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Determinants of plasma branched-chain amino acids and associations with cardiometabolic biomarkers and proteomic signatures

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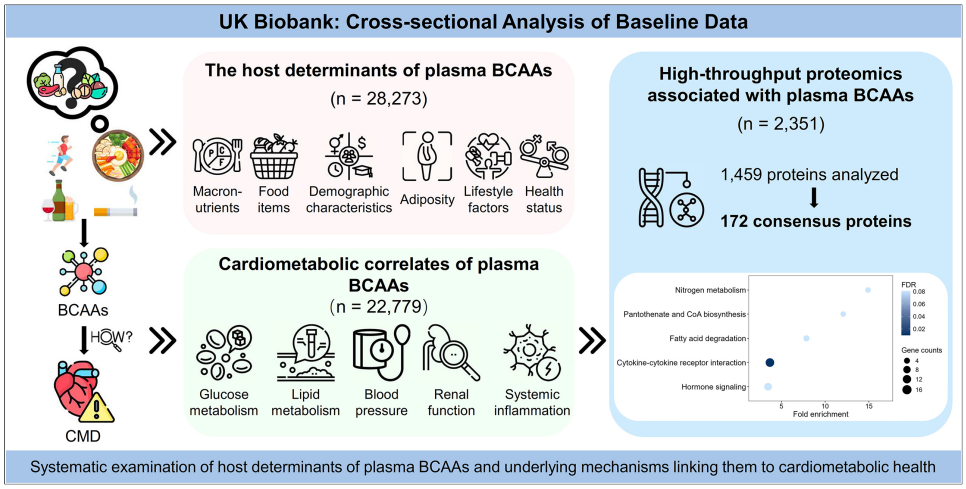

Abstract

Aim: We aimed to identify dietary and non-dietary determinants of plasma branched-chain amino acids (BCAAs), evaluate their associations with comprehensive biomarkers of cardiometabolic health (CMH), and characterize BCAA-related proteomic signatures to clarify potential biological pathways underlying these associations.

Methods: This observational study utilized baseline data from the UK Biobank. Multivariable linear regression models were applied to evaluate associations of dietary and non-dietary factors with plasma BCAAs among 28,273 participants. Subsequent models assessed relationships between these amino acids and comprehensive cardiometabolic biomarkers in 22,779 participants. Using high-throughput proteomics in a subcohort of 2,351 individuals, proteins associated with BCAAs were identified, followed by pathway enrichment and protein-protein interaction analyses.

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Results: Plasma BCAAs were positively associated with animal-based foods and total animal protein intake, but inversely associated with plant-based dietary components. Adiposity exhibited strong positive associations, whereas older age, female sex, and higher physical activity correlated with lower BCAAs. Elevated BCAAs were consistently associated with adverse glycemic and lipid profiles alongside impaired renal function, with the strongest association observed for triglycerides. Proteomic analyses identified 172 consensus proteins converging on core metabolic pathways, with LEP as a central hub. Beyond these shared signatures, individual BCAA species exhibited distinct functional networks: leucine-specific proteins (centered on CXCL8 and SDC1) were primarily associated with chemotaxis and cell migration, whereas valine-specific proteins (centered on F10 and PLAT) were associated with coagulation and fibrinolysis.

Conclusion: Circulating BCAAs are significantly influenced by modifiable host factors, particularly dietary components. They are associated with cardiometabolic risk through distinct proteomic signaling cascades.

INTRODUCTION

Branched-chain amino acids (BCAAs), including isoleucine, leucine, and valine, are essential nutrients primarily derived from dietary sources due to the lack of *de novo* synthesis in humans. Beyond their structural role in muscle, circulating BCAAs are increasingly recognized as active metabolic modulators[1] and important intermediate phenotypes linking food quality analysis and post-consumption metabolism[2,3]. Notably, emerging evidence links plasma BCAAs to cardiometabolic health (CMH), serving as early biomarkers of increased risks for cardiovascular disease and type 2 diabetes[4].

