mellitus consisted only of 3 patients, that is too low for complex disease; therefore, the obtained results may be discussed only as a case report. Third, only 2 major traits of atherosclerotic cellular phenotype (namely, cholesterol accumulation and proliferative activity) were examined, and others were not studied, like enhanced synthesis of protein and matrix components, and proinflammatory response. Finally, insulin action in cell culture studies was not controlled in experiments using insulin receptor knockout/knockdown cells.

Biologically, from a functional point of view, insulin overlaps with various much more potent growth factors, e.g. somatomedins, platelet-derived growth factor and epidermal growth factor, which may disguise the action of insulin, especially in epidemiological surveys. So, one may speculate that hyperinsulinemia in diabetic patients might play an atherogenic role but in indirect manner or be tightly accompanied by other atherogenic factors, which are responsible for the initiation of atherogenesis and further development of atherosclerotic lesions.

DECLARATIONS

Authors' contributions

Concept and experimental studies, data analysis and statistical analysis, and manuscript editing: I.A. Sobenin

Literature search and patients' recruitment: V.A. Orekhova

Clinical examination and clinical data acquisition: A.V. Grechko

General coordination and supervision of the research project, and manuscript editing: A.N. Orekhov

Financial support and sponsorship

The study was supported by the Ministry of Education and Sciences of the Russian Federation (Grant # 14.W02.16.6995-Scientific_School).

Conflict of interests

There are no conflicts of interests.

Patient consent

All participants gave their written informed consent prior to their inclusion in the study.

Ethics approval

This study was kept in accordance with the Helsinki Declaration of 1975 as revised in 1983. It was approved by the local ethics committee of the Institute for Atherosclerosis Research, Skolkovo Innovation Center, Moscow, Russia.

REFERENCES

1. Yahagi K, Kolodgie FD, Lutter C, Mori H, Romero ME, Finn AV, Virmani R. Pathology of human coronary and carotid artery atherosclerosis and vascular calcification in diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2017;37:191-204.
2. Heier M, Margeirsdottir HD, Gaarder M, Stensæth KH, Brunborg C, Torjesen PA, Seljeflot I, Hanssen KF, Dahl-Jørgensen K. Soluble RAGE and atherosclerosis in youth with type 1 diabetes: a 5-year follow-up study. *Cardiovasc Diabetol* 2015;14:126.
3. Hunt KJ, Baker NL, Cleary PA, Klein R, Virella G, Lopes-Virella MF; DCCT/EDIC Group of investigators. Longitudinal association between endothelial dysfunction, inflammation, and clotting biomarkers with subclinical atherosclerosis in type 1 diabetes: an evaluation of the DCCT/EDIC cohort. *Diabetes Care* 2015;38:1281-9.
4. Yoon HJ, Lee YH, Kim SR, Rim TH, Lee EY, Kang ES, Cha BS, Lee HC, Lee BW. Glycated albumin and the risk of micro- and macrovascular complications in subjects with type 1 diabetes. *Cardiovasc Diabetol* 2015;14:53.
5. Heier M, Margeirsdottir HD, Brunborg C, Hanssen KF, Dahl-Jørgensen K, Seljeflot I. Inflammation in childhood type 1 diabetes; influence of glycemic control. *Atherosclerosis* 2015;238:33-7.
6. Zeadin MG, Petlura CI, Werstuck GH. Molecular mechanisms linking diabetes to the accelerated development of atherosclerosis. *Can J Diabetes* 2013;37:345-50.
7. Shah MS, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res* 2016;118:1808-29.
8. Grundy SM. Metabolic syndrome update. *Trends Cardiovasc Med* 2016;26:364-73.
9. Tune JD, Goodwill AG, Sassoon DJ, Mather KJ. Cardiovascular consequences of metabolic syndrome. *Transl Res* 2017;183:57-70.
10. Stout RW. Hyperinsulinemia and atherosclerosis. *Diabetes* 1996;45 Suppl 3:S45-6.
11. Gwinup G, Elias AN. Hypothesis: insulin is responsible for the vascular complications of diabetes. *Med Hypotheses* 1991;34:1-6.
12. Bansilal S, Farkouh ME, Fuster V. Role of insulin resistance and hyperglycemia in the development of atherosclerosis. *Am J Cardiol* 2007;99:B6-14.
13. Stout RW. Insulin as a mitogenic factor: role in the pathogenesis of cardiovascular disease. *Am J Med* 1991;90:S62-5.
14. Escudero CA, Herlitz K, Troncoso F, Guevara K, Acurio J, Aguayo C, Godoy AS, González M. Pro-angiogenic role of insulin: from physiology to pathology. *Front Physiol* 2017;8:204.
15. Rask-Madsen C, Buonomo E, Li Q, Park K, Clermont AC, Yerokun O, Rekhter M, King GL. Hyperinsulinemia does not change atherosclerosis development in apolipoprotein E null mice. *Arterioscler Thromb Vasc Biol* 2012;32:1124-31.
16. Jarrett RJ. Is insulin atherogenic? *Diabetologia* 1988;31:71-5.
17. Durrington PN. Is insulin atherogenic? *Diabet Med* 1992;9:597-600.
18. Radermecker RP, Renard E, Scheen AJ.Circulating insulin antibodies: influence of continuous subcutaneous or intraperitoneal insulin infusion, and impact on glucose control. *Diabetes Metab Res Rev* 2009;25:491-501.
19. American Diabetes Association. (2) Classification and diagnosis of diabetes. *Diabetes Care* 2015;38 Suppl:S8-16.
20. Sobenin IA, Galitsyna EV, Grechko AV, Orekhov AN. Small dense and desialylated low density lipoprotein in diabetic patients. *Vessel Plus* 2017;1:29-37.
21. Tertov VV, Orekhov AN, Ryong LH, Smirnov VN. Intracellular cholesterol accumulation is accompanied by enhanced proliferative activity of human aortic intimal cells. *Tissue Cell* 1988;20:849-54.
22. Adams DO. Macrophages. In: Jakoby WB, Pastan JA, editors. Methods in Enzymology. New York: Academic Press;1979. p. 494-506.
23. Slavina ES, Madanat AYa, Pankov YuA, Syrkin AL, Tertov VV, Orekhov AN. Diabetes mellitus and atherosclerosis. *N Engl J Med* 1987;317:836.
24. Sobenin IA, Tertov VV, Koschinsky T, Bünting CE, Slavina ES, Dedov II, Orekhov AN. Modified low density lipoprotein from diabetic patients causes cholesterol accumulation in human intimal aortic cells. *Atherosclerosis* 1993;100:41-54.
25. Sobenin IA, Tertov VV, Orekhov AN. Atherogenic modified LDL in diabetes. *Diabetes* 1996;45 Suppl 3:S35-9.
26. Lyons TJ, Klein RL, Baynes JW, Stevenson HC, Lopes-Virella MF. Stimulation of cholesteryl ester synthesis in human monocyte-derived macrophages by low-density lipoproteins from type 1 (insulin-dependent) diabetic patients: the influence of non-enzymatic glycosylation of low-density lipoproteins. *Diabetologia* 1987;30:916-23.
27. Lopes-Virella MF, Virella G. Pathogenic role of modified LDL antibodies and immune complexes in atherosclerosis. *J Atheroscler Thromb* 2013;20:743-54.
28. Stout RW. Insulin and atheroma. 20-yr perspective. *Diabetes Care* 1990;13:631-54.
29. Savage PJ, Saad MF. Insulin and atherosclerosis: villain, accomplice, or innocent bystander? *Br Heart J* 1993;69:473-5.
30. Fontbonne AM, Eschwège EM. Insulin and cardiovascular disease. Paris prospective study. *Diabetes Care* 1991;14:461-9.
31. Yosefy C. Hyperglycaemia and its relation to cardiovascular morbidity and mortality: has it been resolved? *Acta Diabetol* 2003;40 Suppl 2:S380-8.
32. Stout RW. Insulin stimulation of cholesterol synthesis by arterial tissue. *Lancet* 1970;1:248.
33. Wahlqvist ML, Stout RW. The effect of insulin on the incorporation of sodium (1-14C)-acetate into the lipids of the rat aorta. *Diabetologia* 1972;8:305-6.
34. Stout RW. Diabetes and atherosclerosis--the role of insulin. *Diabetologia* 1979;16:141-50.
35. Sato Y, Shiraishi S, Oshida Y, Ishiguro T, Sakamoto N. Experimental atherosclerosis-like lesions induced by hyperinsulinism in Wistar rats. *Diabetes* 1989;38:91-6.
36. Abe H, Bandai A, Makuuchi M, Idezuki Y, Nozawa M, Oka T, Osuga J, Watanabe Y, Inaba T, Yamada N. Hyperinsulinaemia accelerates accumulation of cholesterol ester in aorta of rats with transplanted pancreas. *Diabetologia* 1996;39:1276-83.
37. Chattopadhyay DP, Martin JM. Effect of insulin on the in vitro synthesis of sterol and fatty acid by aorta and liver from diabetic rats. *J Atheroscler Res* 1969;10:131-4.
38. Chobanian AV, Gerritsen GC, Brecher PI, McCombs L. Aortic glucose metabolism in the diabetic Chinese hamster. *Diabetologia* 1974;10 Suppl:589-93.
39. Christensen S, Jensen J. Uptake of labelled cholesterol from plasma by aortic intima-media in control and insulin-injected rabbits. *J Atheroscler Res* 1965;5:258-9.

40. Capron L, Jarnet J. Effects of injury and insulin on lipid synthesis from glucose by the rat thoracic aorta. *Arterioscler Thromb* 1991;11:91-6.
41. Pfeifle B, Ditschuneit HH, Ditschuneit H. Insulin as a cellular growth regulator of rat arterial smooth muscle cells in vitro. *Horm Metab Res* 1980;12:381-5.
42. King GL, Buzney SM, Kahn CR, Hetu N, Buchwald S, Macdonald SG, Rand LI. Differential responsiveness to insulin of endothelial and support cells from micro- and macrovessels. *J Clin Invest* 1983;71:974-9.
43. Ledet T. Growth hormone stimulating the growth of arterial medial cells in vitro. Absence of effect of insulin. *Diabetes* 1976;25:1011-7.
44. Baandrup U, Ledet T, Rasch R. Experimental diabetic cardiopathy preventable by insulin treatment. *Lab Invest* 1981;45:169-73.
45. Ridray S, Ktorza A, Picon L, Capron L. In vivo effect of insulin on the acute proliferative response of the rat aorta to injury. *Arterioscler Thromb* 1992;12:633-8.
46. Joseph JJ, Donner TW. Long-term insulin glargine therapy in type 2 diabetes mellitus: a focus on cardiovascular outcomes. *Vasc Health Risk Manag* 2015;11:107-16.
47. Diabetes Control and Complications Trial Research Group; Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:977-86.
48. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005;353:2643-53.
49. Cleary PA, Orchard TJ, Genuth S, Wong ND, Detrano R, Backlund JY, Zinman B, Jacobson A, Sun W, Lachin JM, Nathan DM; DCCT/EDIC Research Group. The effect of intensive glycemic treatment on coronary artery calcification in type 1 diabetic participants of the diabetes control and complications trial/epidemiology of diabetes interventions and complications (DCCT/EDIC) study. *Diabetes* 2006;55:3556-65.
50. Polak JF, Backlund JY, Cleary PA, Harrington AP, O'Leary DH, Lachin JM, Nathan DM; DCCT/EDIC Research Group. Progression of carotid artery intima-media thickness during 12 years in the diabetes control and complications trial/epidemiology of diabetes interventions and complications (DCCT/EDIC) study. *Diabetes* 2011;60:607-13.
51. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998;352:837-53.
52. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008;359:1577-89.
53. Action to Control Cardiovascular Risk in Diabetes Study Group; Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH Jr, Probstfield JL, Simons-Morton DG, Friedewald WT. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008;358:2545-59.
54. Advance Collaborative Group; Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008;358:2560-72.
55. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD; VADT Investigators. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009;360:129-39.
56. Riddle MC. Effects of intensive glucose lowering in the management of patients with type 2 diabetes mellitus in the action to control cardiovascular risk in diabetes (ACCORD) trial. *Circulation* 2010;122:844-6.
57. Fang HJ, Zhou YH, Tian YJ, Du HY, Sun YX, Zhong LY. Effects of intensive glucose lowering in treatment of type 2 diabetes mellitus on cardiovascular outcomes: a meta-analysis of data from 58,160 patients in 13 randomized controlled trials. *Int J Cardiol* 2016;218:50-8.

Sazonova *et al. Vessel Plus* 2017;1:182-91
DOI: 10.20517/2574-1209.2017.16

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New markers of atherosclerosis: a threshold level of heteroplasmy in mtDNA mutations

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How to cite this article: Sazonova MA, Ryzhkova AI, Sinyov VV, Galitsyna EV, Orekhova VA, Melnichenko AA, Orekhov AN, Ravani AL, Sobenin IA. New markers of atherosclerosis: a threshold level of heteroplasmy in mtDNA mutations. *Vessel Plus* 2017;1:182-91.

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Article history:
Received: 26 May 2017
Accepted: 11 Sep 2017
Published: 28 Dec 2017

Key words:
Threshold heteroplasmy level,
mutation,
mitochondrial genome,
mitochondrial DNA,
atherosclerosis,
gene,
marker

ABSTRACT

Aim: The aim of the present article was the detection of threshold heteroplasmy level of mitochondrial DNA mutations, above which a patient is at increased risk of atherosclerotic lesions. Besides, this parameter was detected for mutations, in which after reaching threshold heteroplasmy level, a protective antiatherogenic effect started to appear. **Methods**: The participants of the study were 700 women and men from the Moscow region. Fragments of DNA, amplified by polymerase chain reaction, were analyzed with pyrosequencing technology. Then on the basis of pyrograms' peaks in the samples, the heteroplasmy level of the investigated mitochondrial genome mutations was detected. **Results**: The threshold heteroplasmy level of 11 investigated mutations (m.5178C>A, m.15059G>A, m.652delG, m.13513G>A, m.14846G>A, m.652insG, m.12315G>A, m.3336T>C, m.1555A>G, m.14459G>A, m.3256C>T) in individuals with atherosclerotic plaques or thickening of the intima-medial layer of carotid arteries was detected. **Conclusion**: Using the method developed in our laboratory, the authors managed to determine threshold heteroplasmy levels of 11 mitochondrial genome mutations associated

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with atherosclerosis. The authors suggest that threshold heteroplasmy levels of these mutations is a new criterion for evaluation of predisposition to the occurrence and development of atherosclerotic lesions in human arteries.

INTRODUCTION

Atherosclerosis of human major vessels is often a morphological basis of mortality from cardiovascular diseases. In this pathology intima of arteries is damaged, luminal occlusion occurs and blood supply to organs deteriorates[1-5]. Atherosclerosis is difficult to recognize in the early stages. Molecular genetic markers could help the diagnostics of this disease. Unfortunately, compared to traditional single risk factors of atherosclerosis, nuclear genome mutations have rather low diagnostic and prognostic significance. The relative risk of one such mutation is 1.06-1.40. The total risk of cardiovascular diseases caused due to all known mutations in the nuclear genome is approximately 5%[6-10].

According to the literature, a variety of diseases is associated with some mutations in mitochondrial DNA (mtDNA). These mutations often correlate with pathologies which often occur together with atherosclerosis[11-15]. The penetrance of mtDNA mutations depends on the percentage of normal and mutant copies of genome, i.e. the heteroplasmy level of mitochondrial mutations. That is why, during the analysis of the linkage of mitochondrial genome mutations with human diseases, the value of heteroplasmy level above which in a person begins the occurrence and development of pathologies or begins to show a protective effect caused by mutations is determined. The information about the threshold heteroplasmy level of mitochondrial genome mutations associated with certain diseases, can help in assessing the predisposition and the early diagnosis of these pathologies.

In preliminary studies, a total of 11 of 42 mtDNA mutations associated with different pathologies was found to have an association with atherosclerosis[16-19]. In view of these facts, the aim of the present article was the detection of threshold heteroplasmy level of mtDNA mutations, above which atherosclerotic lesions were found in patients. Besides, this parameter was detected for mutations, in which after reaching the threshold heteroplasmy level started to appear a protective antiatherogenic effect.

The estimation of the mutations' threshold heteroplasmy level was carried out in the following samples of the study participants: (1) with atherosclerotic plaques; (2) with an increased intima-medial thickness of carotid arteries (IMT CA); (3) with a normal carotid intima.

It should be emphasized that at the present time there are no published studies which have investigated the threshold heteroplasmy level of mitochondrial genome mutations in atherosclerosis. Therefore, this article, dedicated to the identification of this parameter in patients with atherosclerotic lesions in blood vessels' wall, is quite relevant, well-timed and novel.

METHODS

Inclusion criteria

The present research was carried out according to the Helsinki Declaration of 1975 as revised in 1983. It was endorsed by the local ethics committees of the Institute for Atherosclerosis Skolkovo Innovation Center, Moscow, Russia and Research Institute of General Pathology and Pathophysiology, Moscow. Prior to their inclusion in the study, all the study participants gave an informed consent in written form.

The participants in the study were enrolled consecutively from the number of patients at Moscow municipal outpatient clinics No. 202, who were examined for cardiovascular risk factors (mainly arterial blood pressure and blood cholesterol). The investigated sample included 700 study participants. The age of men was over 40-year-old, and the age of women was over 50[20,21]. The average age of the participants in the sample was 62.3 years (SD = 8.7), the ratio of men and women was 362:338 (51.7%/48.3%). As a result of ascertaining the clinical diagnosis of atherosclerosis, the sample was divided into 2 groups: (1) conventionally healthy participants without ultrasonographic signs of atherosclerosis (339 people, or 48.4% of the sample); (2) patients without clinical manifestations of atherosclerosis who have ultrasonographic signs of preclinical atherosclerosis: the presence of lesions in the carotid artery lumen (more than 10% of the artery lumen) (361 people, or 51.6% of the sample).

The criteria for exclusion were: anatomical organization of the neck and carotid arteries which prevented qualitative ultrasonography, serious life-threatening diseases and the refusal to sign an informed consent to the investigation. Individuals with a history of, or a diagnoses of the following diseases were excluded from the study: breast cancer or nodal form of mastopathy; stroke or coronary heart disease; liver dysfunction; chronic kidney disease; type 2 diabetes; obesity (body

mass index > 30 kg/m^2); untreated high blood pressure; cigarette smoking; pulmonary embolism or deep vein thrombosis[20,21].

The presence of increased IMT CA or atherosclerotic plaques was detected by high-resolution B-mode ultrasound using a SSI-1000 scanner (SonoScape, China) equipped with a 7.5-MHz linear array probe. Carotid ultrasonography was performed by the same researcher throughout the study. The far walls of the right and left common carotid arteries, the bifurcation, the internal and the external carotid arteries were visualized. The c-IMT of the first centimeter of the common carotid artery was measured in three different projections (anterior, posterior and lateral), and analyses carried out with the use of dedicated software M'Ath (Metris, SRL France). The average value of these measures was considered as an integral indicator of intima-medial thickness[22-25]. The measurements were carried out in accordance with the Mannheim criteria and the criteria of the investigation Improve[26,27].

Materials

The materials for the study were blood leukocyte samples of participants. Blood for genetic analysis was taken after an overnight fast in the amount of 9 mL from the ulnar vein in a 10 mL plastic tube containing sodium salt of Na_2-ethylenediaminetetraacetic (EDTA) acid as an anticoagulant. A mother liquor of 0.1 mol/L Na-EDTA in water (pH 8.0) was used, to which fresh blood was added in a ratio of 9:1 to obtain a final Na-EDTA concentration of 10 mmol/L. Samples were stored at -20 °C.

Procedure

It is noteworthy that in previous research from our laboratory, we haven't found any significant differences in heteroplasmy levels of the investigated mutations between leukocytes and platelets (blood cells having mitochondrial genome), so DNA samples for this study were isolated from whole blood[16-19,28]. This method was developed in our laboratory on the basis of technology of Maniatis[29]. For DNA isolation we used a lysis buffer (0.32 mol/L sucrose; 5 mmol/L $MgCl_2$; 100% Triton X-100; 0.01 mol/L Tris-HCl, pH 7.6), and then deproteinization buffer (25 mmol/L EDTA pH 8.0; 75 mmol/L NaCl) and proteinase K solution (20 mg/mL DNA purification from admixtures was carried out using phenol and chloroform. Precipitation of DNA was carried out using isopropanol, followed by washing in ethanol. The DNA precipitate was dissolved in 300 μL of TE buffer (10 mmol/L Tris-HCl, pH 8.0, 1 mmol/L EDTA). The DNA concentration in the obtained sample was identified by nanospectrophotometer IMPLEN NanoPhotometer™. After measuring the concentration, the DNA samples were stored at -20 °C. For operating with a collection of DNA samples, the samples were diluted in TE buffer to a concentration of 0.03 μg/μL.

The concentration of DNA solution in ng/μL was measured using IMPLEN NanoPhotometer™ nanospectrophotometer with the use of a LabelGuard™ microtubule in "DS DNA" mode at a wavelength of 260 nm[16,17].

The DNA samples of the study participants were used for carrying out the polymerase chain reaction (PCR) of fragments containing the region of 11 investigated mutations[11-15].

Electrophoresis of isolated DNA samples and PCR-fragments was carried out in a horizontal apparatus of Helicon company in agarose gel using 0.5 × tris-borate-EDTA (TBE) buffer. The concentration of agarose ("Fluka") was 0.8% (for DNA samples) and 1.5-2.0% (for PCR-fragments). The gel was stained by the addition of an ethidium bromide solution (0.5 μg/mL). As a colorant, a solution of bromophenol blue (1 μL for 10 μL of sample) was used[16-18]. The composition of 10 × TBE: TrisHCl (108 g), boric acid (55 g), 0.5 mol/L EDTA, pH 8.0 (40 mL per 1 liter of buffer).

For the evaluation of molecular weight of the investigated PCR fragments, DNA markers of 1 Kb (13 fragments from 0.25 to 10 Kb) and 100 bp (10 fragments from 100 to 1,000 bp) were used[16-18].

For conducting PCR, DNA with a concentration of 0.1 μg/mL diluted with μQ and primers at a concentration of 10 pmol/μL were taken[16-18]. The reaction mixture and PCR conditions were the following: μQ (H_2O): 4.6 μL; a mixture of dNTPs 10×: 2 mmol/L deoxyadenosine triphosphate, 2 mmol/L dTTP, 2 mmol/L dGTP, 2 mmol/L dCTP: 4 μL; 10× PCR buffer (16.6 μmol/L $(NH_4)_2SO_4$, 67 mmol/L Tris-HCl (pH 8.8): 4 μL; $MgCl_2$: 25 mmol/L: 4 μL (at the required concentration of 2.5 mmol/L); 2.4 μL (at the required concentration of 1.5 mmol/L); Taq polymerase: 1.33 μL; Matrix DNA: 4 μL; Primer F (+): 2.7 μL; Primer R (-): 2.7 μL. The reaction was carried out in 40 μL of the reaction mixture[16,17].

One of the PCR primers was biotinylated with the aim of the subsequent pyrosequencing of the PCR fragment. The study was carried out using amplifier "PTC DNA Engine 200". After that pyrosequencing of PCR fragments was conducted using the apparatus (PSQ96MA)[30-34]. The pyrosequencing reaction included 4 stages[30-34].

Stage 1: the sequence primer was hybridized to a

single-stranded PCR amplicon, which is used as a template. The obtained fragment was incubated with the following enzymes: DNA polymerase, ATP-sulfurylase, luciferase and apyrase; and it was also incubated with the substrate: adenosine-5-phosphosulfate and luciferin.

Stage 2: the first nucleotide was added to the reaction mixture. DNA polymerase completes it to the DNA strand, in accordance with the principle of complementarity. In this case, the reaction is accompanied by the release of pyrophosphate (PPi) in an amount equimolar to the sum of the included nucleotides.

Stage 3: ATP-sulphurylase converts PPi to ATP in the presence of adenosine-5-phosphosulfate. In this case, luciferin is converted into oxyluciferin, which generates visible light in proportion to the amount of ATP. A pyrosequenator detects this light and converts it into the corresponding peak on the pyrogram. The height of each peak is proportional to the intensity of the flash of light, and, consequently, to the number of analyzed nucleotides in the DNA strand. For example, if there is one nucleotide C in a particular position of the DNA strand, a peak corresponding to a single flash will be seen on the pyrogram, and if there are three nucleotides C in succession, a triple flash will be seen (i.e. the peak will be three times higher).

Stage 4: apyrase, a nucleotide-destroying enzyme, constantly removes the remaining nucleotides which were not attached and ATP. Afterwards another nucleotide is added to the reaction mixture.

To carry out the above-described investigation, it was necessary to conduct sample preparation: a mixture of primers for the sequence should be added to the studied single-chain biotinylated PCR-fragments. Streptavidin-sepharose particles were attached to these biotinylated PCR-fragments, which makes it possible to operate with single-strand PCR-fragments using streptavidin interacting with biotin.

The algorithm of sample preparation for pyrosequencing was as follows: (1) 2 × Binding buffer [per 100 mL of the solution: $C_4H_{11}NO_3$ (0.121 g), NaCl (11.7 g), EDTA (0.0292 g), Tween 20 (100 µL). PH was adjusted to 7.6, 1 mol/L HCl]; (2) 1 × Annealing buffer [per 100 mL of the solution: $C_4H_{11}NO_3$ (0.242 g), $C_4H_6MgO_4 \times 4H_2O$ (0.043 g). PH was adjusted to 7.6, 4 mol/L CH_3COOH]; (3) washing buffer [per 100 mL of solution: $C_4H_{11}NO_3$ (0.121 g). PH was adjusted to 7.6, 4 mol/L CH_3COOH]; and (4) 0.2 mol/L NaOH (per 100 mL solution: NaCl 0.8 g).

Then, a solution of streptavidin-sepharose was prepared for 1 sample: 40 µL 2 × BB, 3 µL of streptavidin-sepharose particles, 7 µL of µQ H_2O. After that, the mode in the computer program for pyrosequencing was set. Into each test tube, 50 µL of streptavidin-sepharose solution were added, previously shaking it with vortex. Samples were put in a shaker for 5 min. At this time, 39 µL of 1 × AB and 3 µL of corresponding probe primer with a concentration of 2 optical units were added to each well of the pyrosequencing dish and the prepared pyrosequencing dish was placed in the runners of a vacuum sample preparation station. The dishes were placed into the appropriate sample niches in the sample preparation station, the following reagents were poured into all the baths: washing buffer (WB), 0.2 mol/L NaOH, rectified alcohol 70%, µQ H_2O. The nozzle with filters was placed into the dish with µQ H_2O and vacuum was switched on at the sample preparation station. In 20 s the nozzle was removed from the dish and dried in an upright position for 10 s. Then the nozzle with filters was placed in the 96-well pyrosequencing plate with samples for 30 s. Then the filters with samples were lowered into reagent dishes: rectified alcohol 70% (for 5 s), then into 0.2 mol/L NaOH (for 5 s), then into WB (for 10 s) and then into µQ H_2O (for 5 s). Then the nozzle with filters was raised and held in the upright position for 10 s. After this procedure, a nozzle with filters was placed above the pyrosequencing plate (without touching the primer solution with the nozzle filters), the vacuum was turned off and only after that the nozzle filters were lowered into the plate. In 2 min the nozzle with filters was removed from the pyrosequencing plate. The pyrosequencing plate was placed on a thermostat at 80 °C for 2 min. Before placing the plate into the sequencer, it was given time to cool down. During the cooling of the plate, the reagents E (enzyme) and S (substrate) were diluted with µQ H_2O, E, S, and individual dNTPs were poured into the cuvette, according to the instructions, at the amount indicated for setting the mode for sequencing. After that, the cuvette with the reagents and the samples was put into the sequencer and the program was launched.

The sequences of primers for pyrosequence were given in the article of Sazonova *et al.*[16] Visualization of the results was carried out with the use of a computer program, which was completed with the pyrosequenator. The heteroplasmy level of every mitochondrial genome mutation was assessed on the basis of the pyrogram of a DNA sample from each study participant, using a formula previously developed by Sazonova *et al.*[16,17] with colleagues: $P = (h - N)/(M - N) \times 100$, where "P" is the heteroplasmy percentage of the studied mutations; "h" is the height of the peak of the investigated nucleotide; "N" is the height of the peak

of the investigated nucleotide corresponding to the presence of 100% normal copies of the mitochondrial genome in the sample; "M" is the height of the peak of the investigated nucleotide corresponding to the presence of 100% mutant copies of the mitochondrial genome in the sample.

It should be emphasized that, alongside the genetic screening, a number of biochemical indicators was investigated in blood of the study participants: total cholesterol, triglycerides blood sugar level, low and high density lipoproteins[20,21].

RESULTS

At the first stage of the study, demographic characteristics of 700 study participants were obtained [Table 1]. The data in this table are presented by means of an average value with the standard deviation (in parentheses).

In the group of conventionally healthy participants, women predominated, while in the group of patients with pre-clinical atherosclerosis, men predominated [Table 1]. Patients with pre-clinical atherosclerosis were characterized by a significantly older age, and also by higher levels of total cholesterol and low-density lipoproteins cholesterol in men ($P \leq 0.01$). This group showed a tendency to an increase in the frequency of smoking ($P \leq 0.1$). In terms of body mass index, blood pressure and triglyceride levels, there were no significant differences between the studied groups.

At the second stage of the study the threshold heteroplasmy level of 11 investigated mutations (m.5178C>A, m.15059G>A, m.652delG, m.13513G>A, m.14846G>A, m.652insG, m.12315G>A, m.3336T>C, m.1555A>G, m.14459G>A and m.3256C>T) in individuals with atherosclerotic plaques or an increased IMT CA was detected. It should be noted that the percentage of mitochondrial genome copies with higher than the threshold level was associated with the occurrence and development of atherosclerotic lesions in patients. At the same time, achieving and exceeding the threshold heteroplasmy level of antiatherogenic mutations is thought to have the effect of lowering incidence of this disease.

The evaluation results of the threshold heteroplasmy level of mutations in atherosclerotic plaques and an increased IMT CA of the carotid arteries of the study participants are shown in Table 2.

The selection of the optimal threshold heteroplasmy level for each investigated mutation was detected using ROC-curve analysis. It was based on simultaneous maximizing of the sensitivity values (Y-axis) and minimizing the value (1-specificity) (X-axis). The example of such a ROC-curve analysis of a mutation m.12315G>A connection with the occurrence of atherosclerotic plaques in carotid arteries is shown [Figure 1 and Table 3].

Therefore, the threshold heteroplasmy level for mitochondrial genome mutation m.12315G>A was found to be a rather strong predictor of the atherosclerotic plaques occurrence in carotid arteries. The area of a ROC-curve for this mutation was found to be 0.577. The optimal threshold value of heteroplasmy level for m.12315G>A was 10.5% (sensitivity was 0.6; specificity was 0.53).

As can be seen from the Table 2, the levels of heteroplasmy of investigated mitochondrial mutations in a sample of patients with atherosclerotic plaques and in a sample of patients with an increased IMT CA are approximately the same.

Table 1: Demographic characteristics of the study participants

Value	Conventionally healthy study participants	Patients with preclinical atherosclerosis	Significance
Systolic blood pressure (mmHg)	129 (15.7)	141 (14.1)	0.26
Body-weight index (kg/sq.m)	25.6 (7.3)	29.3 (6.4)	0.45
Male:female (number of persons)	159:180	203:158	0.002**
Smoking (%)	20 (10.6)	42 (9.5)	0.10*
Cholesterol LDL (mmol/L)	4.03 (1.07)	4.38 (1.09)	0.001**
Diastolic blood pressure (mmHg)	78 (16.7)	89 (17.3)	0.38
Age (years)	53 (8.5)	64 (8.9)	0.003**
Total cholesterol (mmol/L)	6.39 (1.21)	6.76 (1.09)	0.001**
Triglycerides (mmol/L)	1.48 (0.61)	1.51 (0.64)	0.290
Cholesterol HDL (mmol/L)	1.70 (0.48)	1.53 (0.52)	0.002**

*: the differences between conventionally healthy study participants and patients with atherosclerosis are at the significance level $P \leq 0.1$; **: significant differences between conventionally healthy study participants and patients with atherosclerosis; LDL: low-density lipoproteins; HDL: high-density lipoproteins

Table 2: The threshold value of heteroplasmy level of mtDNA mutations associated with atherosclerosis

Gene	Mutation	Threshold value of heteroplasmy level in atherosclerotic plaques (%)	Threshold value of heteroplasmy level in thickened IML CA (%)
MT-TL1	m.3256C>T	15.5	16.5
MT-RNR1	m.652delG	20.5	21.5
MT-CYTB	m.14846G>A*	17.5	17.5
MT-TL2	m.12315G>A	7.5	10.5
MT-ND1	m.3336T>C	6.5	7.5
MT-RNR1	m.652insG	20.0	20.0
MT-CYTB	m.15059G>A	24.5	26.5
MT-RNR1	m.1555A>G*	17.5	19.5
MT-ND5	m.13513G>A*	32.5	33.5
MT-ND2	m.5178C>A	6.5	6.5
MT-ND6	m.14459G>A	4.5	4.5

*: antiatherosclerotic mutations; IML CA: intima-medial layer of carotid arteries

Table 3: Detection of the optimal threshold heteroplasmy level value for m.12315G>A on the basis of ROC-analysis

Coordinates of the curve		
Threshold heteroplasmy level	Sensitivity	1 - Specificity
4.50	0.825	0.795
5.50	0.789	0.667
6.50	0.719	0.643
7.50	0.684	0.608
8.50	0.632	0.544
9.50	0.614	0.485
10.50*	0.596	0.468
11.50	0.579	0.468
12.50	0.561	0.433
13.50	0.544	0.415
14.50	0.544	0.398
16.00	0.526	0.363
17.50	0.474	0.339
18.50	0.421	0.310
19.50	0.404	0.287
20.50	0.386	0.269
21.50	0.386	0.246
22.50	0.351	0.24
23.50	0.333	0.228
24.50	0.333	0.216
25.50	0.316	0.193
26.50	0.298	0.187
27.50	0.298	0.181

ROC: receiver operating characteristic

It is necessary to mention that during the analysis of all the 11 investigated mutations in patients, it was possible to explain more than 84% of cases of atherosclerotic plaques occurrence and thickening of intima-medial layer of carotid arteries.

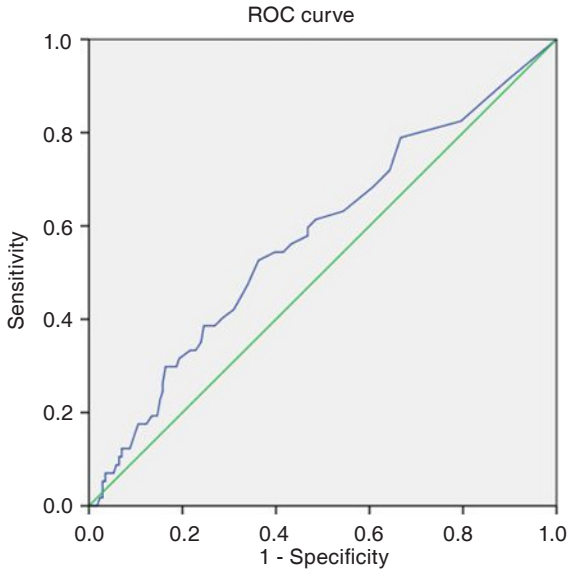

Figure 1: ROC-curve for the analysis of the link of the heteroplasmy level in mitochondrial genome mutation m.12315G>A with the occurrence of atherosclerotic plaques in carotid arteries: area under the curve 0.577 (standard error 0.015); $P \leq 0.042$. ROC: receiver operating characteristic

DISCUSSION

Atherosclerosis of human major vessels is often a cause of mortality from cardiovascular diseases. In this pathology, the intima of arteries is damaged, luminal occlusion occurs and blood supply to organs deteriorates[1-5]. Atherosclerosis is difficult to recognize in the early stages. Molecular genetic markers could help the diagnostics of this disease. Unfortunately, compared to traditional single risk factors of atherosclerosis, nuclear genome mutations have rather low diagnostic and prognostic significance.

According to the literature, a variety of diseases is associated with some mutations in mtDNA. These mutations often correlate with pathologies which often occur together with atherosclerosis[11-15]. The penetrance of mtDNA mutations depends on the percentage of normal and mutant copies of genome, i.e. the heteroplasmy level of mitochondrial mutations. That is why, during the analysis of the linkage of mitochondrial genome mutations with human diseases, the value of heteroplasmy level above which a person begins the occurrence and development of pathologies or begins to show a protective effect of mutations needs to be determined. The information about the threshold heteroplasmy level of mitochondrial genome mutations, associated with certain diseases, can help in assessing the predisposition and the early diagnosis of these pathologies.

As a working hypothesis for the present study, we used the monoclonal hypothesis according to which, if a mutation occurs in the mitochondrial genome, the ATP synthesis is disturbed. The cells containing mitochondria with mutant mtDNA begin to suffer a shortage of energy and proliferate unlimitedly. The number of defective mitochondrial genome copies becomes greatly increased. Finally a mutation reaches the threshold heteroplasmy level and the pathological process starts. This results in the occurrence and development of atherosclerotic lesions. When the threshold heteroplasmy level is reached by antiatherogenic mutations, they begin to show a protective effect. That is why, in people with a high heteroplasmy level for antiatherogenic mutations, atherosclerosis is absent.

For heteroplasmy level evaluation of investigated samples, a new original method of quantitative assessment of mutant allele in mitochondrial genome, based on pyrosequencing technology, was developed by the author and her colleagues[16]. With the use of this method, it is possible to measure the heteroplasmy level of both hereditary and somatic mitochondrial genome mutations, occurring during the lifetime of an individual or in pathologic processes. The method of quantitative assessment of the mutant allele of mitochondrial genome has a number of significant advantages compared to other quantitative methods such as invasive cleavage of the oligonucleotide probe (Invader), high-performance liquid chromatography, heteroduplex analysis, the analysis of heteroplasmy using Surveyor nuclease, ARMS, SNaPshot, HRM, TGGE, Sanger sequencing, NGS using 454/Roche equipment, Applied Biosystems SOLiD, Illumina equipment series used for the analysis of mutations[35-39].

Pyrosequencing has the smallest number of defects and the greatest number of advantages, compared to other methods of measuring the percentage of heteroplasmy of mitochondrial genome[40-44]. It provides a unique opportunity to analyze a very short DNA fragment containing the region of the investigated mutation. The size of such a DNA fragment is, on average, 5-10 bp, it significantly reduces the probability of mistakes made during the analysis. As a result, the method developed by the author with colleagues, based on pyrosequencing technology, can serve as the “gold standard” for all other methods of determining the percentage of mitochondrial genome heteroplasmy and it should be used to verify the heteroplasmy level of mutations detected by other methods.

With the aim of subsequent molecular genetic diagnosis of atherosclerosis in blood cells of the study participants, the threshold value of the heteroplasmic percentage for each mutation was detected, after which the occurrence and development of atherosclerotic lesions begins in an individual, and for anti-atherogenic mutations, an antipathological effect begins to manifest. It should be emphasized that the choice of the optimal threshold value was based on simultaneous maximization of sensitivity and specificity. The revealed good agreement of the threshold heteroplasmy percentage value in the 11 mitochondrial genome mutations for atherosclerotic plaques and the thickening of the carotid intimal-medial layer can be indicative of general pathophysiological mechanisms of the formation of lesions in intimal carotid arteries.

The investigated mutations are localized in the coding region of mitochondrial genome. Let’s consider each of them.

Mutation m.652delG was localized in the *MT-RNR1* gene. It causes a structural defect of the 12S rRNA subunit, which can lead to a partial or complete dysfunction of the mitochondrial ribosome. A consequence of this mutation may be a decrease in synthesis of protein subunits of the enzymes of the mitochondrial respiratory chain. As a result, the synthesis of ATP may decrease, leading to an energy failure in the mitochondria and intimal cells of arteries. It can, as a compensatory mechanism, lead to unlimited proliferation of mitochondria and cells. The result of this process can be the occurrence of atherosclerotic plaques and thickening of the intima-medial layer of human arteries.

Probably, mutation m.652insG (*MT-RNR1* gene) associated with the absence of atherosclerosis stabilizes subunit 12S of rRNA and improves the functions of ribosomes. Perhaps, in case of occurrence of this insertion, the synthesis of protein chains in enzymes of mitochondrial respiratory chains increases. It leads to an increase in the synthesis of ATP and protects mitochondria and cells from oxidative stress. Therefore, this mutation can perform protective functions.

Mitochondrial genome mutation m.1555A>G is also encoded by *MT-RNR1* gene.The mutation has an anti-atherogenic effect. It also leads by far the most to the stabilization of the mitochondrial ribosome.

In case of a single-nucleotide substitution of m.3256C>T (*MT-TL1* gene), transport RNA-Leucine dysfunction (recognition codon UUR) can occur. If leucine is the last in the protein subunit of the enzyme, then without this tRNA, the protein cannot be separated from the ribosome. It can lead to a dysfunction of

some ribosomes in mitochondria and to a decrease in synthesis of respiratory chain enzymes.

