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Review

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Varicose vein disease in the context of insulin resistance

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Abstract

The prevalence of insulin resistance (IR) is growing every year, which determines the risks of developing type 2 diabetes and cardiovascular diseases. Currently, IR is not recognized as a risk factor for the development of varicose veins (VVs), but the connection between the two is tacitly obvious because obesity and diabetes are risk factors for VVs. In this review, we have attempted to highlight the common nature of these two conditions in the context of mitochondrial dysfunction, inflammation, endothelial dysfunction, and tissue hypertrophy, and spotlight the role of IR in the development of VVs. We conclude that IR can contribute to the appearance of VVs.

Keywords: Insulin resistance, varicose veins, mitochondrial dysfunction, inflammation, endothelial dysfunction, tissue hypertrophy

INTRODUCTION

Insulin resistance (IR) is associated with many health problems, particularly cardiovascular diseases[1,2]. IR can be triggered by genetic factors[3], but most often, it arises from an unhealthy lifestyle. Key factors in acquired IR include sedentary lifestyle and obesity, both of which are strongly associated with obesity and diabetes[4]. IR is thought to be an adaptive protective mechanism of cells against the toxic effects of

nutrients[5], with its primary impact on adipose tissue, muscle tissue, and the liver[6]. The intimal layer of blood vessels is responsible for the reaction of the vascular wall to blood factors. Endotheliocytes lining the inner surface of blood vessels respond to insulin by interacting with insulin receptors on their surface[7]. IR in the context of lipid metabolism disorders is considered a cause of atherosclerosis[8,9]. Additionally, IR has been linked to thrombogenic processes[10]. It is also associated with increased blood pressure[11] and inflammatory processes in blood vessels[12,13]. Notably, IR can affect not only individuals with common risk factors like obesity and age but also lean, young individuals[14].

The connection between IR and disorders of the cardiovascular system cannot be overlooked[1,15]. Typically, when discussing IR, pathologies such as microvascular disorders, atherosclerosis, and heart disease are highlighted as the primary cardiovascular manifestations of metabolic syndrome[16]. However, an essential component of blood circulation - the venous system - is often neglected. Although insulin has been shown to reduce the risk of varicose veins (VVs)[17], there are no other literature data available on this topic. In this review, we compare the processes associated with the two conditions - IR and varicose vein disease to highlight the potential role of IR in the development of VVs. To accomplish this, in February-September 2024 we conducted keyword searches using databases such as PubMed, Google Scholar, Scopus, and Research Gate.

COMMON INFORMATION ABOUT IR

Insulin resistance is a decreased sensitivity of tissues to insulin, requiring the pancreas to secrete more insulin to maintain normal blood glucose levels[18]. IR is a common condition underlying "metabolic syndrome"[19], which encompasses IR, visceral obesity, atherogenic dyslipidemia, and endothelial dysfunction[20]. While the hyperinsulinemic-euglycemic clamp is considered the gold standard for assessing insulin resistance, its complexity often leads to the use of the HOMA-IR (homeostasis model assessment of insulin resistance) instead. The HOMA-IR index is calculated as fasting insulin (μU/dL) multiplied by fasting blood glucose (mmol/L)/22.5[21].

The prevalence of IR varies significantly, ranging from 15.5% (520 out of 3,354 Danish residents aged 19-72 years, HOMA-IR > 2.5) to 46.5% (943 out of 2,026 Venezuelan residents with a mean age of 39.69 ± 15.37 years, HOMA2-IR ≥ 2)[22]. In a study by Fahed *et al.*, 38.0% of 286 employees at the University of Notre Dame were found to have IR (HOMA-IR ≥ 2.5)[23]. IR is associated with several conditions, such as obesity, non-alcoholic fatty liver disease, prediabetes and type 2 diabetes, and polycystic ovary syndrome[24]. In cases of prediabetes, blood glucose levels range from 110 to 125 mg/dL (with 70-99 mg/dL considered normal). While these levels are not sufficient for a diabetes diagnosis, approximately 70% of individuals with prediabetes progress to diabetes[25]. According to statistics from CDC (Centers for Disease Control and Prevention, USA), 97.6 million Americans (38.0%) over the age of 18 have prediabetes, and 38.4 million Americans (11.6% of the US population) have type 2 diabetes[26]. In 2021, the global prevalence of diabetes was estimated at 10.5% (approximately 536.6 million people), projected to reach 12.2% by 2045[27].