Given their implications for CMH, identifying modifiable determinants of circulating BCAAs is of substantial public health importance. As systemic BCAAs reflect the metabolic processing of food rather than just direct dietary ingredients, their circulating concentrations are highly responsive to dietary quality and nutritional modifications. For instance, altering macronutrient composition or specific amino acid ratios in protein-restricted models can partially restore BCAA homeostasis[5], and structured dietary interventions have been shown to improve circulating BCAA profiles[6]. In addition to dietary intake, plasma BCAA levels are regulated by a constellation of host factors. Longitudinal studies reveal that BCAA levels tend to increase from young adulthood to midlife, although trajectories vary; males and individuals with obesity or dyslipidemia are more likely to exhibit sustained increases or persistently elevated levels[7]. Lifestyle behaviors also play an important role. Persistent smoking and alcohol consumption may perturb BCAA levels by disrupting mitochondrial energy metabolism[8,9], whereas regular physical activity promotes BCAA catabolism[10]. Despite these observations, population-level evidence evaluating these determinants remains limited. Most previous studies have considered a narrow range of host factors[11], and dietary analyses have largely emphasized dietary patterns or protein intake, failing to characterize contributions of specific food groups to BCAA homeostasis[6,12].

Although BCAAs have been implicated in the development of cardiometabolic diseases, systematic evaluations encompassing a broad panel of CMH biomarkers, spanning glycemic control, lipid metabolism, and systemic inflammation, remain scarce. In addition, while current research suggests that plasma BCAAs influence CMH through specific cellular mechanisms, including platelet activation or mitochondrial dysfunction[13,14], the broader systemic molecular pathways in humans remain poorly characterized. There

remains limited evidence from large-scale population cohorts utilizing high-throughput proteomics to systematically evaluate associations between plasma BCAAs and circulating proteomic biomarkers. This gap hinders a comprehensive understanding of the biological pathways and potential mechanisms underlying the associations between BCAAs and CMH.

To address these knowledge gaps, we utilized the large-scale UK Biobank (UKB) cohort to systematically examine the population-level associations of dietary and non-dietary factors with plasma BCAA levels, and evaluated the relationships between plasma BCAA levels and a broad panel of CMH biomarkers. High-throughput proteomics was further leveraged to explore key biological pathways and identify central hub protein markers within these networks.

METHODS

Study population

This study utilized baseline data from the UKB (https://www.ukbiobank.ac.uk/), a large-scale prospective cohort of approximately 500,000 participants recruited from the general population across 22 assessment centers in the United Kingdom between 2006 and 2010[15]. At baseline, participants completed self-administered touchscreen questionnaires to provide detailed sociodemographic and lifestyle information, underwent standardized physical measurements, and provided biological samples. The UKB was approved by the Northwest Multicenter Research Ethics Committee (REC reference: 21/NW/0157), and all participants provided informed consent.

Participant selection was conducted sequentially according to three analytical objectives [Supplementary Figure 1]. Among the total UKB cohort, 274,119 participants had available baseline plasma BCAA measurements. For analyses of BCAA determinants, we excluded 101,538 individuals with missing data on essential non-dietary factors and additional covariables, and 144,308 who did not complete the dietary questionnaire, yielding a sample of 28,273 participants. For analyses examining associations between plasma BCAAs and cardiometabolic biomarkers, we further excluded 5,494 participants with incomplete biomarker data, resulting in a final sample of 22,779 participants. For subsequent proteomic analyses, an additional 20,428 individuals were excluded because they had more than 50% of protein measurements missing. In accordance with standardized UKB protocols, proteomic data preprocessing included principal component analysis, as well as median and interquartile range-based approaches, to detect outliers, though none were removed[16]. The final analytical sample comprised 2,351 participants.

Plasma BCAAs

Plasma BCAAs in this study comprised isoleucine, leucine, valine, and total BCAA concentration. The three individual BCAA species were quantified using a high-throughput nuclear magnetic resonance platform, and total BCAA concentration was calculated as the sum of these three amino acids. All sample analysis procedures were carried out in accordance with the standard operating protocols belonging to Nightingale Health's EN ISO 13485-certified Quality Management System[17].