Single nucleotide substitution m.3336T>C (*MT-ND1* gene) is a silent mutation. In case of its occurrence, there is no replacement of the amino acid in the first protein subunit of NADH dehydrogenase. Meanwhile, the mutation is associated with atherosclerotic lesions. Probably, this mutation is linked to an atherogenic haplotype.

Mutation m.5178C>A (*MT-ND2* gene) results in the replacement of an amino acid (Leu>Met). Although in some earlier literary sources[45] it is reported that m.5178C>A is anti-atherogenic, in this study, in a sample of 700 study participants, the association of this mutation with atherosclerotic lesions was found. Probably in earlier studies, an undersampling has played a role.

The single nucleotide replacement of guanine by adenine at the position of the mitochondrial genome 12315 results in a change in the tertiary structure of the transport RNA-Leu (recognition codon CUN). It can lead to a dysfunction of this tRNA, leading to a decrease in synthesis of the protein chains in respiratory chain enzymes, which contain amino acid leucine.

Mutation m.13513G>A leads to the replacement of the amino acid (Asp>Asn) in the 5th protein subunit of the respiratory chain enzyme NADH-dehydrogenase. Perhaps, as a result of this mutation, the efficiency of complex 1 of the respiratory chain, leading to an increase in the production of energy in the cell, is improved. Our research team discovered the anti-atherogenic effect of this mutation.

Mutation m.14459G>A (*MT-ND6* gene) results in amino acid substitution (Ala>Val) at position 72 of the 6th protein subunit of NADH dehydrogenase. Probably, this mutation causes a defect in the enzyme, leading to the appearance of atherosclerotic lesions.

The single nucleotide substitution m.14846G>A (*MT-CYTB* gene) results in the amino acid substitution (Gly>Ser) of cytochrome B. Probably, this mutation stabilizes the complex of the III respiratory chain, into which this cytochrome is included. The result of this mutation may be an increase in the synthesis of ATP in the cell. It can protect cells and tissues from the occurrence of atherosclerosis.

Mutation m.15059G>A, localized in the *MT-CYTB* gene, leads to a formation of a stop codon. This stops the synthesis of cytochrome B, which becomes 244 amino acids shorter. Perhaps, this mutation leads to the dysfunction of complex III of the respiratory chain. As a result of this process, atherosclerotic lesions can develop in the cells and arteries in general.

It is necessary to note the novelty and uniqueness of this study. According to the analysis of literary sources, there is not any scientific article in the world where the threshold heteroplasmy level of mitochondrial genome mutations and their association with predisposition to atherosclerosis has been studied. In addition, there are not any similar works for other human diseases either.

Information about the threshold heteroplasmy level in mitochondrial genome mutations, associated with atherosclerosis and its risk factors, can help in assessing the predisposition and the early diagnostics of these pathologies. The obtained data can be used to create test systems for atherosclerosis diagnostics.

In conclusion, the article dedicated to detection of a threshold heteroplasmy level of mitochondrial genome mutations in patients with atherosclerotic lesions in blood vessels, is quite relevant and well-timed. This work is characterized by a high degree of novelty, because similar works in world literature are absent. Using the method, developed in our laboratory[16,17], we managed to determine threshold heteroplasmy levels of 11 mitochondrial genome mutations associated with atherosclerosis. We suppose that threshold heteroplasmy levels of these mutations is a new criterion for evaluation of predisposition to the occurrence and development of atherosclerotic lesions in human arteries.

DECLARATIONS

Authors' contributions

Study design: M.A. Sazonova
Data collection: M.A. Sazonova, E.V. Galitsyna, V.A. Orekhova
Experimental studies: M.A. Sazonova, A.I. Ryzhkova, V.V. Sinyov
Literature search: A.A. Melnichenko
Statistical analysis: I.A. Sobenin
Manuscript writing, preparation and editing: M.A. Sazonova
Manuscript review: A.N. Orekhov, A.L. Ravani

Financial support and sponsorship

This work was supported by the Russian Scientific Foundation (Grant #14-14-01038).

Conflicts of interest

There are no conflicts of interest.

Patient consent

All the study participants gave an informed consent in written form.

Ethics approval

It was endorsed by the local ethics committees of the Institute for Atherosclerosis Skolkovo Innovation Center, Moscow, Russia and Research, Institute of General Pathology and Pathophysiology, Moscow, previous to their inclusion in the study.

REFERENCES

1. Saho T, Onishi H. Evaluation of the impact of carotid artery bifurcation angle on hemodynamics by use of computational fluid dynamics: a simulation and volunteer study. *Radiol Phys Technol* 2016;9:277-85.
2. Orekhov AN, Melnichenko AA, Sobenin IA. Approach to reduction of blood atherogenicity. *Oxid Med Cell Longev* 2014;2014:738679.
3. Nus M, Mallat Z. Immune-mediated mechanisms of atherosclerosis and implications for the clinic. *Expert Rev Clin Immunol* 2016;12:1217-37.
4. Sobenin IA, Galitsyna EV, Grechko AV, Orekhov AN. Small dense and desialylated low density lipoprotein in diabetic patients. *Vessel Plus* 2017;1:29-37.
5. Wu GC, Leng RX, Lu Q, Fan YG, Wang DG, Ye DQ. Subclinical atherosclerosis in patients with inflammatory Bowel diseases: a systematic review and meta-analysis. *Angiology* 2017;68:447-61.
6. Maruthur NM, Li M, Halushka MK, Astor BC, Pankow JS, Boerwinkle E, Coresh J, Selvin E, Kao WH. Genetics of plasma soluble receptor for advanced glycation end-products and cardiovascular outcomes in a community-based population: results from the atherosclerosis risk in communities study. *PLoS One* 2015;10:e0128452.
7. Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial infarction. *World J Cardiol* 2016;8:1-23.
8. Pokharel Y, Sun W, Polfus LM, Folsom AR, Heiss G, Sharrett AR, Boerwinkle E, Ballantyne CM, Hoogeveen RC. Lipoprotein associated phospholipase A2 activity, apolipoprotein C3 loss-of-function variants and cardiovascular disease: the Atherosclerosis Risk In Communities Study. *Atherosclerosis* 2015;241:641-8.
9. Yu B, Li AH, Muzny D, Veeraraghavan N, de Vries PS, Bis JC, Musani SK, Alexander D, Morrison AC, Franco OH, Uitterlinden A, Hofman A, Dehghan A, Wilson JG, Psaty BM, Gibbs R, Wei P, Boerwinkle E. Association of rare loss-of-function alleles in HAL, serum histidine: levels and incident coronary heart disease. *Circ Cardiovasc Genet* 2015;8:351-5.
10. Inanloorahatloo K, Zand Parsa AF, Huse K, Rasooli P, Davaran S, Platzer M, Fan JB, Amini S, Steemers F, Elahi E. Mutation in CYP27A1 identified in family with coronary artery disease. *Eur J Med Genet* 2013;56:655-60.
11. Han CB, Ma JM, Xin Y, Mao XY, Zhao YJ, Wu DY, Zhang SM, Zhang YK. Mutations of mitochondrial 12S rRNA in gastric carcinoma and their significance. *World J Gastroenterol* 2005;11:31-5.
12. Zhelankin A, Khasanova Z, Barinova L, Sazonova M, Postnov A, Sobenin I. 1A.06: mitochondrial DNA haplogroup H is associated with subclinical carotid atherosclerosis in russian population. *J Hypertens* 2015;33:e2.
13. Solano A, Roig M, Vives-Bauza C, Hernandez-Peña J, Garcia-Arumi E, Playan A, Lopez-Perez MJ, Andreu AL, Montoya J. Bilateral striatal necrosis associated with a novel mutation in the mitochondrial ND6 gene. *Ann Neurol* 2003;54:527-30.
14. Jeppesen TD, Schwartz M, Hansen K, Danielsen ER, Wibrand F, Vissing J. Late onset of stroke-like episode associated with a 3256C-->T point mutation of mitochondrial DNA. *J Neurol Sci* 2003;214:17-20.
15. Horváth R, Scharfe C, Hoeltzenbein M, Do BH, Schröder C, Warzok R, Vogelgesang S, Lochmüller H, Müller-Höcker J, Gerbitz KD, Oefner PJ, Jaksch M. Childhood onset mitochondrial myopathy and lactic acidosis caused by a stop mutation in the mitochondrial cytochrome c oxidase III gene. *J Med Genet* 2002;39:812-6.
16. Sazonova M, Budnikov E, Khasanova Z, Sobenin I, Postnov A, Orekhov A. Studies of the human aortic intima by a direct quantitative assay of mutant alleles in the mitochondrial genome. *Atherosclerosis* 2009;204:184-90.
17. Sazonova MA, Sinyov VV, Barinova VA, Ryzhkova AI, Zhelankin AV, Postnov AY, Sobenin IA, Bobryshev YV, Orekhov AN. Mosaicism of mitochondrial genetic variation in atherosclerotic lesions of the human aorta. *Biomed Res Int* 2015;2015:825468.
18. Sazonova MA, Chicheva MM, Zhelankin AV, Sobenin IA, Bobryshev YV, Orekhov AN. Association of mutations in the mitochondrial genome with the subclinical carotid atherosclerosis in women. *Exp Mol Pathol* 2015;99:25-32.
19. Sobenin IA, Sazonova MA, Ivanova MM, Zhelankin AV, Myasoedova VA, Postnov AY, Nurbaev SD, Bobryshev YV, Orekhov AN. Mutation C3256T of mitochondrial genome in white blood cells: novel genetic marker of atherosclerosis and coronary heart disease. *PLoS One* 2012;7:e46573.
20. Sobenin IA, Surnin SA, Karagodin VP, Miasoedova VA, Kirichenko TV, Chuprakova OV, Kozhevnikova IuA, Kovalevskaia LO, Orekhov AN. Variability of intima-media thickness of the common carotid arteries in Moscow city population without clinical symptoms of atherosclerosis. *Ter Arkh* 2011;83:58-62. (in Russian)
21. Sobenin IA, Myasoedova VA, Anisimova EV, Pavlova XN, Möhlenkamp S, Schmermund A, Seibel R, Berenbein S, Lehmann N, Moebus S, Jöckel K, Orekhov AN, Erbel R. Blood serum atherogenicity and coronary artery calcification. *Curr Pharm Des* 2014;20:5884-8.
22. Myasoedova VA, Kirichenko TV, Melnichenko AA, Orekhova VA, Ravani A, Poggio P, Sobenin IA, Bobryshev YV, Orekhov AN. Anti-atherosclerotic effects of a phytoestrogen-rich herbal preparation in postmenopausal women. *Int J Mol Sci* 2016;17:E1318.
23. Kirichenko TV, Myasoedova VA, Orekhova VA, Ravani AL, Nikitina NA, Grechko AV, Sobenin IA, Orekhov AN. Phytoestrogen-rich natural preparation for treatment of climacteric syndrome and atherosclerosis prevention in perimenopausal women. *Phytother Res* 2017;31:1209-14.
24. Aguilera I, Dratva J, Caviezel S, Burdet L, de Groot E, Ducret-Stich RE, Eeftens M, Keidel D, Meier R, Perez L, Rothe T, Schaffner E, Schmit-Trucksäss A, Tsai MY, Schindler C, Künzli N, Probst-Hensch N. Particulate matter and subclinical atherosclerosis: associations between different particle sizes and sources with carotid intima-media thickness in the SAPALDIA study. *Environ Health Perspect* 2016;124:1700-06.
25. Liu X, Lian H, Ruan Y, Liang R, Zhao X, Routledge M, Fan Z. Association of exposure to particular matter and carotid intima-media thickness: a systematic review and meta-analysis. *Int J Environ Res Public Health* 2015;12:12924-40.
26. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Hernandez Hernandez R, Jaff M, Kownator S, Naqvi T, Prati P, Rundek T, Sitzer M, Schminke U, Tardif JC, Taylor A, Vicaut E, Woo KS. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update

on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. *Cerebrovasc Dis* 2012;34:290-6.
27. Amato M, Veglia F, de Faire U, Giral P, Rauramaa R, Smit AJ, Kurl S, Ravani A, Frigerio B, Sansaro D, Bonomi A, Tedesco CC, Castelnuovo S, Mannarino E, Humphries SE, Hamsten A, Tremoli E, Baldassarre D; IMPROVE study group. Carotid plaque-thickness and common carotid IMT show additive value in cardiovascular risk prediction and reclassification. *Atherosclerosis* 2017;263:412-9.
28. Sobenin IA, Zhelankin AV, Mitrofanov KY, Sinyov VV, Sazonova MA, Postnov AY, Orekhov AN. Mutations of mitochondrial DNA in atherosclerosis and atherosclerosis-related diseases. *Curr Pharm Des* 2015;21:1158-63.
29. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning : a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory; 1982.
30. Zhang XX, Li MR, Cao Y, Zhang RW, Zhang Y, Li F, Xi HL, Xu XY. Dynamics of genotypic mutations of the hepatitis B virus associated with long-term entecavir treatment determined with ultradeep pyrosequencing: a retrospective observational study. *Medicine* (*Baltimore*) 2016;95:e2614.
31. Harrington CT, Lin EI, Olson MT, Eshleman JR. Fundamentals of pyrosequencing. *Arch Pathol Lab Med* 2013;137:1296-303.
32. Kreutz M, Schock G, Kaiser J, Hochstein N, Peist R. PyroMark® instruments, chemistry, and software for Pyrosequencing® analysis. *Methods Mol Biol* 2015;1315:17-27.
33. Rajatileka S, Luyt K, Williams M, Harding D, Odd D, Molnár E, Váradi A. Detection of three closely located single nucleotide polymorphisms in the EAAT2 promoter: comparison of single-strand conformational polymorphism (SSCP), pyrosequencing and Sanger sequencing. *BMC Genet* 2014;15:80.
34. Simou M, Kouskouni E, Vitoratos N, Economou E, Creatsas G. Polymorphisms of platelet glycoprotein receptors and cell adhesion molecules in fetuses with fetal growth restriction and their mothers as detected with pyrosequencing. *In Vivo* 2017;31:243-9.
35. Mahmood U, Imran M, Naik SI, Cheema HA, Saeed A, Arshad M, Mahmood S. Detection of common mutations in the GALT gene through ARMS. *Gene* 2012;509:291-4.
36. Fondevila M, Børsting C, Phillips C, de la Puente M, Consortium EN, Carracedo A, Morling N, Lareu MV. Forensic SNP genotyping with SNaPshot: technical considerations for the development and optimization of multiplexed SNP assays. *Forensic Sci Rev* 2017;29:57-76.
37. Salimullah M, Hamano K, Tachibana M, Inoue K, Nishigaki K. Efficient SNP analysis enabled by joint application of the muTGGE and heteroduplex methods. *Cell Mol Biol Lett* 2005;10:237-45.
38. Sazonova MA, Shkurat TP, Demakova NA, Zhelankin AV, Barinova VA, Sobenin IA, Orekhov AN. Mitochondrial genome sequencing in atherosclerosis: what's next? *Curr Pharm Des* 2016;22:390-6.
39. Ladouceur M, Zheng HF, Greenwood CM, Richards JB. Empirical power of very rare variants for common traits and disease: results from sanger sequencing 1998 individuals. *Eur J Hum Genet* 2013;21:1027-30.
40. Georghiou SB, Seifert M, Lin SY, Catanzaro D, Garfein RS, Jackson RL, Crudu V, Rodrigues C, Victor TC, Catanzaro A, Rodwell TC. Shedding light on the performance of a pyrosequencing assay for drug-resistant tuberculosis diagnosis. *BMC Infect Dis* 2016;16:458.
41. Yeo MK, Jung MK, Lee SY, Lee YM, Hur GM, Kim JM. The usefulness of a novel fully automated PCR-based Idylla test for detection of the BRAF V600E mutation in thyroid tissue: comparison with PNA-clamping PCR, real-time PCR and pyrosequencing. *J Clin Pathol* 2017;70:260-5.
42. Mohamed S, Bourliere M, Benali S, Oules V, Castellani P, Khiri H, Camus C, Penaranda G, Chiche L, Gonzalez D, Sayada C, Olive D, Halfon P. Clinical relevance of the HCV protease inhibitor-resistant mutant viral load assessed by ultra-deep pyrosequencing in treatment failure. *J Clin Virol* 2016;78:36-43.
43. Pu D, Mao C, Cui L, Shi Z, Xiao P. Pyrosequencing with di-base addition for single nucleotide polymorphism genotyping. *Anal Bioanal Che*m 2016;408:3113-23.
44. Kuscu C, Kuscu C. Pyrosequencing analysis for breast cancer DNA methylome. *Methods Mol Biol* 2016;1406:89-104.
45. Matsunaga H, Tanaka Y, Tanaka M, Gong JS, Zhang J, Nomiyama T, Ogawa O, Ogihara T, Yamada Y, Yagi K, Kawamori R. Antiatherogenic mitochondrial genotype in patients with type 2 diabetes. *Diabetes Care* 2001;24:500-3.

Nikiforov *et al. Vessel Plus* 2017;1:192-5
DOI: 10.20517/2574-1209.2017.21

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Topic: Atherosclerosis and Related Diseases **Open Access**

Proinflammatory monocyte polarization in type 2 diabetes mellitus and coronary heart disease

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How to cite this article: Nikiforov NG, Galstyan KO, Nedosugova LV, Elizova NV, Kolmychkova KI, Ivanova EA. Proinflammatory monocyte polarization in type 2 diabetes mellitus and coronary heart disease. *Vessel Plus* 2017;1:192-5.

Article history:
Received: 10 Jun 2017
Accepted: 20 Sep 2017
Published: 28 Dec 2017

Key words:
Type 2 diabetes mellitus, coronary heart disease, M1/M2 monocyte polarization, oxidative stress, atherosclerosis, inflammatory

ABSTRACT

Aim: Type 2 diabetes mellitus (T2DM) is associated with rapid progression of atherosclerosis. There is no doubt that inflammation is involved in atherogenesis. Recent studies showed the relationship between the development of atherosclerotic plaque formation and the amount of pro-inflammatory (M1) activated macrophages *in situ*. **Methods**: The authors studied the ability of circulating monocytes isolated from patients with diabetes (n = 28), coronary heart disease (CHD) (n = 27) and healthy subjects (n = 50) to be activated into M1 phenotype *in vitro*. **Results**: Increased levels of basal and stimulated secretion of tumor necrosis factor alpha (TNF-α) was observed in diabetic patients compared with healthy subjects. On the contrary, in patients with CHD, decreased secretion of the pro-inflammatory cytokine, TNF-α, was found. A direct correlation between glycated hemoglobin (HbA1c) levels in T2DM patients and basal secretion of TNF-α from monocytes was observed. **Conclusion**: The authors found diametrically different responses of monocytes from T2DM and CHD under pro-inflammatory stimuli.

INTRODUCTION

Increased prevalence of type 2 diabetes mellitus (T2DM) around the globe has been deemed a "non-infectious epidemic" and according to the International Diabetes Federation, it is predicted that the number of patients with T2DM will reach 642 million people by 2040[1].

T2DM patients are more likely to exhibit an accelerated development of atherosclerosis and have a 3-4-fold increased risk for cardiovascular mortality[2]. Hyperglycemia is currently considered the main culprit for vascular lesion progression. A meta-analysis of 20 different studies involving 95,783 patients, followed for 12 years, led to the conclusion that glucose is the common underlying risk factor for atherosclerosis,

acute cardiovascular mortality and elevations in circulating cholesterol and blood pressure[3]. It is likely that this phenomenon arises from excessive formation of reactive oxygen species in mitochondria due to oxidation of glucose under hyperglycemic blood conditions[4,5]. We have previously observed a 10-25-fold increase in the oxidative modification of lipoproteins in patients with T2DM, compared to healthy subjects[6]. Macrophages capturing modified low density lipoproteins (LDL) through scavenger-receptors accumulate lipids and become lipid-rich foam cells, thereby leading to atherogenesis[7].

Signs of local and systemic non-specific inflammatory processes in atherosclerosis trace to the earliest stages of vascular lesion development[8]. Increased oxidation of LDL contributes to their diffusion into the subendothelial space and activation of nuclear factor kappa-B (NF-kB) through interaction with toll-like receptors. This process leads to production of inflammatatory mediators[9]. However, activation of NF-kB also activates genetic programs necessary for resolution of inflammation[10]. Recent studies showed the relationship between the progression of atherosclerotic plaque formation and the ratio of pro-inflammatory (M1) and anti-inflammatory (M2) activated macrophages[11]. The role of polarization of macrophages in T2DM is still unclear. To address this, we studied the basal and induced secretion of the pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α), by monocytes isolated from the blood of patients with newly diagnosed T2DM, compared to those of patients with coronary heart disease.

METHODS

Primary cultures of human monocytes were obtained from peripheral blood of 28 non-treated patients with newly diagnosed diabetes 2 (11 men, 17 women) and 27 patients (20 men, 7 women) with coronary heart disease (CHD) without disturbances in carbohydrate metabolism. As a control group, 50 healthy volunteers without disturbances in carbohydrate and lipid metabolism were examined (25 men, 25 women) [Table 1].

The levels of glucose in the blood serum were determined by hexokinase (Thermo Scientific). The levels of glycated hemoglobin in red blood cells were determined by the instrument immune inhibition Bekman Coulter AU 680.

None of the patients had any clinical symptoms of systemic inflammation. The study was approved by the local ethics committee and was carried out in agreement with the Declaration of Helsinki. Written informed consent was obtained from all patients.

In order to evaluate the ability of monocytes to be activated, cells were isolated from whole blood using magnetic CD14-positive separation with MACS CD14-positive microbeads (MiltenyiBiotec) and MACS separation columns (MiltenyiBiotec). Monocytes were seeded into sterile 24-well culture plates at a density of 10^6 cells per well with X-Vivo serum-free medium (Lonza) and cultured at 37 °C in CO_2-incubator (95% air and 5% CO_2).

Once cultured, monocytes were stimulated with 100 ng/mL of interferon-γ. Secretion of TNF-α was considered a marker of the pro-inflammatory response. The concentration of TNF-α in the culture medium was determined by enzyme-linked immunosorbent assay (ELISA) on day 1 after cell isolation.

Statistical processing was performed using the SPSS package (SPSS Inc., USA). *T*-test was used to compare between groups.

RESULTS

As a model for investigation of changes in the immune system of studied patients, we used primary culture of monocytes isolated from the blood and evaluated cellular responses to pro-inflammatory stimuli. We revealed possible bias of monocyte polarization towards the M1 phenotype. This procedure was applied to non-treated T2DH patients (n = 28), CHD patients (n = 27) and healthy subjects (n = 50) (their clinical characteristics are given in Table 1). Cells were stimulated with interferon-γ (100 ng/mL). Secretion of TNF-α was measured by ELISA.

Monocytes from subjects with CHD were characterized by a low degree of basal secretion of TNF-α [Table 2]. Moreover, monocytes lacked the ability to be activated in response to both pro- and anti-inflammatory stimuli. On the contrary, monocytes from subjects with T2DM exhibited a significant 3-fold increase in the basal secretion of TNF-α as well as a 3-4-fold increase in the production of TNF-α in response to stimuli.

In this study, we attempted to explore the relationship between glycated hemoglobin (HbA1c) levels in patients with T2DM and susceptibility of their monocytes to activation. The obtained data indicated that there was an obvious trend towards a direct association between HbA1c levels and the monocyte basal secretion of TNF-α [Figure 1].

Table 1: Clinical characteristics of studied groups of patients

	Healthy subjects (*n* = 50)	T2DM patients (*n* = 28)	CHD patients (*n* = 27)
Age (years)	60 (9)	62 (12)	67 (7)
Gender (male/female)	25/25	11/17	20/7
HbA1c (%)	5.2 (0.3)	9.7 (2.4)*	5.0 (0.4)
Body mass index (kg/m^2)	27.5 (2.2)	32.1 (4.3)*	28.2 (5.01)
Cholesterol (mmol/L)	4.7 (0.5)	5.0 (1.2)	5.6 (1.0)*
Triglicerides (mmol/L)	0.94 (0.18)	1.0 (1.8)	1.6 (0.5)*

Values listed are means (standard deviation). *Significant difference from healthy subjects, $P < 0.05$. T2DM: type 2 diabetes mellitus; CHD: coronary heart disease

Table 2: Proinflammatory activation of monocytes from T2DM and CHD patients

	TNF-α (pg/mL)	
	Basal	Stimulated
Healthy subjects (*n* = 50)	270 ± 75	378 ± 92
T2DM patients (*n* = 28)	750 ± 92*	1,571 ± 111*
CHD patients (*n* = 27)	151 ± 70*,#	139 ± 51*,#

*Significant difference from healthy subjects, $P < 0.05$; #significant difference from T2DM patients, $P < 0.05$. T2DM: type 2 diabetes mellitus; CHD: coronary heart disease

DISCUSSION

Diabetes mellitus is associated with a rapid development of atherosclerosis. Previously it was demonstrated that oxidative stress under hyperglycemic conditions promotes lipid infiltration to the vascular wall caused by increased oxidation of lipoproteins[12].

Furthermore, metabolic syndrome, which encompasses T2DM, is characterized by chronic systemic inflammation. Mechanisms underlying these pathological processes remain unclear[13-15]. In response to the development of obesity, adipocytes and endothelial cells reduce their insulin sensitivity, promoting the development of T2DM and cardiovascular complications. In turn, development of hyperglycemia and hyperinsulinemia provoke oxidative stress and cause multiple inflammatory reactions[16].

Polarization of monocytes may reflect the status of the innate immune system. We studied the ability of circulating monocytes from patients with T2DM, CHD and healthy subjects to be activated into M1 and M2 phenotypes *in vitro*. Increased levels of basal and stimulated secretion of the pro-inflammatory cytokine, TNF-α, were observed in diabetic patients, compared to healthy subjects.

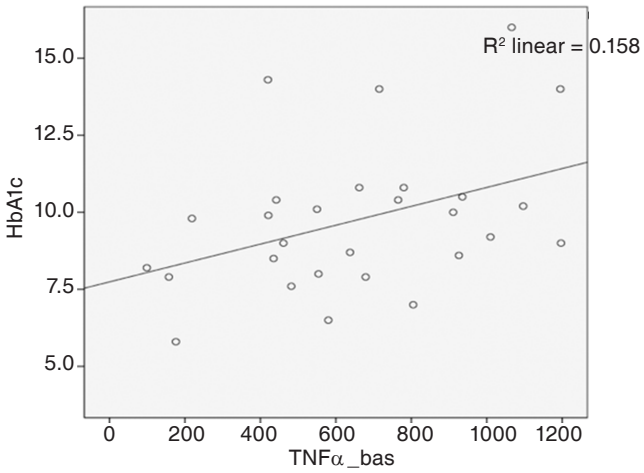

Figure 1: Correlation plot between HbA1c level and the monocyte basal secretion of TNF-α. TNF-α: tumor necrosis factor alpha

Such strong polarization of monocytes caused by pro-inflammatory stimuli may be associated with the activation of many transcription factors, particularly NF-kB. Activation of NF-kB in T2DM is associated with hyperglycaenmia-induced mitochondrial superoxide overproduction, leading to the development of oxidative stress[4,17]. Abundance of superoxide partially inhibits the glycolytic enzyme, GAPDH, thus diverting upstream metabolites from glycolysis into pathways of glucose over-utilization. This leads to increased flux of dihydroxyacetone phosphate to diacylglycerol, an activator of PKC, and of triose phosphates to methylglyoxal, the main intracellular advanced glycation end (AGE) products precursor. In its turn, protein kinase C is responsible for the activation of NF-kB[17] contributing to improvements in adhesion of monocytes to the vascular wall[17,18]. Jin *et al.*[19] found that AGEs not only predominantly induce macrophages to secrete inflammatory cytokines, but also induce M1 polarization. Moreover, AGEs activate the RAGE/NF-kB pathway, whereas the blockade of RAGE or NF-kB can attenuate macrophage activation[19,20]. We also found a direct correlation between the levels of HbA1c and TNF-α in patients with newly diagnosed diabetes [Figure 1]. This observation may be associated not only with more severe oxidative stress in conditions of hyperglycemia, but also with obesity in patients[21].

On the contrary, monocytes from CHD patients lacked the ability to be activated in response to pro-inflammatory stimuli. Recently, similar a phenomenon was found in patients with atherosclerosis[22].

In conclusion, this study aimed to establish the cause of atherosclerosis in patients with diabetes in the context

of chronic inflammation. Unexpectedly, we found diametrically different abilities of monocytes from T2DM and CHD to respond under pro-inflammatory stimulus.

DECLARATIONS

Authors' contributions

Concept and performed experimental studies, general coordination, supervision of the research projectand wrote the manuscript: N.G. Nikiforov
Literature search and patients' recruitment: L.V. Nedosugova
Clinical examination and clinical data acquisition: K.O. Galstyan
Cell culture experiments: N.V. Elizova, K.I. Kolmychkova
Manuscript editing: E.A. Ivanova

Financial support and sponsorship

This work was supported by Russian Science Foundation (Grant # 14-15-00112).

Conflicts of interest

There are no conflicts of interest.

Patient consent

All participants gave their written informed consent prior to their inclusion in the study.

Ethics approval

It was approved by the local ethics committee of the Institute for Atherosclerosis Research, Skolkovo Innovation Center, Moscow, Russia.

REFERENCES

1. IDF Diabetes Atlas 7th Edition (2015). Available from: http://www.idf.org/e-library/epidemiology-research/diabetes-atlas.html. [Last accessed on 29 Sep 2017]
2. Kanter JE, Bornfeldt KE. Inflammation and diabetes-accelerated atherosclerosis: myeloid cell mediators. *Trends Endocrinol Metab* 2013;24:137-44.
3. Coutinho M, Gerstein HC,Wang Y, Yusuf S. The relationship between glucose and incident of cardiovascular events. A meta regression analysis of published data from 20 studies of 93,7883 individuals followed for 12.5 years. *Diabetes Care* 1999;22:233-40.
4. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414:813-20.
5. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 2002;23:599-622.
6. Lankin VZ, Lisina MO, Arzamastseva NE, Konovalova GG, Nedosugova LV, Kaminnyi AI, Tikhaze AK, Ageev FT, Kukharchuk VV, Belenkov YN. Oxidative stress in atherosclerosis and diabetes. *Bull Exp Biol Med* 2005;140:41-3.
7. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, Lerman A. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation* 2007;115:2715-21.
8. Tousoulis D, Davies G, Stefanadis C, Toutouzas P, Ambrose JA. Inflammatory and thrombotic mechanisms in coronary atherosclerosis. *Heart* 2003;89:993-7.
9. Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004;25:280-8.
10. Lawrence T, Gilroy DW. Chronic inflammation: a failure of resolution? *Int J Exp Pathol* 2007;88:85-94.
11. MantovaniA,Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002;2:549-55.
12. Arzamastseva NE, Lankin VZ, Konovalova GG, Tikhaze AK, Ageev FT, Lapina YV, Narusov OY, Mareev VY, Belenkov YN. Oxidative stress in patients with chronic heart failure and type 2 diabetes mellitus. *Bull ExpBiol Med* 2007;143:207-9.
13. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005;115:1111-9.
14. Neels JG, Olefsky JM. Inflamed fat: what starts the fire? *J Clin Invest* 2006;116:33-5.
15. Sobenin IA, Galitsyna EV, Grechko AV, Orekhov AN. Small dense and desialylated low density lipoprotein in diabetic patients. *Vessel Plus* 2017;1:29-37.
16. Fernandez-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev* 2003;24:278-301.
17. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, King GL. Characterisation of the mechanism for the chronic activation of diacylglycerol-protein kinase C in diabetes and hypergalactosaemia. *Diabetes* 1994;43:1122-9.
18. Ceolotto G, Gallo A, Miola M, Sartori M, Trevisan R, Del Prato S, Semplicini A, Avogaro A. Protein kinase C activity is acutely regulated by plasma glucose concentration in human monocytes in vivo. *Diabetes* 1999;48:1316-22.
19. Jin X, Yao T, Zhou Z, Zhu J, Zhang S, Hu W, Shen C. Advanced glycation end products enhance macrophages polarization into M1 phenotype through activating RAGE/NF-kB pathway. *Bio Med Res Int* 2015;2015:732450.
20. Bouhlel MA, Derudas B, Rigamonti E, Dièvart R, Brozek J, Haulon S, Zawadzki C, Jude B, Torpier G, Marx N, Staels B, Chinetti-Gbaguidi G. PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab* 2007;6:137-43.
21. Kraakman MJ, Murphy AJ, Jandeleit-Dahm K, Kammoun HL. Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? *Front Immunol* 2014;470:1-6.
22. Ivanova EA, Orekhov AN. Monocyte activation in immunopathology: cellular test for development of diagnostics and therapy. *J Immunol Res* 2016;2016:4789279.

Bilovol *et al. Vessel Plus* 2017;1:196-201
DOI: 10.20517/2574-1209.2017.17

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Relationship between peculiarities of atrial fibrillation, body mass index and adipokines levels

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How to cite this article: Bilovol O, Shaposhnikova Y, Ilchenko I, Shalimova A. Relationship between peculiarities of atrial fibrillation, body mass index and adipokines levels. *Vessel Plus* 2017;1:196-201.

Article history:
Received: 8 Jun 2017
Accepted: 11 Sep 2017
Published: 28 Dec 2017

Key words:
Atrial fibrillation,
obesity,
metabolically healthy obesity,
metabolically unhealthy obesity,
serum leptin,
high-molecular-weight adiponectin

ABSTRACT

Aim: To assess the value of body mass index (BMI) and adipokine levels in predicting development of atrial fibrillation (AF) in the general population. **Methods:** Three hundred and ninety eight patients were examined for the presence of phenotype metabolically healthy obesity (MHO), according to the Wildman criteria; adipokine levels were assessed by enzyme immunoassay method; AF was assessed by electrocardiography (ECG) and/or by ECG diurnal monitoring. **Results:** Obesity (group 1) and overweight (group 2) was present in 23.7% and 42.0% of participants; while 21.1% were normal body weight (group 3) and 13.1% had a BMI < 19.9 kg/m² (group 4). Phenotype MHO was detected in 19.6% patients. At follow-up, 32.4% of participants developed AF. Adiponectin levels were significantly higher in MHO patients as compared to metabolically unhealthy patients with abdominal obesity (AO). High molecular weight adiponectin (HMVAN) levels were significantly decreased in patients of groups 1 and 4, as compared to groups 2 and 3. Correlation between AF and HMWAN was determined by regressive analysis in patients of 1st and 4th groups ($\beta = -0.24$, $P = 0.003$ and $\beta = -0.26$, $P = 0.002$, respectively). **Conclusion:** The probability of developing AF increases with AO and decreased BMI, which is accompanied by a change in HMVAN levels. In MHO patients, the probability of AF developing is identical with persons having normal BMI.

INTRODUCTION

The likelihood of being overweight or obese in adulthood has been increasing in epidemic proportions in the last few decades[1]. We have witnessed a parallel rise in the incidence of atrial fibrillation (AF), which is the most common sustained cardiac arrhythmia and a significant cause of cardiovascular (CV) morbidity and mortality. It can be explained by advances in the treatment of coronary heart disease (CHD) and heart failure (HF) improving life expectancy and consequently the prevalence of AF (since the incidence of atrial fibrillation increases with age)[2].

Abdominal obesity (AO) and excess body weight are associated with the development of cardiovascular

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disease (CVD), including an increased risk of incidence of sudden cardiac death, atrium fibrillation and other form of arrhythmias[1].

In contrast, a large review from Flegal *et al.*[3] analyzed 97 studies of 2.9 million individuals, including > 270,000 deaths, and demonstrated that optimal survival occurred in "overweight" patients [body mass index (BMI): 25 to 30 kg/m^2], who had a significant 6% lower mortality than the "normal-weight" BMI cohort (BMI: 18.5 to 25 kg/m^2)[3].

Patients with elevated BMI, but normal insulin sensitivity, lipid profiles and blood pressure (BP), are considered to be metabolically healthy obesity (MHO)[4]. Some epidemiological data suggest that MHO carries a lower risk of CVD, lower risk of AF development and less all-cause mortality than being normal weight yet metabolically unhealthy[5]. The precise mechanism that induces AF in obesity is still unknown. Metabolic comorbidities are common in obese people and could be the main reason for increased AF risk and total cardiovascular risk, apart from obesity itself. There are several proposed mechanisms that connect obesity and AF[6-10].

Enhanced neurohormonal activation, impaired insulin tolerance, dyslipoproteinemia, hypertension and pathological changes in circulating renin-angiotensin-aldosterone system accompanies obesity and may contribute to left atrial enlargement and electrical instability, which may result in AF development[11].

Studies on the mechanisms by which obesity induces AF show that obesity causes atrial arrhythmogenic remodeling. Progression of atrial fibrosis is a key event in the pathogenesis of AF[12,13], and can be caused by aging, underlying cardiac diseases, systemic diseases or inflammatory processes, or AF itself. Obese individuals, even without cardiovascular disease, have left atrium (LA) enlargement[14] and different electrical properties, such as slower conduction from the LA entering the pulmonary vein (PV) and a significantly shorter effective refractory period in the LA and PV[15].

Adipokines are bioactive proteins produced by the adipose compartment that have wide-ranging effects across organs and tissues. Leptin and adiponectin are both closely related with obesity and have recently been implicated with AF. These hormones are secreted primarily by adipocytes, while the stromovascular tissue surrounding fat secretes tumor necrosis factor-alpha (TNF-α) and macrophages secrete resistin[16,17]. These adipokines have been proposed as links between adiposity and insulin resistance, glucose deregulation, and CVD. Adiponectin is reduced in obesity, CHD, HF, AF and T2DM, and increased following weight loss. Adiponectin levels were independently associated with the incident of AF[16,17]. However, it remains unclear whether these are markers of failed regulatory pathways that lead to AF or are directly involved in AF pathogenesis.

Our study aimed to elucidate the causal relationship between AF and BMI or adipokine levels, specifically serum leptin and high-molecular-weight adiponectin (HMWAN), among the general population.

METHODS

This study comprised 398 patients randomly selected while visiting the out-patient unit due to any reason, except acute heart pathology, provided they had not had heart rate disorders in their past medical history.

Among allotted patients there were 142 with arterial hypertension (AH) (35.7%), 118 suffering from ischemic heart disease (29.6%), 73 with diabetes mellitus II type (DM-2) (18.3%), provided DM-2 was compensated.

Also excluded were those patients who had developed kidney, liver diseases, heart diseases, heart failure (more than 2 functional class according to New York Heart Association), chronic obstructive lung pathology, malignant neoplasms or alcoholism.

Patients' age ranged from 37 to 56 years old (mean age: 41.4 ± 2.3 year). All patients involved in this study were examined according to the same protocol. Anthropometry was assessed with measurements of waist circumference and BMI calculated by Kettle formula. Additionally, BP was measured along with a panel of laboratory tests: fasting serum glucose (glucosoxidative approach); serum lipoproteins [serum cholesterol, triglycerides (TG), cholesterol of high-density lipoprotein (HDL) by enzyme colorimetric approach with kit of "Human" (Germany)]; C-reactive protein (CP) by solid phase enzyme immunoassay (EIA) (NycoCard CRP, Axis-Shield); serum Leptin by EIA kit Leptin ELISA [Diaghostics Biochem Canada Inc (DBC Inc) cat № ABIN362629 CAN-L-4260]; and serum HMWAN by EIA with use of kit DRG (USA). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated: HOMA-IR = insulin, mcU/mL × glucose, mmol/L ÷ 22.5).

Overweight patients (BWI: 25-29.9 kg/m^2) and those with AO and BMI ≥ 30 kg/m^2 were classified according to Wildman criteria (2008) as "MHO" and "metabolically

unhealthy obesity (MUO)."

At the screening stage, echocardiography was performed on the apparatus Toshiba-SSH-60 A (Japan) using standard methods (in M and B regimens) by the recommendations of the American Echocardiogram Society.

The study included patients who initially had minimal differences in echocardiography indicators as compared to the control group for minimization of the impact of other risk factors at the onset of atrial fibrillation. Careful selection of patients made it possible to reliably estimate the effect of the selected parameters - BMI, leptin and high molecular weight adiponectin on the probability of atrial fibrillation, according to the goal of the study.

The phenotype MHO was assessed according to Wildman criteria: the presence of 0-1 factors indicating metabolic health[18]. Patients with AO were considered as MHO who had met the following criteria: systolic BP and diastolic BP below 130 mmHg and 85 mmHg respectively without antihypertensive therapy; serum TG ≤ 1.70 mmol/L; serum HDL cholesterol ≥ 1.04 mmol/L for males and ≥ 1.30 for females without lipid-reducing treatment; fasting serum glucose ≤ 5.55 mmol/L without hypoglycaemic therapy; serum CP ≤ 4.72 mg/L; HOMA-IR ≤ 4.81.