Insulin is a pancreatic hormone that maintains blood glucose homeostasis[28]. By activating intracellular signaling pathways, insulin promotes glycogen synthesis in the liver[28,29] and muscles[30], inhibits lipolysis[31], and facilitates protein synthesis, as well as cell growth and proliferation[32]. It is secreted by the β-cells of the pancreas in response to rising blood glucose levels until these levels normalize. Typically, cells with insulin receptors effectively respond to this signal and absorb glucose[33]. However, certain genetic pathologies[3] and unfavorable environmental factors[34] can disrupt this process. Some researchers suggest that IR may be a protective response of cells against the toxic effects of excess nutrients[5]. According to this perspective, acquired IR results from nutrient overabundance, which diminishes cellular sensitivity to insulin and

disrupts the processes regulated by it. Insulin is vital for normal energy metabolism[35], wound healing[36], and the proper functioning of the nervous system[37,38] and cardiovascular system[39], as well as for lipid metabolism[31,40] and calcium homeostasis[41].

A reduced cellular response to insulin may indicate an organism's susceptibility to diseases such as atherosclerosis[42], thromboembolism[43,44], cardiomyopathy[45], Alzheimer's disease[46], multiple sclerosis[47], dementia[48], visual impairment[49], lipodystrophy[19], non-alcoholic fatty liver disease[50,51], rheumatoid arthritis[52,53], polycystic ovary syndrome[54], breast cancer[55], and pancreatic cancer[56]. IR is a common condition that is often diagnosed controversially[21]. The following causes of acquired IR are assumed: physical inactivity[57], obesity[58], potential hormonal changes[59], and excessive consumption of saturated fat[60]. Once type 2 diabetes develops, patients face an increased risk of cardiovascular mortality[61], and this condition is a leading cause of lower limb amputation[62]. Conditions associated with IR (such as metabolic syndrome) impact patients' quality of life[16] and escalate healthcare costs[63,64].

COMMON INFORMATION ABOUT VVS

Chronic venous disorders include the entire spectrum of morphological and functional disorders within the venous system. Chronic venous disease (CVeD) refers to long-term morphological and functional disorders that manifest through symptoms and/or signs necessitating examination and/or care[65]. The prevalence of CVeD is high; a review by Salim *et al.* analyzed pooled data from 19 studies across different continents, revealing the following prevalence rates of clinical classes (according to the CEAP classification[66]): C0 (no visible or palpable signs of venous disease): 9%, C1 (telangiectasias or reticular veins): 26%, C2 (VVs): 19%, C3 (edema): 8%, C4 (skin and subcutaneous tissue changes secondary to chronic venous disease): 4%, C5 (healed venous ulcers): 1%, and C6 (active venous ulcers): 0.42%[67]. VVs are characterized by twisted and dilated veins in the lower extremities. If left untreated, this condition can lead to complications such as superficial vein thrombosis, deep vein thrombosis, and venous ulcers[68]. The progression rate of stage C2 is 22%, while the annual incidence of VVs ranges from 0.2% to 2.3%[69]. Classes C3-C6 are designated as chronic venous insufficiency, which indicates advanced CVeD characterized by functional disorders of the venous system, leading to swelling, skin changes, or venous ulcers[65]. In this review, the term VVs encompasses not only the C2 class of CVeD but also represents the broader concept of varicose transformation of the veins in the lower extremities.

Blood flow from the lower extremities is facilitated by a complex system of veins, which function effectively due to the presence of bicuspid valves, muscle contraction that counteracts gravity, and high hydrostatic venous pressure (up to 100 mm Hg)[70]. The venous wall consists of three layers[71]: the *tunica (t.) intima* - the inner layer, composed of endothelial cells that line the basement membrane. This layer is in direct contact with the blood and ensures selective permeability of the venous wall. The *t. media* - the middle layer, made up of smooth muscle cells (SMCs), which regulate pressure within the vein lumen. The *t. adventitia* - the outer layer, primarily consisting of fibroblasts walled up in a large amount of extracellular matrix. This layer provides mechanical support to the vessel and facilitates contact with surrounding tissues. Researchers have identified several abnormalities accompanying with VVs, including hypoxia due to blood stasis, mechanical stretching of the vein, generalized thickening of the venous wall, increased rigidity, valvular dysfunction, and impaired permeability. Additionally, hyperplasia of the intimal layer with degradation of endothelial cells, migration of SMCs, and exposure of the basement membrane, disruptions in the ratios of collagen types and metalloproteases, immune cell infiltration, changes in the phenotype of SMCs from contractile to secretory, alterations in the distribution of cell markers[68], and dysregulation of SMC apoptosis (increased in the early stages of VVs and decreased later) have also been observed[72]. Disruption in apoptotic regulation may contribute to the proliferative phenotype of SMCs observed in VVs.