Dietary assessments and macronutrient calculation

Dietary intakes were assessed using the Oxford WebQ, a web-based 24 h recall questionnaire[18]. To minimize potential biases, we restricted our analyses to dietary records collected at the baseline assessment. The validity of the Oxford WebQ has been evaluated against interview-administered 24 h recalls, showing a mean Spearman correlation coefficient of 0.61 (range 0.54-0.66) for macronutrients[18]. In the UKB, the reliability of the 24 h recall questionnaire data was enhanced by screening for total energy intake of ≥ 1,000 kJ and ≤ 20 MJ (males) and ≤ 15 MJ (females). Additionally, it was ensured that the questionnaire was completed in ≥ 5 min, and statistical adjustments were applied after data collection[19].

Nutrient intakes were estimated by linking reported food items to nutrient composition data from the UK Nutrient Databank[20]. We evaluated specific macronutrients, including carbohydrates, plant and animal protein, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and fiber. In addition, we quantified the daily consumption of major food groups, including red meat, poultry, processed meat, eggs, dairy products, oily fish, non-oily fish, fruits, vegetables, nuts, legumes, cereals, and sugar-sweetened beverages (SSB). Detailed definitions are provided in Supplementary Table 1.

Non-dietary factor assessments

Non-dietary factors were obtained from baseline questionnaires and physical measurements. These included demographic characteristics (age, sex, and race/ethnicity), adiposity indicators [body mass index (BMI) and waist circumference], smoking status, alcohol consumption, physical activity, and reproductive history (menopausal status and hormone therapy use). BMI was categorized as underweight (< 18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obesity (≥ 30.0 kg/m^2). Central obesity was defined according to European waist circumference cut-offs (≥ 102 cm in males and ≥ 88 cm in females)[21]. Smoking status was categorized as never, ever, or current. Alcohol consumption was grouped as none, low-risk (≤ 1 drink/day in females and ≤ 2 drinks/day in males), moderate-risk (2-4 drinks/day in females and 3-5 drinks/day in males), or high-risk (> 4 drinks/day in females and > 5 drinks/day in males)[22]. Physical activity was categorized as favorable (≥ 1,200 MET-min/week), moderate (600-1,199 MET-min/week), or unfavorable (< 600 MET-min/week)[23]. Detailed definitions are provided in Supplementary Table 1.

Cardiometabolic biomarkers

Cardiometabolic biomarkers covered key domains of glucose metabolism, lipid metabolism, blood pressure, renal function, and systemic inflammation. Glucose-related indicators included blood glucose (mmol/L) and glycated hemoglobin (HbA1c, mmol/mol), which were quantified by the hexokinase assay and high-performance liquid chromatography, respectively. For lipid profiling, total cholesterol (TC, mmol/L), high-density lipoprotein cholesterol (HDL-C, mmol/L), low-density lipoprotein cholesterol (LDL-C, mmol/L), apolipoprotein A1 (ApoA1, g/L), and apolipoprotein B (ApoB, g/L)[17] were quantified using a high-throughput nuclear magnetic resonance platform. Triglycerides (TG) (mmol/L) were measured using the glycerol phosphate oxidase-peroxidase method. In addition, the ApoB/ApoA1 ratio was calculated to characterize the atherogenic lipid profile. Blood pressure was measured using an Omron HEM-7015IT automated sphygmomanometer. Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice (mmHg)[24], and the mean of the two readings was used to reduce random measurement variability. Renal function was evaluated using cystatin C (mg/L) and creatinine (μmol/L), measured by latex-enhanced immunoturbidimetry and enzymatic assays, respectively. The estimated glomerular filtration rate (eGFR) was calculated using the 2021 CKD Epidemiology Collaboration (CKD-EPI) creatinine equation[25]. Systemic inflammation was assessed with C-reactive protein (CRP, mg/L) measured by immuno-turbidimetric assay.