Every patient was checked by electrocardiography (ECG) and/or by ECG diurnal monitoring (DM-ECG). Time of surveillance varied from 3.4 to 6.2 years (mean span 3.8 ± 1.2 years).

In order to evaluate obtained results they were compared with values of 20 people regarded as healthy (nothing abnormal was revealed) matching by age and gender. All statistical analyses were performed using the program (STATISTICA® for Windows 10.0) and *t*-criterion of Student ($P < 0.05$); the minimum level of statistical significance was assumed at $P < 0.05$. Continuous variables were presented as means ± standard deviation. To determine the differences among the groups, Student's *t*-test was used for continuous variables, the correlation analysis was used to calculate the Pearson correlation coefficient and the Spearman rank correlation coefficient.

RESULTS

According to the goal of the study, all patients were allotted to 4 groups considering BMI [Table 1]. Echocardiographic characteristic of patients were given in Table 2. Characteristics of patients of different groups included in the study, according to the main physical and laboratory parameters are presented in Table 3. Data for phenotype MHO assessment were accessible in 97 participants. Phenotype MHO (Wildman) was recognized in 19 people (19.6%) that corresponded to the global estimates. Characteristics of MHO-type patients were given in Table 4. Assessment of serum adipokines revealed some peculiarities of leptin and HMWAN rate with regard to different BMI. HMWAN values were significantly raised in MHO-type patients comparatively to MUO type ones with AO: respectively 11.32 mcg/mL and 7.87 mcg/mL ($P < 0.01$). Leptin was in the same range in patients of all subsets. HMWAN was significantly lower in patients of 1st and 4th groups comparatively to 2nd and 3rd groups [Table 5]. AF was identified in 129 patients (32.4%) throughout the span of surveillance. Patients with obesity and body weight deficiency (1st and 4th groups) developed AF more frequently compared to other groups. There were different types of AF: AF paroxysms spontaneously resolved into sinus rhythm or restored to sinus rhythm by medicated cardioversion, long-lasting AF of persistent or permanent type. Analysis of AF incidence among patients involved in the study was shown in Table 6.

Table 1: Subsets of patients considering BMI ($n = 398$)

Groups of the patients (%)	BMI (kg/m²)
Group 1 ($n = 95$) (23.9)	≥ 30
Group 2 ($n = 167$) (42.0)	25-29.9
Group 3 ($n = 84$) (21.1)	20-24.9
Group 4 ($n = 52$) (13.0)	< 19.9

BMI: body mass index

Correlation between AF and HMWAN was determined by regressive analysis in patients of 1st and 4th groups ($\beta = -0.24$, $P = 0.003$ and $\beta = -0.26$, $P = 0.002$ respectively).

The probability of developing AF increases with AO and decreased BMI, which is accompanied by a change in HMVAN levels. In MHO patients probability of AF developing is identical with normal BMI individuals.

DISCUSSION

The obtained results revealed some peculiarities of leptin and HMWAN levels in relation to various BMI. It was established that the level of leptin did not differ significantly in patients with normal body weight or with an increase or decrease BMI. At the same time, HMWAN value changes were more significant and had specific features in individuals with different body weight. We found a reduction of HMWAN with

Table 2: Echocardiographic characteristic of patients

Indices	Control (*n* = 20)	Group 1 (*n* = 95)	Group 2 (*n* = 167)	Group 3 (*n* = 84)	Group 4 (*n* = 52)
LA (sm)	2.9 ± 0.2	3.1 ± 0.2	3.3 ± 0.4	3.0 ± 0.2	2.6 ± 0.3
EDV LV (mL)	98.5 ± 4.3	108.6 ± 3.8	110.8 ± 4.2	84.5 ± 4.9	96.6 ± 3.8
ESV LV (mL)	38.4 ± 2.2	41.7 ± 2.4	42.8 ± 3.6	37.0 ± 3.1	38.6 ± 3.8
EF (%)	64.4 ± 4.2	62.8 ± 3.8	57.2 ± 4.2	59.6 ± 5.3	61.6 ± 3.8
TPWd (mm)	8.4 ± 0.4	9.2 ± 1.2	9.0 ± 1.4	8.6 ± 1.1	8.2 ± 0.8
TIVSd (mm)	8.2 ± 0.6	9.2 ± 1.8	9.1 ± 1.2	8.5 ± 1.2	8.2 ± 0.8

LA: left atrium; EDV LV: end-diastolic volume of the left ventricle; ESV LV: end-systolic volume of the left ventricle; EF: ejection fraction; TPWd: thickness of the posterior wall of the left ventricle in diastole; TIVSd: the thickness of the interventricular septum in diastole

Table 3: Baseline characteristics of the study population with different BMI

Index	Control group (*n* = 20)	Examined patients (*n* = 398)			
		Group 1 (*n* = 95)	Group 2 (*n* = 167)	Group 3 (*n* = 84)	Group 4 (*n* = 52)
WC (sm)	78.2 ± 5.4	101.4 ± 6.2*,**	96.8 ± 4.4*,**	82.6 ± 6.4$^{\wedge,\#,**}$	64.8 ± 2.6$^{\wedge,\#}$
HC (sm)	92.4 ± 6.3	142.8 ± 8.4$^{*,\&,**}$	124.6 ± 7.4*,**	96.5 ± 6.51$^{\wedge,\#,**}$	80.2 ± 3.4$^{\wedge,\#}$
WC/HC	0.76 ± 0.04	0.82 ± 0.08	0.80 ± 0.07	0.80 ± 0.06	0.78 ± 0.06
SAD (mmHg)	120.4 ± 2.6	142.4 ± 6.8*,**	138.8 ± 5.2*,**	132.5 ± 3.5$^{\wedge,**}$	118.8 ± 4.6
DAD (mmHg)	78.4 ± 2.2	88.4 ± 4.5*,**	86,2 ± 3.4*,**	82.4 ± 6.2$^{\wedge,**}$	74.6 ± 3.8$^{\wedge,\#}$
Total cholesterol (mmol/L)	4.42 ± 0.08	6.68 ± 1.07*,**	6.24 ± 1.02*,**	5.16 ± 1.29	4.14 ± 0.08$^{\wedge,\#}$
Triglycerides (mmol/L)	1.34 ± 0.06	3.22 ± 0.08*,**	3.12 ± 0.06*,**	2.16 ± 0.07$^{\wedge,\#,**}$	1.04 ± 0.03$^{\wedge,\#}$
HDL cholesterol (mmol/L)	1.40 ± 0.04	0.72 ± 0.06*,**	0.84 ± 0.04*,**	1.05 ± 0.02$^{\wedge,\#}$	1.02 ± 0.02$^{\wedge,\#}$
Fasting glucose (mmol/L)	4.60 ± 0.22	6.28 ± 0.36*,**	6.02 ± 0.16*,**	5.12 ± 0.08$^{\wedge,\#,**}$	3.72 ± 0.18$^{\wedge,\#}$
HOMA-IR	1.88 ± 0.22	5.12±0.32$^{*,\&,**}$	4.98 ± 0.28$^{*,\&,**}$	2.32 ± 0.24$^{\wedge,\#}$	1.14 ± 0.06$^{\wedge,\#}$
C-reactive protein (mg/L)	2.24 ± 0.12	5.38 ± 1.24*,**	4.42 ± 1.12*,**	3.02 ± 0.79$^{\wedge}$	2.68 ± 0.32$^{\wedge,\#}$
Serum leptin (mcg/mL)	21.6 ± 1.2	22.5 ± 1.6	23.5 ± 1.8	22.4 ± 1.4	21.9 ± 1.2
HMWAN (mcg/mL)	14.74 ± 1.22	4.42 ± 1.06$^{*,\#,\&}$	13.82 ± 1.12$^{\wedge,**}$	14.23 ± 1.07$^{\wedge,**}$	3.75 ± 0.18$^{*,\#,\&}$

*: reliability of differences ($P \leq 0.05$) compared with control group; ^: reliability of differences ($P \leq 0.05$) compared with group 1; #: reliability of differences ($P < 0.05$) compared with group 2; &: reliability of differences ($P < 0.05$) compared with group 3; **: reliability of differences ($P < 0.05$) compared with group 4; WC: waist circumference; HC: hip circumference; SAD: systolic blood pressure; DAD: diastolic blood pressure; HDL: high-density lipoprotein; HOMA-IR: homeostasis model of assessment insulin resistance; BMI: body mass index

BMI deviations greater and smaller from normal values. Thus, there was significant decrease of serum HMWAN in patients with obesity of group 1 with a BMI of more than 30 kg/m^2, and with a body mass deficit in patients of group 4 with BMI less than 19.9 kg/m^2. It is also important to note that the difference in the HMWAN value reduction in patients with obesity and body weight deficit was insignificant, while significantly different from those with normal and increased body weight with a BMI within 25-29.9 kg/m^2.

During the observation period, AF most often developed in patients who had the most significant initial HMWAN reduction - in patients with more prominent obesity and in patients with body mass deficiency in groups 1 and 4. Less often, AF developed in individuals with increased body weight, even without obesity and only in isolated cases in patients with normal body weight. The most frequent variant of AF was paroxysmal AF, while the other variants - persistent or permanent type, developed much less frequently.

These changes have been confirmed by established correlation dependence of the development of AF and HMWAN reduction in groups 1 and 4 of the patients. It was found that significant BMI deviation in both directions is a prognostically unfavorable factor for the development of AF in patients, even without any other manifestations of CV disease or metabolic abnormality. It was found that the deviation of BMI

Table 4: Characteristics of MHO-type patients by Wildman criteria

Index	Wildman criteria	MHO-type patients	*P*
SAD (mmHg)	≤ 130	123.4 ± 2.4	0.05
DAD (mmHg)	≤ 85	78.8 ± 2.2	0.05
Triglycerides (mmol/L)	≤ 1.70	1.46 ± 0.18	0.05
HDL cholesterol: men, women (mmol/L)	≥ 1.04 ≥ 1.30	1.15 ± 0.08 1.42 ± 0.11	0.05 0.05
Fasting blood glucose (mmol/L)	≤ 5.55	4.08 ± 0.06	0.05
C-reactive protein (mg/L)	< 4.72	2.18 ± 0.06	0.05
HOMA-IR	< 4.81	2.62 ± 0.12	0.05

MHO: metabolically healthy obesity; SAD: systolic blood pressure; DAD: diastolic blood pressure; HDL: high-density lipoprotein; HOMA-IR: homeostasis model of assessment insulin resistance

Table 5: Serum leptin and high-molecular-weight adiponectin values in patients with different body weight

Index	Control group	Examined patients (*n* = 398)			
	(*n* = 20)	Group 1 (*n* = 95)	Group 2 (*n* = 167)	Group 3 (*n* = 84)	Group 4 (*n* = 52)
Serum leptin (mcg/mL)	21.6 ± 1.2	22.5 ± 1.6	23.5 ± 1.8	22.4 ± 1.4	21.9 ± 1.2
HMWAN (mcg/mL)	14.74 ± 1.22	4.42 ± 1.06$^{*,\#,\&}$	13.82 ± 1.12$^{\&,**}$	14.23 ± 1.07$^{\&,**}$	3.75 ± 0.18$^{*,\#,\&}$

*: reliability of differences ($P \leq 0.05$) compared with control group; ^: reliability of differences ($P \leq 0.05$) compared with group 1; #: reliability of differences ($P < 0.05$) compared with group 2; &: reliability of differences ($P < 0.05$) compared with group 3; **: reliability of differences ($P < 0.05$) compared with group 4

Table 6: The incidence of various forms of atrial fibrillation in patients examined during the observation period (3.8 ± 1.2 years)

Index	Examined patients (*n* = 398) (%)			
	Group 1 (*n* = 95)	Group 2 (*n* = 167)	Group 3 (*n* = 84)	Group 4 (*n* = 52)
Paroxysmal atrial fibrillation (AFpx)	24 (25.3)	26 (15.6)	7 (8.3)	13 (25.0)
Persistent atrial fibrillation (AFps)	17 (17.9)	24 (14.4)	4 (4.8)	9 (17.3)
Permanent atrial fibrillation (AFpm)	11 (11.6)	19 (11.4)	2 (2.4)	7 (13.5)

both in the direction of increase and decrease is a prognostically unfavorable factor for the development of AF in patients even without any other manifestations of CV disease.

These results indicate that obesity not only leads to different metabolic, hormonal and hemodynamic changes in the body that affect the heart muscle, causing its structural and functional changes, but the same changes are taking place in the patient with a body weight deficit, causing similar pathogenesis, particularly AF development.

Adiponectin plays an important role in many metabolic processes; it has a protective effect, especially for development of endothelial dysfunction, atherosclerosis and other vascular diseases, the progression of which is reflected in heart rhythm disturbances, in particular, the onset of AF[19]. Adiponectin is under the influence and itself influences the action of many pathophysiological mechanisms, including pathological TNF-α, interleukin-6, C-reactive protein, insulin, body weight, blood pressure deviation, and progression of many chronic diseases[20].

Adiponectin is involved in cardiac remodeling, not only through direct action on the cardiac muscle, but indirectly through the effect on endothelial function, atherogenesis, and vascular inflammation. In addition, adiponectin penetrates the blood-brain barrier and affects the function of the heart through the central nervous system[21].

Adipose tissue can be related to the lipotoxicity of cardiomyocytes, which is carried out through unknown factors with the participation of FATP4 and CD36 transporters, and cardiomyocytes can maintain the stability of adipose tissue, affecting the secretion of adipokines[22].

Insulin resistance - this is the main pathological mechanism that binds metabolic, anthropometric and clinical indicators with the increasing risk of CVD and DM2. Adipocytokine imbalance, with a low level of adiponectin, can act as a triggering mechanism for the development of hyperinsulinemia, impaired glucose tolerance, dyslipidemia, endothelial dysfunction, arterial hypertension, abdominal obesity, fibrosis and atrial fibrillation[23].

DECLARATIONS

Authors' contributions

Study design: O. Bilovol, I. Ilchenko
Development of methodology: O. Bilovol
Collection of data: O. Bilovol, Y. Shaposhnikova, I. Ilchenko, A. Shalimova
Analysis and/or interpretation of data: O. Bilovol, Y. Shaposhnikova, I. Ilchenko, A. Shalimova
Writing (not revising) all or sections of the manuscript: O. Bilovol, Y. Shaposhnikova, I. Ilchenko, A. Shalimova
Manuscript review: O. Bilovol, I. Ilchenko
Supervision: O. Bilovol

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Each patient was informed the study and gave their consent.

Ethics approval

The study protocol was supported by the Ethics Committee of the Kharkiv National Medical University.

REFERENCES

1. Ortega FB, Lavie CJ, Blair SN. Obesity and cardiovascular disease. *Circ Res* 2016;118:1752-70.
2. Asghar O, Alam U, Hayat SA, Aghamohammadzadeh R, Heagerty AM, Malik RA. Obesity, diabetes and atrial fibrillation; epidemiology, mechanisms and interventions. *Curr Cardiol Rev* 2012;8:253-64.
3. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA* 2013;309:71-82.
4. Lee H, Choi EK, Lee SH, Han KD, Rhee TM, Park CS, Lee SR, Choe WS, Lim WH, Kang SH, Cha MJ, Oh S. Atrial fibrillation risk in metabolically healthy obesity: a nationwide population-based study. *Int J Cardiol* 2017;240:221-7.
5. Payvar S, Kim S, Rao SV, Krone R, Neely M, Paladugu N, Daggubati R. In-hospital outcomes of percutaneous coronary interventions in extremely obese and normal-weight patients: findings from the NCDR (National Cardiovascular Data Registry). *J Am Coll Cardiol* 2013;62:692-6.
6. Wanahita N, Messerli FH, Bangalore S, Gami AS, Somers VK, Steinberg JS. Atrial fibrillation and obesity--results of a meta-analysis. *Am Heart J* 2008;155:310-5.
7. Wang TJ, Parise H, Levy D, D'Agostino RB Sr, Wolf PA, Vasan RS, Benjamin EJ. Obesity and the risk of new-onset atrial fibrillation. *JAMA* 2004;292:2471-7.
8. Guglin M, Maradia K, Chen R, Curtis AB. Relation of obesity to recurrence rate and burden of atrial fibrillation. *Am J Cardiol* 2011;107:579-82.
9. Frost L, Hune LJ, Vestergaard P. Overweight and obesity as risk factors for atrial fibrillation or flutter: the Danish diet, cancer, and health study. *Am J Med* 2005;118:489-95.
10. Murphy NF, MacIntyre K, Stewart S, Hart CL, Hole D, McMurray JJ. Long-term cardiovascular consequences of obesity: 20-year follow-up of more than 15 000 middle-aged men and women (the Renfrew-Paisley study). *Eur Heart J* 2006;27:96-106.
11. Goette A, Staack T, Röcken C, Arndt M, Geller JC, Huth C, Ansorge S, Klein HU, Lendeckel U. Increased expression of extracellular signal-regulated kinase and angiotensin-converting enzyme in human atria during atrial fibrillation. *J Am Coll Cardiol* 2000;35:1669-77.
12. Dzeshka MS, Lip GY, Snezhitskiy V, Shantsila E. Cardiac fibrosis in patients with atrial fibrillation: mechanisms and clinical implications. *J Am Coll Cardiol* 2015;66:943-59.
13. Nattel S, Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. *J Am Coll Cardiol* 2014;63:2335-45.
14. Ito K, Date T, Kawai M, Nojiri A, Narui R, Hioki M, Tanigawa S, Yamashita S, Tokuda M, Inada K, Matsuo S, Yamane T, Yoshimura M. Morphological change of left atrium in obese individuals. *Int J Cardiol* 2011;152:117-9.
15. Munger TM, Dong YX, Masaki M, Oh JK, Mankad SV, Borlaug BA, Asirvatham SJ, Shen WK, Lee HC, Bielinski SJ, Hodge DO, Herges RM, Buescher TL, Wu JH, Ma C, Zhang Y, Chen PS, Packer DL, Cha YM. Electrophysiological and hemodynamic characteristics associated with obesity in patients with atrial fibrillation. *J Am Coll Cardiol* 2012;60:851-60.
16. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011;11:85-97.
17. Van de Voorde J, Pauwels B, Boydens C, Decaluwé K. Adipocytokines in relation to cardiovascular disease. *Metabolism* 2013;62:1513-21.
18. Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, Sowers MR. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). *Arch Intern Med* 2008;168:1617-24.
19. Macheret F, Bartz TM, Djousse L, Ix JH, Mukamal KJ, Zieman SJ, Siscovick DS, Tracy RP, Heckbert SR, Psaty BM, Kizer JR. Higher circulating adiponectin levels are associated with increased risk of atrial fibrillation in older adults. *Heart* 2015;101:1368-74.
20. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci* 2014;15:6184-223.
21. Kizer JR, Benkeser D, Arnold AM, Djousse L, Zieman SJ, Mukamal KJ, Tracy RP, Mantzoros CS, Siscovick DS, Gottdiener JS, Ix JH. Total and high-molecular-weight adiponectin and risk of coronary heart disease and ischemic stroke in older adults. *J Clin Endocrinol Metab* 2013;98:255-63.
22. Anan M, Uchihashi K, Aoki S, Matsunobu A, Ootani A, Node K, Toda S. A promising culture model for analyzing the interaction between adipose tissue and cardiomyocytes. *Endocrinology* 2011;152:1599-605.
23. Colwell JA. Type 2 diabetes, pre-diabetes, and the metabolic syndrome. *JAMA* 2011;306:215.

Wang *et al. Vessel Plus* 2017;1:202-12
DOI: 10.20517/2574-1209.2017.29

Vessel Plus
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Review **Open Access**

Adipose tissue-derived cytokines, CTRPs as biomarkers and therapeutic targets in metabolism and the cardiovascular system

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How to cite this article: Wang YJ, Zhao JL, Lau WB, Liu J, Guo R, Ma XL. Adipose tissue-derived cytokines, CTRPs as biomarkers and therapeutic targets in metabolism and the cardiovascular system. *Vessel Plus* 2017;1:202-12.

Article history:
Received: 4 Aug 2017
First Decision: 12 Sep 2017
Revised: 1 Oct 2017
Accepted: 21 Oct 2017
Published: 28 Dec 2017

Key words:
Adipose tissue-derived cytokines, adipokines, C1q/tumor necrosis factor-related proteins, metabolism, cardiovascular system, biomarker, therapeutic target

ABSTRACT

Increasing evidence indicates that adipose tissue-originated cytokines mediate communication between obesity-related exogenous molecules and the molecular events that initiate the metabolic syndrome and the inflammatory responses in the cardiovascular system (CVS). Adipose tissue-derived cytokines including the C1q/tumor necrosis factor-related proteins (CTRPs), a 15-member family of novel adipokines, has attracted much interest due to its metabolic regulatory and anti-inflammatory effect and appear to play a pathophysiological role in metabolism and immunity. To date, 15 members of CTRP family have been identified and they also play a role in the CVS, especially as potent biomarkers or therapeutic targets for modulating metabolic or inflammatory functions. Therefore, this review will focus on the characteristics of CTRPs that influence cardiovascular function and on the potential of CTRPs as biomarkers and therapeutic targets.

INTRODUCTION

During the last decade, a mounting number of adipocyte-originated hormones (adipokines) have been recognized and documented to be discriminately regulated during the onset of metabolic syndrome, obesity, and the inflammatory processes. By acting systemically as circulating hormones or locally on diverse cell types, adipokines are involved in the regulation of many physiological functions, including energy storage, metabolism, and the development of obesity-associated disorders (type 2 diabetes mellitus,

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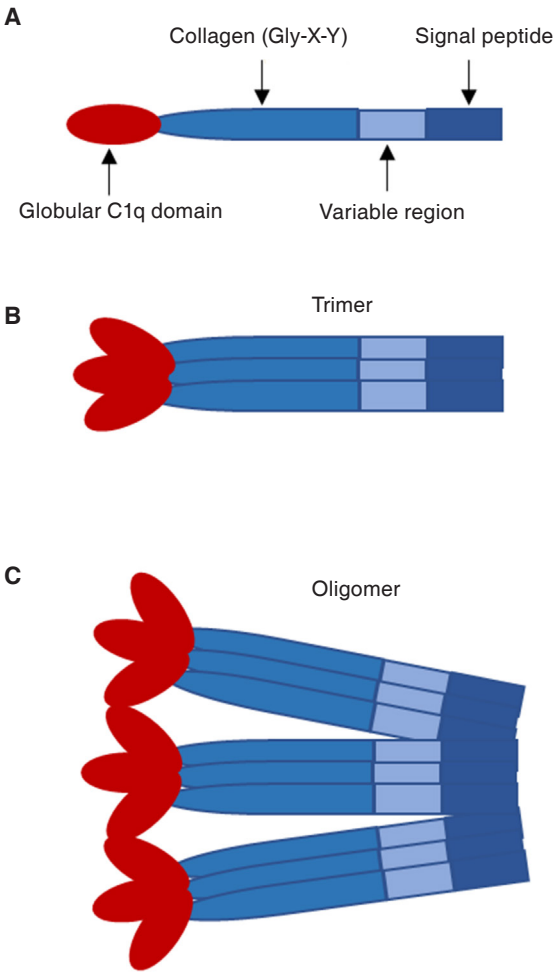

Figure 1: Structural organization of the CTRPs. A: domain structure of CTRP monomeric protein; B: homotrimeric CTRP protein structure. C: CTRP trimeric proteins form higher-order three-dimensional structures. CTRPs: C1q/tumor necrosis factor-related proteins

cardiovascular disease, the inflammatory process).

Adiponectin, discovered as the first member of the C1q/tumor necrosis factor-related proteins (CTRPs) family, has been broadly investigated because of its metabolic regulatory and cardiovascular protective effects. To date, additional CTRP family members, a newly discovered novel family of adipokines, have been identified that may play a role in metabolism and cardiovascular system path-physiological regulation. Hence, in future, the studies on these novel adipokines will provide new understandings into the physiological/pathological mechanisms including the communication of different organs based on energy homeostasis, homeostasis of the internal environment, and prevention of inflammation.

The current review focuses on the main roles of CTRPs in the context of pathophysiology with particular attention to their potential biomarker features and therapeutic roles in the metabolic energy balance regulation, inflammatory responses and in obesity-associated disorders.

WHAT ARE CTRPS?

CTRPs, first identified by the Harvey Lodish laboratory, are considered a new family of secreted proteins from various organs. These 15 secreted proteins (CTRP1 to CTRP15) share common structural and functional characteristics with adiponectin: a N-terminal signal peptide, a short variable region, a collagen domain containing collagen triples (Gly-X-Y), and a C-terminal globular complement factor C1q domain [Figure 1][1,2]. CTRPs display distinct biological and signaling properties by occurring in the circulation as trimers and assembling with their basic structural unit into hexameric and high molecular weight oligomeric complexes [Figure 1]. Most CTRPs are expressed by adipose tissue and circulate in plasma. Their plasma levels vary with the genetic background, age, gender, and metabolic states. In terms of CTRPs sexually dimorphic patterns, female mice express higher levels of CTRP13, CTRP11, CTRP9, CTRP5, and CTRP3 compared to male animals/humans[1-5]. The exact mechanism for these differences is still unclear.

CTRPs are expressed and distributed in a variety of ways that may determine their diverse biological functions [Table 1]. To date, metabolic functions have been established for CTRP1, CTRP2, CTRP3, CTRP5, and CTRP9[2]. Although the functional receptors for CTRPs have not yet been recognized, vascular, liver, muscle, heart and adipose tissue are the likely targets of CTRPs. They share similar structure and function with adiponectin[6], including the regulation of balance of body energy metabolism through enhancing insulin sensitivity in the liver and muscle, exhibition of anti-inflammatory responses, and protection of the cardiovascular system (CVS). Thus, the CTRP family may have parallel implications for energy homeostasis and provides new pharmacological targets in the obesity-related diseases and type 2 diabetes. Meanwhile, they consist of many family members distributed in various organs, hence they have the potential to serve as biomarkers in the obesity-related diseases and may serve as detectable parameters for early prognosis and diagnosis.

CTRPS IN CIRCULATION

Endogenous CTRPs can be detected in the blood by enzyme-linked immunosorbent assay (ELISA), even although they circulate at 1-2 orders of magnitude less than adiponectin. The distribution of CTRPs levels

Table 1: Overview of the function of CTRP family

CTRP	Expression	Function
1	Heart, liver, muscle, kidney, sexual gland	Metabolic regulation: glucose and lipid hemostasis; antithrombotic effects; attenuate plaque formation; increases aldosterone production[18,54,55]
2	Adipose tissue	Metabolic regulation: improve insulin and lipid tolerance[18]
3 (3A,3B)	Kidney, brain, small intestine, adipose tissue	Regulates glucose and lipid metabolism regulates systemic inflammation in obesity and insulin resistance; modulates mitochondrial biogenesis; attenuate liver fibrosis; biomarker for diabetic retinopathy; negative regulator of osteoclastogenesis; independent predictor for atherosclerosis[47,56-60]
4	Hepatic cells	Modulates energy metabolism; novel nutrient-responsive central regulator of food intake and energy balance; reduces colitis; suppresses the pyroptosis of trophoblasts derived from rats with preeclampsia[61-63]
5	Adipose tissue, ocular tissue	Inhibits pro-metabolic insulin signaling; related to the degree of obesity and is associated with obesity-related at alterations; associated with ISR after PCI with DES implantation; associated with obesity and diabetes; Alleviates insulin resistance; circulating markers of metabolic disease, diabetes, nonalcoholic fatty liver disease and age-related macular degeneration[64-67]
6	Adipose tissue, human synoviocytes	Novel metabolic/immune regulator; modulating both inflammation and insulin sensitivity; regulates fat development; alleviates AngII-induced hypertension and vascular endothelial dysfunction; potential therapeutic target for the prevention of skin fibrosis; endogenous complement regulator[5,29,68,69]
7	Adipose tissue, lung	Improves insulin sensitivity; beneficial metabolic outcomes in the setting of obesity and diabetes[9,54]
8 (8B)	Lung, testis, absent in mice	Blocks glioblastoma dissemination within the brain[14,70]
9 (9A,9B)	Cardiac tissue, adipose tissue	Important in the development of type 2 diabetes; novel metabolic regulator and a new component of the metabolic network that links adipose tissue to lipid metabolism in skeletal muscle and liver; prevents vascular restenosis after angioplasty, hepatic steatosis and hypertension; stabilizes plaque, improves endothelial cell survival and function[12,20,48,71-73]
10	Eye, adipose tissue	Regulates metabolism, adipose tissue homeostasis[3,23]
11	Adipose tissue	New regulator of adipogenesis; maintains adipose tissue homeostasis[3,74]
12	Adipose tissue	Novel biomarkers for the prediction and early diagnosis of T2DM; regulates glucose and lipid metabolism and whole-body glucose homeostasis[24,50,51,75,76]
13	Adipose tissue, brain	Associated with increased risk of T2DM and coronary artery disease and non-alcoholic fatty liver disease; negative association with metabolism; modulates whole-body energy balance[2,24,27,77,78]
14	Brain, adipose tissue	Promotes tissue regeneration, and recovery of ischemic heart disease; maintains adipose tissue homeostasis by generating complexes with CTRP11[74]
15	Skeletal muscle	Modulates energy homeostasis and metabolic circuit; modulates inter-tissue crosstalk[21,79]

ISR: in-stent restenosis; PCI: percutaneous coronary intervention; DES: drug-eluting-stent; CTRP: C1q/tumor necrosis factor-related protein; T2DM: type 2 diabetes mellitus

Table 2: C1q/TNF-related proteins traits

Characteristic	Description
Metabolism	Regulation of insulin sensitivity, glucose and lipid metabolism; expression of C1q/TNF-related proteins is various in conditions of obesity and diabetes; promotion of insulin sensitivity, promoting insulin resistance; action is local, action is systemic, exertion of effects via central neuron mechanisms or action on peripheral tissues
Muscle and liver	Regulation of AMPK signaling pathway Inflammation; inhibition of monocyte chemoattractant releasing; inhibition of LPS-induced basic and common proinflammatory pathways
Obesity and type 2 diabetes	Potent inhibition of LPS-induced systemic inflammation; regulation of TLR9 signaling pathway in chronic inflammation[80], biomarker in reflecting the degree of inflammatory response

LPS: lipopolysaccharide; TLR: toll-like-receptor; TNF: tumor necrosis factor; AMPK: AMP-activated protein kinase

varies and is influenced by metabolic hormones, significant signaling and inflammatory states, meanwhile, they can exist in autocrine, paracrine, and endocrine manners that influence the individual susceptibility to the disease and therefore, it provides the possibility that they timely reflect the processing of insulin resistance, obesity, and type 2 diabetes. Accumulated studies reveal that circulating levels of CTRP3, CTRP6, CTRP7, CTRP9, CTRP12, and CTRP15 are reduced in diet-induced diabetes or obese mice or humans[4,5,7-9]. CTRP1 and CTRP5 concentrations are higher in obese and diabetic rodents[10]. The characteristics of CTRPs in various situations are listed [Table 2]. While different CTRP oligomers have distinct distribution properties, the functional significance of CTRPs still remains largely undefined.

CTRPS PERTURBATIONS AND METABOLISM

Inter-organ communication in the organism is necessary to maintain the integrated control of

metabolism and this crosstalk can be realized by the well-orchestrated secreted hormones. The newly discovered CTRPs family has profound significance aiding better understanding of hormonal control of the energy homeostasis based upon the reports from the research group lead by Wong and other researchers in the last decade[11]. They discovered the contributions of various CTRPs hormones to whole-body glucose and lipid metabolic regulation.

CTRPs can dynamically modulate the response to the alterations in short-term nutritional states or the changes in long-term metabolic status. However, the signaling pathways modulated by CTRPs are frequently disrupted by the metabolic perturbations of excess caloric uptake in obesity- and inflammation-induced disorders. Consequently, the hormone dysregulation leads to broad metabolic disorders such as insulin resistance, diabetes, and obesity. Those disruptions include the alterations of structural organization, and post-translational modifications of CTRPs.

For CTRPs structural organization, it has been reported that all CTRPs form trimers, but accumulating reports reveal that CTRP3, CTRP5, CTRP9, CTRP6, CTRP8, CTRP10, CTRP11, CTRP12, CTRP13 and CTRP15 can further assemble into multimeric complexes mediated by the N-terminal Cysteine residues or with the aid of oxidoreductase. Adiponectin, as an insulin sensitizer, assembles with CTRP9 to form heterotrimers[12], by which CTRP9 and adiponectin share the same receptor to exert their cardiovascular protective function[13]. In addition to forming the homo-oligomers, CTRP6/CTRP1, CTRP7/CTRP2, and CTRP2/adiponectin form heterotrimers generating functionally distinct ligands to provide new framework for the action of this family of secreted glycoproteins in normal and diseases states[11]. CTRP9 has 2 isoforms 9A and 9B, whereas CTRP9B requires physical interaction with CTRP9A and adiponectin for completing its function[14]. CTRP8 in addition to forming homotrimers, forms heteromeric complexes with CTRP1, CTRP9 and CTRP10. It effectively activates GPCR relaxin/insulin like family peptide receptor (RXFP1 receptor) to enhance the motility of the glioblastoma through regulating the activity of cathepsin B[15].

CTRPs, as secreted hormones are subjected to multiple functionally relevant post-translational modifications at their highly conserved residues. CTRP12 is glycosylated on the 39th Asparagine amino acid and the 85th Cysteine modified with oligosaccharides which mediates the assembly of oligomeric structure in human embryonic kidney (HEK) 293T cells. However, the exact modification mechanisms are still under investigation[16]. In addition, the two isoforms of CTRP12 differ from the oligomeric structure and are distinct in the regulation of function. Full length CTRP12 preferentially stimulates the Akt signaling in adipocytes, whereas the globular form activates the mitogen-activated protein (MAP) kinase signaling[16]. CTRP9, as a secreted glycoprotein, can be multiple post-translationally modified in multiple ways in its collagen domain. However, since the CTRP9 globular domain is closely similar to that of adiponectin, the interaction between CTRP9 and adiponectin does not need their collagen domains and is independent of posttranslational modification to activate the downstream signaling pathways[12]. Here, we highlight the characteristics of CTRPs and their isoforms; modification of CTRPs could account for the functional diversity of CTRPs.

The circulating levels of CTRPs tend to fluctuate according to sex, age, and alterations in the metabolic states and are sensitive to different responses in mammals. Reports have been made that CTRP6, a protein with fundamentally different modes of action as opposed to other CTRPs characterized to date, is a negative physiological regulator of glucose metabolism in adipocytes, skeletal muscle and liver to control the systemic energy balance. Expression of CTRP6 is upregulated in leptin deficient animals and increases in diabetic animals[8]. However, other researchers have reported that CTRP6 induces fatty acid oxidation in myocytes via AMPK activation[17]. These studies suggest that the newly discovered CTRPs still need to be investigated further in depth to understand their diverse functions. In addition, other CTRPs functional analysis reveals various roles of CTRPs. CTRP2 exerts fatty acid oxidation and glycogen deposition in myotubes[18]. CTRP1 transcript levels are augmented by rosiglitazone in mice, which enhance the insulin sensitizing action on the skeletal muscle via activation of the AMPK and Akt pathways[19]. CTRP3 can be a secreted plasma hormone and regulates hepatic gluconeogenesis. The metabolic regulatory action of CTRP3 is the downregulation of its plasma concentration which indicates that CTRP3 has a significant role in regulation of the lipid metabolism although the exact mechanism has not yet been established. However, CTRP3 deficiency reduces the liver size which indicates that its target organ appears to be the liver. Additionally, reduced circulating level of CTRP3 alters the inflammatory responses in the obese animals and the short-term daily administration of CTRP3 in diet-induced obese mice has been sufficient to improve the fatty liver phenotype, as evidenced by the suppressed triglyceride content and

expression of the triglyceride synthesis genes[1,20]. Hence, CTRP3 shows potent action on the regulation of metabolism. Loss of CTRP5 improves the insulin action states and hepatic steatosis. Deletion of CTRP7 attenuates the obesity-linked glucose intolerance of cytokine expression and circulating levels of cytokine[8]. Overexpression of CTRP9 modestly downregulates the serum glucose and insulin levels. CTRP9 and CTRP15 are considered as cardiokine (the heart-derived proteins are termed cardiokines) due to their high profile in the cardiac tissue and for their protective and preventive actions against cardiovascular injury. Although the CTRP9's functional receptor has not been thoroughly investigated, cadherin family appears to be a potential candidate[21]. Loss of CTRP12 affects the glucose and lipid metabolism in the obese and insulin-resistant mouse models[22]. CTRP13 regulates the metabolism through AMPK activation and decrease of fatty acid-induced JNK signaling[23]. CTRP15 links skeletal muscle and liver to systemic lipid homeostasis[21]. We summarize current understanding of CTRPs metabolic functions and provide insight into the dynamic regulatory role of CTRP on metabolic balance. Although much has been acknowledged since CTRPs were initially defined, many more questions remain to be addressed.

Of all the CTRPs, CTRP1, CTRP3, CTRP9, CTRP12, CTRP13 have been reported to exhibit positive metabolic regulation and cardiovascular effects in animal models. However, in human studies, circulating levels of CTRP1 are elevated, while CTRP12 displayed a decrease in type-2 diabetes patients. Thus, circulating CTRP1 and CTRP12 can serve as potential novel biomarkers for the early diagnosis of type-2 diabetes in humans[24,25]. The measurement of circulating CTRPs in serum is through commercially available ELISA kits provide by Aviscera company (Santa Clara, USA). CTRP3, in recent human studies, was increased in subjects with the cardio-metabolic syndrome and is associated with various cardio-metabolic risk factors including the triglycerides, high-density lipoprotein-cholesterol, waist-to-hip ratio, and eGFR, which indicate the decreased CTRP3 levels that may serve as a predictor of coronary artery disease, while CTRP13 cannot serve as a predictor candidate since there is evidence that CTRP13 mRNA expression is increased in the setting of obesity[26,27]. This contradiction may be attributed to different nature of human and rodent studies.

Collectively, based upon properties of the known and characterized CTRPS, a strategy designed for screening the biomarkers to predict the metabolic-related disease reveals that CTRP1 and CTRP12 can serve as potential novel biomarkers for the prediction and early diagnosis of type-2 diabetes, and furthermore, they represent a new treatment strategy. CTRP3 can be a better predictor for coronary artery disease than other CTRPs.

CTRPS AND INFLAMMATION

Metabolic syndrome such as obesity is associated with the chronic low-level inflammation, and therefore the metabolic regulators connecting obesity and diabetes to the inflammatory response have attracted much attention. In addition, it has been gradually recognized that the imbalance of anti-inflammatory adipokines and pro-inflammatory factors leads to the progression of obesity-related diseases. Disrupted anti-inflammatory adipokines participate in the systemic or local inflammatory reactions contributing to the initiation and development of metabolic dysfunction and cardiovascular events. In this regard, to define the imbalance of anti-inflammatory adipokines (CTRPs) and proinflammatory factors (risks factor) would be valuable in studying the obesity-associated complications. Hence, this section will emphasize the possible associations of CTRPs with cardiovascular inflammation.

CTRPs as a priming potential biomarker for predicting the dysfunctional metabolic status and therapeutic target has drawn much attention. Thus, their capability of regulation of metabolism has been widely investigated and documented. However, the association between CTRPs and inflammation, insulin resistance/obesity-linked inflammation, and pro-inflammatory cytokines needs to be thoroughly investigated even although these relationships between those disorders besides CTRPs have been well documented.

CTRPs protect against the complications of obesity such as cardiovascular diseases (CVDs) via their anti-inflammatory properties. Obesity is characterized by chronic inflammation leading to the obesity-related diseases including hypertension, atherosclerosis, and diabetes, while CTRPs as secretory proteins circulating in the organism can easily reach the site of infection to exert its anti-inflammatory characteristics.

Recent studies have shown new insights into CTRP6. For example, upregulation of CTRP6 in the leptin knock-out mice and under diabetic conditions shows distinct roles of CTRP6 in modulating inflammation[5]. In contrast to other CTRPs, CTRP6 expression is obviously elevated in adipose tissue and vascular cells in obese, diabetic patients and mouse models. Overexpression of CTRP6 not only impairs glucose

disposal in response to glucose challenge in animal models, but also augments local inflammation by targeting the inflammatory cells in production of TNF-α and IL-10[28]. Circulating inflammatory cytokines and pro-inflammatory macrophages were suppressed in the CTRP6-deficient mouse[5]. In addition, CTRP6 exhibits an immune-regulatory role in the complement system as it specifically suppresses the alternative pathway by binding of the finalizing factor-B to treat arthritis[29], where CTRP6 displays ability to cure the arthritis by intra-articulate injection in mice model[29].