Risk factors for VVs include family history (genetic factors), advanced age, female gender, pregnancy, obesity, physical inactivity, smoking, hypertension, hormone replacement therapy/oral contraceptives, diabetes mellitus, orthopedic injuries, lifestyles characterized by prolonged standing or sedentary behavior, as well as superficial vein thrombosis and low fiber intake[73-75]. The researchers highlight the connections between type 2 diabetes (characterized by impaired glucose tolerance and insulin resistance) and CVeD, including thrombogenesis, proinflammatory state, collagen structure disorders, and endothelial dysfunction[76,77]. Studies indicate that patients with CVeD are twice as likely to have diabetes mellitus compared to the general population; however, a higher incidence of more severe stages of CVeD (specifically C5 and C6, according to the CEAP) has not yet been established[76]. A cohort study in Taiwan showed that the overall incidence of venous thromboembolism is higher in patients with diabetes than in the general population, with these patients also facing a higher risk of developing deep vein thrombosis and pulmonary embolism[78].

The additive genetic component of CVeD is 17.3%[79], suggesting that other risk factors contribute 82.7% to the development of the disease. Additionally, the influence of factors such as gender and age on the progression of clinical status is estimated to account for 10.7%[79].

MITOCHONDRIA AND CALCIUM METABOLISM IN IR AND VVS

Mitochondrial dysfunction significantly contributes to IR[80]. Researchers relate IR to disruptions in mitochondrial dynamics and a decrease in the number of functional mitochondria[81], which are provoked by the changes in mitochondrial calcium homeostasis[82]. Endoplasmic reticulum stress, potentially arising from hypoxia or oxidative damage, leads to calcium release, causing cell death or, if the stimulus is insufficient, mitochondrial dysfunction[83]. In IR, apoptotic processes are intensified[84] and provoked by fatty acids (FAs) metabolites (such as diacylglycerol and ceramide)[85], alongside an observed accumulation of Ca^{2+} in the cytoplasm, disrupting mitochondrial dynamics[81]. Mitochondria typically undergo fission, a process that allows for the removal of dysfunctional parts of these organelles through mitophagy. However, in the case of IR, dysfunctional mitochondria persist, hindering the development of new, healthy mitochondria. Mitochondrial fission reduces the activity of the p38 MAPK pathway and increases the activity of insulin receptor substrate 1 (IRS-1) and AKT serine/threonine kinase 1 (AKT). In this case, biogenesis is disrupted and the formation of new competent mitochondria is reduced, likely linked to reduced expression of PGC-1α, a transcription coactivator that regulates genes involved in energy metabolism[81].

Due to a decrease in insulin's ability to regulate lipolysis, FAs accumulate in the inner mitochondrial membrane, leading to their oxidation in peroxisomes and microsomes. In turn, increases the production of reactive oxygen species (ROS)[85], which is presumed to cause IR[86]. When energy substrates are in excess during IR, cells experience a deficiency in adenosine triphosphates (ATPs). This reduction in ATP levels during IR is probably associated with an adaptive thermoregulatory mechanism activated by excess FAs in the body, which favors heat production over ATP synthesis (uncoupling of the oxidation and phosphorylation processes)[85].

We have demonstrated that in varicose veins, compared to non-varicose veins, the amount of mitochondrial DNA is decreased, and the mitochondrial membrane potential is reduced[87]. A single mitochondrion can contain multiple copies of DNA; however, the overall amount of mitochondrial DNA is generally indicative of the number of mitochondria within a cell. These findings suggest an impairment in both mitochondrial biogenesis and function in VVs, potentially classifying VVs as a form of secondary mitochondrial dysfunction. Additionally, differential expression of several genes regulating the