Proteomic assessments

Plasma proteomic profiles were characterized using the Olink Explore 3072 PEA platform within the UKB Pharma Proteomics Project. Assessments of the protein batch, plate effects, and abnormalities in protein coefficients of variation (CVs) suggested that these factors had minimal effects on proteins. Intraindividual protein CVs across duplicate samples spanned 1.8%-27.2% (median 6.7%), with high cross-panel correlations for the same proteins[16]. We focused on a prespecified subset of 1,472 proteins from the Cardiometabolic (I & II) and Inflammation (I & II) panels[16]. The protein PCOLCE was excluded due to an excessive missingness rate (62.0%), and duplicate biomarkers across panels were removed by retaining the first occurrence according to the predefined panel order. After deduplication, 1,459 unique proteins were included in downstream analyses [Supplementary Table 2]. Protein levels were expressed as $\log_2$-normalized

protein expression (NPX) values. Missing protein measurements were imputed by k-nearest-neighbor (k = 10) imputation using the impute R package (version 1.82.0)[26]. Detailed information on the normalization procedures is available in the UKB Olink Normalization Guide.

Covariate assessments

Considering the effect of social factors and the history of cardiometabolic diseases on BCAAs, additional covariates included education, the Townsend deprivation index, and baseline medical history. Education level was obtained from the baseline touchscreen questionnaire. The Townsend deprivation index, an area-based proxy of socioeconomic status[27], was automatically assigned to each participant according to their residential postal code. Medical history at baseline, including diabetes, hypertension, hyperlipidemia, and metabolic dysfunction-associated steatotic liver disease (MASLD), was ascertained using self-reported information and hospital inpatient records coded using the International Classification of Diseases, 9th (ICD-9) and 10th (ICD-10) Revision. Detailed diagnostic code lists are provided in Supplementary Table 3.

Statistical analysis

Prior to analysis, plasma BCAAs and cardiometabolic biomarkers were log-transformed and Z-score standardized. For proteomic data, NPX values were directly Z-score standardized. Baseline participant characteristics were summarized across quartiles of total BCAA concentrations. Continuous variables are reported as mean ± standard deviation (SD), and categorical variables as frequencies and percentages.

Multivariable linear regression models were used to assess the independent associations of each dietary and non-dietary factor with plasma BCAA levels. Associations are presented as regression coefficients (β) with corresponding 95% confidence intervals (CI). Covariates were prespecified and adjusted for across three models. Model 1 was adjusted for age, sex, and ethnicity; Model 2 further adjusted for education, Townsend deprivation index, adiposity (BMI and central obesity), smoking, alcohol consumption, physical activity, menopausal status and hormone therapy (in females), and major food group intakes; Model 3 additionally adjusted for medical history, including diabetes, hypertension, hyperlipidemia, and MASLD. All evaluated determinants were mutually adjusted for. For dietary analyses, participants with implausible total energy intake (exceeding the mean ± 5 SD) were excluded, and total energy intake was included as a covariate. Food intake was modeled as an increment of one serving per day, macronutrients as increments of 5% of energy intake, and fiber as increments of 5 g per day. Similar linear regression models were applied to evaluate associations of plasma BCAAs with CMH biomarkers and proteomic biomarkers. These associations are presented as standardized regression coefficients with 95%CIs.

To evaluate potential effect modification, we conducted subgroup analyses stratified by demographic characteristics and disease history. The statistical significance of interactions was assessed by incorporating cross-product terms between the stratifying variables and plasma BCAAs into Model 3, with *P* values for interaction derived from the Wald test. The robustness of the associations between host factors and plasma BCAAs, as well as between BCAAs and CMH biomarkers, was examined through three sensitivity analyses: (1) excluding participants who reported atypical dietary intake (24 h recall diet differed from habitual pattern); (2) additionally adjusting for eGFR to account for the impact of renal function on BCAAs and CMH biomarkers; and (3) excluding participants with hypertension.

To elucidate the shared and specific biological mechanisms underlying the association between BCAAs and CMH, we focused on proteomic biomarkers associated with these BCAAs. Specifically, we identified consensus proteins that were significantly associated with both total BCAA and each of the three individual BCAA species, while proteins uniquely associated with individual BCAAs were classified as species-specific proteins. The consensus and species-specific protein sets were subsequently subjected to functional

enrichment analyses using the Kyoto Encyclopedia of Genes and Genomes (KEGG)[28] and Gene Ontology (GO)[29] databases. Proteins involved in significantly enriched pathways were then used to construct a protein-protein interaction (PPI) network, retrieved from the STRING database with a confidence score ≥ 0.7. To identify key mediators within this network, hub proteins were defined as nodes with the highest degree centrality.