Differing from CTRP6, other CTRPs exhibit the inflammatory regulatory role in a distinct way. In contrast to suppression of CTRP6 expression by cytokines, cytokines IL-1β and TNF-α elevate the expression of CTRP1 adaptively in the adipose tissue by which CTRP1 suppresses inflammation[30]. Meanwhile, CTRP1 impedes collagen-induced platelet coagulation, indicating its potent therapeutic value for treating vascular disorders during the inflammatory process[31]. CTRP3 has been shown to be capable of decreasing the secretion of pro-inflammatory mediators by primary human leukocytes, suggesting strongly an anti-inflammatory function, comparable to adiponectin[32-34]. CTRP5 appears to be a better biomarker than CTRP3 for predicting the severity of obstruction of airflow and systemic inflammation in patients with COPD although concentration alteration in response to the obese women[35,36]. CTRP4 can modulate tumor-promoting inflammation in cancer[37]. CTRP12 has been identified as an anti-inflammatory molecule by Enomoto *et al.*[38] in 2013. Even among the diverse types of cells, CTRP12 exhibits similar anti-inflammatory actions through the MAPK/JNK-dependent regulatory pathway to modulate TNF-α production.

Various CTRPs exert beneficial actions on the obesity-associated complications including inflammatory disorders through their anti-inflammatory actions. But, numerous functional, physiological, and mechanistic questions regarding the role of CTRPs in inflammation remain to be answered. The establishment and availability of transgenic and knock-out mice models for investigating inflammatory response of CTRPs will advance the knowledge of functions and mechanisms of action of CTRPs. Meanwhile, the receptor(s) responsible for the CTRP-mediated signal transduction should be thoroughly characterized and established.

CTRPS AND CARDIOVASCULAR SYSTEM

Reports show that CTRPs have beneficial function in the CVS and it is well established that the circulating levels of CTRPs are negatively correlated with the severity of CVDs without or with obesity in animals. Clinic studies indicate close association between CTRPs levels and CVDs. Conversely, the expression of inflammatory mediators in human tissues has been observed to be inversely regulated related to the levels of CTRPs[13,39]. Hence, the reciprocal relationship among CTRPs and the inflammatory mediators may present a dynamic crosstalk in the development of obesity and diabetic complications. Furthermore, the dysregulated production of CTRPs shown in obesity is associated with the pathogenesis of CVDs[40].

In contrast to adiponectin, which is a well-established adipokine for its anti-inflammatory and insulin sensitizer characteristics, CTRPs show diverse biological activities in the setting of normal metabolic condition and CVDs [Figure 2]. In the past decades, adiponectin has been recognized as a member of the CTRP family as it contains the collagen tail and C1q like globular domain. Recent studies reported that CTRPs exhibit stronger effective and sensitive response than adiponectin to different CVDs. Therefore, in this section, we mainly focus on the characteristics of anti-inflammatory and metabolic regulatory actions of the CTRPs in the onset of metabolic dysfunction-related CVDs.

Cardiovascular metabolism plays a pivotal role in the CVDs and the metabolic cascade is particularly important to investigate in the normal and disease processes. Cardiovascular metabolic dysfunction contributes to the progression of obesity-linked cardiovascular events and marks the fate of treatment application. Accumulating studies show that increased levels of CTRP1 are closely associated with the initiation and severity of the coronary arterial disease and can serve as a marker for myocardial infarction[10]. Meanwhile, the inhibition of CTRP1 may slow down the pathogenesis of early stage atherosclerosis and prevent the development of pathological vascular remodeling[41,42]. Circulating levels of CTRP 3 is elevated in patients with dysfunction of glucose metabolism and is associated with numerous cardiovascular metabolic risk factors[26]. It also can serve as a biomarker to predict proliferative retina disorder (PDR) and has beneficial actions for preventing CVDs, providing a promising strategy of vascular remodeling[43]. The levels of CTRP5 are associated with in-stent restenosis after percutaneous coronary intervention (PCI) with drug-eluting-stent implantation. CTRP9 can reflect the pathophysiology of renal involvement and abnormal glucose metabolism besides impairing vasorelaxation in type 2 diabetics[44]. CTRP15, a newly identified myokine, can link skeletal muscle to lipid homeostasis

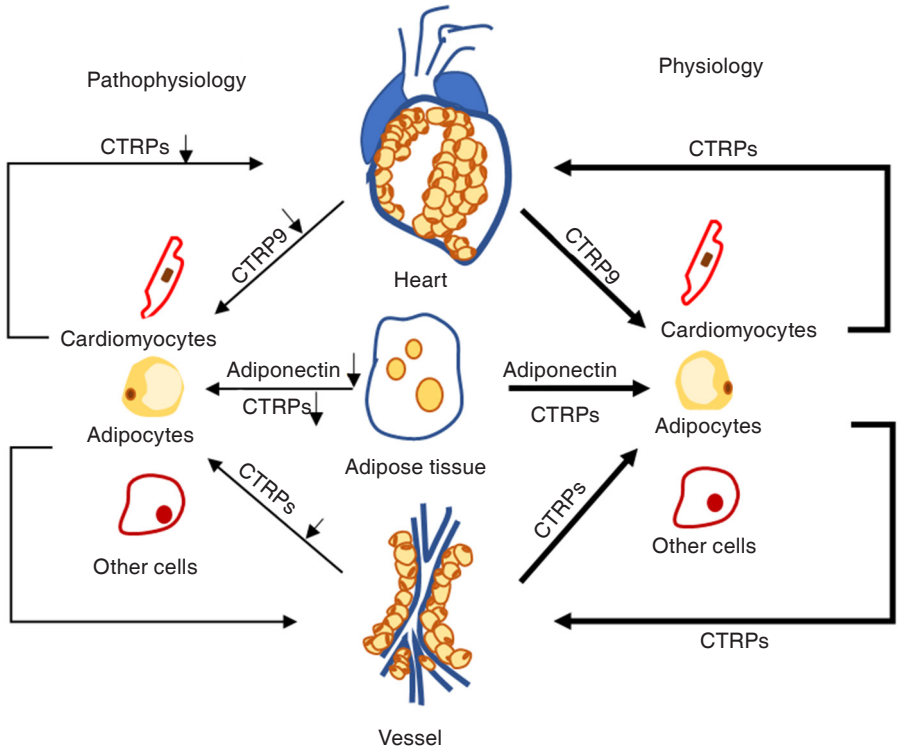

Figure 2: Pathophysiology of CTRPs in cardiovascular system. CTRPs exerts protective effects in cardiovascular system in physiological condition, but unbalanced levels of CTRPs in pathophysiological condition contributes to the development of cardiovascular relevant disease. CTRPs: C1q/tumor necrosis factor-related proteins

in response to alterations in energy state and predicting changes in the metabolic circuit[21].

Therefore, among the CTRPs, CTRP1 displays the potential to serve as a marker and therapeutic target in the vascular system and CTRP3 exhibits biomarker features in patients with diabetic-related PDR[45]. CTRP5 appears to have a greater potential to serve as a biomarker to predict the risks in PCI following the clinical operations.

CTRPS AND CARDIOVASCULAR INFLAMMATION

Inflammation happens in the cardiac tissue and vasculature as an injurious signal in response to the CVDs or risk factors including hypertension, smoking and diabetes. Those situations combined with metabolic dysfunction will amplify the harmful effects to initiate chronic inflammatory reactions resulting in rupture, thrombosis and vulnerable plaque. Basic research and epidemiological clinical studies showed that consistent association between parameters of inflammation and risks of impending cardiovascular event. Emerging advanced techniques can detect locally occurring inflammation by means of screening more reliable and accessible marker systemically, which may provide the best identification of individual at risk for cardiovascular disorders and benefit to prognosis and future treatment.

The alteration in levels of CTRPs is associated with increased risks of cardiovascular event and reflect the progression of CVDs. For better establishment of the relationship between inflammation and CVDs, we will address the recently discovered response of CTRPs to inflammation in the cardiac and vascular systems, respectively to lead the researchers to obtain in depth understanding for the novel strategy for cardiovascular risk.

The levels and actions of circulating CTRPs (CTRP1, CTRP3, CTRP6, CTRP9, CTRP12 and CTRP13) in normal subjects and diabetic patients have been examined in a cross- sectional clinical study which reveals that in chronic inflammatory condition. CTRP1 and CTRP12 show opposite changes in the concentration, while CTRP3 may have a beneficial effect on the prevention of vascular remodeling through anti-inflammatory actions providing a promising strategy for the therapy of vascular disease linked to obesity[42,24]. CTRP6, although it has no effect on collagen or fibroblasts proliferation, does suppress

the cell migration induced by inflammatory factors[46]. CTRP9 has been reported to exert positive effects on endothelium-dependent vasorelaxation in aortic vessel ring with the vasorelaxative effects induced via the activation of nitric oxide synthase. Besides that, CTRP9 inhibits the remodeling of vascular wall in the mouse wire-injured model through attenuating vascular smooth muscle proliferation. It is notable that those actions have been mediated by CTRP9 through sharing the adiponectin receptor[47,48]. Those studies highlight the potential protective roles of CTRPs in response to the inflammatory stress.

It has been reported that CTRP3 may act as an inflammatory molecule to improve the post-ischemic cardiac function and cardiac remodeling in animal model via the ability to reduce apoptosis. Whereas, CTRP6 may affect the inflammatory states in the context of obesity by stimulating the activation of p42/44 mitogen-activated protein kinase-dependent pathway[28]. Meanwhile, it can increase the production of anti-inflammatory cytokine IL-10 in monocyte-derived macrophages in human patients. In protecting against the inflammatory response of oxidative stress, CTRP9 can reduce the myocardial ischemia injury-induced superoxide generation to suppress apoptosis and shrink the infarct size[49]. Although CTRP12 has been reported to be lower in an obese rodent model, the systemic administration of CTRP12 inhibits the macrophage pro-inflammatory factor and attenuates the infiltration of cells in the obese mice. Hence, CTRP12 not only modulates the glucose homeostasis, but also leads to the suppression of the inflammatory response of white blood cells[50,51].

Those new investigations and the numerous epidemiological studies indicate that the CTRPs family has the capability to modulate the metabolic and related inflammation to maintain the cardiovascular homeostasis. This sheds light on the biomarker features of CTRPs in the assessment of obesity relevant diseases or as the therapeutic link for the treatment of metabolic dysfunction-associated disorders. Even so, large-scale clinical trials to investigate the effects/actions of CTRPs on cardiovascular events are required for a better understanding of the potential risks and beneficial roles in the prevention of cardiovascular risks.

LIMITATIONS

Although investigations on the CTRP receptors are ongoing, much work required to be done to understand them better. Adiponectin receptor1 may in part be involved in the effects of CTRP9 on cardiomyocytes and vascular endothelial cells, but research has been limited on the RNA interference approaches. Solid evidence of the CTRP9 interaction with the adiponectin receptor on the cell surface is lacking. A yeast-based assay system for the progestin and adipoQ receptor family (PAQR) receptor activity reveals that the adiponectin receptor is identified as the category of PAQR1 and PAQR2. Adiponectin is identified as an agonist for PAQR3[52,53]. Since CTRP family members share many characteristics with adiponectin, it has been speculated that the CTRP receptors belong to the PAQR family members or share similarity with the adiponectin receptors.

The number of members in the C1q/TNF-related superfamily keeps growing along with the progression of research. C1q engages a broad range of ligands via its globular domain and modulates cells via the collagen region. The members of this new family are involved in processes as diverse as inflammation, metabolism, energy conversation/expenditure, and beyond, including cell differentiation and proliferation. Therefore, the CTRP family is vastly underestimated and its functions need to be further investigated.

CONCLUSION

Research on CTRPs has provided insights into their metabolic roles and their characteristics of vascular protection. Although abundant knowledge has been gained since the CTRPs were first discovered, additional questions still need to be answered. CTRPs possess unique and shared functions that are supported by research using the advanced manipulative techniques, such as the genetic mouse models and gene engineering, and by population research on obese and diabetic patients. Future studies will reveal novel insights into the physiological and pathological functions of CTRPs, their metabolic behavior, and possible redundancy of CTRPs in health and disease. Additionally, studying the CTRPs receptors and the downstream signals that transduce the actions of CTRP in the cell are major challenges but the research will provide tremendous insights into drug design and identification of biomarker.

DECLARATIONS

Authors' contributions

Structure design, and finalized the manuscript: Y.J. Wang

Collected data and worked on the revision: W.B. Lau, X.L. Ma

Composed the first draft of the article: J.L. Zhao

Drew figures and collated literatures: J. Liu, R. Guo

Financial support and sponsorship

This work was supported by the following grants: Shanxi Key Subjects Construction; Innovative Talents of Higher Learning Institutions of Shanxi; American Diabetes Association 1-14-BS-218, 1-17-IBS-297; Natural Science Foundation of China, 81670278, 31322026 (to Y.J. Wang), and 81270185, 81470020 (to J.L. Zhao).

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. Peterson JM, Wei Z, Wong GW. C1q/TNF-related protein-3 (CTRP3), a novel adipokine that regulates hepatic glucose output. *J Biol Chem* 2010;285:39691-701.
2. Byerly MS, Swanson R, Wei Z, Seldin MM, McCulloh PS, Wong GW. A central role for C1q/TNF-related protein 13 (CTRP13) in modulating food intake and body weight. *PLoS One* 2013;8:e62862.
3. Wei Z, Seldin MM, Natarajan N, Djemal DC, Peterson JM, Wong GW. C1q/tumor necrosis factor-related protein 11 (CTRP11), a novel adipose stroma-derived regulator of adipogenesis. *J Biol Chem* 2013;288:10214-29.
4. Wolf RM, Steele KE, Peterson LA, Magnuson TH, Schweitzer MA, Wong GW. Lower circulating C1q/TNF-related protein-3 (CTRP3) levels are associated with obesity: a cross-sectional study. *PLoS One* 2015;10:e0133955.
5. Lei X, Seldin MM, Little HC, Choy N, Klonisch T, Wong GW. C1q/TNF-related protein 6 (CTRP6) links obesity to adipose tissue inflammation and insulin resistance. *J Biol Chem* 2017;292:14836-50.
6. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 1995;270:26746-9.
7. Wolf RM, Steele KE, Peterson LA, Zeng X, Jaffe AE, Schweitzer MA, Magnuson TH, Wong GW. C1q/TNF-related protein-9 (CTRP9) levels are associated with obesity and decrease following weight loss surgery. *J Clin Endocrinol Metab* 2016;101:2211-7.
8. Lei X, Rodriguez S, Petersen PS, Seldin MM, Bowman CE, Wolfgang MJ, Wong GW. Loss of CTRP5 improves insulin action and hepatic steatosis. *Am J Physiol Endocrinol Metab* 2016;310:E1036-52.
9. Petersen PS, Lei X, Wolf RM, Rodriguez S, Tan SY, Little HC, Schweitzer MA, Magnuson TH, Steele KE, Wong GW. CTRP7 deletion attenuates obesity-linked glucose intolerance, adipose tissue inflammation, and hepatic stress. *Am J Physiol Endocrinol Metab* 2017;312:E309-25.
10. Wang H, Wang R, Du D, Li F, Li Y. Serum levels of C1q/TNF-related protein-1 (CTRP-1) are closely associated with coronary artery disease. *BMC Cardiovasc Disord* 2016;16:92.
11. Wong GW, Krawczyk SA, Kitidis-Mitrokostas C, Revett T, Gimeno R, Lodish HF. Molecular, biochemical and functional characterizations of C1q/TNF family members: adipose-tissue-selective expression patterns, regulation by PPAR-gamma agonist, cysteine-mediated oligomerizations, combinatorial associations and metabolic functions. *Biochem J* 2008;416:161-77.
12. Wong GW, Krawczyk SA, Kitidis-Mitrokostas C, Ge G, Spooner E, Hug C, Gimeno R, Lodish HF. Identification and characterization of CTRP9, a novel secreted glycoprotein, from adipose tissue that reduces serum glucose in mice and forms heterotrimers with adiponectin. *FASEB J* 2009;23:241-58.
13. Zheng Q, Yuan Y, Yi W, Lau WB, Wang Y, Wang X, Sun Y, Lopez BL, Christopher TA, Peterson JM, Wong GW, Yu S, Yi D, Ma XL. C1q/TNF-related proteins, a family of novel adipokines, induce vascular relaxation through the adiponectin receptor-1/AMPK/eNOS/nitric oxide signaling pathway. *Arterioscler Thromb Vasc Biol* 2011;31:2616-23.
14. Peterson JM, Wei Z, Wong GW. CTRP8 and CTRP9B are novel proteins that hetero-oligomerize with C1q/TNF family members. *Biochem Biophys Res Commun* 2009;388:360-5.
15. Klonisch T, Glogowska A, Thanasupawat T, Burg M, Krcek J, Pitz M, Jaggupilli A, Chelikani P, Wong GW, Hombach-Klonisch S. Structural commonality of C1q TNF-related proteins and their potential to activate relaxin/insulin-like family peptide receptor 1 signalling pathways in cancer cells. *Br J Pharmacol* 2017;174:1025-33.
16. Wei Z, Lei X, Seldin MM, Wong GW. Endopeptidase cleavage generates a functionally distinct isoform of C1q/tumor necrosis factor-related protein-12 (CTRP12) with an altered oligomeric state and signaling specificity. *J Biol Chem* 2012;287:35804-14.
17. Lee W, Kim MJ, Park EJ, Choi YJ, Park SY. C1qTNF-related protein-6 mediates fatty acid oxidation via the activation of the AMP-activated protein kinase. *FEBS Lett* 2010;584:968-72.
18. Peterson JM, Seldin MM, Tan SY, Wong GW. CTRP2 overexpression improves insulin and lipid tolerance in diet-induced obese mice. *PLoS One* 2014;9:e88535.
19. Peterson JM, Aja S, Wei Z, Wong GW. CTRP1 protein enhances fatty acid oxidation via AMP-activated protein kinase (AMPK) activation and acetyl-CoA carboxylase (ACC) inhibition. *J Biol Chem* 2012;287:1576-87.
20. Peterson JM, Wei Z, Seldin MM, Byerly MS, Aja S, Wong GW. CTRP9 transgenic mice are protected from diet-induced obesity and metabolic dysfunction. *Am J Physiol Regul Integr Comp Physiol* 2013;305:R522-33.
21. Seldin MM, Peterson JM, Byerly MS, Wei Z, Wong GW. Myonectin (CTRP15), a novel myokine that links skeletal muscle to systemic lipid homeostasis. *J Biol Chem* 2012;287:11968-80.
22. Tan SY, Little HC, Lei X, Li S, Rodriguez S, Wong GW. Partial deficiency of CTRP12 alters hepatic lipid metabolism. *Physiol Genomics* 2016;48:936-49.
23. Wei Z, Peterson JM, Wong GW. Metabolic regulation by C1q/TNF-related protein-13 (CTRP13): activation OF AMP-activated protein kinase and suppression of fatty acid-induced JNK signaling. *J Biol Chem* 2011;286:15652-65.
24. Bai B, Ban B, Liu Z, Zhang MM, Tan BK, Chen J. Circulating C1q complement/TNF-related protein (CTRP) 1, CTRP9, CTRP12 and CTRP13 concentrations in type 2 diabetes mellitus: in vivo regulation by glucose. *PLoS One* 2017;12:e0172271.
25. Chalupova L, Zakovska A, Adamcova K. Development of a novel enzyme-linked immunosorbent assay (ELISA) for measurement of serum CTRP1: a pilot study: measurement of serum CTRP1 in healthy donors and patients with metabolic syndrome. *Clin Biochem* 2013;46:73-8.
26. Choi KM, Hwang SY, Hong HC, Yang SJ, Choi HY, Yoo HJ, Lee KW, Nam MS, Park YS, Woo JT, Kim YS, Choi DS, Youn BS, Baik SH. C1q/TNF-related protein-3 (CTRP-3) and pigment epithelium-derived factor (PEDF) concentrations in patients with type 2 diabetes and

metabolic syndrome. *Diabetes* 2012;61:2932-6.
27. Fadaei R, Moradi N, Baratchian M, Aghajani H, Malek M, Fazaeli AA, Fallah S. Association of C1q/TNF-related protein-3 (CTRP3) and CTRP13 serum levels with coronary artery disease in subjects with and without type 2 diabetes mellitus. *PLoS One* 2016;11:e0168773.
28. Kim MJ, Lee W, Park EJ, Park SY. C1qTNF-related protein-6 increases the expression of interleukin-10 in macrophages. *Mol Cells* 2010;30:59-64.
29. Murayama MA, Kakuta S, Inoue A, Umeda N, Yonezawa T, Maruhashi T, Tateishi K, Ishigame H, Yabe R, Ikeda S, Seno A, Chi HH, Hashiguchi Y, Kurata R, Tada T, Kubo S, Sato N, Liu Y, Hattori M, Saijo S, Matsushita M, Fujita T, Sumida T, Iwakura Y. CTRP6 is an endogenous complement regulator that can effectively treat induced arthritis. *Nat Commun* 2015;6:8483.
30. Kim KY, Kim HY, Kim JH, Lee CH, Kim DH, Lee YH, Han SH, Lim JS, Cho DH, Lee MS, Yoon S, Kim KI, Yoon DY, Yang Y. Tumor necrosis factor-alpha and interleukin-1beta increases CTRP1 expression in adipose tissue. *FEBS Lett* 2006;580:3953-60.
31. Lasser G, Guchhait P, Ellsworth JL, Sheppard P, Lewis K, Bishop P, Cruz MA, Lopez JA, Fruebis J. C1qTNF-related protein-1 (CTRP-1): a vascular wall protein that inhibits collagen-induced platelet aggregation by blocking VWF binding to collagen. *Blood* 2006;107:423-30.
32. Kopp A, Bala M, Weigert J, Buchler C, Neumeier M, Aslanidis C, Scholmerich J, Schaffler A. Effects of the new adiponectin paralogous protein CTRP-3 and of LPS on cytokine release from monocytes of patients with type 2 diabetes mellitus. *Cytokine* 2010;49:51-7.
33. Hofmann C, Chen N, Obermeier F, Paul G, Buchler C, Kopp A, Falk W, Schaffler A. C1q/TNF-related protein-3 (CTRP-3) is secreted by visceral adipose tissue and exerts antiinflammatory and antifibrotic effects in primary human colonic fibroblasts. *Inflamm Bowel Dis* 2011;17:2462-71.
34. Schmid A, Kopp A, Hanses F, Karrasch T, Schaffler A. C1q/TNF-related protein-3 (CTRP-3) attenuates lipopolysaccharide (LPS)-induced systemic inflammation and adipose tissue Erk-1/-2 phosphorylation in mice in vivo. *Biochem Biophys Res Commun* 2014;452:8-13.
35. Choi HY, Park JW, Lee N, Hwang SY, Cho GJ, Hong HC, Yoo HJ, Hwang TG, Kim SM, Baik SH, Park KS, Youn BS, Choi KM. Effects of a combined aerobic and resistance exercise program on C1q/TNF-related protein-3 (CTRP-3) and CTRP-5 levels. *Diabetes Care* 2013;36:3321-7.
36. Li D, Wu Y, Tian P, Zhang X, Wang H, Wang T, Ying B, Wang L, Shen Y, Wen F. Adipokine CTRP-5 as a potential novel inflammatory biomarker in chronic obstructive pulmonary disease. *Medicine (Baltimore)* 2015;94:e1503.
37. Li Q, Wang L, Tan W, Peng Z, Luo Y, Zhang Y, Zhang G, Na D, Jin P, Shi T, Ma D, Wang L. Identification of C1qTNF-related protein 4 as a potential cytokine that stimulates the STAT3 and NF-kappaB pathways and promotes cell survival in human cancer cells. *Cancer Lett* 2011;308:203-14.
38. Enomoto T, Ohashi K, Shibata R, Kambara T, Uemura Y, Yuasa D, Kataoka Y, Miyabe M, Matsuo K, Joki Y, Hayakawa S, Hiramatsu-Ito M, Ito M, Murohara T, Ouchi N. Transcriptional regulation of an insulin-sensitizing adipokine adipolin/CTRP12 in adipocytes by Kruppel-like factor 15. *PLoS One* 2013;8:e83183.
39. Qu H, Deng M, Wang H, Wei H, Liu F, Wu J, Deng H. Plasma CTRP-3 concentrations in Chinese patients with obesity and type II diabetes negatively correlate with insulin resistance. *J Clin Lipidol* 2015;9:289-94.
40. Seldin MM, Tan SY, Wong GW. Metabolic function of the CTRP family of hormones. *Rev Endocr Metab Disord* 2014;15:111-23.
41. Liu ZH, Li C, Chen JW, Shen Y, Gao J, Shen WF, Zhang RY, Wang XQ, Lu L. C1q/TNF-related protein 1 promotes endothelial barrier dysfunction under disturbed flow. *Biochem Biophys Res Commun* 2017;490:580-6.
42. Kanemura N, Shibata R, Ohashi K, Ogawa H, Hiramatsu-Ito M, Enomoto T, Yuasa D, Ito M, Hayakawa S, Otaka N, Murohara T, Ouchi N. C1q/TNF-related protein 1 prevents neointimal formation after arterial injury. *Atherosclerosis* 2017;257:138-45.
43. Lin S, Ma S, Lu P, Cai W, Chen Y, Sheng J. Effect of CTRP3 on activation of adventitial fibroblasts induced by TGF-beta1 from rat aorta in vitro. *Int J Clin Exp Pathol* 2014;7:2199-208.
44. Fujita T, Watanabe H, Murata Y, Hemmi S, Yabuki M, Fuke Y, Satomura A, Soma M. Plasma C1q/TNF-related protein 9: a promising biomarker for diabetic renal vascular injury. *Minerva Urol Nefrol* 2017;69:195-200.
45. Yan Z, Zhao J, Gan L, Zhang Y, Guo R, Cao X, Lau WB, Ma X, Wang Y. CTRP3 is a novel biomarker for diabetic retinopathy and inhibits HGHL-induced VCAM-1 expression in an AMPK-dependent manner. *PLoS One* 2017;12:e0178253.
46. Lei H, Wu D, Wang JY, Li L, Zhang CL, Feng H, Fu FY, Wu LL. C1q/tumor necrosis factor-related protein-6 attenuates post-infarct cardiac fibrosis by targeting RhoA/MRTF-A pathway and inhibiting myofibroblast differentiation. *Basic Res Cardiol* 2015;110:35.
47. Yang Y, Li Y, Ma Z, Jiang S, Fan C, Hu W, Wang D, Di S, Sun Y, Yi W. A brief glimpse at CTRP3 and CTRP9 in lipid metabolism and cardiovascular protection. *Prog Lipid Res* 2016;64:170-7.
48. Uemura Y, Shibata R, Ohashi K, Enomoto T, Kambara T, Yamamoto T, Ogura Y, Yuasa D, Joki Y, Matsuo K, Miyabe M, Kataoka Y, Murohara T, Ouchi N. Adipose-derived factor CTRP9 attenuates vascular smooth muscle cell proliferation and neointimal formation. *FASEB J* 2013;27:25-33.
49. Appari M, Breitbart A, Brandes F, Szaroszyk M, Froese N, Korf-Klingebiel M, Mohammadi MM, Grund A, Scharf GM, Wang H, Zwadlo C, Fraccarollo D, Schrameck U, Nemer M, Wong GW, Katus HA, Wollert KC, Muller OJ, Bauersachs J, Heineke J. C1q-TNF-related protein-9 promotes cardiac hypertrophy and failure. *Circ Res* 2017;120:66-77.
50. Tan BK, Lewandowski KC, O'Hare JP, Randeva HS. Insulin regulates the novel adipokine adipolin/CTRP12: in vivo and ex vivo effects. *J Endocrinol* 2014;221:111-9.
51. Wei Z, Peterson JM, Lei X, Cebotaru L, Wolfgang MJ, Baldeviano GC, Wong GW. C1q/TNF-related protein-12 (CTRP12), a novel adipokine that improves insulin sensitivity and glycemic control in mouse models of obesity and diabetes. *J Biol Chem* 2012;287:10301-15.
52. Garitaonandia I, Smith JL, Kupchak BR, Lyons TJ. Adiponectin identified as an agonist for PAQR3/RKTG using a yeast-based assay system. *J Recept Signal Transduct Res* 2009;29:67-73.
53. Gonzalez-Velazquez W, Gonzalez-Mendez R, Rodriguez-del Valle N. Characterization and ligand identification of a membrane progesterone receptor in fungi: existence of a novel PAQR in Sporothrix schenckii. *BMC Microbiol* 2012;12:194.
54. Rohrbach S, Aurich AC, Li L, Niemann B. Age-associated loss in adiponectin-activation by caloric restriction: lack of compensation by enhanced inducibility of adiponectin paralogs CTRP2 and CTRP7. *Mol Cell Endocrinol* 2007;277:26-34.
55. Shabani P, Emamgholipour S, Doosti M. CTRP1 in liver disease. *Adv Clin Chem* 2017;79:1-23.
56. Cheng C, Yu S, Kong R, Yuan Q, Ma Y, Yang W, Cao G, Xie L. CTRP3 attenuates hepatic stellate cell activation through transforming growth factor-beta/Smad signaling pathway. *Biomed Pharmacother* 2017;89:1387-91.
57. Wolf RM, Lei X, Yang ZC, Nyandjo M, Tan SY, Wong GW. CTRP3 deficiency reduces liver size and alters IL-6 and TGFbeta levels in obese mice. *Am J Physiol Endocrinol Metab* 2016;310:E332-45.
58. Petersen PS, Wolf RM, Lei X, Peterson JM, Wong GW. Immunomodulatory roles of CTRP3 in endotoxemia and metabolic

stress. *Physiol Rep* 2016;4:e12735.
59. Feng H, Wang JY, Zheng M, Zhang CL, An YM, Li L, Wu LL. CTRP3 promotes energy production by inducing mitochondrial ROS and up-expression of PGC-1alpha in vascular smooth muscle cells. *Exp Cell Res* 2016;341:177-86.
60. Kim JY, Min JY, Baek JM, Ahn SJ, Jun HY, Yoon KH, Choi MK, Lee MS, Oh J. CTRP3 acts as a negative regulator of osteoclastogenesis through AMPK-c-Fos-NFATc1 signaling in vitro and RANKL-induced calvarial bone destruction in vivo. *Bone* 2015;79:242-51.
61. Luo Y, Wu X, Ma Z, Tan W, Wang L, Na D, Zhang G, Yin A, Huang H, Xia D, Zhang Y, Shi X, Wang L. Expression of the novel adipokine C1qTNF-related protein 4 (CTRP4) suppresses colitis and colitis-associated colorectal cancer in mice. *Cell Mol Immunol* 2016;13:688-99.
62. Tan WF, Wang LL, Li Q, Luo Y, Na DX, Ma Z, Wang L. Prokaryotic expression and polyclonal antibody preparation of human novel gene CTRP4. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2012;28:614-7. (in Chinese)
63. Duan L, Liu Z, Wang L, Ma B, Fan Y, Xu Y, Guo F. C1q and tumor necrosis factor related protein 4 (CTRP4) suppresses caspase-1/IL-1beta inflammatory pathway in trophoblasts of rat models with preeclampsia. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2016;32:1441-5. (in Chinese)
64. Schwartze JT, Landgraf K, Spielau U, Rockstroh D, Loffler D, Kratzsch J, Kiess W, Korner A. Adipocyte C1QTNF5 expression is BMI-dependently related to early adipose tissue dysfunction and systemic CTRP5 serum levels in obese children. *Int J Obes (Lond)* 2017;41:955-63.
65. Shen Y, Li C, Zhang RY, Zhang Q, Shen WF, Ding FH, Lu L. Association of increased serum CTRP5 levels with in-stent restenosis after coronary drug-eluting stent implantation: CTRP5 promoting inflammation, migration and proliferation in vascular smooth muscle cells. *Int J Cardiol* 2017;228:129-36.
66. Emamgholipour S, Moradi N, Beigy M, Shabani P, Fadaei R, Poustchi H, Doosti M. The association of circulating levels of complement-C1q TNF-related protein 5 (CTRP5) with nonalcoholic fatty liver disease and type 2 diabetes: a case-control study. *Diabetol Metab Syndr* 2015;7:108.
67. Chavali VR, Khan NW, Cukras CA, Bartsch DU, Jablonski MM, Ayyagari R. A CTRP5 gene S163R mutation knock-in mouse model for late-onset retinal degeneration. *Hum Mol Genet* 2011;20:2000-14.
68. Wu W, Zhang J, Zhao C, Sun Y, Pang W, Yang G. CTRP6 regulates porcine adipocyte proliferation and differentiation by the AdipoR1/MAPK signaling pathway. *J Agric Food Chem* 2017;65:5512-22.
69. Chi L, Hu X, Zhang W, Bai T, Zhang L, Zeng H, Guo R, Zhang Y, Tian H. Adipokine CTRP6 improves PPARgamma activation to alleviate angiotensin II-induced hypertension and vascular endothelial dysfunction in spontaneously hypertensive rats. *Biochem Biophys Res Commun* 2017;482:727-34.
70. Glogowska A, Kunanuvat U, Stetefeld J, Patel TR, Thanasupawat T, Krcek J, Weber E, Wong GW, Del Bigio MR, Hoang-Vu C, Hombach-Klonisch S, Klonisch T. C1q-tumour necrosis factor-related protein 8 (CTRP8) is a novel interaction partner of relaxin receptor RXFP1 in human brain cancer cells. *J Pathol* 2013;231:466-79.
71. Jia Y, Luo X, Ji Y, Xie J, Jiang H, Fu M, Li X. Circulating CTRP9 levels are increased in patients with newly diagnosed type 2 diabetes and correlated with insulin resistance. *Diabetes Res Clin Pract* 2017;131:116-23.
72. Jung TW, Hong HC, Hwang HJ, Yoo HJ, Baik SH, Choi KM. C1q/TNF-related protein 9 (CTRP9) attenuates hepatic steatosis via the autophagy-mediated inhibition of endoplasmic reticulum stress. *Mol Cell Endocrinol* 2015;417:131-40.
73. Li Y, Geng X, Wang H, Cheng G, Xu S. CTRP9 ameliorates pulmonary arterial hypertension through attenuating inflammation and improving endothelial cell survival and function. *J Cardiovasc Pharmacol* 2016;67:394-401.
74. Liu F, Tan A, Yang R, Xue Y, Zhang M, Chen L, Xiao L, Yang X, Yu Y. C1ql1/Ctrp14 and C1ql4/Ctrp11 promote angiogenesis of endothelial cells through activation of ERK1/2 signal pathway. *Mol Cell Biochem* 2017;424:57-67.
75. Mehrdadi P, Kolahdouz Mohammadi R, Alipoor E, Eshraghian MR, Esteghamati A, Hosseinzadeh-Attar MJ. The effect of coenzyme q10 supplementation on circulating levels of novel adipokine adipolin/CTRP12 in overweight and obese patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2017;125:156-62.
76. Enomoto T, Shibata R, Ohashi K, Kambara T, Kataoka Y, Uemura Y, Yuasa D, Murohara T, Ouchi N. Regulation of adipolin/CTRP12 cleavage by obesity. *Biochem Biophys Res Commun* 2012;428:155-9.
77. Afrookhteh A, Emamgholipour S, Alipoor B, Moradi N, Meshkani R, Nasli-Esfahani E, Rahimipour A, Shanaki M. The circulating levels of complement-C1q/TNF-related protein 13 (CTRP13) in patients with type 2 diabetes and its association with insulin resistance. *Clin Lab* 2017;63:327-33.
78. Shanaki M, Fadaei R, Moradi N, Emamgholipour S, Poustchi H. The circulating CTRP13 in type 2 diabetes and non-alcoholic fatty liver patients. *PLoS One* 2016;11:e0168082.
79. Seldin MM, Wong GW. Regulation of tissue crosstalk by skeletal muscle-derived myonectin and other myokines. *Adipocyte* 2012;1:200-2.
80. Karrasch T, Schmid A, Kopp A, Obermeier F, Hofmann C, Schaffler A. Impact of toll-like-receptor-9 (TLR9) deficiency on visceral adipose tissue adipokine expression during chronic DSS-induced colitis in mice. *Exp Clin Endocrinol Diabetes* 2015;123:80-7.

Singh *et al. Vessel Plus* 2017;1:213-29
DOI: 10.20517/2574-1209.2017.26

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Review **Open Access**

An overview of different methods of myocardial protection currently employed peri-transplantation

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How to cite this article: Singh SSA, Das De S, Spadaccio C, Berry C, Al-Attar N. An overview of different methods of myocardial protection currently employed peri-transplantation. *Vessel Plus* 2017;1:213-29.

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Article history:
Received: 26 Jul 2017
First Decision: 7 Sep 2017
Revised: 17 Sep 2017
Accepted: 20 Sep 2017
Published: 28 Dec 2017

Key words:
Myocardial protection,
cardiac surgery,
cardiac transplantation

ABSTRACT

Myocardial protection is integral to the functioning of hearts in day to day cardiac surgery. However, due to the longer ischaemic times, it becomes pivotal in the management of organs during transplantation. There are many different strategies employed to ensure diligent and judicious myocardial protection during donor management, transportation of the heart and the post-operative period. Given the limited supply of organs and the increasing waiting lists for heart transplants worldwide, it has become an area of renewed interest with many innovations and inventions using the principles of basic sciences to improve outcomes of transplanted hearts. The heart procurement process encompasses several of the different myocardial protection strategies in tandem to provide the greatest benefit to the recipients. This review looks at the different modalities employed, which include different types of cardioplegia, the role of biomarkers, the cutting-edge novel therapies, hormonal therapies and ischaemic conditioning strategies.

Quick Response Code:

INTRODUCTION

Cardiac surgery is a rapidly evolving specialty when compared to other forms of surgery. In the 19th century, professor Theodor Billroth, one of the pioneers of modern abdominal surgery once stated: "A surgeon who tries to suture a heart wound deserves to lose the esteem of his colleagues" in 1896[1]. In 1951, Russian physiologist Vladimar Petrovich Demikhov performed the first thoracic transplants recorded. He transplanted the heart and lungs of a male dog into a 3-year-old female dog. The dog survived 6 days exhibiting normal behaviour but succumbed to respiratory complications. This was the first recorded history of any animal surviving any length of time with a transplanted heart supporting its circulation[2].

Stanford University Medical Center led the field in cardiac transplantation. Norman Shumway from the University of Minnesota was well known for his work on myocardial protection. Along with Lillehei, they would later lead the team that refined the technique of orthotopic heart transplantation. They reported a series of orthotopic heart transplantations in dogs and perfected a method that had been use previously by Cass and Brock by preserving the recipient atrial cuffs[3]. They had also first introduced the idea of cold cardioplegia. These experiments were successful but Shumway that host immunologic mechanisms were destroying the graft and survival could be prevented by addressing these[4].

Shumway searched for biomarkers to monitor transplant rejection. Initial experiments with enzymes (hypertensin I) were unsuccessful. He settled on electrocardiography as a monitor but noted its limitations and pointed out that atrial fibrillation within 7-21 days of transplant was an early sign of rejection[5]. After working with transplant nephrologists, he assessed the potential benefits of immunosuppression (6-mercaptopurine, azathioprine, and prednisolone) and increased survival by up to 250 days in dogs receiving orthotopic transplants. Dr. Christiaan Barnard extended their pioneering work. Barnard undertook the first successful human-to-human heart transplant in December 1967[6], before Shumway's group followed shortly afterwards in January 1968.

However, the initial reported 1-year survival was only 22%, resulting in most centers abandoning this procedure[7]. Shumway's group at Stanford along with other contemporaries (Cabrol's group at Paris, Lower's group at Virginia) persevered and following introduction of cyclosporine, 1-year survival increased to approximately 80% by the 80s.

The number of heart transplants performed worldwide continued to increase until the shortage of donors in the 1990s became a limiting factor[8]. In 2005, almost half (48%) of patients had spent at least 2 years on the heart transplant waiting list worldwide[9] leading to challenging decisions about the eligibility of donor hearts. Abuanzeh *et al.*[10] described steps utilized by their retrieval team to optimize donors. This was included standardizing protocols which includes insertion of a pulmonary artery catheter and performance of cardiac output studies, weaning other vasopressors and commencing arginine vasopressin, and administration of hormone replacement. This included tri-iodothyronine, methylprednisolone and insulin. They managed to retrieve 14 additional hearts in that time frame (56% of the borderline hearts) after aggressive donor management and optimization[10].

Despite recent advances, myocardial protection during heart transplantation remains a challenging avenue. With the increasing usage of borderline donor hearts as described, we review some of the myocardial protection strategies and potential markers for perioperative myocardial infarction.

CARDIOPLEGIA

Ringer first noted the relationship between potassium and sodium concentration and the heart rate in 1883[11]. Experimentations with potassium citrate were initially unsuccessful due to the excessive osmolality due to the high-concentration potassium citrate[12]. Most cardioplegic solutions today employ a more physiological osmolality and potassium concentration.

Cardioplegic solutions are defined as intracellular and extracellular solutions based on the concentrations of sodium and potassium ions. Extracellular solutions contain high levels of potassium, magnesium and sodium, while intracellular solutions contain low electrolyte levels. Intracellular solutions mimic the high potassium/low sodium conditions reducing potential concentration gradients across the plasma membrane, thereby halting potassium efflux. This reduction in membrane potential resting state prevents generation of action potentials. The function of the Na^+/K^+ ATPase channel is reduced in hypothermic conditions, therefore permitting the intracellular concentrations to persist[13]. Extracellular cardioplegia on the other hand works by preventing repolarization of myocytes. Potassium rich perfusate in the extracellular space reduces the membrane voltage difference causing

depolarization. Intracellular calcium sequestration occurs via active transport across an ATP-dependent pump allowing relaxation of the myocardium in diastole. Repolarization however is prevented by the high potassium concentration of the cardioplegic solution. Both intracellular and extracellular cardioplegic solutions have similar long-term outcomes[14].