mitochondrial function has been observed in VVs. For example, a decrease in *OXA1L* gene mRNA levels has been reported in VVs compared to non-varicose veins[88]. The OXA1L protein participates in the assembly of mitochondrial respiratory chain complexes by inserting proteins encoded by mitochondria and the nucleus into the mitochondrial membrane. This process is required for the oxidative phosphorylation mechanism[89,90]. Furthermore, an increase in the expression of the *BCL-2* gene and a decrease in the expression of the *BAX* gene at the mRNA and protein levels have been observed in varicose SMCs compared to non-varicose SMCs[91]. The BCL-2 protein is localized in the membranes of mitochondria, endoplasmic reticulum, and the nucleus, where it helps preventing oxidative damage. Conversely, the BAX protein interacts with the mitochondrial voltage-dependent anion channel, leading to a loss of the membrane potential and the release of cytochrome C[92]. The BCL-2/BAX ratio determines cell survival or death after an apoptotic stimulus[93]. The absence of differential expression of each of those genes in whole VV segments (regardless of cell type related to a certain layer of the vein wall)[88] may reflect their multidirectional expression in different cell types. In patients with polycystic ovary syndrome (the condition related to IR), who did not receive growth hormone treatment, the expression of the *BAX* and *BCL-2* genes in granulosa cells is increased and decreased, respectively[94]. Furthermore, *BNIP-3* gene expression at the mRNA level was shown to be increased in VVs compared to non-varicose veins[95]. BNIP-3 is a mitochondrial protein and a proapoptotic factor associated with mitochondrial dysfunction[96].

The loci rs2911463 (*PIEZO1*), rs2861819 (*PPP3R1*), and rs28558138 (*STIM2*) are associated with VVs[97]. PIEZO1 is a cation channel activated by a mechanical stimulus: tension or shear stress. Its activation has been shown to enhance mitochondrial respiration and glycolysis in endothelial cells, stimulating ATP production[98]. In addition, PIEZO1 has been implicated in the regulation of insulin sensitivity[99]. PPP3R1 plays an important role in the adaptive regulation of body weight and energy metabolism to a high-fat, high-sugar diet and exercise[100]. It has also been found to increase Ca^{2+} influx, which promotes the aging of mesenchymal stem cells[101] and is involved in the pathogenesis of Alzheimer's disease[102], a condition linked to IR condition. STIM2 is a key regulator of cytosolic calcium concentration[103] and is also involved in the development of Alzheimer's disease[104].

We cannot definitely claim that mitochondrial dysfunction in VVs is directly related to IR, but both conditions (IR and VVs) share similarities in mitochondrial biogenesis, calcium homeostasis, and energy metabolism.

INFLAMMATION IN IR AND VVS

Inflammation is a key indicator and contributing factor of IR[105]. One of the primary signs of IR is inflammation within adipose tissue, where hypertrophy and hyperplasia are accompanied by the secretion of cytokines and increased recruitment of immune cells[106]. Obesity-induced lipid accumulation activates the JNK and NF-kB pathways[107], leading adipocytes to secrete proinflammatory cytokines such as CCL2[108], TNF-α[109], and IL-6[110]. High levels of CCL2 promote the infiltration of monocytes into adipose tissue, where these cells differentiate into adipose tissue macrophages[111]. In turn, macrophages secrete TNF-α, IL1β, IL-6, and IL-8, which contribute to increased lipolysis (release of FAs), induction of matrix metalloproteinases (MMPs), and inhibition of type 2 collagen synthesis[112,113]. Beyond macrophages, various other immune cells, including mast cells, neutrophils, CD-34 cells, B cells, Th1, and CD8 T cells, are implicated in IR[106]. In a human induced pluripotent stem cell model of hepatic IR, TNFα and IL1β have been shown to promote inflammation and IR[114]. TNF-α increases serine phosphorylation of IRS1/2, which reduces GLUT4 expression[115] and increases IL-6 secretion. IL-6, in turn, inhibits the transcription of the *IRS-1*, *GLUT-4* and *PPAR-γ* (peroxisome proliferator-activated receptor gamma) genes, thereby impairing insulin-stimulated glucose transport. IL-6 expression in fat cells increases approximately 15-fold during

IR[113]. In human skeletal muscle myoblasts, IL-6 has been found to enhance the expression of Toll-like receptor 4 (TLR-4), which triggers the innate immune response through STAT3 activation[116]. Interestingly, the inhibition of PIEZO1 in mouse adipocytes has been shown to promote TLR4-mediated inflammation and induce IR[99]. A pathway for hepatic IR has been proposed, in which STAT3 is activated through mTOR, which, in turn, increases the expression of SOCS3 (suppressor of cytokine signaling 3), promoting the inhibition of insulin signaling[117]. Moreover, IFNγ has been identified as a type 2 diabetes-specific atherogenic factor that suppresses the antiatherogenic proteins APOE and C3, predisposing macrophages to increased cholesterol accumulation[118].