All statistical analyses were conducted using R 4.5.1. PPI networks were visualized in Cytoscape 3.10.4. To account for multiple testing, *P* values were corrected using the Benjamini-Hochberg method. For the KEGG pathway enrichment analysis of consensus proteins, statistical significance was defined at a false discovery rate (FDR) < 0.1, whereas a stricter threshold of FDR < 0.05 was applied for other analyses.

RESULTS

A total of 28,273 participants were included in the primary analyses of determinants of plasma BCAAs. Baseline characteristics were stratified by quartiles of total BCAA concentrations [Table 1]. In multivariable-adjusted models, plasma BCAAs exhibited specific association patterns with dietary macronutrients and food groups [Figure 1A and Supplementary Tables 4-7]. Overall, plasma BCAA levels were inversely associated with carbohydrate intake and positively associated with animal protein and MUFA consumption (all $P < 0.05$). At the food group level, positive associations were observed between plasma BCAAs and red meat, poultry, eggs, dairy products, and non-oily fish. In contrast, plant-based food showed mostly weak or non-significant associations with plasma BCAAs. Notably, individual BCAA species displayed unique dietary signatures. Isoleucine demonstrated a distinctive positive relationship with PUFA intake, whereas leucine concentrations were negatively associated with the consumption of plant protein, dietary fiber, oily fish, and fruits. Regarding non-dietary factors [Figure 1B and Supplementary Tables 8-11], older age was associated with lower plasma BCAA levels. Marked sex differences were observed, with males exhibiting higher levels than females. Among females, postmenopausal status and hormone therapy use were both positively associated with plasma BCAAs. Adiposity showed robust associations with BCAAs: underweight individuals had lower levels, whereas participants with obesity, defined by either BMI or waist circumference (central obesity), had substantially higher concentrations. Compared with other ethnic groups, White participants had lower levels of isoleucine and leucine. For lifestyle behaviors, lower levels of physical activity were associated with higher plasma BCAA concentrations. Current smoking was associated with lower plasma BCAAs. Although associations across alcohol consumption categories were generally not significant, BCAA levels tended to decrease with increasing alcohol intake, and high-risk drinking was significantly associated with lower isoleucine levels. These associations remained highly consistent in sensitivity analyses. Excluding participants with atypical dietary reports or further adjusting for eGFR did not materially alter the effect estimates [Supplementary Tables 12-21].

Cross-sectional analyses revealed that elevated plasma BCAAs were consistently associated with unfavorable glycemic and lipid profiles [Figure 2 and Supplementary Table 22]. The strongest positive associations were observed for TG (total BCAA: β = 0.288, 95%CI: 0.276-0.300; isoleucine: β = 0.249, 95%CI: 0.237-0.261; leucine: β = 0.234, 95%CI: 0.222-0.247; valine: β = 0.307, 95%CI: 0.295-0.319; all $P < 0.001$). Weaker, yet statistically significant, positive associations were identified for TC, ApoB, and LDL cholesterol, whereas associations with ApoA1 and HDL-C were largely null. Notably, plasma BCAAs were inversely associated with eGFR, suggesting an association between higher BCAA levels and compromised renal function. In the linear regression models, plasma BCAA levels were inversely associated with both SBP and DBP. However, restricted cubic spline (RCS) models revealed a non-linear inverted U-shaped association between plasma BCAAs and blood pressure metrics [Supplementary Figures 2 and 3]. While the three individual BCAAs exhibited broadly concordant associations across CMH domains, species-specific nuances were evident: isoleucine and leucine showed more pronounced associations with blood pressure and renal function, whereas valine demonstrated stronger associations for lipid metabolism markers. Sensitivity analyses confirmed the robustness of these findings, as excluding participants with atypical dietary reports and

hypertension, and further adjusting for eGFR, did not materially attenuate the observed associations [Supplementary Tables 23-25].