Norman Shumway first noted the use of topical hypothermia to reduce myocardial metabolic requirements in 1960 when chemical cardioplegia fell out of favour[15]. Denton Cooley then attempted intermittent aortic occlusion and utilized Kay's work with intracoronary blood perfusion with cross clamp fibrillation[16-18].

Clinical cardioplegia was reintroduced in the 1970s by using a low-sodium solution by Bretschneider *et al.*[19] with potassium chloride[20] that was reported as safe and allowed safer aortic cross-clamping allowing cold crystalloid cardioplegic solutions to be in favour for general cardiac surgery. Blood was later introduced as medium for cardioplegia by Buckberg as it was later discovered that reperfusion injuries occurred in crystalloid cardioplegia due to the associated influence of calcium and oxygen as described by Buckberg[21] and Hearse *et al.*[22]. There are intracellular and extracellular types of crystalloid cardioplegia which have become the gold standard for cardiac preservation [Table 1][23].

Histidine-tryptophan-ketoglutarate (custodiol/bretschneider)

Reichenspurner *et al.*[24] studied the effect of histidine-tryptophan-ketoglutarate (HTK) in a cohort of 600 (524 male:76 female) patients undergoing heart transplantation over a 10-year period (1981-1991). They reported good results provided the ischaemic times were less than 4 h.

Sung *et al.*[25] compared Bretscheinder's HTK solution (18 patients) and cold blood cardioplegia (CBC) (49 patients) for myocardial protection in donor heart preservation. Cold HTK solution was infused at low perfusion pressure after procurement and the donor heart was placed in a sterile bag containing HTK solution. The CBC group heart was placed in St Thomas' Hospital (StH). The heart was covered with ice-cold saline for topical cooling and packed in a container filled with ice. Two patients (11.1%) in the HTK group died within 30 days of surgery due to right heart failure and pneumonia with septic shock. There were 4 deaths (8.2%) in the CBC group due to acute rejection (n = 2), right heart failure and pneumonia with septic shock. There was no statistically significant difference between thebypass time, ischaemic time short term outcomes, creatine kinase/CKMB/troponin I values, length of ICU stay, and hospital stay between

Table 1: Comparison of cardioplegic solutions contents

	HTK[15]	University of Wisconsin[16]	Celsior[14]	Eurocollins[17]	St Thomas's Solution[18]
Intracellular/extracellular	Extracellular	Intracellular	Extracellular	Intracellular	Extracellular
Na^+	10	25	100	10	120
K^+	10	120	15	115	16
Ca^{2+}	0.015	0	0.25	0	1.2
Mg^{2+}	4	5	13	0	16
Cl^-	50	20	0	15	160
Glucose	–	0	0	180	0
Others	α-KG	Adenosine	0		0
Glucose	0	0	0	195	0
Impermeant/colloid					
Hydroxyl-Ethyl Starch (g/L)	0	0	50	0	0
Lactobionate	0	0	100	80	0
Mannitol	30	0		60	0
Raffinose	0	0	30	0	0
Buffer					
Phosphate	0	25	0	100	0
Bicarbonate	0	0	0	10	10
Histidine	180	0	30		00
Osmolarity (mOsm/L)	310	330	320	375	320
Anti-oxidants					
Glutothione	0	2	3	0	0
Allopurinol	0	1	0	0	0
Tryptophan	2	0	0	0	0

All units expressed in mmol/L unless otherwise indicated

the 2 groups. There was however a higher inotropic score in the HTK group at 24 h (P = 0.03). Multivariate analysis revealed a significantly reduced bypasstime in the HTK group (P = 0.002). They concluded that HTK was superior due to the reduced pumping time albeit with a higher post-operative inotrope score. A single dose of HTK provided similar myocardial protection as repeated doses of CBC solution in donated hearts. Minami *et al.*[26] noted an increment in troponin and CK-MB levels if the ischaemic times were > 4 h using HTK solution but concluded that it was still within acceptable limits when compared to other crystalloid cardioplegias CK-MB 25 IU and troponin I 21 pg/mL, with ischaemic time of 263 min).

HTK is an intracellular type of cardioplegic solution. It lowers concentrations of sodium and calcium thereby inducing cardiac arrest by deprivation of extracellular sodium thus preventing depolarisation of the action potential. Calcium channels open leading to increased cytosolic calcium and potentially aggravating cellular injury, indirectly reducing the calcium concentration. Histidine in the HTK solution, acts as a buffer enhancing the efficiency of anaerobic glycolysis. This has been quoted by several sources to be its primary advantage, with its buffering capacity allowing effective myocardial preservation. The Ketoglutarate (α-KG) component serves as a high energy ATP provider during reperfusion. Tryptophan stabilises the cell membranes. Mannitol, an osmotic diuretic is added to reduce cellular oedema as it has free radical scavenging properties thus reducing the extent of ischaemic injury[27].

St Thomas's solution

St Thomas's solution (StH) is an extracellular type of cardioplegic solution that induces rapid cardiac arrest by high potassium and magnesium concentrations alongside the membrane stabilising effect of procaine[28]. Addition of a buffer and reduction of calcium concentrations resulted in the formation of No. 2 (Plegisol, Abbott Laboratories, North Chicago, III.). In a rat model, Plegisol was shown to be superior to its predecessor with lower rates of post-operative ventricular fibrillation, increased left ventricular pressure and recovery of aortic flow[29]. Addition of procaine in this solution reduces the incidence of post-declamping ventricular fibrillation. Luciani *et al.*[30] group performed a prospective single blinded randomised control trial comparing cold blood cardioplegia to StH (crystalloid cardioplegia). Spontaneous sinus rhythm was significantly higher in the blood cardioplegia group (11% *vs.* 40%) (P = 0.02) with a higher creatine kinase (P = 0.01) and CK-MB (144 ± 90 IU *vs.* 102 ± 59 IU) (P = 0.06). They performed a follow up study 12 years later and revealed no difference in terms of mortality (46% *vs.* 42%, P = 0.7) and cause of death (chronic rejection: 50% *vs.* 50%; neoplasia: 33% *vs.* 25%, P = 0.8). Survival at 12 years was 50 ± 12% *vs.* 52 ± 11% (P = 0.9). Follow-up echocardiogram showed similar mean left ventricular ejection fraction (LVEF; 47 ± 12% *vs.* 49 ± 11%, P = 0.7) and prevalence of LVEF < 35% (21% *vs.* 18%, P = 0.8). The prevalence of chronic rejection was similar in both groups (42% *vs.* 32%, P = 0.1), but severe allograft vasculopathy was more prevalent in the St Thomas cardioplegia group (64% *vs.* 17%, P = 0.04). There were no other between-group differences[31].

Eurocollins

Collins *et al.*[32] designed an "intracellular" organ preservation solution and is credited to being one of the first solutions to attempt the advancement of organ preservation based on changes that occur during cell hypothermia. The predecessor to the Eurocollins solution, Collins solution, provided reliable preservation and was the organ preservation fluid of choice in abdominal organ transplantation especially renal preservation.

Collins solution has a high potassium content alongside a glucose osmotic barrier. Despite achieving relatively long storage times for abdominal organs, hearts were more susceptible to ischaemic injury and the low protective properties of glucose compounded by the acidotic conditions resulting from glucose conversion to lactate resulted in the addition of mannitol or sucrose instead of glucose as the impermeant[33]. Euro-Collins solution however fell out of favour due to the variability of recovery of hearts at non-uniform temperatures[34].

University of Wisconsin (Belzer UW/Viaspan)

University of Wisconsin Solution (UW) was formulated by James *et al.*[35] in the late 1980s as the preservation fluid of choice for pancreas preservation. Prior to its introduction, abdominal organs preserved in Collins solution would have limited ischaemic tolerance of about 8 h. Belzer and Southard developed UW solution initially to prolong liver and pancreas preservation. Their initial experiments of canine pancreases were encouraging and used it for liver and kidneys with similarly encouraging results[36-38].

UW solution became the "gold standard" of preservation fluids as it first highlighted the lack of equilibrium achieved by Na^+/K^+ ratios during cold preservation[39]. It contains lactobionate and raffinose, which are metabolically inert, making it suitable for multiorgan usage. Both these substances which are osmotically active prevent organ oedema. Addition of adenosine provides precursor of an energy source (ATP).

Allopurinol with glutathione act as antioxidants[40,41]. UW limited ischaemic damage from prolonged storage and improved myocardial function in the early posttransplant period, thus allowing transplantation of organs with ischaemic times > 300 min[42]. Jeevanandam *et al.*[43] performed a study comparing University of Wisconsin solution with crystalloid cardioplegia to saline storage and noted a significant improvement in mean time from reperfusion to achieving a stable rhythm, need for intraoperative defibrillation, need for cardiac pacing and CK-MB release over 48 h. They however also reported higher CK-MB levels (335 IU) post-operatively despite a relatively shorter ischaemic time of 153 min when compared to the HTK group of Minami's cohort[26].

Celsior

Dr Menasche and colleagues developed Celsior solution[44]. They utilised lactobionate and mannitol as impermeants. Celsior also uses histidine as a buffer and glutamate as an energy substance alongside magnesium to stabilise calcium levels. Unlike UW which has a high potassium content, Celsior had a lower potassium content and a high sodium concentration. In canine models, Celsior had a similar cardioprotective profile as UW. Higher concentrations of potassium results in increase coronary vascular resistance secondary to endothelial distension[45,46]. De Santo *et al.*[46] compared the results of "high risk" grafts *vs.* "standard" grafts using Celsior. They followed up 200 consecutive heart recipients with 73 in the high-risk group (defined as 2 or more of the following: age > 45, female, high pre-retrieval inotropic support, size mismatch > 20%, and ischaemia time > 180 min) and 127 in the standard group. There was no difference noted between the two groups in terms of 1-year mortality, hospital mortality, histological findings and patterns of enzyme release[47].

Comparison of cardioplegic solutions

Lee *et al.*[48] combined both the intracellular and extracellular cardioplegic solutions (HTK and StH). In their cohort of 31 patients, they demonstrated non-inferiority to other approaches. The theoretical benefits include the quick initial arrest from StH alongside the prolonged effect of HTK alongside its buffering mechanism. The effectiveness of HTK has resulted in lower CK and lactate dehydrogenase levels in non-transplant cardiac surgery [Table 2][48,49].

Comparisons between the different crystalloid cardioplegia solutions are difficult to extrapolate due to the lack of direct comparisons. Several smaller animal studies however do suggest potential superiority of HTK cardioplegia over the rest.

BIOMARKERS

Cardiac troponins have largely replaced cardiac muscle enzymes (CK-MB) for the diagnosis of myocardial infarction. Cardiac troponin T (cTnT) and troponin I (cTnI) are cardiac regulatory proteins that control the calcium mediated interaction between actin and myosin. cTnT is also expressed in small amounts in skeletal muscles as well. The role of post-operative troponin release as a prognostic factor for mid- and short-term all-cause mortality after adult cardiac surgery is accepted albeit cut-off values are difficult to establish due to the variety of timing of the Tn testing, Tn subunit and Tn assays[59]. Its prognostic value in a transplant setting however has not been clearly understood. CK-MB and troponin I are released immediately after transplantation and depends on myocardial ischaemic damage, which is related to ischaemic time[60].

De Santo *et al.*[60] investigated troponin release after cardiac transplantation. Data from 362 consecutive recipients were collated over 11 years. Target outcomes included factors determining troponin release, early graft failure, rise in creatinine and operative death. This study depicted the largest group of adult cardiac transplantation patients who had cTnI levels correlated with perioperative morbidity and mortality reported in the literature thus far. The pattern of troponin release observed was similar to that reported by Minami. cTnI release > 10 μg/L proved to be an independent predictor for early graft dysfunction which in turn was a determinant of hospital mortality. Factors that predicted this rise included previous cardiac surgery, left ventricular hypertrophy, increased ischaemic time and transplant status 2B. Troponin proteins are intracellular proteins released primarily from cardiac myocytes undergoing cellular necrosis. Perhaps surprisingly, ischaemia/reperfusion injury following cardiac transplantation may not cause cellular necrosis and occasionally troponin concentrations may not be increased[61].

Brain natriuretic peptide (BNP) is actively synthesized and released from cardiac myocytes in response to ischaemia and inflammation[62]. It is not directly stimulated by surgical manipulation or cardiopulmonary bypass, hence its role as a biomarker for ischaemic reperfusion injury in non-transplant cardiac surgery to predict post-operative dysfunction[63,64]. McIlroy *et al.*[65] studied the role of BNP as marker for myocardial ischaemic reperfusion in 25 consecutive patients following cardiac transplantation. The median preoperative troponin-I concentrations were almost three-fold the upper limit of normal in both the donor and recipient. The donor BNP levels centred around

Table 2: Comparison of studies comparing cardioplegic agents

Study	Year of study	Type	Solution	Cases (*n*)	Findings	Comment
Vega *et al.*[50]	2001	Open Label RCT (Humans)	Celsior *vs.* Standard of care (UW, STh, others)	Celsior (64) SOC (67)	Fewer patients in the Celsior group experienced at least one cardiac-related serious adverse event	Younger recipients in control group
Wieselthaler *et al.*[51]	1999	Open Label RCT (Humans)	HTK *vs.* Celsior	HTK (24) Celsior (24)	Increased Ischaemic time for HTK group (*P*-values not given) 2 cases of Acute graft failure in HTK cohort	Similar outcomes
Cannata *et al.*[52]	2012	Retrospective cohort (humans)	Celsior *vs.* HTK *vs.* STh	HTK (61) Celsior (38) STh (34)	Similar outcomes	Similar outcomes
George *et al.*[53]	2012	Retrospective cohort	UW *vs.* Celsior	UW (42) Celsior (134)	UW is associated with less acute ischaemic necrosis (on pathology) than CS	
George *et al.*[54]	2011	Retrospective cohort	UW *vs.* Celsior	UW (3,107) Celsior (1,803)	In high-risk allografts, UW was associated with improved survival	UW recipient cohort had significantly better haemodynamic readings pre-operatively
Kofler *et al.*[55]	2009	Retrospective cohort (humans)	UW *vs.* HTK	UW (118) HTK (222)	UW demonstrated a significantly better survival	HTK group was derived from historic control
Garlicki *et al.*[56]	1999	Retrospective cohort	UW *vs.* Celsior *vs.* HTK	UW (64) CEL (28) HTK (132)	HTK had highest mortality	Statistical significance uncertain as *P*-values unavailable
Lee *et al.*[57]	2011	Prospective cohort Lewis donor rat	HTK *vs.* Celsior	NA	HTK = Significant reduction in serum troponin I & creatine phosphokinase HTK = reduction in upregulation of mRNA for interleukin-6, intercellular adhesion molecule-1, and tumor necrosis factor-α, fewer infiltrating cells, less apoptosis, and less phosphorylated adenosine monophosphate-activated protein kinase	HTK- superior protective effects against ischemia-reperfusion in older donors
Ackemann *et al.*[58]	2002	Prospective cohort 28 to 35 kg adult fox hound dogs	HTK *vs.* Celsior	19 (HTK) *vs.* 19 (Celsior)	HTK = more ATP after 8 and 12 h of ischemia	HTK = better LV function, less prone to arrhythmic events

NA: not applicable; LV: left ventricular; HTK: histidine-tryptophan-ketoglutarate

the upper limit of normal, while in recipients, it was the levels were all markedly elevated with greater heterogeneity. Posto-peratively, BNP had a moderate correlation with ischaemic time (ρ = 0.52, *P* = 0.01), donor BNP (ρ = 0.45, *P* = 0.03), and donor troponin-I (ρ = 0.49, *P* = 0.01). The post-operative concentrations of BNP were significantly higher in patients requiring increasing doses of inotropic support. However, there was no correlation between post-operative troponin I and the measured parameters.

The role of microRNAs (miRNAs) as a biomarker for cardiac disease is rapidly expanding due to its rapid release kinetics, cardio-selectivity and plasma stability[66]. It negatively regulates gene expression by prohibiting complementary messenger RNAs (mRNAs) translation into functional proteins. Wang *et al.*[67] attempted to monitor initial injury to the myocardium and post-operative recovery by detecting levels of circulating muscle-specific miRNAs in 7 consecutive patients. Fourteen controls were also included in their study. Samples were obtained at daily intervals post transplantation. They also collected cTnI levels. Their results showed significant correlation between miRNAs and cTnI ($P < 0.05$) and the circulating concentrations of both proteins were strongly correlated with bypass time. Circulating miR-133b correlated well with parameters of heart function such as central venous. It also had strong correlations with ventilation time ($r > 0.99$, $P < 0.001$) and length of ICU stay ($r > 0.92$, $P < 0.05$). They

concluded that cardiac muscle specific miRNAs could detect early myocardial injury and possibly predict graft dysfunction and recovery post-operatively.

NOVEL THERAPIES

The current standard of care for organ preservation of hearts post explant is cold preservation (usually in an icebox). Perfusion of the heart with cold preservative solution is then followed by explantation and storage of the heart at 4 °C. The choice of cardioplegic solution is primarily based on experience of individual centres. The generally acceptable time for cold preservation is about 4 h with ISHLT data suggesting that ischaemic times > 6 h associated with primary graft dysfunction[68]. One of the contributing factors to primary graft dysfunction may be suboptimal organ preservation alongside the role of ischaemia reperfusion injury.

Goldsmith *et al.*[69] group analysed the potential benefits of reducing the ischaemic time (IT). They analysed survival rates beyond 20 years' post-transplantation. The study showed that median survival post-transplantation was between 10-11 years. Every additional hour of donor organ IT, conferred a 25% increased risk of death after heart transplantation in the first year after transplant, with a 5% increase thereafter ($P < 0.001$). On average, recipients surviving a decade post-transplantation could potentially gain 0.4 life-years if IT was reduced to 1 h. This worked out to almost 3 life years saved if IT was reduced to 1 h if someone had IT > 6 h[69].

To overcome this limitation, Hassanein *et al.*[70] proposed the use of a makeshift continuous perfusion device to permit prolonged storage of allografts.

At 2 h of reperfusion, the hearts that were continuously perfused had higher LV generated pressures and lower lactate levels (myocardial acidosis) compared to the controls in cold storage. Based on Hassanein's findings, the Organ Care System (OCS), a continuous perfusion device developed by TransMedics, Inc., Andover, MA, USA, was then used in two phase 1 trials, the prospective multi-centre European trial to evaluate the safety and performance of the Organ Care System for heart transplants (PROTECT) trial based in Europe and the Prospective Multicentre Safety and Effectiveness Evaluation of the Organ Care System Device for Heart Use (PROCEED) trial based in the United States. The OCS consists of a miniature pulsatile pump with an inbuilt inline heater. It is also permits monitoring of cardiac output, coronary flow and blood pressure via the attached monitor. A specific perfusion solution consisting of part crystalloid, part glucose and amino acids, physiological extracellular electrolyte concentrations, free-radical scavengers, antibiotics, and calculated levels of catecholamines and insulin alongside oxygenated warm blood with a haematocrit of 20-25%; thus simulating a more "physiological" environment[71].

The PROTECT trial[72] was a prospective study of 20 patients who received donor hearts that had been maintained by the OCS in a perfused and physiologic beating state for a mean time of 3.7 h. The graft survival rate of 100% at 30 days and the percentage of cardiac related complications was 23%. Additionally, OCS was associated with earlier recovery with a shorted ventilation time and shorter ICU stay. The PROCEED[73] trial was a 20 patient, single arm, non-randomized, Food and Drug Administration approved safety and performance study. This study highlighted the importance of lactate concentration during OCS use. Hamed's group concluded that when using the OCS for donor heart maintenance, the final serum lactate concentrationis the most powerful predictor of graft failure post heart transplant with high sensitivity and specificity[74].

PROCEED II[75] was the first prospective, open-label, multicentre, randomised non-inferiority trial comparing OCS to current standard of care (cold hypothermic static preservation) at ten heart-transplant centres in the USA and Europe. Eligible adult heart-transplant candidates were randomly assigned (1:1) to receive donor hearts preserved with either the Organ Care System or standard cold storage. One hundred and thirty patients were recruited and randomised to Organ Care System group ($n = 67$) or the standard cold storage group ($n = 63$). The 30-day patient and graft survival rates were 94% ($n = 63$) in the Organ Care System group and 97% ($n = 61$) in the standard of care ($P = 0.45$). Eight (13%) patients in the Organ Care System group and 9 (14%) patients in the standard cold storage group had cardiac-related serious adverse events. The results were consistent with non-inferiority of OCS *vs.* standard of care in terms of short term outcomes. Donor hearts in the OCS group had a significantly longer preservation (out-of-body) time, but shorter cold ischemia time compared to standard of care. The longest preservation time with the OCS was 9 h and 7 min thought to be due to the extra time needed to instrument the donor heart into the Organ Care System circuit and optimise the perfusion characteristics.

Donation following cardiac death however is a new avenue which resulted in an increase of available organs. Initially used primarily for kidney transplantation, donor after circulatory death (DCD) was first split into four categories

Table 3: Modified Maastricht Classification of DCD[76]

Classification	Descriptions
I	Dead on arrival and have not been resuscitated
II	Unsuccessfully resuscitated
III	Typical controlled DCD, with planned cardiac arrest
IV	Planned DBD that suddenly arrest during or after the brain death determination

DCD: donor after circulatory death; DBD: donations following brain death

by the Maastricht group [Table 3][76].

Of these, type I, II and IV are regarded uncontrolled DCD. For these donors, cardiopulmonary resuscitation is typically conducted until organ recovery procedures are employed.

Iyer *et al.*[77] conducted a porcine orthotopic heart transplant using a DCD asphyxia model. Following 30 min of warm ischaemia, the hearts were allocated to either OCS preservation of SOC with Celsior solution. Following preservation, the OCS group demonstrated acceptable lactate profiles and all hearts out of this group were successfully transplanted whereas none of the hearts in the SOC group could be weaned off bypass.

Dhital *et al.*[78] then piloted the first case series of Maastrict group III DCD cardiac transplants at St Vincent's Hospital (Australia) using the OCS. The 3 recipients (2 men and 1 woman; mean age 52 years) received the transplants. After periods of warm ischaemia < 30 min, *ex-vivo* perfusion was done with the OCS device to resuscitate, assess, and transport the donor hearts. Of these patients, 1 required mechanical circulatory support for 72 h post-operatively, with all 3 patients showing normal cardiac function within a week post-transplantation. Follow up data shows patients are still making a good recovery at 176, 91, and 77 days after transplantation.The cohort included a fourth donor, a trauma victim, who was excluded as the warm ischaemic time was > 30 min (which did not meet the inclusion criteria).

DCD donation however was not pioneered by this group. In fact the first ever cardiac transplant by Barnard was a DCD heart. Boucek *et al.*[79] highlighted the first case series of DCD donations in the paediatric population owing to the higher waiting list mortality compared to adults. They successfully performed 3 transplants in the paediatric population and found no late deaths (3.5 years post-operatively) with functional and immunologic outcomes similar to those of controls.

In March 2015, the first DCD heart transplant in Europe was performed at Papworth Hospital[80]. While it is quoted that this may potentially increase the donor pool by about 25% in the UK alone, several ethical issues arise from DCD heart procurement. These include the definition of death. While the needs and feelings of the donors and their families are noted, organ viability should be maintained and maximized. Organ donation in itself should not be the reason for donor death[81].

Another point noted by the Australian group was the potential use of OCS for resuscitating marginal donors. An estimated 60% of hearts offered are rejected for transplantation and the introduction of OCS may therefore on paper at least, increase the number of suitable organs[82].

HORMONAL THERAPY

As alluded to, the increasing recipient waiting list has led to the recruitment of so-called "marginal" donors. Brain death usually succeeds a period of variable intracranial pressure in which the term "coning" is often used. The classic Cushing's reflex of increased blood pressure and reduced heart rate is often discernible through monitoring and can lead to deleterious effects on multiple organ systems if not managed appropriately. There is a compensatory arterial hypertension and bradycardia (Cushing's reflex) that is followed by sympathetic stimulation with vasoconstriction, raised systemic vascular resistance and tachycardia (a triad called the catecholamine storm)[83]. There is a redistribution of blood volume that prompts visceral ischaemia and in one study, revealed that myocardial injury occurs in 20-25% of DBD donors[84], with echocardiographic imaging of cardiac dysfunction evident in up to 40% of DBD donors[85]. Following this catecholamine storm phenomena, there is a profound hypotension that results from a reduction in sympathetic tone and peripheral vasodilation causing mass hypoperfusion of all organs, potentially resulting in more organ dysfunction[86].

Cooper *et al.*[87] and Novitzky *et al.*[88] noted that several animal model studies carried out in South Africa in the 1980s demonstrated the catecholamine storm phenomena followed by profound hypotension occurred with reduction in cortisol, insulin, thyroid, and antidiuretic hormone levels, a switch from aerobic to anaerobic metabolism and increases in inflammation markers and cytokines. Hormonal replacement resulted in recovery of cardiac function in both experimental animals and humans, thus protecting the donor organs. Registry multivariate studies on hormonal treatment of brain-dead donors also

revealed significant increases in organs transplanted and in 1-year survival of kidneys and hearts. Cardiac transplant centres then formed specific teams for the purpose of "optimizing" the donors with the "Papworth Cocktail"[89] of hormones[90]. Multiple studies showed increased organ procurement and a reduction in primary graft dysfunction in transplanted hearts when triple therapy with thyroid hormone, corticosteroids and arginine vasopressin was used[91,92].

Cortisol

It has been postulated that haemodynamic instability in DBD donors is caused by adrenal insufficiency. Nicolas-Robin *et al.*[93] revealed in their cohort of brain-dead patients, adrenal insufficiency was present in almost 90%. They also noted that hydrocortisone supplementation enhanced systemic haemodynamics and decreased norepinephrine dose by more than 30% in more than half of brain-dead patients with haemodynamic instability.

They also identified several pathophysiological groups based on cortisol levels and Adrenocorticotropic Hormone (ACTH) response. (1) ACTH responders with low plasma cortisol: this reflected a hypothalamic-pituitary failure (secondary adrenal insufficiency) resulting from direct insult from intracranial hypertension causing ischaemia of the hypothalamus and pituitary gland, impairing the release of corticotropin-releasing hormone and ACTH as described by Novitzky *et al.*[94]. This group responded to tetracosactrin (synthetic ACTH) administration; (2) ACTH non-responders with normal baseline cortisol - suggesting primary adrenal failure; (3) ACTH non-responders with low baseline cortisol: this was probably caused by a hypothalamic-pituitary-adrenal insufficiency; and (4) ACTH responders with normal plasma cortisol: no pathology within the hypothalamic-pituitary-adrenal axis.

Ironically, Nicolas-Robin *et al.*[93]'s study demonstrated that hydrocortisone infusion was more often efficient in enhancing haemodynamic stability in ACTH non-responders than in ACTH responders suggesting that exogenous steroids have a higher likelihood of producing a haemodynamic response when there is no endogenous response to ACTH stimulation.

It is also postulated that the effects of corticosteroid administration could be due to the re-sensitization of α- and β-adrenoceptors pathway which are often altered by down-regulation and later by desensitization in patients with shock treated with catecholamines[95,96]. Another explanation is the "consumption" of cortisol following its initial release by the hyper-stimulated hypothalamic-pituitary-adrenal axis adrenal gland induced by brain death may be unable to replenish cortisol stores. Nicolas-Robin *et al.*[93]'s group also noted the effectiveness of hydrocortisone infusion on norepinephrine dose decrease was observed in 25% of ACTH non-responders with low baseline cortisol. The anti-inflammatory properties of hydrocortisone could also have an effect as adrenal insufficiency results from the release of several cytokines such as tumor necrosis factor α, interleukin-1, interleukin-6, and overexpression of cell adhesion molecules such as intercellular adhesion molecule-1 and E-selectin[97-99].

A French study involving 22 ICUs during 15 months to compare two different resuscitation strategies: systematic hydrocortisone supplementation (steroid group) or no supplementation (control group) in brain-dead patients who were potential organ donors was conducted. Eleven centres administered standard-care, low-dose hydrocortisone to brain-dead patients before organ procurement the remaining 11 did not administer corticosteroids[100].

Adrenal insufficiency was noted in almost 80% of brain-dead patients. The average number of episodes ofhypotension and vascular filling volume per hour were similar in the two groups. Although more patients in the steroid group received norepinephrine before brain death, the mean dose of vasopressor administered after brain death was significantly lower than in the control group, duration of vasopressor support use was shorter than in control group and norepinephrine weaning before aortic clamping was more frequent. This decrease in number of vasopressors used following steroid administration was seen in other studies as well[101].

Dhar *et al.*[102] looked at 132 consecutive brain-dead donors managed before and after changing the steroid protocol from 15 mg/kg methylprednisolone (high dose) to 300 mg hydrocortisone (low dose) and found that the only significant differences were lower final insulin requirements and faster weaning off insulin infusions in the low dose group.

Steroid therapy was not associated with improvements in the recovery of primary graft function in these studies.

Thyroxine

Another change that occurs with brain death is the reduction of plasma-free triiodothyronine (T3) resulting in impaired aerobic metabolism. This causes a reduction of myocardial energy stores and an increased tissue lactate as a result of increased anaerobic metabolism.

Novitzky *et al.*[103] performed one of the pioneering studies in the role of T3 in transplants. One hundred and sixteen consecutive potential donors were treated, alongside 70 recipients with good immediate cardiac function in all but 3 patients, who recovered within 24 h of mechanical circulatory support. They also conducted 2 randomized trials in patients undergoing myocardial revascularization on cardiopulmonary bypass, and administration of post-operative T3 therapy was associated with a reduced need for inotropic support and diuretic therapy in the first study and improved cardiac output in the second study. Chen *et al.*[104]'s study on rat models suggested that T3 can protect myocytes against ischemia-induced apoptosis, which may be mediated by Akt signalling.

In a study by Jeevanandam *et al.*[105], donor hearts with statistically higher filling pressures, lower EF on echocardiograms, and higher inotrope requirements were resuscitated with T3 and compared to normal donors not receiving T3. All patients survived the immediate post-operative period, and at 1 week and 6 months there were no significant differences in systolic blood pressure, diastolic blood pressure, heart rate, cardiac index, central venous pressure, pulmonary capillary wedge pressure, or LVEF on echocardiography. It should also be noted that the donors also received furosemide and dopamine, both increasing renal perfusion thereby being partially responsible for the fall in pre-load and increased mean arterial pressure[87].

Novitzky *et al.*[106] then conducted a retrospective review on 63,593 donors (2000-2009) who were administered T3/T4. They noted a 12.8% increment of organs from T3/T4-treated donors compared to untreated donors ($P < 0.0001$). In study 2, a 15.3% increase was noted ($P < 0.0001$). T3/T4 therapy was associated with procurement of significantly greater numbers of hearts, lungs, kidneys, pancreases, and intestines, but not livers. Multivariate analysis indicated a beneficial effect of T3/T4 independent of other factors ($P < 0.0001$)[105]. Apart from Novitzky's work however, there have been mixed reviews on the efficacy of T3 administration. A recent systematic review conducted by Macdonald *et al.*[107] noted that all case series and retrospective audits reported a beneficial effect of thyroid hormone administration but all seven randomized controlled trials reported no benefit of thyroid hormone administration either alone or in combination with other hormonal therapies. In four placebo-controlled trials, administration of thyroid hormone had no significant effect on donor cardiac index (pooled mean difference, 0.15 L/min/m²; 95% confidence interval -0.18 to 0.48). They noted that there was a lack of consideration of confounding factors in case series and retrospective audits. However, it also notes that of the few randomized controlled trials conducted, the number of patients who were hemodynamically unstable or marginal in other ways, who would have possibly benefited from T3 administration was too small to exclude a benefit of thyroid hormone in this subgroup. A randomized trial by Venkateswaran *et al.*[108] allocated 80 donors to four treatment groups; A control group, T3 monotherapy, Methylprednisolone monotherapy, T3 and Methylprednisolone and placebo. Pulmonary Artery Catheters were used to guide management, with vasopressin infusion commenced while weaning catecholamines at the commencement of blinded trial medication. The study found no difference in outcomes in patients from all 4 groups. They concluded that detailed donor haemodynamic measurement and management is possibly the most important criteria in increasing the yield of transplantable hearts. In animal models, administration of T3 was shown to improve haemodynamic function before and after transplantation[108-110].

However, this has yet to be seen in prospective randomized studies in human donors. The stance taken by most centres in the UK is to replace T3 only when there is evidence of thyroid hypofunction.

Antidiuretic hormone (arginine-vasopressin)

Antidiuretic hormone is synthesized by magnicellular neurons at the supraoptic and paraventricular nuclei, stored in neurosecretory granules in the axons that project into the posterior pituitary[111,112]. Given its anatomical location, rising intracranial pressures from the Cushing reflex as described earlier plays a part in the depletion of ADH. Yoshioka *et al.*[113] first described the role of vasopressin and epinephrine *vs.* epinephrine alone in 16 brain-dead patients improving mean survival from 1 day to 23 days. The rise in ICP is a cause for neurogenic diabetes insipidus (DI) which is very commonly found (in some studies up to 77% of solid organ donors[113]), and hormone replacement with vasopressin, an effective treatment for DI, would have resolved the haemodynamic instability.

Blaine *et al.*[114] noted that aggressive resuscitation with crystalloid solutions may instigate intravascular to intracellular fluid shifts thus contribute to the development of both interstitial and intracellular oedema, and ultimately result in profound hypoperfusion of end organs causing the rejection of the organs for transplantation. They conducted their study of an animal model of a brain-dead organ donor, in which polyuria, hypernatremia, and hyperosmolality developed. Low-dose (2-10 microU/kg/min) vasopressin was continuously infused to maintain plasma sodium and osmolality within normal range over the course of the

experiments. Cardiovascular function remained stable in both control and experimental vasopressin-infusion groups, with the only significant difference being a moderate rise in pulmonary artery pressure.

Rostron *et al.*[115] conducted a similar study looking at the effects of arginine vasopressin in preventing neurogenic vasoplegia which exacerbates lung injury. They induced brain death in Wistar rats by inflating an intracranial balloon mimicking coning. They noted an increment in pulmonary capillary permeability, wet/dry lung weight ratios, neutrophil integrin expression and pro-inflammatory cytokines in serum (TNF-α, IL-1β, CINC-1 and CINC-3), bronchoalveolar lavage (TNF-α, IL-1β,) and lung tissue (IL-1β and CINC-1) in braindead animals compared to controls. These effects were corrected by administration of arginine vasopressin (AVP) and norepinephrine to correct the neurogenic hypotension.

Another study conducted by Chen *et al.*[116] investigated vasopressin deficiency and hypersensitivity as a potential contributing factor to hypotension in organ donors. In their cohort of 50 organ donors, 10 patients were treated with a continuous infusion of vasopressin (0.04 to 0.1 U/min). Mean arterial pressure (MAP), catecholamine requirements, serum vasopressin, and serum osmolality were obtained before and after vasopressin administration. An increment of MAP allowed complete discontinuation of catecholamine pressors in 40% of patients and a decrement in pressor dose in another 40%. Plasma vasopressin levels (2.9 ± 0.8 pg/mL) were notably low for the degree of hypotension. It is likely that haemodynamically unstable organ donors may not only display diabetes insipidus but also have a defect in baroreflex-mediated secretion of vasopressin, for which supplementation would permit catecholamine sparing. The catecholamine sparing effects of vasopressin were also noted by Pennefather *et al.*[117] in which 24 DBD donors were randomised to receive either saline or low dose AVP. The AVP group had a decreased plasma hyperosmolality ($P < 0.05$), improved blood pressure ($P < 0.01$), and reduced inotrope use ($P < 0.01$), while maintaining cardiac output. Myocardial ATP levels were higher in the AVP than the control group (NS). Kinoshita *et al.*[118] studied the effects of epinephrine and arginine vasopressin in 10 brain dead patients. Patients maintained haemodynamic stability for more than a week with an initial rise in ST wave changes that was reversible. This was confirmed by a normal level of CK-MB and normal or slightly swollen mitochondria on cardiac biopsy specimens, highlighting the role of both arginine vasopressin and epinephrine in maintaining haemodynamic stability post brain death. Papadopoulos *et al.*[119] found that vasopressin administration reduced the dose of requirements of catecholamines and contributed to prevention of the post-cardiotomy vasoplegic shock in the patient with low ejection fraction (30-40%) on ACE inhibitors in a double blind randomized controlled trial whereby the group A who were infused with low dose vasopressin and the group B who were infused with normal saline intraoperatively and for the 4 post-operative hours. This further illustrated the benefits of vasopressin out with the correction of neurogenic diabetes insipidus.

Glucose-Insulin-Potassium

Calva *et al.*[120] first conducted experiments on canines by inducing myocardial infarctions via coronary artery ligation noting the extent of damage to the mitochondria with and without glucose-insulin-potassium regimes in the 60s. Opie *et al.*[121] conducted similar studies with baboons and noted similar findings, a reduction in mitochondrial damage and decreased infarction and reduced ST segment depression on EKG. Multiple studies have since been done to study the effect of glucose-potassium-insulin (GKI) on myocyte function. Human studies were first pioneered by Sodi-Pallares *et al.*[122] in which 10 patients with acute myocardial infarction and 20 patients with chronic coronary insufficiency, with 3 patients showing improvements but 2 patients worsening and a general improvement in the chronic patients. A meta-analysis however revealed no reduction in mortality in patients receiving GKI in randomised studies[123]. They concluded that while it may have had a potential benefit in the pre-revascularisation and thrombolysis era, its benefits are not clearly evident now. Sun *et al.*[124] investigated the role of GKI for prevention of oxygen free radical injury during reperfusion of ischaemic stored hearts. Comparing known free radical scavengers (superoxide dismustase and catalase) alone and combination with GKI in rat models, they noted that there was no added benefit of GKI infusion in reduction reperfusion injury once reperfusion was commenced, but noted an improvement in the superoxide dismustase and catalase infusion group. A significant improvement however was noted when GKI and the free radical scavengers were combined showing improvement in left ventricular end-diastolic pressure, myocardial blood flow. They concluded that free radical scavengers in the presence of glucose-insulin-potassium significantly improve functional recovery in the setting of heart transplantation.Myocardial dysfunction that occurs post brain death is a phenomenon that is ubiquitously reported but not fully understood. It is thought to be related to direct myocardial injury from sympathetic activation[125], potential reduction in oxidative metabolism from the reduction in T3, variability

of loading conditions, endothelial dysfunction and impairment of coronary blood flow[126].

Nicolas-Robin *et al.*[127] looked at GKI infusion in comparison to dobutamine for in DBD donors. They found that a GKI infusion significantly improved the systolic dysfunction comparably to dobutamine without its inherent side effects of peripheral vasodilation, potential arrhythmogenesis, and tachycardia. This was thought to be possibly due to an adaptive hibernating state of the myocardium to reduce myocardial oxygen demand, thereby allowing a longer period of ischaemia without necrosis[128]. Hence the rationale behind GKI infusion is by replenishing the energy supply of the failing heart, by switching metabolism from oxidation of fatty acids (glycogenolysis) to oxidation of glucose (glycolysis) and lactate[129]. This allows restoration of calcium homeostasis and replenishment of glycogen stores by increasing the rate of ATP[130]. Although GKI was not shown to improve mortality in acute myocardial infarction as mentioned above, there may be a role for it in the ischaemic myocardium.

Cottin *et al.*[131] demonstrated that their cohort of patients with heart failure (Ejection Fraction < 45%), GKI infusion reduced Wall Motion Score Index and increased ejection fraction significantly in their small study. Similar findings were noted in several other studies, including a reduction in BNP concentrations[132-135].

ISCHAEMIC CONDITIONING

Reperfusion injury is postulated to be a key contributing factor for primary graft dysfunction, thus the role of ischaemic conditioning whilst still in its trialling phase may be of benefit. The lack of evidence of benefit in large scale studies such as RIPheart[136] and ERICCA[137] clarified that this intervention does not confer any benefits to patients undergoing CABG.

Animal models have shown potential benefits and cardioprotective mechanisms, but while biochemical improvements were noted by the ERICCA trial (reduced troponin levels), its relevance remains to be seen. Remote ischaemic preconditioning (RIC) and remote ischaemic post conditioning (PostC) work on the premise that brief episodes of ischemia and reperfusion to the remote organ protect the heart by a paracrine or neural-reflex mechanism while avoiding additional stress on the heart itself[138].