Inflammation in the venous wall can occur due to the activation of endothelial cells, triggered by changes in shear stress and the onset of initial inflammatory processes. In response, the endothelium increases the adhesion and migration of leukocytes through the venous wall[119]. Compared to normal endothelial cells, venous endothelial cells from patients with VVs show increased expression of cell surface adhesion molecules, such as CD146 and ICAM-1[120]. Interestingly, in patients with diabetes and obesity who lost weight through exercise and a balanced low-calorie diet, the levels of adhesion molecules ICAM-1 and VCAM-1 decrease[121]. It has also been demonstrated that senescent human umbilical vein endothelial cells treated with serum from patients with VVs produce increased amounts of ICAM-1, VCAM-1, P-selectin, uPA, PAI-1, and ET-1 via TGF-β[122]. PAI-1, a fibrinolysis inhibitor, is elevated in IR due to visceral fat accumulation, and this effect may be further amplified by TNF-α and TGF-β. Elevated PAI-1 levels are also linked to atherothrombosis in the context of IR[123]. ICAM-1, a cell surface glycoprotein on endothelial cells, is activated in response to inflammatory stimuli, facilitating leukocyte transmigration across the endothelium. ICAM-1 expression is induced by TNF-α or IL-1β[124]. VCAM-1 also mediates the binding of leukocytes to the endothelium, which may be enhanced by increased levels of circulating cytokines (TNF-α, IL-6) and lipoproteins[13]. Leukocytes adhering to the vein wall release TGF-β and proinflammatory cytokines, triggering pathological cascades[119]. TGF-β regulates cell proliferation, differentiation, and growth, and plays a role in diabetic fibrosis across various tissues[125]. Blood samples from the site of VVs show significantly increased concentrations of IL-6, IL-8, and CCL2, suggesting that the surrounding tissue (including fat) may activate inflammation in the vein wall[126].

In addition to the aforementioned information, an increase in the expression of the *CX3CR1* gene[88] and a decrease in the expression of the *IL-8* gene[127], which is associated with inflammation, were observed in VVs. CX3CR1 is a transmembrane protein and chemokine involved in leukocyte adhesion and migration, and its expression is elevated in diabetic patients[128]. IL-8, a key mediator of the inflammatory response that directs neutrophils to infection sites, plays a role in the endothelial dysfunction observed in IR[129]. There is also a genetic link between VVs and inflammation, involving the previously mentioned variants: rs2861819 (*PPP3R1*), rs11135046 (*EBF1*), rs9880192 (*GATA2*), and rs12625547 (*NFATC2*)[97]. EBF1 is a transcription factor that stimulates B cell differentiation and is associated with cardiovascular and metabolic risk[130]. GATA2, another transcription factor, is involved in hematopoiesis and the regulation of endocrine cell lines. Activation of the Wnt pathway in human adipocytes has been shown to induce *GATA2* expression, while reducing *GLUT4* expression, which characterizes IR[131]. NFATC2, a nuclear factor expressed by activated T cells, plays a critical role in the induction of gene transcription during immune responses. In *TLR4* knockout models, NFATC2 promotes mitochondrial metabolic reprogramming through its translocation to mitochondria, potentially alleviating oxidative stress and chronic inflammation during IR[132].

ENDOTHELIAL DYSFUNCTION IN IR AND VVS

The endothelium, which lines the entire inner surface of blood vessels, plays a crucial role in regulating blood circulation. Its functions include maintaining the barrier between the bloodstream and surrounding tissues, controlling vascular tone, regulating hemostasis, promoting the formation of new blood vessels, facilitating hormone transport, and recruiting neutrophils. Endothelial dysfunction is marked by decreased vasodilation and increased proinflammatory and prothrombic properties. The damaged endothelium becomes excessively permeable, allowing harmful substances (such as toxins, *etc*.) to infiltrate the surrounding tissues[133].