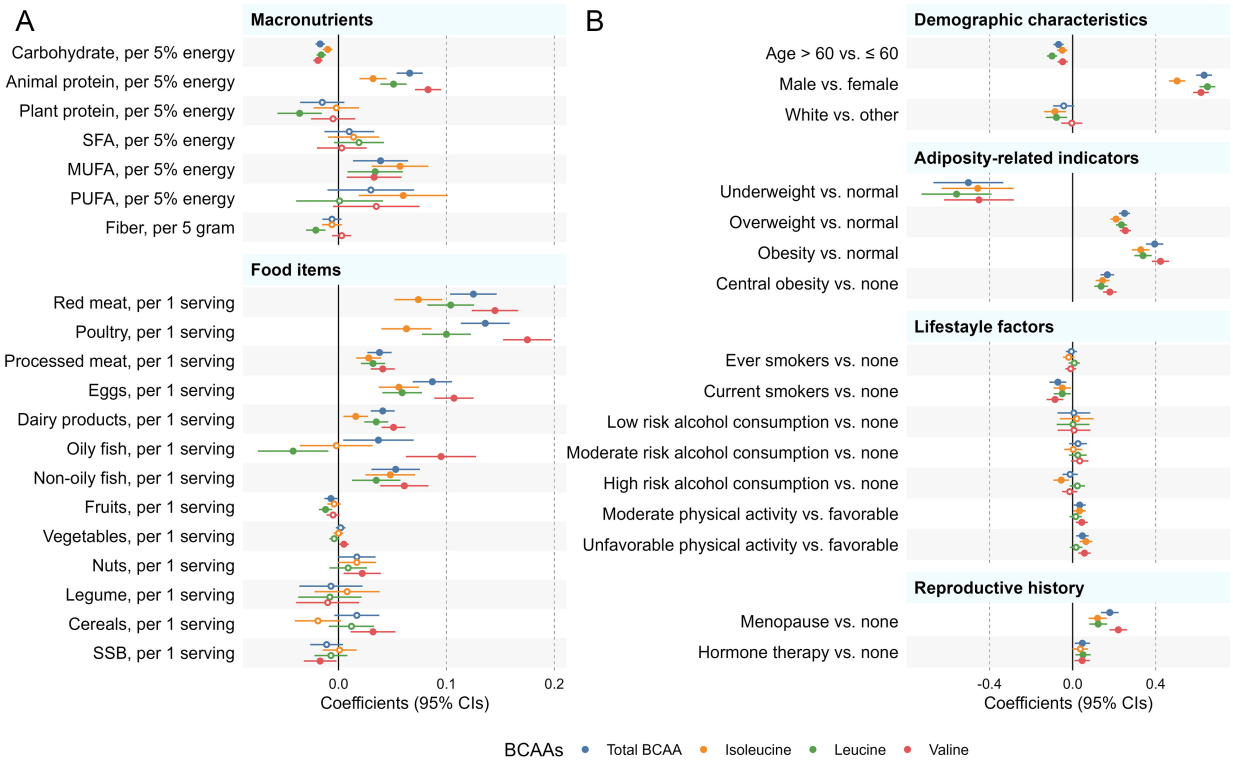

Figure 1. Dietary and non-dietary determinants of plasma BCAAs. (A) Associations of plasma BCAAs with dietary factors; (B) Associations of plasma BCAAs with non-dietary factors. Coefficients represent the SD unit change in log-transformed BCAAs. Multivariable linear regression models were adjusted for age, sex, race/ethnicity, education, Townsend deprivation index, adiposity (BMI and central obesity), smoking status, alcohol consumption, physical activity, menopausal status and hormone therapy (in females), history of diabetes, hypertension, dyslipidemia, and MASLD, as well as intakes of major food groups. All evaluated determinants were adjusted for each other. Dietary analyses were additionally adjusted for total energy intake. Solid circles indicate FDR < 0.05. BCAAs: Branched-chain amino acids; BMI: body mass index; CIs: confidence intervals; FDR: false discovery rate; MASLD: metabolic dysfunction-associated steatotic liver disease; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SD: standard deviation; SFA: saturated fatty acids; SSB: sugar-sweetened beverages.