Thielmann *et al.*[139] conducted the first single centre randomised, double blind controlled trial of RIC in 329 patients from 2008-2012. They found a significantly lower troponin level (cTnI) in the RIC group compared to the control group and the all-cause mortality was assessed over 1.54 (SD 1.22) years and was lower with remote ischaemic preconditioning than without (ratio 0.27, 95% CI 0.08-0.98, P = 0.046)[137]. Hong *et al.*[140] however failed to demonstrate any difference between the groups but it should be noted that he included RIC with PostC in 1280 patients and had a much broader composite of outcomes. Hong's group also failed to record any biomarkers, limiting the end-points to solely clinical outcomes.

Sachdeva *et al.*[141] noted no subsequent benefit in both remote preconditioning and postconditioning alone or in combination and observed that they failed to attenuate infarct size in an anesthetized rat model with myocardial infarction. There was also no recovery of LV dysfunction induced by ischemia-reperfusion injury.

However, the recently concluded randomized LipsiaConditioning[142] trial studied the effects of RIC and PostC revealed conflicting evidence to this. Using cardiac magnetic resonance to quantify myocardial injury, they showed that combined intra-hospital RIC and PostC significantly increases myocardial salvage when compared with conventional PCI, whereas PostC alone failed to demonstrate a cardioprotective effect in STEMI patients undergoing primary PCI.

Another article by Pichot *et al.*[143] however revealed PostC had a significant effect in reducing myocardial injury independently of traditional cardiovascular risk factors in patients with STEMI.

Ischaemic conditioning has garnered a lot of interest in recent times with almost 500 articles published every year, and 53 clinical trials (phase I to IV) available on PubMed. Of these 37 clinical trials are specific to cardiac surgery alone[144]. A lot of the RIC data in other studies have focused on biomarkers of cardiac injury and not outcomes, which were the endpoints for both RIPheart and ERICCA. To date, no adequately powered and randomised trial has looked at the effect of RIC and PostC in transplantation, and given the recent findings of large trials in CABGs, an adequately powered trial in transplant cannot be justified. The negative results have generated more discussion and questions with better discourse into methodology. For example, in Kottenberg *et al.*[145]'s study, propofol was a potential confounding factor. Propofol interferes with the activation of the signal transducer and activator of transcription 5 (STAT5) pathway. A recent study by Kleinbongard *et al.*[146] looking at confounders that may affect the efficacy of RIC found that patients with an aortic cross-clamp time of < 56 min had no protection by RIC whereas there was solid protection

by RIC at cross-clamp times of 57-75 min (ratio of RIC/control = 0.757; P = 0.0348) and of ≥ 76 min (ratio of RIC/control = 0.735; P = 0.0277)[146]. RIC theoretically confers protection against ischaemia but not trauma during surgery (cannulation/handling); thus making it plausible that in longer cross-clamp times, the protection conferred by RIC becomes more overt due to the effect of ischaemic/reperfusion injury. Another recent study what studied the effect of preconditioning with cyclosporine-A (which prevents MPTP opening at the onset of reperfusion, thereby also reducing the incidence of ischaemic-reperfusion injury[147] in patients undergoing elective CABG revealed similar findings, with the cardioprotective benefits noted at longer cross-clamp times (85-120 min) but not in the shorter (50-85 min) group[148].

Iyer *et al.*[149] utilised PostC in DCD hearts using a porcine asphyxia model and subjected them to warm ischaemic times of 20-40 min prior to flushing with Celsior solution. The solution was supplemented with erythropoietin[150], glyceryl trinitrate[151,152] and zoniporide (Cs)[153], a combination that activates ischaemic postconditioning pathways.

Hearts were assessed for functional, biochemical and metabolic recovery on an *ex-vivo* working heart apparatus. Hearts with postconditioning pathways activated demonstrated complete recovery up to 20-min of warm ischaemia time after which a rapid decline ensued.

CONCLUSION

There have been multiple recent advances in recent times with specific interest in myocardial protection. The search for biomarkers however continue to persist and may provide a gauge to quantify myocardial damage. Continuous normothermic organ perfusion remains an interesting prospect that allows transport and working assessment of the heart prior to transplantation. This has revolutionised DCD transplantation thus added more organs to the potential donor pool. Short term outcomes of DCD hearts have been good, however long-term outcomes need to be studied to allow widespread use. The role of ischaemic pre- and post-conditioning remain uncertain in the field of cardiac transplantation. A summation of myocardial protection strategies may be the way forward.

DECLARATIONS

Authors' contributions

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Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1 Ravishankar V. March of cardiac surgery through the decades. *Med J Armed Forces India* 2014;70:3-4.
2. Konstantinov IE. At the cutting edge of the impossible: a tribute to Vladimir P. Demikhov. *Tex Heart Inst J* 2009;36:453-8.
3. Lower RR, Stofer RC, Hurley EJ, Dong E Jr, Cohn RB, Shumway NE. Successful homotransplantation of the canine heart after anoxic preservation for seven hours. *Am J Surg* 1962;104:302-6.
4. Dong E Jr, Hurley EJ, Lower RR, Shumway NE. Performance of the heart two years after autotransplantation. *Surgery* 1964;56:270-4.
5. Shumway NE, Lower RR. Special problems in transplantation of the heart. *Ann N Y Acad Sci* 1964;120:773-7.
6. Barnard CN. The operation. A human cardiac transplant: an interim report of a successful operation performed at Groote Schuur Hospital, Cape Town. *S Afr Med J* 1967;41:1271-4.
7. Lansman SL, Ergin MA, Griepp RB. The history of heart and heart-lung transplantation. *Cardiovasc Clin* 1990;20:3-19.
8. Stehlik J, Edwards LB, Kucheryavaya AY, Aurora P, Christie JD, Kirk R, Dobbels F, Rahmel AO, Hertz MI. The registry of the international society for heart and lung transplantation: twenty-seventh official adult heart transplant report--2010. *J Heart Lung Transplant* 2010;29:1089-103.
9. Lietz K, Miller LW. Improved survival of patients with end-stage heart failure listed for heart transplantation: analysis of organ procurement and transplantation network/U.S. United Network of Organ Sharing data, 1990 to 2005. *J Am Coll Cardiol* 2007;50:1282-90.
10. Abuanzeh R, Hashmi F, Dimarakis I, Khasati N, Machaal A, Yonan N, Venkateswaran RV. Early donor management increases the retrieval rate of hearts for transplantation in marginal donors. *Eur J Cardiothorac Surg* 2015;47:72-7; discussion 77.
11. Ringer S. A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. *J Physiol* 1883;4:29-42.
12. Tyers GF, Todd GJ, Niebauer IM, Manley NJ, Waldhausen JA.. The mechanism of myocardial damage following potassium citrate (Melrose) cardioplegia. *Surgery* 1975;78:45-53.
13. Maathuis MH, Leuvenink HG, Ploeg RJ. Perspectives in organ preservation. *Transplantation* 2007;83:1289-98.
14. tHart NA, Leuvenink HGD, Ploeg RJ. New solutions in organ preservation. Available from: http://www.sciencedirect.com/science/article/pii/S0955470X02800027. [Last accessed on 20 Oct 2017]
15. Shumway NE, Lower RR. Topical cardiac hypothermia for extended periods of anoxic arrest. *Surg Forum* 1960;10:563-6.

16. Blaisdell FW, Cooley DA. The mechanism of paraplegia after temporary thoracic aortic occlusion and its relationship to spinal fluid pressure. *Surgery* 1962;51:351-5.
17. Kay EB, Head LR, Nogueira C. Direct coronary artery perfusion for aortic valve surgery; report of technique. *J Am Med Assoc* 1958;168:1767-8.
18. Senning A. Ventricular fibrillation during hypothermia, used as a method to facilitate intracardiac operations. *Acta Chir Scand* 1955;109:303-9.
19. Bretschneider HJ, Hübner G, Knoll D, Lohr B, Nordbeck H, Spieckermann PG. Myocardial resistance and tolerance to ischemia: physiological and biochemical basis. *J Cardiovasc Surg (Torino)* 1975;16:241-60.
20. Hearse DJ, Stewart DA, Braimbridge MV. Cellular protection during myocardial ischemia: the development and characterization of a procedure for the induction of reversible ischaemic arrest. *Circulation* 1976;54:193-202.
21. Buckberg GD. Recent progress in myocardial protection during cardiac operations. *Cardiovasc Clin* 1987;17:291-319.
22. Hearse DJ, Humphrey SM, Nayler WG, Slade A, Border D. Ultrastructural damage associated with reoxygenation of the anoxic myocardium. *J Mol Cell Cardiol* 1975;7:315-24.
23. Chambers DJ, Fallouh HB. Cardioplegia and cardiac surgery: pharmacological arrest and cardioprotection during global ischemia and reperfusion. *Pharmacol Ther* 2010;127:41-52.
24. Reichenspurner H, Russ C, Uberfuhr P, Nollert G, Schlüter A, Reichart B, Klövekorn WP, Schüler S, Hetzer R, Brett W. Myocardial preservation using HTK solution for heart transplantation. A multicenter study. *Eur J Cardiothorac Surg* 1993;7:414-9.
25. Sung SY, Lin CY, Song JY, Tsai YT, Kao CH, Lee CY, Lin YC, Hsu PS, Tsai CS. Myocardial protection in donor heart preservation: a comparison between Bretschneider's histidine-tryptophan-ketoglutarate solution and cold blood cardioplegia. *Transplant Proc* 2014;46:1077-81.
26. Minami K, Omoto T, Böthig D, Tenderich G, Wlost S, Schütt U, Körfer R. Creatine kinase and troponin after myocardial preservation using HTK solution (Custoidol) for clinical heart transplantation. *J Heart Lung Transplant* 2003;22:192-4.
27. Larsen M, Webb G, Kennington S, Kelleher N, Sheppard J, Kuo J, Unsworth-White J. Mannitol in cardioplegia as an oxygen free radical scavenger measured by malondialdehyde. *Perfusion* 2002;17:51-5.
28. Cordell AR. Milestones in the development of cardioplegia. *Ann Thorac Surg* 1995;60:793-6.
29. Chambers DJ, Sakai A, Braimbridge MV, Kosker S, Manzanera G, Kind PR, Jupp RA, Smith LD, Slavin B.Clinical validation of St. Thomas' hospital cardioplegic solution No. 2 (Plegisol). *Eur J Cardiothorac Surg* 1989;3:346-52.
30. Luciani GB, Faggian G, Montalbano G, Casali G, Forni A, Chiominto B, Mazzucco A. Blood versus crystalloid cardioplegia for myocardial protection of donor hearts during transplantation: a prospective, randomized clinical trial. *J Thorac Cardiovasc Surg* 1999;118:787-95.
31. Luciani GB, Forni A, Rigatelli G, Chiominto B, Cardaioli P, Mazzucco A, Faggian G. Myocardial protection in heart transplantation using blood cardioplegia: 12-year outcome of a prospective randomized trial. *J Heart Lung Transplant* 2011;30:29-36.
32. Collins GM, Hartley LC, Clunie GJ. Kidney preservation for transportation. Experimental analysis of optimal perfusate composition. *Br J Surg* 1972;59:187-9.
33. Andrews PM, Bates SB. Improving Euro-Collins flushing solution's ability to protect kidneys from normothermic ischemia. *Miner Electrolyte Metab* 1985;11:309-13.
34. Hendry PJ, Labow RS, Keon WJ. A comparison of intracellular solutions for donor heart preservation. *J Thorac Cardiovasc Surg* 1993;105:667-73.
35. James H. Southard MDa, Folkert O. Belzer MD. ORGAN PRESERVATION. *Ann Rev Med* 1995;46:235-47.
36. Ploeg RJ, Goossens D, McAnulty JF, Southard JH, Belzer FO. Successful 72-hour cold storage of dog kidneys with UW solution. *Transplantation* 1988;46:191-6.
37. Todo S, Nery J, Yanaga K, Podesta L, Gordon RD, Starzl TE. Extended preservation of human liver grafts with UW solution. *JAMA* 1989;261:711-4.
38. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DG, Southard JH, Belzer FO. Preservation of the canine liver for 24-48 hours using simple cold storage with UW solution. *Transplantation* 1988;46:517-22.
39. Kozlova I, Khalid Y, Roomans GM. Preservation of mouse liver tissue during cold storage in experimental solutions assessed by x-ray microanalysis. *Liver Transpl* 2003;9:268-78.
40. Vreugdenhil PK, Belzer FO, Southard JH. Effect of cold storage on tissue and cellular glutathione. *Cryobiology* 1991;28:143-9.
41. Biguzas M, Jablonski P, Howden BO, Thomas AC, Walls K, Scott DF, Marshall VC. Evaluation of UW solution in rat kidney preservation. II. The effect of pharmacological additives. *Transplantation* 1990;49:1051-5.
42. Kur F, Beiras-Fernandez A, Meiser B, Uberfuhr P, Reichart B. Clinical heart transplantation with extended preservation time (>5 hours): experience with University of Wisconsin solution. *Transplant Proc* 2009;41:2247-9.
43. Jeevanandam V, Barr ML, Auteri JS, Sanchez JA, Fong J, Schenkel FA, Marboe CC, Michler RE, Smith CR, Rose EA.University of Wisconsin solution versus crystalloid cardioplegia for human donor heart preservation. A randomized blinded prospective clinical trial. *J Thorac Cardiovasc Surg* 1992;103:194-8; discussion 198-9.
44. Mankad P, Slavik Z, Yacoub M. Endothelial dysfunction caused by University of Wisconsin preservation solution in the rat heart. The importance of temperature. *J Thorac Cardiovasc Surg* 1992;104:1618-24.
45. Saitoh Y, Hashimoto M, Ku K, Kin S, Nosaka S, Masumura S, Nakayama K. Heart preservation in HTK solution: role of coronary vasculature in recovery of cardiac function. *Ann Thorac Surg* 2000;69:107-12.
46. De Santo LS, Amarelli C, Romano G, Della Corte A, Maiello C, Giannolo B, Marra C, De Feo M, Scardone M, Cotrufo M. High-risk heart grafts: effective preservation with Celsior solution. *Heart Vessels* 2006;21:89-94.
47. Careaga G, Salazar D, Téllez S, Sánchez O, Borrayo G, Argüero R. Clinical impact of histidine-ketoglutarate-tryptophan (HTK) cardioplegic solution on the perioperative period in open heart surgery patients. *Arch Med Res* 2001;32:296-9.
48. Lee KC, Chang CY, Chuang YC, Sue SH, Yang HS, Weng CF, Lee YT, Huang WS, Chen IC, Wei J.Combined St. Thomas and histidine-tryptophan-ketoglutarat solutions for myocardial preservation in heart transplantation patients. *Transplant Proc* 2012;44:886-9.
49. Careaga G, Salazar D, Téllez S, Sánchez O, Borrayo G, Argüero R.Clinical impact of histidine-ketoglutarate-tryptophan (HTK) cardioplegic solution on the perioperative period in open heart surgery patients. *Arch Med Res* 2001;32:296-9.
50. Vega JD, Ochsner JL, Jeevanandam V, McGiffin DC, McCurry KR, Mentzer RM Jr, Stringham JC, Pierson RN 3rd, Frazier OH, Menkis AH, Staples ED, Modry DL, Emery RW, Piccione W Jr, Carrier M, Hendry PJ, Aziz S, Furukawa S, Pham SM. A multicenter, randomized, controlled trial of Celsior for flush and hypothermic storage of cardiac allografts. *Ann Thorac Surg* 2001;71:1442-7.
51. Wieselthaler GM, Chevtchik O, Konetschny R, Moidl R, Mallinger R, Mares P, Griessmacher A, Grimm M, Wolner E, Laufer G. Improved graft function using a new myocardial preservation solution: Celsior. Preliminary data from a randomized prospective study. *Transplant Proc* 1999;31:2067-8.
52. Cannata A, Botta L, Colombo T, Russo CF, Taglieri C, Bruschi G,

Merlanti B, Frigerio M, Martinelli L. Does the cardioplegic solution have an effect on early outcomes following heart transplantation? *Eur J Cardiothorac Surg* 2012;41:e48-52; discussion e52-3.
53. George TJ, Arnaoutakis GJ, Beaty CA, Shah AS, Conte JV, Halushka MK. A novel method of measuring cardiac preservation injury demonstrates University of Wisconsin solution is associated with less ischaemic necrosis than Celsior in early cardiac allograft biopsy specimens. *J Heart Lung Transplant* 2012;31:410-8.
54. George TJ, Arnaoutakis GJ, Baumgartner WA, Shah AS, Conte JV. Organ storage with University of Wisconsin solution is associated with improved outcomes after orthotopic heart transplantation. *J Heart Lung Transplant* 2011;30:1033-43.
55. Kofler S, Bigdeli AK, Kaczmarek I, Kellerer D, Müller T, Schmoeckel M, Steinbeck G, Uberfuhr P, Reichart B, Meiser B. Long-term outcomes after 1000 heart transplantations in six different eras of innovation in a single center. *Transpl Int* 2009;22:1140-50.
56. Garlicki M, Kołcz J, Rudziński P, Kapelak B, Sadowski J, Wójcik S, Pietrzyk E, Frasik W, Drukała J, Dziatkowiak A. Myocardial protection for transplantation. *Transplant Proc* 1999;31:2079-83.
57. Lee S, Huang CS, Kawamura T, Shigemura N, Billiar TR, Nakao A, Toyoda Y. Histidine-tryptophan-ketoglutarate or celsior: which is more suitable for cold preservation for cardiac grafts from older donors? *Ann Thorac Surg* 2011;91:755-63.
58. Ackemann J, Gross W, Mory M, Schaefer M, Gebhard MM. Celsior versus custodiol: early postischemic recovery after cardioplegia and ischemia at 5 degrees C. *Ann Thorac Surg* 2002;74:522-9.
59. Lurati Buse GA, Koller MT, Grapow M, Bolliger D, Seeberger M, Filipovic M. The prognostic value of troponin release after adult cardiac surgery - a meta-analysis. *Eur J Cardiothorac Surg* 2010;37:399-406.
60. De Santo LS, Torella M, Romano G, Maiello C, Buonocore M, Bancone C, Della Corte A, Galdieri N, Nappi G, Amarelli C. Perioperative myocardial injury after adult heart transplant: determinants and prognostic value. *PLoS One* 2015;10:e0120813.
61. Gross GJ, Kersten JR, Warltier DC. Mechanisms of postischemic contractile dysfunction. *Ann Thorac Surg* 1999;68:1898-904.
62. Dengler TJ, Gleissner CA, Klingenberg R, Sack FU, Schnabel PA, Katus HA. Biomarkers after heart transplantation: nongenomic. *Heart Fail Clin* 2007;3:69-81.
63. Masuda M, Morita S, Tomita H, Kurisu K, Nishida T, Tominaga R, Yasui H. Off-pump CABG attenuates myocardial enzyme leakage but not postoperative brain natriuretic peptide secretion. *Ann Thorac Cardiovasc Surg* 2002;8:139-44.
64. Hajjar L, Galas F, Grande S, Campos M, Silva R, Marques A, Leao W, Auler Jr J. Plasma brain natriuretic peptide and cardiac troponin I concentrations after adult cardiac surgery: association with cardiovascular death, postoperative cardiac, renal and pulmonary dysfunction. Available from: https://ccforum.biomedcentral.com/track/pdf/10.1186/cc6665. [Last accessed on 20 Oct 2017]
65. McIlroy DR, Wallace S, Roubos N. Brain natriuretic peptide (BNP) as a biomarker of myocardial ischemia-reperfusion injury in cardiac transplantation. *J Cardiothorac Vasc Anesth* 2010;24:939-45.
66. Cavarretta E, Frati G. MicroRNAs in coronary heart disease: ready to enter the clinical arena? *Biomed Res Int* 2016;2016:2150763.
67. Wang E, Nie Y, Zhao Q, Wang W, Huang J, Liao Z, Zhang H, Hu S, Zheng Z. Circulating miRNAs reflect early myocardial injury and recovery after heart transplantation. *J Cardiothorac Surg* 2013;8:165.
68. Russo MJ, Chen JM, Sorabella RA, Martens TP, Garrido M, Davies RR, George I, Cheema FH, Mosca RS, Mital S, Ascheim DD, Argenziano M, Stewart AS, Oz MC, Naka Y. The effect of ischaemic time on survival after heart transplantation varies by donor age: an analysis of the United Network for organ sharing database. *J Thorac Cardiovasc Surg* 2007;133:554-9.
69. Goldsmith KA, Demiris N, Gooi JH, Sharples LD, Jenkins DP, Dhital KK, Tsui SS. Life-years gained by reducing donor heart ischaemic times. *Transplantation* 2009;87:243-8.
70. Hassanein WH, Zellos L, Tyrrell TA, Healey NA, Crittenden MD, Birjiniuk V, Khuri SF. Continuous perfusion of donor hearts in the beating state extends preservation time and improves recovery of function. *J Thorac Cardiovasc Surg* 1998;116:821-30.
71. Messer S, Ardehali A, Tsui S. Normothermic donor heart perfusion: current clinical experience and the future. *Transpl Int* 2015;28:634-42.
72. Tenderich G, El-Banayosy A, Rosengard B, Tsui S, Wallwork J, Hetzer R, Koerfer R, Hassanein W. 10: Prospective multi-center European trial to evaluate the safety and performance of the Organ Care System for heart transplants (PROTECT). Available from: http://www.jhltonline.org/article/S1053-2498(06)00824-2/fulltext. [Last accessed on 20 Oct 2017]
73. McCurry K, Jeevanandam V, Mihaljevic T, Couper G, Elanwar M, Saleh H, Ardehali A. 294: Prospective Multi-center safety and effectiveness evaluation of the Organ Care System device for cardiac use (PROCEED). Available from: http://www.jhltonline.org/article/S1053-2498(07)01142-4/abstract. [Last accessed on 20 Oct 2017]
74. Hamed A, Tsui S, Huber J, Lin R, Poggio EC, Ardehali A. 19: Serum lactate is a highly sensitive and specific predictor of post cardiac transplant outcomes using the Organ Care System. *J Heart Lung Transplant* 2009;28:S71.
75. Ardehali A, Esmailian F, Deng M, Soltesz E, Hsich E, Naka Y, Mancini D, Camacho M, Zucker M, Leprince P, Padera R, Kobashigawa J; PROCEED II trial investigators. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. *Lancet* 2015;385:2577-84.
76. Daemen JW, Kootstra G, Wijnen RM, Yin M, Heineman E. Nonheart-beating donors: the Maastricht experience. *Clin Transpl* 1994:303-16.
77. Iyer A, Gao L, Doyle A, Rao P, Cropper JR, Soto C, Dinale A, Kumarasinghe G, Jabbour A, Hicks M, Jansz PC, Feneley MP, Harvey RP, Graham RM, Dhital KK, MacDonald PS. Normothermic ex vivo perfusion provides superior organ preservation and enables viability assessment of hearts from DCD donors. *Am J Transplant* 2015;15:371-80.
78. Dhital KK, Iyer A, Connellan M, Chew HC, Gao L, Doyle A, Hicks M, Kumarasinghe G, Soto C, Dinale A, Cartwright B, Nair P, Granger E, Jansz P, Jabbour A, Kotlyar E, Keogh A, Hayward C, Graham R, Spratt P, Macdonald P. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. *Lancet* 2015;385:2585-91.
79. Boucek MM, Mashburn C, Dunn SM, Frizell R, Edwards L, Pietra B, Campbell D; Denver Children's Pediatric Heart Transplant Team. Pediatric heart transplantation after declaration of cardiocirculatory death. *N Engl J Med* 2008;359:709-14.
80. Walsh F. Europe's first non-beating heart transplant. Available from: http://www.bbc.com/news/health-32056350. [Last accessed on 20 Oct 2017]
81. Truog RD, Miller FG, Halpern SD. The dead-donor rule and the future of organ donation. *N Engl J Med* 2013;369:1287-9.
82. Ullah S, Zabala L, Watkins B, Schmitz ML. Cardiac organ donor management. *Perfusion* 2006;21:93-8.
83. Cuisinier A, Maufrais C, Payen JF, Nottin S, Walther G, Bouzat P. Myocardial function at the early phase of traumatic brain injury: a prospective controlled study. *Scand J Trauma Resusc Emerg Med* 2016;24:129.
84. Novitzky D, Rhodin J, Cooper DK, Ye Y, Min KW, DeBault L. Ultrastructure changes associated with brain death in the human donor heart. *Transpl Int* 1997;10:24-32.
85. Kilic A, Emani S, Sai-Sudhakar CB, Higgins RS, Whitson BA. Donor selection in heart transplantation. *J Thorac Dis* 2014;6:1097-104.
86. Lee H, Cho YH, Sung K, Yang JH, Chung CR, Jeon K, Suh GY. The use of extracorporeal circulation in suspected brain dead organ donors

with cardiopulmonary collapse. *J Korean Med Sci* 2015;30:1911-4.
87. Cooper LB, Milano CA, Williams M, Swafford W, Croezen D, Van Bakel AB, Rogers JG, Patel CB. Thyroid hormone use during cardiac transplant organ procurement. *Clin Transplant* 2016;30:1578-83.
88. Novitzky D, Cooper DK, Rosendale JD, Kauffman HM. Hormonal therapy of the brain-dead organ donor: experimental and clinical studies. *Transplantation* 2006;82:1396-401.
89. Novitzky D, Cooper DK, Wicomb W. Hormonal therapy to the brain-dead potential organ donor: the misnomer of the "Papworth cocktail". *Transplantation* 2008;86:1479.
90. Wheeldon DR, Potter CD, Oduro A, Wallwork J, Large SR. Transforming the "unacceptable" donor: outcomes from the adoption of a standardized donor management technique. *J Heart Lung Transplant* 1995;14:734-42.
91. Wojda TR, Stawicki SP, Yandle KP, Bleil M, Axelband J, Wilde-Onia R, Thomas PG, Cipolla J, Hoff WS, Shultz J. Keys to successful organ procurement: an experience-based review of clinical practices at a high-performing health-care organization. *Int J Crit Illn Inj Sci* 2017;7:91-100.
92. Hahnenkamp K, Böhler K, Wolters H, Wiebe K, Schneider D, Schmidt HH. Organ-protective intensive care in organ donors. *Dtsch Arztebl Int* 2016;113:552-8.
93. Nicolas-Robin A, Barouk JD, Amour J, Coriat P, Riou B, Langeron O. Hydrocortisone supplementation enhances hemodynamic stability in brain-dead patients. *Anesthesiology* 2010;112:1204-10.
94. Novitzky D, Cooper DK, Muchmore JS, Zuhdi N. Pituitary function in brain-dead patients. *Transplantation* 1989;48:1078-9.
95. Barnes PJ. Beta-adrenergic receptors and their regulation. *Am J Respir Crit Care Med* 1995;152:838-60.
96. Clemente G, Tuttolomondo A, Colomba D, Pecoraro R, Renda C, Della Corte V, Maida C, Simonetta I, Pinto A. When sepsis affects the heart: a case report and literature review. *World J Clin Cases* 2015;3:743-50.
97. McLean KM, Duffy JY, Pandalai PK, Lyons JM, Bulcao CF, Wagner CJ, Akhter SA, Pearl JM. Glucocorticoids alter the balance between pro- and anti-inflammatory mediators in the myocardium in a porcine model of brain death. *J Heart Lung Transplant* 2007;26:78-84.
98. Guglin M. How to increase the utilization of donor hearts? *Heart Fail Rev* 2015;20:95-105.
99. Pandit RA, Zirpe KG, Gurav SK, Kulkarni AP, Karnath S, Govil D, Abhram B, Mehta Y, Gupta A, Hegde A, Patil V, Bhatacharya P, Dixit S, Samavedan S, Todi S. Management of potential organ donor: Indian society of critical care medicine: position statement. *Indian J Crit Care Med* 2017;21:303-16.
100. Pinsard M, Ragot S, Mertes PM, Bleichner JP, Zitouni S, Cook F, Pierrot M, Dube L, Menguy E, Lefèvre LM, Escaravage L, Dequin PF, Vignon P, Pichon N. Interest of low-dose hydrocortisone therapy during brain-dead organ donor resuscitation: the CORTICOME study. *Crit Care* 2014;18:R158.
101. Dimopoulou I, Tsagarakis S, Anthi A, Milou E, Ilias I, Stavrakaki K, Charalambidis C, Tzanela M, Orfanos S, Mandragos K, Thalassinos N, Roussos C. High prevalence of decreased cortisol reserve in brain-dead potential organ donors. *Crit Care Med* 2003;31:1113-7.
102. Dhar R, Cotton C, Coleman J, Brockmeier D, Kappel D, Marklin G, Wright R. Comparison of high- and low-dose corticosteroid regimens for organ donor management. *J Crit Care* 2013;28:111.e1-7.
103. Novitzky D, Cooper DK, Chaffin JS, Greer AE, DeBault LE, Zuhdi N. Improved cardiac allograft function following triiodothyronine therapy to both donor and recipient. *Transplantation* 1990;49:311-6.
104. Chen YF, Kobayashi S, Chen J, Redetzke RA, Said S, Liang Q, Gerdes AM. Short term triiodo-L-thyronine treatment inhibits cardiac myocyte apoptosis in border area after myocardial infarction in rats. *J Mol Cell Cardiol* 2008;44:180-7.
105. Jeevanandam V, Todd B, Regillo T, Hellman S, Eldridge C, McClurken J. Reversal of donor myocardial dysfunction by triiodothyronine replacement therapy. *J Heart Lung Transplant* 1994;13:681-7; discussion 685-7.
106. Novitzky D, Mi Z, Sun Q, Collins JF, Cooper DK. Thyroid hormone therapy in the management of 63,593 brain-dead organ donors: a retrospective analysis. *Transplantation* 2014;98:1119-27.
107. Macdonald PS, Aneman A, Bhonagiri D, Jones D, O'Callaghan G, Silvester W, Watson A, Dobb G. A systematic review and meta-analysis of clinical trials of thyroid hormone administration to brain dead potential organ donors. *Crit Care Med* 2012;40:1635-44.
108. Venkateswaran RV, Steeds RP, Quinn DW, Nightingale P, Wilson IC, Mascaro JG, Thompson RD, Townend JN, Bonser RS. The haemodynamic effects of adjunctive hormone therapy in potential heart donors: a prospective randomized double-blind factorially designed controlled trial. *Eur Heart J* 2009;30:1771-80.
109. Novitzky D, Wicomb WN, Cooper DK, Tjaalgard MA. Improved cardiac function following hormonal therapy in brain dead pigs: relevance to organ donation. *Cryobiology* 1987;24:1-10.
110. Votapka TV, Canvasser DA, Pennington DG, Koga M, Swartz MT. Effect of triiodothyronine on graft function in a model of heart transplantation. *Ann Thorac Surg* 1996;62:78-82.
111. Hing AJ, Hicks M, Garlick SR, Gao L, Kesteven SH, Faddy SC, Wilson MK, Feneley MP, Macdonald PS. The effects of hormone resuscitation on cardiac function and hemodynamics in a porcine brain-dead organ donor model. *Am J Transplant* 2007;7:809-17.
112. Zimmerman EA, Nilaver G, Hou-Yu A, Silverman AJ. Vasopressinergic and oxytocinergic pathways in the central nervous system. *Fed Proc* 1984;43:91-6.
113. Yoshioka T, Sugimoto H, Uenishi M, Sakamoto T, Sadamitsu D, Sakano T, Sugimoto T. Prolonged hemodynamic maintenance by the combined administration of vasopressin and epinephrine in brain death: a clinical study. *Neurosurgery* 1986;18:565-7.
114. Blaine EM, Tallman RD Jr, Frolicher D, Jordan MA, Bluth LL, Howie MB. Vasopressin supplementation in a porcine model of brain-dead potential organ donors. *Transplantation* 1984;38:459-64.
115. Rostron AJ, Avlonitis VS, Cork DM, Grenade DS, Kirby JA, Dark JH. Hemodynamic resuscitation with arginine vasopressin reduces lung injury after brain death in the transplant donor. *Transplantation* 2008;85:597-606.
116. Chen JM, Cullinane S, Spanier TB, Artrip JH, John R, Edwards NM, Oz MC, Landry DW. Vasopressin deficiency and pressor hypersensitivity in hemodynamically unstable organ donors. *Circulation* 1999;100:II244-6.
117. Pennefather SH, Bullock RE, Mantle D, Dark JH. Use of low dose arginine vasopressin to support brain-dead organ donors. *Transplantation* 1995;59:58-62.
118. Kinoshita Y, Yahata K, Yoshioka T, Onishi S, Sugimoto T. Long-term renal preservation after brain death maintained with vasopressin and epinephrine. *Transpl Int* 1990;3:15-8.
119. Papadopoulos G, Sintou E, Siminelakis S, Koletsis E, Baikoussis NG, Apostolakis E. Perioperative infusion of low- dose of vasopressin for prevention and management of vasodilatory vasoplegic syndrome in patients undergoing coronary artery bypass grafting-A double-blind randomized study. *J Cardiothorac Surg* 2010;5:17.
120. Calva E, Mújica A, Núñez R, Aoki K, Bisteni A, Sodi-Pallares D. Mitochondrial biochemical changes and glucose-KCl-insulin solution in cardiac infarct. *Am J Physiol* 1966;211:71-6.
121. Opie LH, Bruyneel K, Owen P. Effects of glucose, insulin and potassium infusion on tissue metabolic changes within first hour of myocardial infarction in the baboon. *Circulation* 1975;52:49-57.
122. Sodi-pallares D, Testelli MR, Fishleder BL, Bisteni A, Medrano GA, Friedland C, De Micheli A. Effects of an intravenous infusion of a potassium-glucose-insulin solution on the electrocardiographic signs of myocardial infarction. A preliminary clinical report. *Am J Cardiol* 1962;9:166-81.

123. Fath-Ordoubadi F, Beatt KJ. Glucose-insulin-potassium therapy for treatment of acute myocardial infarction: an overview of randomized placebo-controlled trials. *Circulation* 1997;96:1152-6.
124. Sun SC, Appleyard R, Masetti P, Byrne JG, Laurence RG, Marsh JD, Cohn LH. Improved recovery of heart transplants by combined use of oxygen-derived free radical scavengers and energy enhancement. *J Thorac Cardiovasc Surg* 1992;104:830-7.
125. Pilati CF, Bosso FJ, Maron MB. Factors involved in left ventricular dysfunction after massive sympathetic activation. *Am J Physiol* 1992;263:H784-91.
126. Szabo G, Buhmann V, Bahrle S, Vahl CF, Hagl S. Brain death impairs coronary endothelial function. *Transplantation* 2002;73:1846-8.
127. Nicolas-Robin A, Amour J, Ibanez-Esteve C, Coriat P, Riou B, Langeron O. Effect of glucose-insulin-potassium in severe acute heart failure after brain death. *Crit Care Med* 2008;36:2740-5.
128. Heusch G, Schulz R, Rahimtoola SH. Myocardial hibernation: a delicate balance. *Am J Physiol Heart Circ Physiol* 2005;288:H984-99.
129. Stanley AW Jr, Moraski RE, Russell RO, Rogers WJ, Mantle JA, Kreisberg RA, McDaniel HG, Rackley CE. Effects of glucose-insulin-potassium on myocardial substrate availability and utilization in stable coronary artery disease. Studies on myocardial carbohydrate, lipid and oxygen arterial-coronary sinus differences in patients with coronary artery disease. *Am J Cardiol* 1975;36:929-37.
130. Taegtmeyer H, Goodwin GW, Doenst T, Frazier OH. Substrate metabolism as a determinant for postischemic functional recovery of the heart. *Am J Cardiol* 1997;80: A3-10.
131. Cottin Y, Lhuillier I, Gilson L, Zeller M, Bonnet C, Toulouse C, Louis P, Rochette L, Girard C, Wolf JE. Glucose insulin potassium infusion improves systolic function in patients with chronic ischaemic cardiomyopathy. *Eur J Heart Fail* 2002;4:181-4.
132. Khoury VK, Haluska B, Prins J, Marwick TH. Effects of glucose-insulin-potassium infusion on chronic ischaemic left ventricular dysfunction. *Heart* 2003;89:61-5.
133. Demircan S, Yazici M, Diraman E, Demircan G, Kilicaslan F, Durna K, Acar Z, Eren Z. The effect of glucose-insulin-potassium treatment on myocardial oxidative stress in patients with acute coronary syndromes undergoing percutaneous coronary intervention. *Coron Artery Dis* 2008;19:99-104.
134. Kalay N, Ozdogru I, Gul A, Yucel Y, Cetinkaya Y, Inanc MT, Dogan A, Kaya MG, Eryol NK. Effects of intermittent and long-term glucose-insulin-potassium infusion in patients with systolic heart failure. *Exp Clin Cardiol* 2008;13:85-8.
135. Klein LJ, van Campen CM, Sieswerda GT, Kamp O, Visser FC. Effects of high-dose insulin infusion on left ventricular function in normal subjects. *Neth Heart J* 2010;18:183-9.
136. Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, Stoppe C, Coburn M, Schaelte G, Böning A, Niemann B, Roesner J, Kletzin F, Strouhal U, Reyher C, Laufenberg-Feldmann R, Ferner M, Brandes IF, Bauer M, Stehr SN, Kortgen A, Wittmann M, Baumgarten G, Meyer-Treschan T, Kienbaum P, Heringlake M, Schön J, Sander M, Treskatsch S, Smul T, Wolwender E, Schilling T, Fuernau G, Hasenclever D, Zacharowski K; RIPHeart Study Collaborators. A multicenter trial of remote ischaemic preconditioning for heart surgery. *N Engl J Med* 2015;373:1397-407.
137. Hausenloy DJ, Candilio L, Evans R, Ariti C, Jenkins DP, Kolvekar S, Knight R, Kunst G, Laing C, Nicholas J, Pepper J, Robertson S, Xenou M, Clayton T, Yellon DM; ERICCA Trial Investigators. Remote ischaemic preconditioning and outcomes of cardiac surgery. *N Engl J Med* 2015;373:1408-17.
138. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischaemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 1993;87:893-9.
139. Thielmann M, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, Pasa S, Price V, Tsagakis K, Neuhäuser M, Peters J, Jakob H, Heusch G. Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial. *Lancet* 2013;382:597-604.
140. Hong DM, Lee EH, Kim HJ, Min JJ, Chin JH, Choi DK, Bahk JH, Sim JY, Choi IC, Jeon Y. Does remote ischaemic preconditioning with postconditioning improve clinical outcomes of patients undergoing cardiac surgery? Remote ischaemic preconditioning with postconditioning outcome trial. *Eur Heart J* 2014;35:176-83.
141. Sachdeva J, Dai W, Gerczuk PZ, Kloner RA. Combined remote perconditioning and postconditioning failed to attenuate infarct size and contractile dysfunction in a rat model of coronary artery occlusion. *J Cardiovasc Pharmacol Ther* 2014;19:567-73.
142. Eitel I, Stiermaier T, Rommel KP, Fuernau G, Sandri M, Mangner N, Linke A, Erbs S, Lurz P, Boudriot E, Mende M, Desch S, Schuler G, Thiele H. Cardioprotection by combined intrahospital remote ischaemic perconditioning and postconditioning in ST-elevation myocardial infarction: the randomized lipsia conditioning trial. *Eur Heart J* 2015;36:3049-57.
143. Pichot S, Mewton N, Bejan-Angoulvant T, Roubille F, Rioufol G, Giraud C, Boussaha I, Lairez O, Elbaz M, Piot C, Angoulvant D, Ovize M. Influence of cardiovascular risk factors on infarct size and interaction with mechanical ischaemic postconditioning in ST-elevation myocardial infarction. *Open Heart* 2015;2:e000175.
144. Moscarelli M, Angelini GD, Suleiman S, Fiorentino F, Punjabi PP. Remote ischaemic preconditioning: is it a flag on the field? *Perfusion* 2015;30:438-47.
145. Kottenberg E, Musiolik J, Thielmann M, Jakob H, Peters J, Heusch G. Interference of propofol with signal transducer and activator of transcription 5 activation and cardioprotection by remote ischaemic preconditioning during coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 2014;147:376-82.
146. Kleinbongard P, Neuhäuser M, Thielmann M, Kottenberg E, Peters J, Jakob H, Heusch G. Confounders of cardioprotection by remote ischaemic preconditioning in patients undergoing coronary artery bypass grafting. *Cardiology* 2016;133:128-33.
147. Hausenloy DJ, Boston-Griffiths EA, Yellon DM. Cyclosporin A and cardioprotection: from investigative tool to therapeutic agent. *Br J Pharmacol* 2012;165:1235-45.
148. Hausenloy DJ, Kunst G, Boston-Griffiths E, Kolvekar S, Chaubey S, John L, Desai J, Yellon D. The effect of cyclosporin-A on peri-operative myocardial injury in adult patients undergoing coronary artery bypass graft surgery: a randomised controlled clinical trial. *Heart* 2014;100:544-9.
149. Iyer A, Gao L, Doyle A, Rao P, Jayewardene D, Wan B, Kumarasinghe G, Jabbour A, Hicks M, Jansz PC, Feneley MP, Harvey RP, Graham RM, Dhital KK, Macdonald PS. Increasing the tolerance of DCD hearts to warm ischemia by pharmacological postconditioning. *Am J Transplant* 2014;14:1744-52.
150. Watson AJ, Gao L, Sun L, Tsun J, Jabbour A, Ru Qiu M, Jansz PC, Hicks M, Macdonald PS. Enhanced preservation of the rat heart after prolonged hypothermic ischemia with erythropoietin-supplemented Celsior solution. *J Heart Lung Transplant* 2013;32:633-40.
151. Trahanas JM, Witer LJ, Alghanem F, Bryner BS, Iyengar A, Hirschl JR, Hoenerhoff MJ, Potkay JA, Bartlett RH, Rojas-Pena A, Owens GE, Bocks ML. Achieving 12 hour normothermic ex situ heart perfusion: an experience of 40 porcine hearts. *ASAIO J* 2016;62:470-6.
152. Hing AJ, Watson A, Hicks M, Gao L, Faddy SC, McMahon AC, Kesteven SH, Wilson MK, Jansz P, Feneley MP, Macdonald PS. Combining cariporide with glyceryl trinitrate optimizes cardiac preservation during porcine heart transplantation. *Am J Transplant* 2009;9:2048-56.
153. Gao L, Tsun J, Sun L, Kwan J, Watson A, Macdonald PS, Hicks M. Critical role of the STAT3 pathway in the cardioprotective efficacy of zoniporide in a model of myocardial preservation - the rat isolated working heart. *Br J Pharmacol* 2011;162:633-47.