The activation of insulin receptors on the surface of endotheliocytes is necessary to regulate the function of these cells. Insulin controls blood flow and facilitates its delivery to peripheral tissues[134,135] through the activation of AKT, which subsequently activates endothelial nitric oxide synthase (eNOS)[7]. Nitric oxide (NO), produced by eNOS, is an endogenous vasodilator and inhibitor of thrombogenesis[136], giving insulin vasodilatory properties. Additionally, insulin stimulates angiogenesis and promotes wound healing by stimulating the secretion of vascular endothelial growth factor (VEGF)[137,138], and prevents endothelial cell apoptosis[139]. Studies on murine knockouts of the insulin receptor gene have shown a significant increase in baseline systolic blood pressure and impaired natriuresis[140], indicating the role of IR in arterial hypertension. High blood pressure triggers a proinflammatory and prothrombic state in the endothelium, increases vascular stiffness, and heightens the number and activation of platelets[141]. IR increases the tendency to thrombus formation[10]; endothelial dysfunction provoked by IR can lead to an increased level of circulating PAI-1 in plasma due to its enhanced synthesis in endothelial cells. In addition to being highly correlated with IR, elevated levels of circulating PAI-1 predict the development of diabetes[142]. It is worth noting that deep vein thrombosis is a known risk factor for the development of VVs[143]. Furthermore, IR in other tissues negatively affects endothelial function. For example, IR in adipose tissue is associated with dyslipidemia (an abnormal increase in plasma levels of free fatty acids and triglycerides) characterized by an imbalance between high-density lipoprotein and low-density lipoprotein (LDL), favoring the latter[144]. LDL, when oxidized through interaction with ROS, binds to lectin-like oxidized LDL receptor-1 on the surface of endothelial cells and is absorbed, which enhances the production of MMPs and the adhesion of leukocytes to the endothelium, and downregulates eNOS[1].

In the previous chapter, we discussed endothelial dysfunction in VVs, which is directly related to inflammation. Normally, laminar shear stress enhances the expression and activity of eNOS, leading to increased NO production, which, among other effects, inhibits SMC proliferation. VVs are characterized by reduced shear stress that contributes to endothelial dysfunction and pathological remodeling of the venous wall[145]. Our recent study demonstrated that oscillatory shear stress on the endothelium results in epigenome-wide methylation changes in endothelial cells and other cell types in adjacent layers of the venous wall[146]. Additionally, the hypoxic environment caused by blood stagnation negatively affects the venous endothelium. It has been shown that *HIF-1α* and *HIF-2α* genes encoding the α-subunits of hypoxia-inducible factors are upregulated in VVs[95]. HIF-α is elevated in response to decreased oxygen concentrations. Increased expression of HIF target genes - *GLUT-1*, *CA9*, *BNIP-3*, and *VEGF* - has also been reported[95]. While VEGF normally supports endothelial function and regulates vascular growth, its dysregulation, with abnormally high levels, leads to aberrant angiogenesis[147]. In addition to endothelial damage in VVs, neoangiogenesis is commonly observed[148]. We assume that high circulating insulin levels may also contribute to the upregulation of VEGF in VVs, as a higher HOMA-IR index is predictive of increased serum VEGF levels[149]. Furthermore, studies indicate that VVs exhibit elevated levels of VEGF-A (mRNA and protein) and increased expression of the *VEGFR2* gene encoding the VEGF receptor, which may be a marker of, or lead to, abnormal extracellular matrix metabolism[150,151]. In VVs, increased mRNA

levels of genes encoding proteins from the cellular communication network family -*CCN1*, *CCN2*, and *CCN5* - have been reported[88]. Members of this family play a regulatory role and are involved in endothelial cell adhesion. Conversely, decreased expression of the *VCL* gene, which encodes a protein that protects VE-cadherin junctions in endothelial cells and strengthens the endothelial barrier[127,152]. Additionally, the rs247749 locus associated with the *AGGF1* gene, which encodes an angiogenic factor promoting endothelial cell proliferation, has been identified as a genetic risk factor for VVs[153]. Notably, overexpression of *Aggf1* has been demonstrated to lead to hepatic steatosis in mice, causing IR[154].