Table 1. Baseline characteristics of study participants by total BCAA status

Characteristics		Total BCAA (μmol/L) (*n* = 28,273)	Total BCAA quartiles			
			Q1 (132.6-303.4 μmol/L) (*n* = 7,079)	Q2 (303.4-348.9 μmol/L) (*n* = 7,060)	Q3 (348.9-402.6 μmol/L) (*n* = 7,077)	Q4 (402.6-1,304.6 μmol/L) (*n* = 7,057)
Age, years, mean ± SD		56.21 ± 8.22	55.66 ± 8.47	56.52 ± 8.20	56.40 ± 8.10	56.25 ± 8.10
Sex, *n* (%)	Female	13,833 (48.9%)	5,001 (70.6%)	3,702 (52.4%)	2,785 (39.4%)	2,345 (33.2%)
	Male	14,440 (51.1%)	2,078 (29.4%)	3,358 (47.6%)	4,292 (60.6%)	4,712 (66.8%)
Education, *n* (%)	Lower	12,565 (44.4%)	3,134 (44.3%)	3,152 (44.6%)	3,120 (44.1%)	3,159 (44.8%)
	Higher	15,708 (55.6%)	3,945 (55.7%)	3,908 (55.4%)	3,957 (55.9%)	3,898 (55.2%)
Race/ethnicity, *n* (%)	White	26,819 (94.9%)	6,758 (95.5%)	6,715 (95.1%)	6,694 (94.6%)	6,652 (94.3%)
	Other	1,454 (5.1%)	321 (4.5%)	345 (4.9%)	383 (5.4%)	405 (5.7%)
Townsend deprivation index, mean ± SD		-1.44 ± 2.81	-1.35 ± 2.82	-1.48 ± 2.81	-1.50 ± 2.79	-1.46 ± 2.81