Editor's note

There is a correction to this article.
It is available http://vpjournal.net/article/view/2861

Martínez *et al. Vessel Plus* 2017;1:230-41
DOI: 10.20517/2574-1209.2017.34

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Review **Open Access**

Energetic metabolism in cardiomyocytes: molecular basis of heart ischemia and arrhythmogenesis

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How to cite this article: Martínez MS, García A, Luzardo E, Chávez-Castillo M, Olivar LC, Salazar J, Velasco M, Rojas Quintero JJ, Bermúdez V. Energetic metabolism in cardiomyocytes: molecular basis of heart ischemia and arrhythmogenesis. *Vessel Plus* 2017;1:230-41.

Article history:
Received: 19 Oct 2017
First Decision: 15 Nov 2017
Revised: 29 Nov 2017
Accepted: 29 Nov 2017
Published: 28 Dec 2017

Keywords:
Myocardial metabolism, cardiomyocyte, myocardial infarction, ischemia, arrhythmias

ABSTRACT

Cardiac muscle contraction is a strictly regulated process which conjugates a series of electrophysiological, biochemical and mechanic events, resulting in the pumping of blood to all bodily tissues. These phenomena require a very high energetic demand both for generating the necessary mechanical force, and for maintaining cellular homeostasis during the process. In the myocardium, fatty acids (FA) represent the main energy substrate, although other secondary substrates, such as glucose and ketone bodies, may also be used. Nevertheless, under certain conditions such as heart failure or myocardial ischemia, FA metabolism may become deleterious via mechanisms such as oxidative stress and arrhythmogenesis. In an ischemic milieu, various metabolic changes occur as a consequence of hypoxia, favoring cell necrosis, ventricular arrhythmias, and death. Major events in this context include an increase in extracellular K^+, a decrease in pH, and accumulation of intracellular calcium. This review includes a detailed description of the molecular basis underlying myocardial contraction and energetic metabolism in cardiomyocytes, aiming to promote an integral understanding of the pathophysiology of heart ischemia. This in turn may aid in the development of future, more satisfactory alternative treatments in the management of acute coronary ischemia episodes.

INTRODUCTION

The heart is composed of specialized muscle tissue, the myocardium, which depends on a constant input of energy for adequate functioning, and thus utilizes various sources of carbon for adenosine triphosphate (ATP) production. However, in cardiomyocytes, the majority of the energy produced derives from the mitochondrial oxidation of free fatty acids (FA)[1].

Alterations in myocardial energetic metabolism may lead to conditions such as ischemic heart disease,

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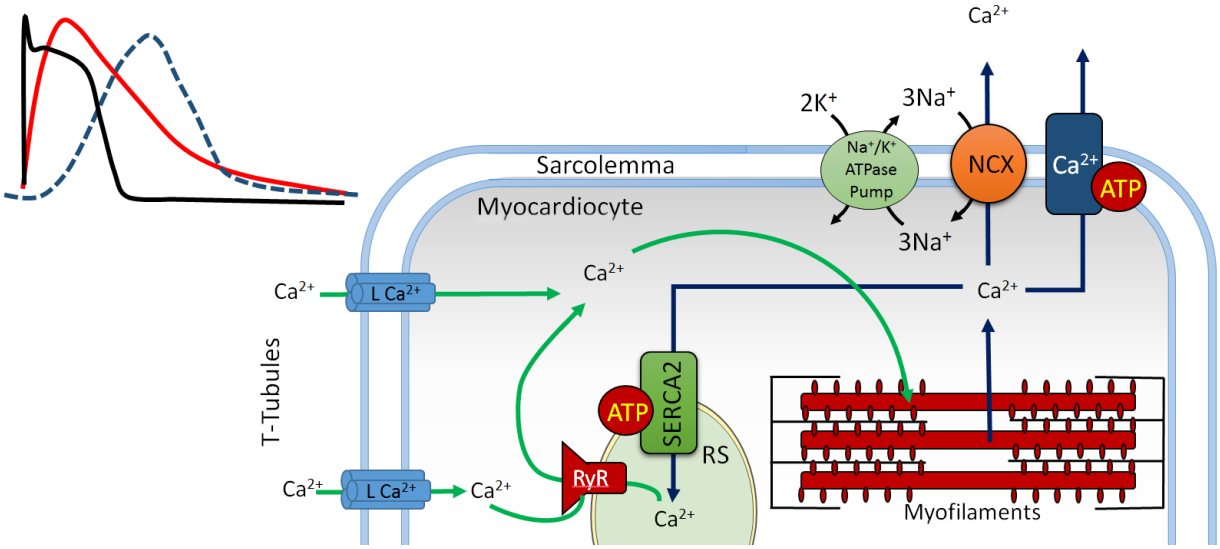

Figure 1: The role of calcium in excitation-contraction coupling. Green arrows: calcium entry; blue arrows: calcium exit; SR: sarcoplasmic reticulum; RyR: ryanodine receptor; NCX: Na^+/Ca^{2+} exchanger

arrhythmias, heart failure, and others. During ischemia, cardiomyocytes are restricted in both their oxygen and nutrient supplies, rendering these cells unable to oxidize energetic substrates, causing a significant depletion in ATP reserves. This leads to deterioration of cardiac contractility and disruptions in the cardiac conduction system, which may culminate in heart failure and arrhythmias, respectively[2]. Therefore, an understanding of myocardial energetic metabolism is essential, in order to comprehend the physiologic basis of the therapeutic management of acute and chronic heart disease.

FUEL FOR MYOCARDIAL CONTRACTION: THE ROLE OF MACROMOLECULES

The heart is the driving force of the circulatory system, pumping blood to all bodily tissues. This organ consists of various layers, the thickest of which is the myocardium. The main component of this layer is contractile cells termed cardiomyocytes[3], with only 2% corresponding to Purkinje fibers. The Purkinje fibers are organized into an arborized structure which originates in the atrioventricular node and constitutes a specialized conduction system that allows quick and synchronic activation of the ventricles[4,5].

The cytosol of cardiomyocytes contains sarcomeres, complex proteic structures composed of thick myosin filaments and thin actin filaments[6]. Sarcomeres occupy most of these cells' cytosol and mediate calcium-dependent cellular contraction [Figure 1][7]. This phenomenon is regulated by the cardiac conduction system through modulation of ionic transport and generation of action potentials[8]. These impulses are synchronically propagated throughout the myocardium via gap junctions and ionic channels which facilitate electrical and chemical communication between cardiomyocytes, allowing these to function as a syncytium[9]. Connexins, especially Cx43, Cx40 and Cx45 are key voltage-gated proteic structures found in these gap junctions, each of which can differentially and dynamically intervene in the propagation of the action potential[10-12].

The myocardium is a form of specialized striated muscle, richly innervated by the autonomic nervous system, which is under uninterrupted activity throughout life. Therefore, it requires a constant and substantial energetic input from macromolecules such as carbohydrates, lipids and proteins in order to sustain the process of contraction and relaxation. Indeed, the cardiomyocyte transforms chemical energy from FA, glucose, ketone bodies and other substrates into mechanical energy[13,14]. The energetic metabolism of the cardiomyocyte consists of three key components: (1) capture and utilization of primary substrates, with the incorporation of their metabolites into the tricarboxylic acid (TCA) cycle; (2) oxidative phosphorylation, which occurs in the respiratory chain within the internal mitochondrial membrane; and (3) the phosphocreatine-creatine kinase energy transference system, a network for phosphate transference from ATP to creatine (an "energy-storing" molecule), through mitochondrial creatine kinase and yielding phosphocreatine, an important source of energy under high-demand conditions[15].

The metabolic machinery of the heart utilizes oxygen

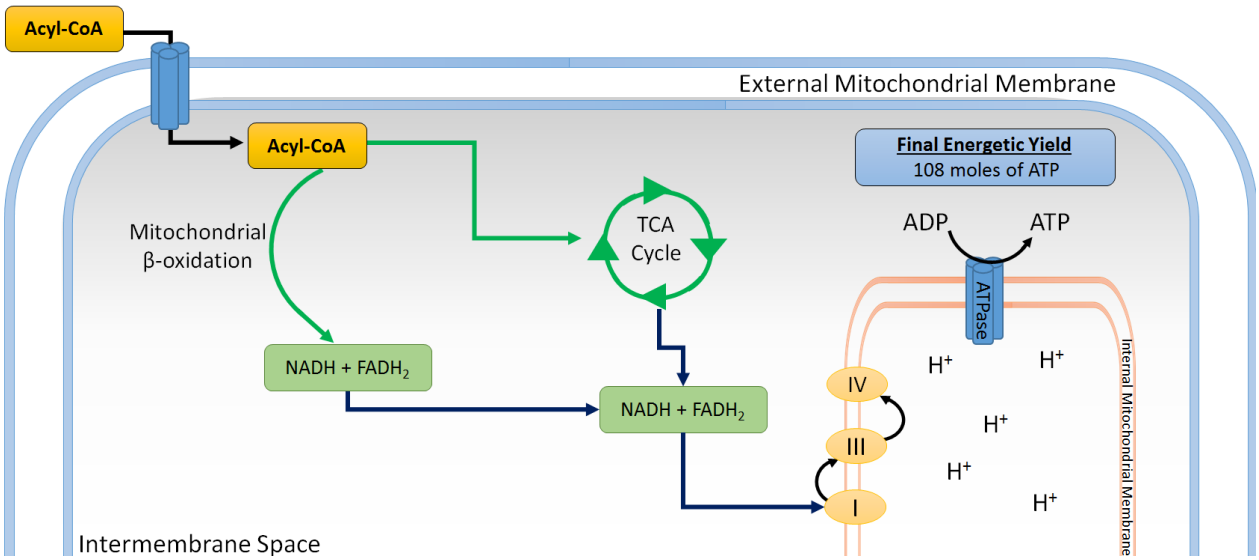

Figure 2: FA metabolism in myocardiocytes. Once inside cells, FA are esterified with Coenzyme A into acyl-CoA, which in turn can be esterified for storage as triacylglycerides, or transported to the external mitochondrial membrane, where it is carried by a carnitine-dependent system into the intermembrane space. In this process, the acyl group is condensed with carnitine and separated from CoA to form acyl-carnitine, via carnitine palmitoyl transferase I. Then, it binds to acyl-carnitine translocase, which transports it into the mitochondrial matrix, and is converted into acetyl CoA, which goes into the TCA cycle. This process also yields NADH and FADH2 and several reducing equivalents, which can be utilized in the respiratory chain to generate ATP through oxidative phosphorylation. FA: fatty acid; TCA: tricarboxylic acid

up to 80%-90% of the maximum capacity of the electron transport chain; however, at a resting state, the heart operates at only 15%-25% of its maximum oxidative capacity[16]. Cardiomyocytes show an elevated rate of ATP hydrolysis, which is strongly linked to oxidative phosphorylation. Because under non-ischemic conditions, over 95% of these cells' ATP is produced in this process[17], it is indispensable in order to assure the full replenishment of the cardiomyocytes' ATP content every 10 s, and thus maintain constant concentrations of this molecule, even under conditions of increased frequency or force of contractions[18]. Of the total energy produced by ATP hydrolysis, approximately 60%-70% serves as fuel for contraction, while the remaining 30%-40% is used by the Ca^{2+}ATPase pumps in the smooth sarcoplasmic reticulum and other ion pumps[19].

FA metabolism in cardiomyocytes

Cardiomyocytes use FA as their main source of energy, yet their synthesizing capacity for these molecules is relatively low[20] as is shown in Figure 2. As a result, these cells depend fundamentally on the influx of FA from the vascular compartment, and thus, the rate of FA consumption by cardiac muscle is principally determined by the concentration of non-esterified FA in plasma[21,22].

The heart obtains these FA chiefly from exogenous substrates, especially free FA bound to albumin, and FA released from triacylglycerides (TAG) contained in chylomicrons and very low-density lipoproteins, later undergoing β-oxidation[23-25]. FA may enter cardiomyocytes by passive diffusion or by active transport through the sarcolemma, involving an FA translocase (CD36) or an FA-binding protein in the cell membrane[26]. This translocation is mediated by intracellular vesicles, and may be promoted by electrical stimulation or high-demand conditions[27].

CD36 is one of the main translocases, an 80 kD integral membrane glycoprotein which is stored in intracellular compartments and transported towards the cell membrane in response to increased energy demands[28]. This protein is found in platelets, immune cells, adipocytes, myocytes, enterocytes and various other cells, yet is most abundantly expressed in cardiomyocytes. It is also the most important FA translocase in the heart and plays a key role in the entry of long-chain FA into cardiomyocytes[29]. The extracellular domain of CD36 has three disulfide bridges in its C-terminus end which contain binding sites for FA, oxidized low-density lipoprotein, thrombospondin and *Plasmodium falciparum*-infected erythrocytes. This domain operates actively by generating transduction signals which interact with multiple tyrosine-kinases and generate various structural modifications which modulate the functions of CD36; including phosphorylation, glycosylation, palmitoylation and ubiquitination[30].

As cardiac activity increases, so does the

cardiomyocyte's metabolic demand, which results in an increase in intracellular concentrations of adenosine monophosphate (AMP) and reactive oxygen species[31]. In turn, this upregulates AMP-activated protein kinase (AMPK), a key metabolic regulator. AMPK is a serine/threonine kinase which acts as a metabolic sensor in cardiomyocytes. It is activated during high energy requirement states, enhancing FA availability, uptake and oxidation in these cells by promoting the expression and activation of lipoprotein lipase and CD36, also known as fatty acid translocase[31]. This favors the entry of long-chain FA into the cell, preserving stable levels of ATP in the face of increased metabolic demand[32]. Nevertheless, excessive expression of CD36 has been associated with impaired cardiac insulin sensitivity, reduced uptake of glucose, and excessive uptake of FA, subsequently causing cardiomyocyte lipotoxicity and retention of GLUT4 in their cytoplasm[33]. Recent *in vitro* studies in cardiomyocytes have shown that use of CD36 blockers or deletion of its coding gene ameliorates contractile dysfunction mediated by lipotoxicity, and reduced lipid-induced damage[34,35]. AMPK can also inhibit acetyl-CoA carboxylase, which enhances mitochondrial FA uptake. In addition, in energy depletion states, AMPK increases GLUT4 expression and inhibits its internalization and also enhances glycolysis by phosphorylation of phosphofructokinase 2. It may also facilitate glycogen storage in adequate ATP supply states[36].

In addition to their plasma concentration, an important long-term regulator of FA β-oxidation is the modulation by peroxisome proliferator-activated receptor (PPAR)[37]. Numerous coactivator proteins, such as PPAR-γ co-activator 1-α can powerfully induce the transcription of PPAR target genes, including those involved in FA storage (such as diacylglycerol acyltransferase, promoted by PPARα), FA oxidation (such as medium-chain acyl-CoA dehydrogenase, promoted by PPARα/β/δ/γ), and glucose metabolism (such as pyruvate dehydrogenase kinase 4, promoted by PPARα)[38,39].

PPAR also plays an important role in the regulation of oxidative stress in the cardiovascular system, with several isoforms implicated in various transcriptional mechanisms for antioxidant genes[40,41]. For example, PPARα and PPARγ promote the transcription and activation of Cu/Zn-superoxide dismutase (SOD1), Mn-superoxide dismutase (SOD2) and catalase in cardiac tissue. Furthermore, PPARα augments IGF-1 transcription, subsequently activating the IGF-1/PI3K pathway, inhibiting apoptosis and protecting cardiomyocytes under ischemic stress[42].

Carbohydrate metabolism in cardiomyocytes

Carbohydrates are also a valuable source of energy in the myocardium, with glucose providing roughly a quarter of the total energy produced in a well-irrigated heart. Of this total ATP, approximately 10%-40% derives from the oxidation of glucose-lactate within the TCA, and only 2% derives from glycolysis[43].

Glucose enters the cardiomyocyte through glucose transporter proteins (GLUT). Fourteen different GLUTs have been described in humans, all of which appear to be able to transport hexoses or polyols, although it is suspected that many other GLUT substrates remain undiscovered[44]. GLUT 1-5 are the most studied to date, and they are well-known to be glucose and/or fructose transporters in various tissues and cell types[45]. In cardiomyocytes, GLUT4 is the main transporter, translocating to the membrane in response to signaling by insulin, increased work demand, or ischemia, with GLUT1 playing an accessory role[14].

The products of glycolysis are utilized in both the TCA cycle and the respiratory chain in order to generate ATP through oxidative phosphorylation[46]. Although only 2% the heart's ATP is produced in glycolysis, it becomes very important under anaerobic or ischemic conditions. Indeed, in heart failure and hypertrophy, there is a metabolic switch towards favoring carbohydrate over FA metabolism in the heart, with a notable change being the acceleration of glycolysis[47]. This increase in the glycolytic flux appears to be due to a functional upregulation in the pathway's enzyme, rather than a clear increase in the expression of glycolytic enzymes[48].

This shift towards utilization of glucose in the hypertrophic myocardium had traditionally been considered a maladaptive change. Nevertheless, recent studies in bioengineering-modified mice have demonstrated glucose-dependence not to be harmful in adult hearts, and a decrease in the utilization of glucose appears to be deleterious in failure and hypertrophy[49]. For example, mice with GLUT1 overexpression - and thus, increased glycolysis - appear to be protected against heart failure and left ventricular dilatation, even when subjected to pressure overload[50,51]. On the other hand, those with deletion of GLUT4 and insulin receptors in the heart failure, and showed worse responses to cardiac hypertrophy-promoting stimuli[52].

The phosphocreatine-creatine kinase system incardiomyocytes

Because both the systole and diastole are active,

highly ATP-dependent processes, the demand for these metabolites in cardiomyocytes is very high, with the heart requiring approximately 20 times its weight in ATP per day in order to sustain its energetic demands[53]. After synthesis, ATP must be transported from the mitochondria to the myofilaments and membrane proton pumps, a process in which the phosphocreatine-creatine kinase system intervenes substantially[54].

Creatine kinase (CK) is a key enzyme for phosphate transference in cells with high energetic demand, and works in harmony with other enzymatic machinery in order to facilitate intracellular energetic communication[55]. CK synthesizes phosphocreatine (PC) from creatine and a phosphate group from ATP in a reversible reaction, acting as a functional ATP reserve. CK associated with myofilaments catalyzes the transference of the phosphate from PC towards adenosine diphosphate (ADP), replenishing ATP in ATPase active sites, such as myosin heads. In cardiomyocytes, CK isoenzymes and the highly diffusible PC are responsible for sustaining the transference of energy from producing centers (mitochondria and glycolysis) towards ATP-consuming sites (myofilaments and ATPase pumps)[15].

The PC-CK system represents the first line of energetic reserves in cardiomyocytes, providing a quick source of ATP and favoring its transportation to its utilization sites, especially myofilaments[56]. In animal models, disruptions in the PC-CK system have been linked to impaired myocardial contractility and increased risk for arrhythmias[57,58]. Moreover, alterations in the functionality of CK have been identified as an independent risk factor for heart failure[59].

CK is composed of dimers, which consist of subunits M and B, and originate three isoenzymes: CK-MM, -MB and -BB. A fourth isoenzyme is found in mitochondria (mi-CK) and accounts for 20%-40% of all CK activity in the heart[60]. CK is not evenly distributed within the cell, and is rather a part of a compartmentalized metabolic pathway, bound to myofilaments and the sarcoplasmic reticulum to form functional complexes which accelerate ATP synthesis[61].

The mi-CK isoform is coupled to the external surface of the internal mitochondrial membrane, near the ATP-ADP translocases, also termed adenine nucleotide translocases (ANT). During oxidative phosphorylation, the ATP generated in the mitochondrial matrix is exported by ANT to the intermembrane space and transphophorylated by mi-CK to PC and ADP, with the latter being immediately available for oxidative phosphorylation, stimulating cellular respiration[55]. Another isoform of CK is associated with myofilaments, acting as a structural protein within M-bands, which is functionally coupled to the myosin ATPase and can transfer phosphate from PC to ATP, providing sufficient energy for maintaining maximum contractility[61].

In a healthy heart, approximately two thirds of all creatine is phosphorylated by CK to yield PC. In heart failure, the level of PC is lower in relation to the concentrations of ATP, with a lower PC/ATP index[62]. Lower values of this index have been related to increased mortality[63]. Human and animal models have demonstrated a progressive reduction in the creatine pool of up to 60% in patients with heart failure, with a directly proportional relationship between the decrease of the index and the severity of the condition[64].

Fatty acids *vs.* glucose as energetic substrates

The selection of energetic substrates in cardiomyocytes is a fundamental step for the constant generation of ATP which depends on the dynamic metabolic milieu in each body at a given time[65]. This flexibility is present during fetal development; however, after birth, FA becomes the preferential substrates, due to the increased availability of oxygen and dietary fats[66-68]. Infants with mutations in genes involved in FA metabolism have been documented to develop cardiomyopathy when under stress, highlighting the essential role of FA in this tissue[66]. Likewise, in heart failure and left ventricular hypertrophy (when the oxidative capacity of mitochondria in cardiomyocytes is diminished), there is a shift towards a predominance for glucose metabolism[69,70].

FA are known to be the main source of energy in cardiomyocytes when the heart is at rest and during fasting periods: most of the acetyl CoA that enters the TCA cycle (60%-90%) originates in the β-oxidation of free FA[13], while the remaining 10%-40% is produced by oxidation of pyruvate, which derives from the oxidation of glucose or lactic acid[71]. Several reports have shown that cardiac efficiency, in terms of oxygen consumption, is greater when oxidizing glucose and lactate rather than FA[72]. Studies using ranolazine - an inhibitor of β-oxidation which induces oxidation of carbohydrates - have found enhanced left ventricular function and improved metabolic efficiency when utilizing glucose as the main energetic substrate[73]. Similarly, potentiating FA use in the heart with

heparin or TAG infusions has been reported to result in a 26% increase in oxygen consumption without changes in mechanical capacity of the left ventricle[74], which suggests a greater functional capacity for this chamber when utilizing glucose[75]. This may be due to the higher level of oxidative stress caused by the oxidation of FA in comparison with carbohydrates, due to the increased oxygen consumption rate in the former[76].

The ATP synthesis/oxygen consumption rates for glucose and lactic acid are 3.17 and 3.00, respectively; whereas they are 2.80 and 2.86 for palmitate and oleate, respectively. Although these are theoretical values which may be lower *in vivo* as a consequence of the constant proton efflux through the mitochondrial membrane, the differences between substrates remain substantial[77]. For example, when comparing palmitate with glucose, the complete oxidation of 1 molecule of palmitate yields 105 ATP molecules and requires 46 oxygen atoms, while 1 molecule of glucose generates 31 ATP molecules and uses 12 oxygen atoms. Thus, despite FA clearly yielding greater amount of ATP, this occurs at the expense of larger oxygen requirements[39]. Furthermore, β-oxidation of FA generates more lipid peroxidation due to increased delivery of NADH and FADH2 to the electron transport chain and production of superoxide anion[78].

In addition, elevated free FA are harmful in the ischemic myocardium, augmenting cell damage. In the first hours of an acute myocardial infarction (AMI), free FA can act as detergents on the cell membrane of cardiomyocytes[79-81]. Moreover, there is increased generation of free radicals, which can inactivate IRS-1 via phosphorylation of serine residues. This directly promotes insulin resistance and simultaneously stimulates the release of proinflammatory cytokines, such as TNF-α and IL-6[82]. Therefore, all conditions of metabolic inefficiency in the heart favor insulin-mediated left ventricular remodeling and diastolic myocardial dysfunction[83].

Considering this, various systemic conditions such as obesity cause elevated serum free FA which can potentiate β-oxidation, and thus increase lipid traffic in cardiomyocytes, prompting a phenomenon termed lipotoxicity[13,84]. This process can lead the cell towards contractile dysfunction, insulin resistance and ultimately apoptosis in association with accumulation of ceramides[85].

On the other hand, pre-clinical and clinical evidence suggests partial inhibition of free FA oxidation in the myocardium can prevent or diminish tissue damage and dysfunction under conditions of ischemia or reperfusion, diabetic cardiomyopathy, and AMI. This occurs because the heart shifts towards glucose as the main source for ATP synthesis, which reduces the oxygen demand by 11%-13% and therefore improves cardiac efficiency and protects mitochondrial function[43,86]. Nonetheless, the real benefits of this partial inhibition remain uncertain when contemplating the potential consequences of excessive lipid accumulation within cardiomyocytes[86].

ARRHYTHMOGENIC METABOLIC CHANGES DURING MYOCARDIAL ISCHEMIA

Cardiac sudden death is responsible for approximately half of all cardiovascular mortality[87]. The majority of these are attributed to ventricular tachyarrhythmias (ventricular tachycardia, ventricular fibrillation), which are frequently caused by myocardial ischemia[88]. The mechanisms through which myocardial ischemia leads to local electrophysiological disorders and arrhythmogenesis have been extensively explored [Figure 3][2,89].

Severe metabolic changes begin a few seconds after coronary occlusion: high-energy phosphates are hydrolyzed, intracellular pH lowers as a consequence of the activation of anaerobic glycolysis, and extracellular potassium levels increase[90,91]. The latter lasts for roughly 10 min, during which the resting membrane potential decreases, approaching the firing threshold potential, thus accelerating electrical conduction[92]. The intracellular acidosis also drives an increase in cytosolic calcium, facilitating early and late depolarization, as well as spatial and temporal fluctuations in the duration of action potentials[93]. Additionally, ischemia leads to dephosphorylation of connexin-43 in gap junctions, which impairs intercellular electrical coupling and anisotropy[94]. Lastly, sympathetic stimulation not only promotes calcium release from the sarcoplasmic reticulum, but it also prompts lipolysis, elevating circulating free FA levels and therefore predisposing to ischemia-induced arrhythmogenesis[95].

Other possible mediators of cardiac arrhythmia include thrombin and endothelin-1. Patients with ST-elevation myocardial infarction (STEMI) complicated with ventricular fibrillation have been found to have higher levels of thrombin activity markers[96]. The production of thrombin at sites of coronary occlusion has been suggested to favor accumulation

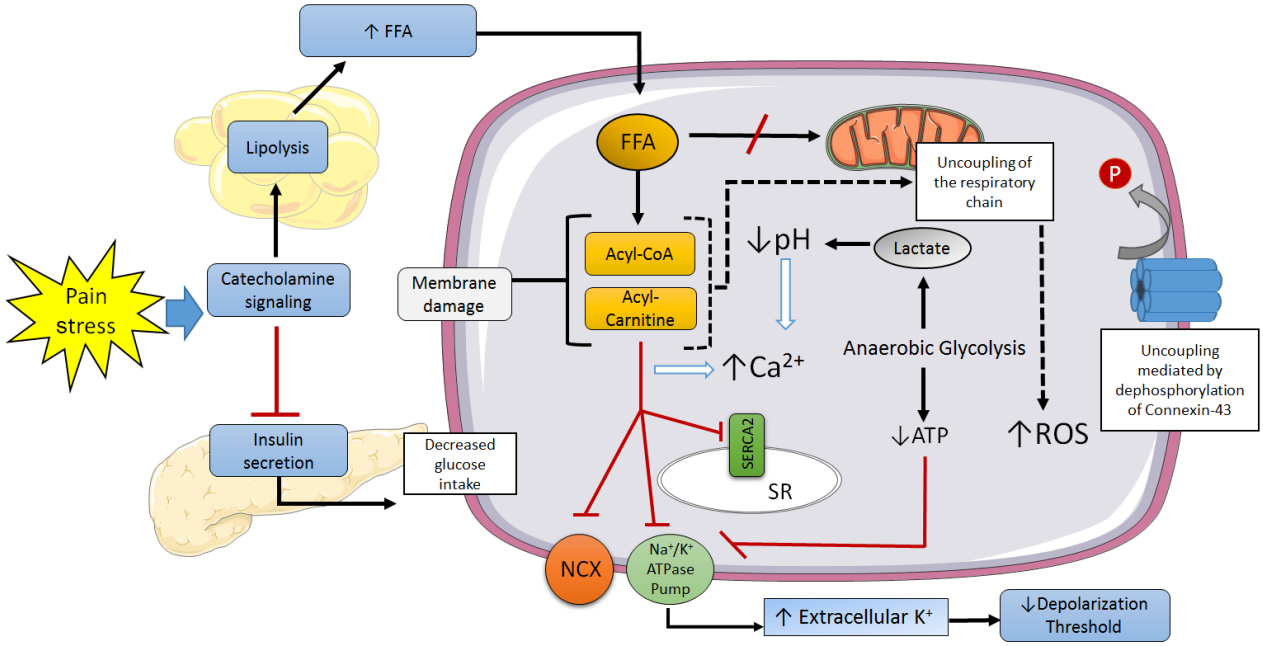

Figure 3: Mechanisms involved in the genesis of ventricular arrhythmias in the ischemic myocardiocyte. SR: sarcoplasmic reticulum; NCX: Na^+/Ca^{2+} exchanger; ROS: reactive oxygen species; FFA: free fatty acids; P: phosphate

of lysophosphatidylcholine, which activates Na^+ channels and Na^+/H^+ exchangers, enhancing the entry of Na^+ into the cell[97]. On the other hand, endothelin-1 activity increases exponentially during acute ischemia, mediating direct and indirect electrophysiological effects through type-A endothelin receptors, and possibly playing a protecting role through type B endothelin receptors during the early stages of ischemia[98].

Acute pro-inflammatory cytokines - including TNF-α, IL-1β, IL-6 - and metalloproteinases are also found in greater proportions in the myocardium following an AMI, and may account for the electrophysiological changes observed in the cardiomyocytes within the border zone of the infarction[99]. This suggests an inflammatory process is involved in the remodeling and arrhythmogenic changes seen after an infarction. Patients with post-AMI arrhythmias tend to display higher levels of pro-inflammatory cytokines than those without arrhythmias[100,101]; and subjects without AMI or structural myocardial damage with systemic inflammation have shown an increased risk of ventricular tachyarrhythmias[102].

This catalogue of arrhythmogenic changes occur heterogeneously along the penumbra of the infarction zone, impairing its functionality as a syncytium and the electrical coupling among cardiomyocytes[103,104]. These phenomena are complex and appear to occur erratically due to the dynamic nature of reperfusion, low pH, oxygen depletion, increased intracellular calcium and potentiated sympathetic signaling[105]. These events, along with the high proportion of electrical decoupling and the auto-oscillatory activity of individual cells lay the foundation for a very variable syncytial behavior[106]. This leads to the continuous generation of mini-reentry circuits from individual ectopic rhythmic foci within the decoupled cell areas, which then spread to the better coupled zones within the penumbra, constituting the genesis of arrhythmias[107]. In concert, these changes are thought to be the triggers and substrates for ventricular arrhythmias, although further research is required to elucidate them in more detail.

LIPOTOXIC PHENOMENA IN ISCHEMIA AND ARRHYTHMIA

In 1968, Oliver *et al.*[108] determined patients with AMI and especially those with elevated free FA levels had a greater incidence of ventricular arrhythmias in comparison with normolipemic patients. This finding has been also been found by various authors ever since[109]. More recently, in the Paris Prospective Study - a research realized in 5,250 male subjects followed during 22 years - high plasma free FA levels have been associated with sudden cardiac death, yet appeared to be unrelated to other causes of AMI[110].

The exact mechanism through which the shifts in FA/glucose metabolism in the cardiomyocyte lead to

arrhythmia remain unclear, yet various alternatives have been proposed[111]. During an infarction, numerous events develop in parallel, with some of the earliest being precordial pain and increased sympathetic activity[112]. Although a moderate increase in catecholamines may aid in maintaining cardiac contractility in the face of oxygen depletion, excessive signaling can augment the energetic demands of the myocardium, impairing functionality[113].

It has been proposed that for the ischemic cardiomyocyte, glucose metabolism is more beneficial, and FA metabolism is deleterious[114]. However, FA availability is much greater: catecholamines induce lipolysis in adipose tissue, abruptly rising circulating FA levels[115], and inhibit insulin signaling, reducing glucose entry into the myocardium[116]. Likewise, FA can inhibit glucose oxidation and potentiate oxygen consumption in ischemic myocardial areas, leading to a preferential utilization of FA over glucose during ischemia[117].

The mechanisms underlying FA-related toxicity appear to be fundamentally linked with adrenergic stimulation[118]. However, FA also exhibit direct arrhythmogenic activity. Even without ischemia, a sufficiently high free FA/albumin molar rate can inhibit β-oxidation, leading to accumulation of acyl-carnitine and acyl-CoA in the cytosol. In turn, acyl-carnitine inhibits Ca^{++} pumps in the sarcoplasmic reticulum, as well as the Na^{+}/Ca^{++} exchanger and the Na^{+}/K^{+} ATPase pump, finalizing in an overload of Ca^{++} in the cytosol[119].

Furthermore, other metabolic processes activated upon elevation of free FA levels, such as membrane lipid peroxidation, inhibition of β-oxidation, uncoupling of proteins in the mitochondrial respiratory chain, accumulation of CoA derivates and extracellular K^{+} with shortening of action potentials, are all harmful for the ischemic cardiomyocyte[120]. On this basis, strong arguments propose the reduction of circulating free FA as a therapeutic measure in AMI, although further evidence is required to encourage this practice. Regarding instrumentation, the glucose-insulin-potassium (GIK) infusion is a well-known and viable alternative, as it promotes glucose entry into the myocardium and inhibits lipolysis, with the metabolic benefits this implies in cardiomyocytes[121]. Despite early clinical trials failing to demonstrate the utility of GIK at decreasing sudden death during the first 3 days following an AMI[122], more recent reports, such as the IMMEDIATE study have shown better outcomes at a 1-year follow-up: in patients with STEMI, 1-year mortality and hospitalizations for heart failure decreased significantly[123]. Theoretically, drugs for rapid inhibition of lipolysis would yield better results, yet research efforts in this line remain scarce and poor.

Quick myocardial FA availability has been closely related with epicardial adipose tissue (EAT) or the epicardial fat pad which is found between the myocardium and the visceral pericardium. This isin close proximity with the coronary arteries and so facilitates the activity of anti-inflammatory and pro-inflammatory adipokines and FA in the myocardium and arterial walls[124,125]. The Framingham Heart Study[126] has identified the EAT volume as a predictor of atrial fibrillation - the most frequent arrhythmia in clinical practice - independent of other methods for the measurement of adiposity such as the body mass index. Other studies have reported the EAT volume to be associated with the prevalence and severity of atrial fibrillation[127].

The EAT has also been found to release pro-inflammatory messengers such as activin A, which induces the expression of the transforming growth factor β1 (TGF-β1) and several metalloproteinases. These are key regulators in the homeostasis of the extracellular matrix - in particular by modifying collagen fibers - and mediate the profibrotic effect of EAT in the atrial myocardium[128]. Many other inflammatory mediators secreted by EAT also intervene in the pathogenesis of atrial fibrillation, including TNF-α, IL-8, and the monocyte chemotactic protein 1 (MCP-1)[129].

Finally, increased EAT has been associated with fatty infiltration in the myocardium, which contributes to tissue disorganization and promotes an arrhythmia-prone environment[130]. At the same time, these deposits favor the proliferation of myofibroblasts and increase the amount of dystrophic cardiomyocytes[130]. In this context, the EAT has been proposed as a novel therapeutic target in the management of cardiovascular disease[125].

CONCLUSION

The cardiomyocyte is the protagonist cell in the heart which allows it to function as a pump. This capacity requires strict electrophysiological and mechanical control and large amounts of ATP. These energetic demands are covered by a dynamic selection of substrates, shifting between carbohydrates and FA depending on various intracellular and systemic circumstances. Myocardial metabolism has been historically described as the “lost child of cardiology”[131], as clinicians have rather focused on

thrombolysis and angioplasty, and very little practical value has been obtained from its study. Nonetheless, the evidence suggesting metabolic causes for arrhythmias grants a "resuscitation" of this cause[120]. Indeed, further research is required in order to explore and exploit the therapeutic implications of myocardial metabolism.