TISSUE HYPERTROPHY IN IR AND VVS

Normally, insulin activates and maintains the balance of two signaling pathways: PI3K-AKT (metabolic arm: glucose transport, protection against apoptosis, oxidative stress and inflammation, inhibition of lipolysis, glycogen and protein synthesis) and MAPK (mitogenic arm: cell growth, hypertrophy and fibrosis, inflammation). However, in the presence of IR, this balance shifts toward the mitogenic arm, leading to disturbances in the cardiovascular system[155]. Both the PI3K-AKT and MAPK pathways are upregulated in patients with venous reflux[74]. Thus, the processes associated with VVs may be linked to both IR of the venous endothelium itself and the hyperinsulinemia caused by IR in other tissues.

There is a connection between cardiac hypertrophy and IR[156], as well as between IR and the hypertrophy and hyperplasia of adipose tissue[157]. VVs are characterized by hypertrophy (with local atrophy) of the venous wall, with disorganization of the *t. media*, thickening of the *t. intima*, and the transition of SMCs to a synthetic and proliferative phenotype[158]. Surendran *et al.* demonstrated hypertrophy and hyperplasia of SMCs in VVs, which was associated with upregulation of the FoxC2-Dll4 pathway[159]. Additionally, evidence indicates that insulin can increase the expression of the FOXC2 protein in mesenchymal stem cells, which regulates the expression of genes associated with IR (*GLUT4*, *PAI-1*, *UCP-1*)[160]. In VVs, there is an increased expression of genes involved in proliferation, including *TIMP1*, *TMEM158*, *CHRDL2*, *EFEMP1*, and *TGF-β*[127]. Levels of Timp1 (tissue inhibitor of matrix metalloproteinase 1) have been found to be elevated in the serum and adipose tissue of diet-induced obese mice[161]. TGF-β plays a crucial role in the pathogenesis of VVs, serving as a link between inflammation and remodeling of the venous wall[74]. However, the study by Bruczko-Goralewska *et al.* reported a significant reduction in TGF-β protein levels in VVs compared to veins of patients with chronic limb ischemia[151]. TGF-β disrupts the regulation of fibronectin and collagen, MMPs and their tissue inhibitors (TIMPs)[74], and influences the expression and activity of growth factors. An imbalance in the MMPs/TIMPs ratio results in decreased elasticity and increased distensibility of the venous wall[162]. In obesity, regulation of MMPs and TIMPs in adipose tissue is disrupted, which plays a significant role in metabolic disorders. Notably, membrane-type 1 matrix metalloproteinase can modulate insulin sensitivity by cleaving insulin receptors[163].

We observed an increase in the expression of the *MFAP5* gene, which encodes a microfibril-associated component of the extracellular matrix, in VVs. This increase correlated with the hypomethylated status of two loci located in the regulatory regions of this gene[88]. Among other layers of the venous wall investigated, the *t. intima* appeared to be the main contributor to hypomethylation at one of these loci in VVs[164]. MFAP5 is known to bind growth factors (TGFβ, BMP)[165]; it promotes angiogenesis[166], enhances the stabilization of type 1 procollagen[167], and correlates with IR markers in adipose tissue[168]. Additionally, perivascular adipose tissue (PVAT), which provides mechanical support and is in close contact with the *t. adventitia*, contributes to the endothelium-independent regulation of vascular tone through vasoactive adipokines[169]. Therefore, with regard to the PVAT surrounding saphenous veins, our findings highlight the role of IR in the development of VVs.

CONCLUSION

In this review, we have highlighted the evident connection between VVs and IR in processes such as mitochondrial dysfunction, inflammation, endothelial dysfunction, and tissue hypertrophy. Consequently, we conclude that IR can contribute to the development of VVs. We believe this original insight will draw researchers' attention to these interrelations and may lead to meaningful concepts. In our opinion, future research on this topic could be performed in a wide variety of scientific fields, including epidemiology, molecular genetics, physiology, and clinical investigations. Ultimately, this research could: (1) enable clinicians to take appropriate and timely measures for patients with VVs (which, in fact, can serve as markers of existing IR, thereby potentially alleviating symptoms of CVeD) to early diagnose IR and reduce the possible risks of further complications; and (2) empower patients to (a) prevent the development of metabolic syndrome by controlling circulating insulin levels; and (b) reduce the risk of VVs by making lifestyle adjustments.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception, conceptualization, and design of the study: Korolenya V, Smetanina M, Filipenko M

Performed literature data acquisition and analysis; wrote the manuscript (original draft): Korolenya V

Wrote the manuscript (review & editing): Korolenya V, Smetanina M

Provided administrative, technical, and material support: Smetanina M, Filipenko M

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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