DECLARATIONS

Authors' contributions

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Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

REFERENCES

1. D'Souza K, Nzirorera C, Kienesberger P. Lipid metabolism and signaling in cardiac lipotoxicity. *Biochim Biophys Acta* 2016;1861:1513-24.
2. Gillespie HS, Lin CC, Prutkin JM. Arrhythmias in structural heart disease. *Curr Cardiol Rep* 2014;16:510.
3. Burton RA, Lee P, Casero R, Garny A, Siedlecka U, Schneider JE, Kohl P, Grau V. Three-dimensional histology: tools and application to quantitative assessment of cell-type distribution in rabbit heart. *Europace* 2014;16 Suppl 4:iv86-95.
4. Maass K, Shekhar A, Lu J, Kang G, See F, Kim EE, Delgado C, Shen S, Cohen L, Fishman GI. Isolation and characterization of embryonic stem cell-derived cardiac Purkinje cells. *Stem Cells* 2015;33:1102-12.
5. Vigmond EJ, Stuyvers BD. Modeling our understanding of the His-Purkinje system. *Prog Biophys Mol Biol* 2016;120:179-88.
6. Yin Z, Ren J, Guo W. Sarcomeric protein isoform transitions in cardiac muscle: a journey to heart failure. *Biochim Biophys Acta* 2015;1852:47-52.
7. Hu LY, Ackermann MA, Kontrogianni-Konstantopoulos A. The sarcomeric M-region: a molecular command center for diverse cellular processes. *Biomed Res Int* 2015;2015:714197.
8. Katz A. Contractile proteins of the heart. *Phys Rev* 2016;50:63-158.
9. Gaztañaga L, Marchlinski F, Betensky B. Mechanisms of cardiac arrhythmias. *Rev Esp Cardiol (Engl Ed)* 2012;65:174-85.
10. Kleber AG, Saffitz JE. Role of the intercalated disc in cardiac propagation and arrhythmogenesis. *Front Physiol* 2014;5:404.
11. Kurtenbach S, Kurtenbach S, Zoidl G. Gap junction modulation and its implications for heart function. *Front Physiol* 2014;5:82.
12. Veeraraghavan R, Poelzing S, Gourdie RG. Intercellular electrical communication in the heart: a new, active role for the intercalated disk. *Cell Commun Adhes* 2014;21:161-7.
13. Rider O, Cox P, Tyler D, Clarke K, Neubauer S. Myocardial substrate metabolism in obesity. *Int J Obes (Lond)* 2012;37:972-9.
14. Carley AN, Taegtmeyer H, Lewandowski ED. Mechanisms linking energy substrate metabolism to the function of the heart. *Circ Res* 2014;114:717-29.
15. Doenst T, Nguyen TD, Abel ED. Cardiac metabolism in heart failure - implications beyond ATP production. *Circ Res* 2013;113:709-24.
16. Rosano G, Vitale C, Spoletini I. Metabolic approach to heart failure: the role of metabolic modulators. *Egyptian Heart J* 2015;67:177-81.
17. Long Q, Yang K, Yang Q. Regulation of mitochondrial ATP synthase in cardiac pathophysiology. *Am J Cardiovasc Dis* 2015;5:19-32.
18. Ingwall JS. Energy metabolism in heart failure and remodelling. *Cardiovasc Res* 2009;81:412-9.
19. Wang J, Guo T. Metabolic remodeling in chronic heart failure. *J Zhejiang Univ Sci B* 2013;14:688-95.
20. Goldberg IJ, Trent CM, Schulze PC. Lipid metabolism and toxicity in the heart. *Cell Metabolism* 2012;15:805-12.
21. Wolf P, Winhofer Y, Krššák M, Krebs M. Heart, lipids and hormones. *Endocr Connect* 2017;6:R59-69.
22. Abumrad NA, Goldberg IJ. CD36 actions in the heart: lipids, calcium, inflammation, repair and more? *Biochim Biophys Acta* 2016;1860:1442-9.
23. Fukushima A, Lopaschuk GD. Cardiac fatty acid oxidation in heart failure associated with obesity and diabetes. *Biochim Biophys Acta* 2016;1861:1525-34.
24. Fukushima A, Milner K, Gupta A, Lopaschuk GD. Acetylation control of cardiac fatty acid β-oxidation and energy metabolism in obesity, diabetes, and heart failure. *Biochim Biophys Acta* 2016;1862:2211-20.
25. Fillmore N, Mori J, Lopaschuk GD. Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy. *Br J Pharmacol* 2014;171:2080-90.
26. Glatz JF, Nabben M, Heather LC, Bonen A, Luiken JJ. Regulation of the subcellular trafficking of CD36, a major determinant of cardiac fatty acidutilization. *Biochim Biophys Acta* 2016;1861:1461-71.
27. Pepino M, Kuda O, Samovski D, Abumrad N. Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. *Ann Rev Nutr* 2014;34:281-303.
28. Glatz JF, Luiken JJ. From fat to FAT (CD36/SR-B2): understanding the regulation of cellular fatty acid uptake. *Biochimie* 2017;136:21-6.
29. Chanda D, Luiken JJ, Glatz JF. Signaling pathways involved in cardiac energy metabolism. *FEBS Lett* 2016;590:2364-74.
30. Kim TT, Dyck JR. The role of CD36 in the regulation of myocardial lipid metabolism. *Biochim Biophys Acta* 2016;1861:1450-60.
31. Kim TT, Dyck JR. Is AMPK the savior of the failing heart? *Trends Endocrinol Metab* 2015;26:40-8.
32. Glatz JF, Angin Y, Steinbusch LK, Schwenk RW, Luiken JJ. CD36 as a target to prevent cardiac lipotoxicity and insulin resistance. *Prostaglandins Leukot Essent Fatty Acids* 2013;88:71-7.
33. Samovski D, Su X, Xu Y, Abumrad N, Stahl P. Insulin and AMPK regulate FA translocase/CD36 plasma membrane recruitment in cardiomyocytes via Rab GAP AS160 and Rab8a RabGTPase. *J Lipid Res* 2012;53:709-17.
34. Angin Y, Steinbusch L, Simons P, Greulich S, Hoebers N, Douma K, van Zandvoort MA, Coumans WA, Wijnen W, Diamant M, Ouwens DM, Glatz JF, Luiken JJ. CD36 inhibition prevents lipid accumulation and contractile dysfunction in rat cardiomyocytes. *Biochem J*

2012;448:43-53.
35. Tate M, Grieve D, Ritchie R. Are targeted therapies for diabetic cardiomyopathy on the horizon? *Clin Sci* 2017;131:897-915.
36. Bairwa S, Parajuli N, Dyck J. The role of AMPK in cardiomyocyte health and survival. *Biochim Biophys Acta* 2016;1862:2199-210.
37. Vázquez-Carrera M. Unraveling the effects of PPARβ/δ on insulin resistance and cardiovascular disease. *Trends Endocrinol Metab* 2016;27:319-34.
38. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC.Myocardial fatty acid metabolism in health and disease. *Physiol Rev* 2010;90:207-58.
39. Huss JM, Kelly DP. Nuclear receptor signaling and cardiac energetics. *Circ Res* 2004;95:568-78.
40. Barlaka E, Galatou E, Mellidis K, Ravingerova T, Lazou A. Role of pleiotropic properties of peroxisome proliferator-activated receptors in the heart: focus on the nonmetabolic effects in cardiac protection. *Cardiovasc Ther* 2016;34:37-48.
41. Barlaka E, Ledvényiová V, Galatou E, Ferko M, Čarnická S, Ravingerová T, Lazou A. Delayed cardioprotective effects of WY-14643 are associated with inhibition of MMP-2 and modulation of Bcl-2 family proteins through PPAR-α activation in rat hearts subjected to global ischaemia-reperfusion. *Can J Physiol Pharmacol* 2013;91:608-16.
42. Sun W, Liu Q, Leng J, Zheng Y, Li J. The role of pyruvate dehydrogenase complex in cardiovascular diseases. *Life Sci* 2015;121:97-103.
43. Mueckler M, Thorens B. The SLC2 (GLUT) family of membrane transporters. *Mol Asp Med* 2013;34:121-38.
44. Deng D, Yan N. GLUT, SGLT, and SWEET: structural and mechanistic investigations of the glucose transporters. *Protein Sci* 2016;25:546-58.
45. Szablewski L. Glucose transporters in healthy heart and in cardiac disease. *Int J Cardiol* 2017;230:70-5.
46. Azevedo PS, Minicucci MF, Santos PP, Paiva SA, Zornoff LA. Energy metabolism in cardiac remodeling and heart failure. *Cardiol Rev* 2013;21:135-40.
47. Lopaschuk GD. Metabolic modulators in heart disease: past, present, and future. *Can J Cardiol* 2017;33:838-49.
48. Fillmore N, Lopaschuk GD. Targeting mitochondrial oxidative metabolism as an approach to treat heart failure. *Biochim Biophys Acta* 2013;1833:857-65.
49. Luptak I, Yan J, Cui L, Jain M, Liao R, Tian R. Long-term effects of increased glucose entry on mouse hearts during normal aging and ischemic stress. *Circulation* 2007;116:901-9.
50. Liao R, Jain M, Cui L, D'Agostino J, Aiello F, Luptak I, Ngoy S, Mortensen RM, Tian R. Cardiac-specific overexpression of GLUT1 prevents the development of heart failure attributable to pressure overload in mice. *Circulation* 2002;106:2125-31.
51. Domenighetti AA, Danes VR, Curl CL, Favaloro JM, Proietto J, Delbridge LM. Targeted GLUT-4 deficiency in the heart induces cardiomyocyte hypertrophy and impaired contractility linked with Ca(2+) and proton flux dysregulation. *J Mol Cell Cardiol* 2010;48:663-72.
52. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. *Circulation* 2007;116:434-48.
53. Varma N, Eberli FR, Apstein CS. Increased diastolic chamber stiffness during demand ischemia: response to quick length change differentiates rigor-activated from calcium-activated tension. *Circulation* 2000;101:2185-92.
54. Guimarães-Ferreira L. Role of the phosphocreatine system on energetic homeostasis in skeletal and cardiac muscles. *Einstein* 2014;12:126-31.
55. Wallimann T, Dolder M, Schlattner U, Eder M, Hornemann T, O'Gorman E, Rück A, Brdiczka D. Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. *Biofactors* 1998;8:229-34.
56. Weiss R, Gerstenblith G, Bottomley P. ATP flux through creatine kinase in the normal, stressed, and failing human heart. *Proc Nat Acad Sci U S A* 2005;102:808-13.
57. Fowler ED, Benoist D, Drinkhill MJ, Stones R, Helmes M, Wüst RC, Stienen GJ, Steele DS, White E. Decreased creatine kinase is linked to diastolic dysfunction in rats with right heart failure induced by pulmonary artery hypertension. *J Mol Cell Cardiol* 2015;86:1-8.
58. Bottomley PA, Panjrath GS, Lai S, Hirsch GA, Wu K, Najjar SS, Steinberg A, Gerstenblith G, Weiss RG. Metabolic rates of ATP transfer through creatine kinase (CK Flux) predict clinical heart failure events and death. *Sci Transl Med* 2013;5:215re3.
59. Kristjansson RP, Oddsson A, Helgason H, Sveinbjornsson G, Arnadottir GA, Jensson BO, Jonasdottir A, Jonasdottir A, Bragi Walters G, Sulem G, Oskarsdottir A, Benonisdottir S, Davidsson OB, Masson G, Magnusson OT, Holm H, Sigurdardottir O, Jonsdottir I, Eyjolfsson GI, Olafsson I, Gudbjartsson DF, Thorsteinsdottir U, Sulem P, Stefansson K. Common and rare variants associating with serum levels of creatine kinase and lactate dehydrogenase. *Nat Commun* 2016;7:10572.
60. Ventura-Clapier R, Garnier A, Veksler V. Energy metabolism in heart failure. *J Physiol* 2003;555:1-13.
61. Kitzenberg D, Colgan SP, Glover LE. Creatine kinase in ischemic and inflammatory disorders. *Clin Transl Med* 2016;5:31.
62. Neubauer S, Horn M, Cramer M, Harre K, Newell JB, Peters W, Pabst T, Ertl G, Hahn D, Ingwall JS, Kochsiek K. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation* 1997;96:2190-6.
63. Nakae I, Mitsunami K, Omura T, Yabe T, Tsutamoto T, Matsuo S, Takahashi M, Morikawa S, Inubushi T, Nakamura Y, Kinoshita M, Horie M. Proton magnetic resonance spectroscopy can detect creatine depletion associated with the progression of heart failure in cardiomyopathy. *J Am Coll Cardiol* 2003;42:1587-93.
64. Hue L, Taegtmeyer H. The Randle cycle revisited: a new head for an old hat. *Am J Physiol Endocrinol Metab* 2009;297:E578-91.
65. Fukushima A, Milner K, Gupta A, Lopaschuk GD. Myocardial energy substrate metabolism in heart failure: from pathways to therapeutic targets. *Curr Pharm Des* 2015;21:3654-64.
66. Aroor A, Mandavia C, Sowers J. Insulin resistance and heart failure: molecular mechanisms. *Heart Fail Clin* 2012;8:609-17.
67. Breckenridge RA, Piotrowska I, Ng KE, Ragan TJ, West JA, Kotecha S, Towers N, Bennett M, Kienesberger PC, Smolenski RT, Siddall HK, Offer JL, Mocanu MM, Yelon DM, Dyck JR, Griffin JL, Abramov AY, Gould AP, Mohun TJ. Hypoxic regulation of hand1 controls the fetal-neonatal switch in cardiac metabolism. *PLoS Biol* 2013;11:e1001666.
68. Wende AR, Brahma MK, McGinnis GR, Young ME. Metabolic origins of heart failure. *JACC Basic Transl Sci* 2017;2:297-310.
69. Wende AR, Kim J, Holland WL, Wayment BE, O'Neill BT, Tuinei J, Brahma MK, Pepin ME, McCrory MA, Luptak I, Halade GV, Litwin SE, Abel ED. Glucose transporter 4 (GLUT4) deficient hearts develop maladaptive hypertrophy in response to physiologic or pathologic stresses. *Am J Physiol Heart Circ Physiol* 2017;313:H1098-108.
70. Wang J, Li Z, Wang Y, Zhang J, Zhao W, Fu M, Han X, Zhou J, Ge J. Qiliqiangxin enhances cardiac glucose metabolism and improves diastolic function in spontaneously hypertensive rats. *Evid Based Complement Alternat Med* 2017;2017:3197320.
71. Korvald C, Elvenes OP, Myrmel T.Myocardial substrate metabolism influences left ventricular energetics in vivo. *Am J Physiol Heart Circ Physiol* 2000;278:H1345-51.

72. Nagoshi T, Yoshimura M, Rosano GM, Lopaschuk GD, Mochizuki S. Optimization of cardiac metabolism in heart failure. *Curr Pharm Des* 2011;17:3846-53.
73. Babalis D, Tritakis V, Floros G, Mouzarou A, Kafkas N, Bampali K, Mertzanos G. Effects of ranolazine on left ventricular diastolic and systolic function in patients with chronic coronary disease and stable angina. *Hellenic J Cardiol* 2015;56:237-41.
74. Burkhoff D, Weiss RG, Schulman SP, Kalil-Filho R, Wannenburg T, Gerstenblith G. Influence of metabolic substrate on rat heart function and metabolism at different coronary flows. *Am J Physiol* 1991;261:H741-50.
75. Sabbah HN, Gupta RC, Rastogi S, Wang M. Dysregulation of mitochondria fission and fusion proteins in explanted failure human hearts. *J Heart Lung Transplant* 2011;30:S137.
76. Carolo dos Santos K, Pereira Braga C, Octavio Barbanera P, Seiva FR, Fernandes Junior A, Fernandes AA. Cardiac energy metabolism and oxidative stress biomarkers in diabetic rat treated with resveratrol. *PLoS One* 2014;9:e102775.
77. Maulucci G, Daniel B, Cohen O, Avrahami Y, Sasson S. Hormetic and regulatory effects of lipid peroxidation mediators in pancreatic beta cells. *Mol Aspects Med* 2016;49:49-77.
78. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* 2014;2014:360438.
79. Gruzdeva O, Uchasova E, Dyleva Y, Belik E, Kashtalap V, Barbarash O. Relationship between free fatty acids, insulin resistance markers, and oxidized lipoproteins in myocardial infarction and acute left ventricular failure. *Diabetes Metab Syndr Obes* 2013;6:103-11.
80. Roy VK, Kumar A, Joshi P, Arora J, Ahanger AM. Plasma free fatty acid concentrations as a marker for acute myocardial infarction. *J Clin Diagn Res* 2013;7:2432-4.
81. Ma P, Han L, Lv Z, Chen W, Hu H, Tu J, Zhou X, Liu SM. In-hospital free fatty acids levels predict the severity of myocardial ischemia of acute coronary syndrome. *BMC Cardiovasc Disord* 2016;16:29.
82. Styskal J, Van Remmen H, Richardson A, Salmon AB. Oxidative stress and diabetes: what can we learn about insulin resistance from antioxidant mutant mouse models? *Free Radic Biol Med* 2012;52:46-58.
83. Abel ED, O'Shea KM, Ramasamy R. Insulin resistance: metabolic mechanisms and consequences in the heart. *Arterioscler Thromb Vasc Biol* 2012;32:2068-76.
84. Drosatos K, Schulze PC. Cardiaclipotoxicity: molecular pathways and therapeutic implications. *Curr Heart Fail Rep* 2013;10:109-21.
85. Park TS, Goldberg IJ. Sphingolipids, lipotoxic cardiomyopathy, and cardiac failure. *Heart Fail Clin* 2012;8:633-41.
86. Steggall A, Mordi IR, Lang CC. Targeting metabolic modulation and mitochondrial dysfunction in the treatment of heart failure. *Diseases* 2017;5:14.
87. Deo R, Albert CM. Epidemiology and genetics of sudden cardiac death. *Circulation* 2012;125:620-37.
88. Hayashi M, Shimizu W, Albert CM. The spectrum of epidemiology underlying sudden cardiac death. *Circ Res* 2015;116:1887-906.
89. Bhar-Amato J, Davies W, Agarwal S. Ventricular arrhythmia after acute myocardial infarction: "the perfect storm". *Arrhythm Electrophysiol Rev* 2017;6:134-9.
90. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. *Int Rev Cell Mol Biol* 2012;298:229-317.
91. Gewirtz H, Dilsizian V. Myocardial viability: survival mechanisms and molecular imaging targets in acute and chronicischemia. *Circ Res* 2017;120:1197-212.
92. Baumeister P, Quinn TA. Altered calcium handling and ventricular arrhythmias in acute ischemia. *Clin Med Insights Cardiol* 2016;10:61-9.
93. Bai J, Yin R, Wang K, Zhang H. Mechanisms underlying the emergence of post-acidosis arrhythmia at the tissue level: a theoretical study. *Front Physiol* 2017;8:195.
94. Kolettis T. Coronary artery disease and ventricular tachyarrhythmia: pathophysiology and treatment. *Curr Opin Pharmacol* 2013;13:210-7.
95. Thackeray JT, Beanlands RS, DaSilva JN. Altered sympathetic nervous system signaling in the diabetic heart: emerging targets for molecular imaging. *Am J Nucl Med Mol Imaging* 2012;2:314-34.
96. Oikonomidis DL, Tsalikakis DG, Baltogiannis GG, Tzallas AT, Xourgia X, Agelaki MG, Megalou AJ, Fotopoulos A, Papalois A, Kyriakides ZS, Kolettis TM. Endothelin-B receptors and ventricular arrhythmogenesis in the rat model of acute myocardial infarction. *Basic Res Cardiol* 2009;105:235-45.
97. Francis Stuart S, De Jesus N, Lindsey M, Ripplinger C. The crossroads of inflammation, fibrosis, and arrhythmia following myocardial infarction. *J Mol Cell Cardiol* 2016;91:114-22.
98. Skovsted GF, Kruse LS, Berchtold LA, Grell AS, Warfvinge K, Edvinsson L. Myocardial ischemia-reperfusion enhances transcriptional expression of endothelin-1 and vasoconstrictor ETB receptors via the protein kinase MEK-ERK1/2 signaling pathway in rat. *PLoS One* 2017;12:e0174119.
99. Streitner F, Kuschyk J, Veltmann C, Ratay D, Schoene N, Streitner I, Brueckmann M, Schumacher B, Borggrefe M, Wolpert C. Role of proinflammatory markers and NT-proBNP in patients with an implantable cardioverter-defibrillator and an electrical storm. *Cytokine* 2009;47:166-72.
100. Maradit-Kremers H, Crowson CS, Nicola PJ, Ballman KV, Roger VL, Jacobsen SJ, Gabriel SE. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum* 2005;52:402-11.
101. Downar E, Janse MJ, Durrer D. The effects of acute coronary artery occlusion on subepicardial transmembrane potentials in the intact porcine heart. *Circulation* 1977;56:217-24.
102. Biktashev VN, Biktasheva IV, Sarvazyan NA. Evolution of spiral and scroll waves of excitation in a mathematical model of ischaemic border zone. *PLoS One* 2011;6:e24388.
103. Solovyova O, Katsnelson L, Kohl P, Panfilov A, Tsaturyan A, Tsyvian P. Mechano-electric heterogeneity of the myocardium as a paradigm of its function. *Prog Biophys Mol Biol* 2016;120:249-54.
104. Robbers LF, Delewi R, Nijveldt R, Hirsch A, Beek AM, Kemme MJ, van Beurden Y, van der Laan AM, van der Vleuten PA, Tio RA, Zijlstra F, Piek JJ, van Rossum AC. Myocardial infarct heterogeneity assessment by late gadolinium enhancement cardiovascular magnetic resonance imaging shows predictive value for ventricular arrhythmia development after acute myocardial infarction. *Eur Heart J Cardiovasc Imaging* 2013;14:1150-8.
105. Pertsov AM, Davidenko JM, Salomonsz R, Baxter WT, Jalife J. Spiral waves of excitation underlie reentrant activity in isolated cardiac muscle. *Circ Res* 1993;72:631-50.
106. Oliver MF. Control of free fatty acids during acute myocardial ischaemia. *Heart* 2010;96:1883-4.
107. Tse G. Mechanisms of cardiac arrhythmias. *J Arrhythm* 2016;32:75-81.
108. Oliver MF, Kurien VA, Greenwood TW. Relation between serum free fatty acids and arrhythmias and death after acute myocardial infarction. *Lancet* 1968;1:710-4.
109. Tansey MJ, Opie LH. Relation between plasma free fatty acids and arrhythmias within the first twelve hours of acute myocardial infarction. *Lancet* 1983;2:419-22.
110. Ouven X, Charles MA, Desnos M, Ducimetiere P. Circulating non-esterified fatty acid level as a predictive risk factor for sudden death in the population. *Circulation* 2001;104:756-61.
111. Yang KC, Kyle JW, Makielski JC, Dudley SC. Mechanisms of sudden cardiac death: oxidants and metabolism. *Circ Res* 2015;116:1937-55.

112. Leach A, Fisher M. Myocardial ischaemia and cardiac pain - a mysterious relationship. *Br J Pain* 2013;7:23-30.
113. Ostrowski SR, Pedersen SH, Jensen JS, Mogelvang R, Johansson PI. Acute myocardial infarction is associated with endothelial glycocalyx and cell damage and a parallel increase in circulating catecholamines. *Crit Care* 2013;17:R32.
114. Kalra BS, Roy V. Efficacy of metabolic modulators in ischemic heart disease: an overview. *J Clin Pharmacol* 2012;52:292-305.
115. Nielsen TS, Jessen N, Jørgensen JO, Møller N, Lund S. Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease. *J Mol Endocrinol* 2014;52:R199-222.
116. Oliver M. Fatty acids and the risk of death during acute myocardial ischaemia. *Clin Sci* 2015;128:349-55.
117. Fragasso G. Deranged cardiac metabolism and the pathogenesis of heart failure. *Card Fail Rev* 2016;2:8-13.
118. Geerling JJ, Boon MR, Kooijman S, Parlevliet ET, Havekes LM, Romijn JA, Meurs IM, Rensen PC. Sympathetic nervous system control of triglyceride metabolism: novel concepts derived from recent studies. *J Lipid Res* 2014;55:180-9.
119. Opie L. Metabolic management of acute myocardial infarction comes to the fore and extends beyond control of hyperglycemia. *Circulation* 2008;117:2172-7.
120. Miller N, Mjøs O, Oliver M. Relationship of epicardial ST segment elevation to the plasma free fatty acid/albumin ratio during coronary occlusion in dogs. *Clin Sci* 1976;51:209-13.
121. Selker HP, Udelson JE, Massaro JM, Ruthazer R, D'Agostino RB, Griffith JL, Sheehan PR, Desvigne-Nickens P, Rosenberg Y, Tian X, Vickery EM, Atkins JM, Aufderheide TP, Sayah AJ, Pirrallo RG, Levy MK, Richards ME, Braude DA, Doyle DD, Frascone RJ, Kosiak DJ, Leaming JM, Van Gelder CM, Walter GP, Wayne MA, Woolard RH, Beshansky JR. One-year outcomes of out-of-hospital administration of intravenous glucose, insulin, and potassium (GIK) in patients with suspected acute coronary syndromes (from the IMMEDIATE [Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency Care] Trial). *Am J Cardiol* 2014;113:1599-605.
122. Mamas MA, Neyses L, Fath-Ordoubadi F. A meta-analysis of glucose-insulin-potassium therapy for treatment of acute myocardial infarction. *Exp Clin Cardiol* 2010;15:e20-4.
123. Evans R, Hauton D. The role of triacylglycerol in cardiac energy provision. *Biochim Biophys Acta* 2016;1861:1481-91.
124. Salazar J, Luzardo E, Mejías JC, Rojas J, Ferreira A, Rivas-Ríos JR, Bermúdez V. Epicardial fat: physiological, pathological, and therapeutic implications. *Cardiol Res Pract* 2016;2016:1-15.
125. Thanassoulis G, Massaro JM, O'Donnell CJ, Hoffmann U, Levy D, Ellinor PT, Wang TJ, Schnabel RB, Vasan RS, Fox CS, Benjamin EJ. Pericardial fat is associated with prevalent atrial fibrillation: the framingham heart study. *Circ Arrhythm Electrophysiol* 2010;3:345-50.
126. Al Chekakie MO, Welles CC, Metoyer R, Ibrahim A, Shapira AR, Cytron J, Santucci P, Wilber DJ, Akar JG. Pericardial fat is independently associated with human atrial fibrillation. *J Am Coll Cardiol* 2010;56:784-8.
127. Hatem SN, Sanders P. Epicardial adipose tissue and atrial fibrillation. *Cardiovasc Res* 2014;102:205-13.
128. Venteclef N, Guglielmi V, Balse E, Gaborit B, Cotillard A, Atassi F, Amour J, Leprince P, Dutour A, Clément K, Hatem SN. Human epicardial adipose tissue induces fibrosis of the atrial myocardium through the secretion of adipo-fibrokines. *Eur Heart J* 2014;36:795-805.
129. Tsao HM, Hu WC, Wu MH, Tai CT, Lin YJ, Chang SL, Lo LW, Hu YF, Tuan TC, Wu TJ, Sheu MH, Chang CY, Chen SA. Quantitative analysis of quantity and distribution of epicardial adipose tissue surrounding the left atrium in patients with atrial fibrillation and effect of recurrence after ablation. *Am J Cardiol* 2011;107:1498-503.
130. Samanta R, Pouliopoulos J, Thiagalingam A, Kovoor P. Role of adipose tissue in the pathogenesis of cardiac arrhythmias. *Heart Rhythm* 2016;13:311-20.
131. Taegtmeyer H. Metabolism--the lost child of cardiology. *J Am Coll Cardiol* 2000;36:1386-8.

Singh *et al. Vessel Plus* 2017;1:242-5
DOI: 10.20517/2574-1209.2017.25

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Case Report **Open Access**

Sternal malunion fixation using a Titanium Sternal Fixation System in a diabetic patient. The first of its kind in Scotland

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How to cite this article: Singh SSA, Capoccia M, Deep K, Al-Attar N. Sternal malunion fixation using a titanium sternal fixation system in a diabetic patient. The first of its kind in Scotland. *Vessel Plus* 2017;1:242-5.

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Article history:
Received: 17 Jul 2017
First Decision: 5 Sep 2017
Revised: 5 Sep 2017
Accepted: 11 Sep 2017
Published: 28 Dec 2017

Key words:
Sternal fixation,
sternal dehiscence,
diabetes,
bilateral internal thoracic artery

ABSTRACT

Sternal non-union is a rare but serious complication post-sternotomy. It is defined as sternal pain with clicking, instability, or both for more than 6 months in the absence of infection and usually presents in an outpatient setting. It may result in multiple admissions to the hospital for surgical debridement and wound care therapies. Several risk factors have been identified in the literature but treatment options are limited. Proven benefits of increased stability and decreased incidence of non-union using the principles of rigid fixation have been described in other surgical specialties such as orthopaedic surgery. Employing this, the authors describe the first successful sternal non-union fixation using the Titanium Sternal Fixation System (DepuySynthes) in Scotland.

INTRODUCTION

A 54-year-old male was referred to our unit following a Non-ST elevation myocardial infarction. His past medical history included type 2 diabetes mellitus for which he was on oral hypoglycaemic agents and subcutaneous insulin. He underwent a coronary angiogram that revealed severe triple vessel disease in:

Quick Response Code:

(1) the left anterior descending artery; (2) intermediate artery; (3) right coronary artery. The patient also had an occluded distal circumflex artery.

At the time of angiography, the patient had an estimated ejection fraction of about 35%. He was referred to the care of the cardiac surgeons for consideration of coronary artery bypass grafting (CABG).

He successfully underwent a quadruple vessel bypass using a bilateral internal thoracic artery (BITA) technique. This involves using both internal thoracic arteries as a Y-graft and sequential anastomosis to the target vessel. The closure of his sternum was performed using stainless steel wires (USP size 6) in a modified Robicsek technique [Figure 1] given his history of diabetes. This involves placing stainless steel wires parasternally on both sides. The initial wire is passed via the manubrium through the second intercostal space, forming a ring. This is continued for the succeeding intercostal spaces. Transverse parasternal wires are then placed proximal to distally allowing horizontal and vertical stabilization. He was discharged on postoperative day 6 after satisfactory progression[1]. On his journey home, the taxi he was involved in a near collision necessitating a sudden stop. He recalled an audible click on the tugging action of the seatbelt and noted increase movement in his sternum. He was referred following concerns of a non-healing sternal wound that was treated conservatively in the community that was worst on lifting objects and affected his sleep.

CASE REPORT

He described abnormal clicking and movement of his sternum when turning in bed. There were no discharging sinuses as the skin had healed well. All other examination findings were normal.

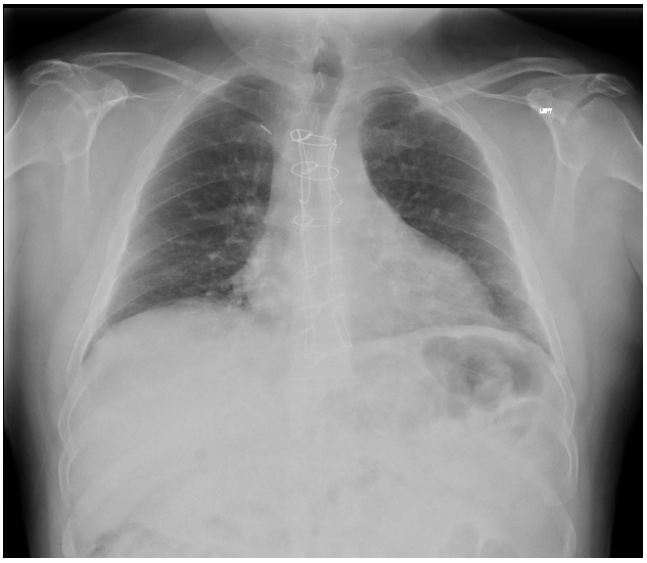

Figure 1: Chest X-ray showing sternal wires

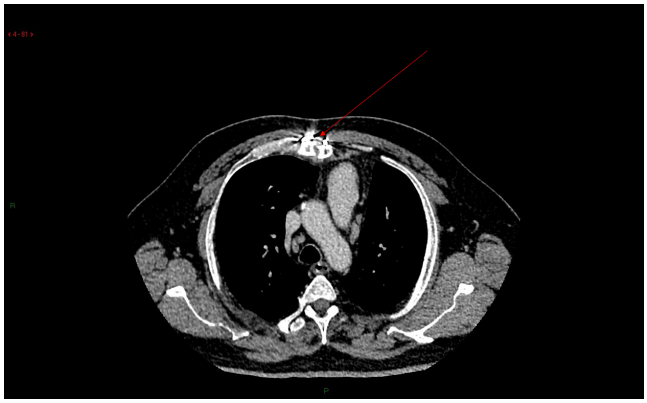

Figure 2: Computed tomography scan of thorax showing non-union of sternum (red arrow - sternal malunion)

His blood results revealed a HbA1c of 110 mmol/mol (normal range 20-42) indicating poor glycemic control. All other blood results were within normal limits.

A computed tomography scan revealed noted the displacement of the two sternal edges with a midline defect confirming malunion of the sternum and he was consented for sternal fixation. He was also referred to a diabetologist for optimisation of his glycemic control [Figure 2].

Procedure

The patient was prepped and draped in standard fashion. A midline incision was made on the skin. The sternal wires were removed and sent for microbiology analysis. Further debridement of devitalised tissues was performed with no use of bone wax. A specimen of bone was sent for microbiology.

Using a depth gauge, the sternal edges adjacent to each rib was measured for placement of the plates. Sternal reducing forceps was then used to reduce the cranial and caudal ends of the sternum. A template was used to gauge the size and contours needed prior to shaping of the eventual plate using bending pliers and a rod cutter. Two other plates were inserted to provide satisfactory fixation of the sternum. Bone grafts from the patients iliac crest was used to enhance the osteosynthesis. The sternum was then irrigated with saline. The pectoral muscles were sutured using interrupted Vicryl sutures, with continuous Vicryl for the subcutaneous layer closure and skin using Monocryl. The patient was extubated in theatre and taken to the wards.

Postoperatively, the patient recovered well with

minimal oral opioids and was discharged home on postoperative day 3 [Figure 3].

Microbiology results revealed inflamed fibrous tissue with a fibrinous reaction from the sternal debridement and no signs of infection or malignancy.

He was seen at the routine follow-up clinic 6 weeks and 1 year postoperatively with no further complaints of pain.

DISCUSSION

Since the advents of the SYNTAX trial (2009)[2], CABGs have been the mainstay of treatment of triple vessel diseases involving the left main stem as it had a lower rate of major adverse cardiac or cerebrovascular event at 1 year. Most CABG patients across the UK (90%) receive a "standard" operation with a single internal thoracic artery and vein grafts for revascularisation with excellent postoperative outcomes[3]. Progressive vein graft failure however is inevitable in these groups[4]. A meta-analysis by Yi *et al.*[5] highlighted that the benefits of BITA that continued to increase with duration of follow-up with freedom from redo surgery and survival[5]. A study by Nasso *et al.*[6] showed the superiority of a dual arterial technique over a single arterial graft technique at 2 years.

Critics of BITA however highlight its limitation with the theoretical increased risk of sternal wound infections due to the devascularisation of the sternum. Grossi *et al.*[7] highlighted the risk of devascularizing the sternum as an independent risk factor for sternal wound infection development[7]. With improving techniques of skeletonising the internal thoracic arteries however, many other studies have shown that there is no increased risk of BITA over single internal thoracic artery even in diabetic patients regardless of whether the surgery was on-pump or off-pump[8-11].

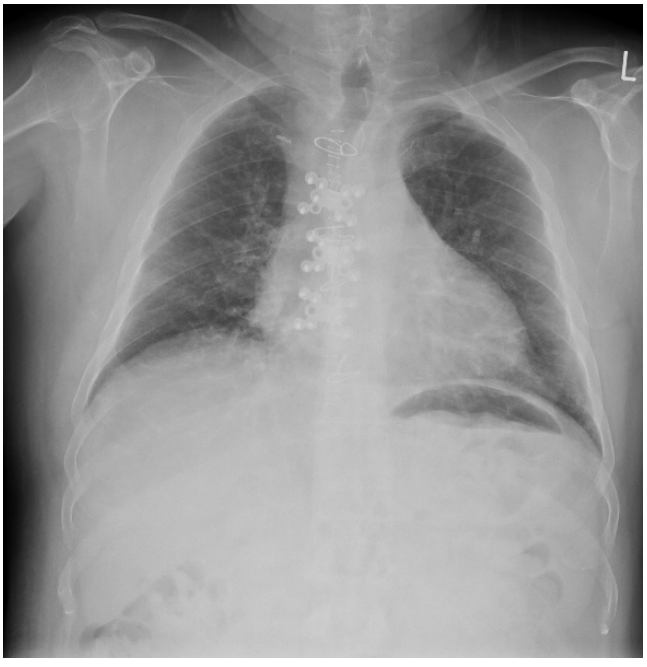

Figure 3: Postoperative chest X-ray showing union and fixation of sternum with titanium sternal fixation system

Another risk factor for sternal dehiscence is poor sternal closure. Sternal closure stability plays a pivotal role in preventing this. Our patient underwent a modified Robicsek closure which is described as sternal closure technique that provides the greatest stability[12]. This consists of placing interlocking steel wires parasternal bilaterally and then including them in transverse sternal wires providing stability against vertical and horizontal forces.

Poor glycemic control in diabetics has also been identified as an independent risk factor for sternal dehiscence. Optimisation of preoperative levels of HbA1c and blood glucose significantly reduced the rate of sternal wound complications in patients undergoing CABG[13].

Sternal non-union is defined as sternal pain with clicking, instability, or both for more than 6 months in the absence of infection as present in outpatient. The use of plating for sternal non-union is relatively new. Hendrickson *et al.*[14] first described its use in a case series of 6 patients with debilitating pain who reported improved quality of life post-plating with radiological evidence of fully healed sternotomies on follow up. Two patients developed bursae which settled on removal of the plates. Since then, multiple instances of sternal plate fixation have been used in the literature. A recent pilot study even advocated its use for primary closure of the sternum[15].

Vos *et al.*[16] conducted a retrospective analysis of patients with sternal dehiscence and compared outcomes of standard repair (steel wire cerclage and pectoralis muscle reconstruction) *vs.* titanium plating. The sternal plating group had greater sternal stability compared to the standard closure.

Kim *et al.*[17] conducted a similar review of their patients. In their cohort of 2,769 patients, 36 patients developed deep sternal wound complications and 17 underwent titanium plate fixation following debridement of the sternum. Almost half of the patients who underwent plating were diabetic (n = 8). Eight patients had undergone conservative therapy (vacuum dressings and soft tissue debridement) prior to internal fixation

with a titanium plate. One patient died following multi-organ failure secondary to mediastinitis that was present prior to plating with 2 requiring wound revisions due to aseptic wound dehiscence confined to the soft tissue. Another patient contracted tuberculosis and warranted an extended in-hospital stay. They concluded that rigid plate sternal fixation was a good option particularly for patients who require aggressive sternal resection.

The titanium plates also cost more than the other traditional methods of management including irrigation debridement and rewiring. However, considering its effectiveness, the shortened postoperative stay, improved outcomes and reduced readmission rates may reap its benefits.

Given the improvements in cardiac surgical techniques, sternal non-union remains an uncommon complication, albeit with significant morbidity. A larger multicentre trial may be warranted to verify its benefits over other modalities.

In conclusion, sternal fixation is a viable method to treat sternal malunion. Larger studies are needed to highlight its benefits compared to conventional therapy.

DECLARATIONS

Authors' contributions

Writing: S.S.A. Singh
Reviewing, editing, supervision: M. Capoccia, K. Deep, N. Al-Attar

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Verbal and written consent was obtained at time of surgery.

Ethics approval

Ethical approval was not needed as this was an FDA and CE-mark approved device that had been used for treatment of sternal non-union (FDA 510 k number: K093 772).

REFERENCES

1. Tavilla G, van Son JA, Verhagen AF, Lacquet LK. Modified Robicsek technique for complicated sternal closure. *Ann Thorac Surg* 1991;52:1179-80.
2. Serruys PW, Morice MC, Kappetein AP, Colombo A, Holmes DR, Mack MJ, Ståhle E, Feldman TE, van den Brand M, Bass EJ, Van Dyck N, Leadley K, Dawkins KD, Mohr FW; SYNTAX Investigators. Percutaneous coronary intervention versus coronary-artery bypass grafting for severe coronary artery disease. *N Engl J Med* 2009;360:961-72.
3. Taggart DP. Bilateral internal mammary artery grafting: are BIMA better? *Heart* 2002;88:7-9.
4. Taggart DP, Aratari C, Wong P, Paul EA, Wright JE. Applicability of intermittent global ischemia for repeat coronary artery operations. *J Thorac Cardiovasc Surg* 1996;112:501-7.
5. Yi G, Shine B, Rehman SM, Altman DG, Taggart DP. Effect of bilateral internal mammary artery grafts on long-term survival: a meta-analysis approach. *Circulation* 2014;130:539-45.
6. Nasso G, Coppola R, Bonifazi R, Piancone F, Bozzetti G, Speziale G. Arterial revascularization in primary coronary artery bypass grafting: direct comparison of 4 strategies--results of the Stand-in-Y Mammary Study. *J Thorac Cardiovasc Surg* 2009;137:1093-100.
7. Grossi EA, Esposito R, Harris LJ, Crooke GA, Galloway AC, Colvin SB, Culliford AT, Baumann FG, Yao K, Spencer FC. Sternal wound infections and use of internal mammary artery grafts. *J Thorac Cardiovasc Surg* 1991;102:342-6; discussion 346-7.
8. De Paulis R, de Notaris S, Scaffa R, Nardella S, Zeitani J, Del Giudice C, De Peppo AP, Tomai F, Chiariello L. The effect of bilateral internal thoracic artery harvesting on superficial and deep sternal infection: The role of skeletonization. *J Thorac Cardiovasc Surg* 2005;129:536-43.
9. Ogawa S, Okawa Y, Sawada K, Goto Y, Yamamoto M, Koyama Y, Baba H, Suzuki T. Continuous postoperative insulin infusion reduces deep sternal wound infection in patients with diabetes undergoing coronary artery bypass grafting using bilateral internal mammary artery grafts: a propensity-matched analysis. *Eur J Cardiothorac Surg* 2016;49:420-6.
10. Hirotani T, Kameda T, Kumamoto T, Shirota S, Yamano M. Effects of coronary artery bypass grafting using internal mammary arteries for diabetic patients. *J Am Coll Cardiol* 1999;34:532-8.
11. Jeong DS, Sung K, Lee YT, Ahn JH, Carriere KC, Kim WS, Park PW. Pure bilateral internal thoracic artery grafting in diabetic patients with triple-vessel disease. *Ann Thorac Surg* 2015;100:2190-7.
12. Schimmer C, Reents W, Elert O. Primary closure of median sternotomy: a survey of all German surgical heart centers and a review of the literature concerning sternal closure technique. *Thorac Cardiovasc Surg* 2006;54:408-13.
13. Hillis LD, Smith PK, Anderson JL, Bittl JA, Bridges CR, Byrne JG, Cigarroa JE, Disesa VJ, Hiratzka LF, Hutter AM Jr, Jessen ME, Keeley EC, Lahey SJ, Lange RA, London MJ, Mack MJ, Patel MR, Puskas JD, Sabik JF, Selnes O, Shahian DM, Trost JC, Winniford MD. 2011 ACCF/AHA guideline for coronary artery bypass graft surgery: a report of the american college of cardiology foundation/american heart association task force on practice guidelines. *Circulation* 2011;124:e652-735.
14. Hendrickson SC, Koger KE, Morea CJ, Aponte RL, Smith PK, Levin LS. Sternal plating for the treatment of sternal nonunion. *Ann Thorac Surg* 1996;62:512-8.
15. Bennett-Guerrero E, Phillips-Bute B, Waweru PM, Gaca JG, Spann JC, Milano CA. Pilot study of sternal plating for primary closure of the sternum in cardiac surgical patients. *Innovations (Phila)* 2011;6:382-8.
16. Vos RJ, Jongbloed L, Sonker U, Kloppenburg GTL. Titanium plate fixation versus conventional closure for sternal dehiscence after cardiac surgery. *Thorac Cardiovasc Surg* 2017;65:338-42.
17. Kim WK, Kim JB, Kim GS, Jung SH, Choo SJ, Chung CH, Lee JW. Titanium plate fixation for sternal dehiscence in major cardiac surgery. *Korean J Thorac Cardiovasc Surg* 2013;46:279-84.

AUTHOR INSTRUCTIONS

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Manuscript Type	Definition	Abstract	Keywords	Main Text Structure
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