Smoking status, *n* (%)	Never	19,442 (68.8%)	5,020 (70.9%)	4,974 (70.5%)	4,776 (67.5%)	4,672 (66.2%)
	Ever	6,523 (23.1%)	1,448 (20.5%)	1,530 (21.7%)	1,719 (24.3%)	1,826 (25.9%)
	Current	2,308 (8.2%)	611 (8.6%)	556 (7.9%)	582 (8.2%)	559 (7.9%)
Alcohol consumption, *n* (%)	None	3,078 (10.9%)	818 (11.6%)	802 (11.4%)	734 (10.4%)	724 (10.3%)
	Low-risk	640 (2.3%)	198 (2.8%)	179 (2.5%)	144 (2.0%)	119 (1.7%)
	Moderate-risk	5,029 (17.8%)	1,608 (22.7%)	1,338 (19.0%)	1,074 (15.2%)	1,009 (14.3%)
	High-risk	19,526 (69.1%)	4,455 (62.9%)	4,741 (67.2%)	5,125 (72.4%)	5,205 (73.8%)
Physical activity, *n* (%)	Favorable	18,675 (66.1%)	4,902 (69.2%)	4,790 (67.8%)	4,557 (64.4%)	4,426 (62.7%)
	Moderate	4,887 (17.3%)	1,167 (16.5%)	1,190 (16.9%)	1,269 (17.9%)	1,261 (17.9%)
	Unfavorable	4,711 (16.7%)	1,010 (14.3%)	1,080 (15.3%)	1,251 (17.7%)	1,370 (19.4%)
BMI, *n* (%)	Normal	9,781 (34.6%)	3,599 (50.8%)	2,693 (38.1%)	1,999 (28.2%)	1,490 (21.1%)
	Underweight	118 (0.4%)	83 (1.2%)	20 (0.3%)	8 (0.1%)	7 (0.1%)
	Overweight	12,237 (43.3%)	2,572 (36.3%)	3,061 (43.4%)	3,335 (47.1%)	3,269 (46.3%)
	Obesity	6,137 (21.7%)	825 (11.7%)	1,286 (18.2%)	1,735 (24.5%)	2,291 (32.5%)
Central obesity, *n* (%)	No	19,585 (69.3%)	5,712 (80.7%)	5,105 (72.3%)	4,682 (66.2%)	4,086 (57.9%)
	Yes	8,688 (30.7%)	1,367 (19.3%)	1,955 (27.7%)	2,395 (33.8%)	2,971 (42.1%)
Menopause, *n* (%)	No	18,601 (65.8%)	3,796 (53.6%)	4,418 (62.6%)	5,065 (71.6%)	5,322 (75.4%)
	Yes	9,672 (34.2%)	3,283 (46.4%)	2,642 (37.4%)	2,012 (28.4%)	1,735 (24.6%)
Hormone replacement therapy, *n* (%)	No	23,810 (84.2%)	5,587 (78.9%)	5,851 (82.9%)	6,153 (86.9%)	6,219 (88.1%)
	Yes	4,463 (15.8%)	1,492 (21.1%)	1,209 (17.1%)	924 (13.1%)	838 (11.9%)
Diabetes, *n* (%)	No	26,899 (95.1%)	6,894 (97.4%)	6,835 (96.8%)	6,702 (94.7%)	6,468 (91.7%)
	Yes	1,374 (4.9%)	185 (2.6%)	225 (3.2%)	375 (5.3%)	589 (8.3%)
Hypertension, *n* (%)	No	20,808 (73.6%)	5,608 (79.2%)	5,263 (74.5%)	5,080 (71.8%)	4,857 (68.8%)
	Yes	7,465 (26.4%)	1,471 (20.8%)	1,797 (25.5%)	1,997 (28.2%)	2,200 (31.2%)
Dyslipidemia, *n* (%)	No	27,171 (96.1%)	6,863 (96.9%)	6,818 (96.6%)	6,790 (95.9%)	6,700 (94.9%)
	Yes	1,102 (3.9%)	216 (3.1%)	242 (3.4%)	287 (4.1%)	357 (5.1%)
MASLD, *n* (%)	No	28,240 (99.9%)	7,073 (99.9%)	7,053 (99.9%)	7,071 (99.9%)	7,043 (99.8%)
	Yes	33 (0.1%)	6 (0.1%)	7 (0.1%)	6 (0.1%)	14 (0.2%)
Red meat, serving/day, mean ± SD		0.32 ± 0.54	0.26 ± 0.49	0.30 ± 0.52	0.33 ± 0.55	0.37 ± 0.59
Poultry, serving/day, mean ± SD		0.27 ± 0.51	0.23 ± 0.45	0.25 ± 0.49	0.28 ± 0.52	0.31 ± 0.57
Eggs, serving/day, mean ± SD		0.28 ± 0.60	0.24 ± 0.55	0.26 ± 0.58	0.28 ± 0.60	0.34 ± 0.68
Fish, serving/day, mean ± SD		0.32 ± 0.59	0.33 ± 0.58	0.33 ± 0.58	0.31 ± 0.58	0.32 ± 0.60
Dairy products, serving/day, mean ± SD		1.07 ± 1.04	1.03 ± 1.00	1.08 ± 1.03	1.07 ± 1.04	1.11 ± 1.08
BCAA blood concentration, μmol/L, mean ± SD	Isoleucine	49.44 ± 16.97	33.47 ± 6.64	42.75 ± 6.11	51.03 ± 6.73	70.57 ± 16.66
	Leucine	102.53 ± 27.00	74.19 ± 10.39	92.14 ± 7.58	106.83 ± 8.42	137.04 ± 23.68
	Valine	207.72 ± 41.67	161.66 ± 16.26	191.80 ± 11.32	216.16 ± 12.57	261.39 ± 32.73

BCAA: Branched-chain amino acid; BMI: body mass index; MASLD: metabolic dysfunction-associated steatotic liver disease; *n*: number of participants; Q1: first quartile; Q2: second quartile; Q3: third quartile; Q4: fourth quartile; SD: standard deviation.

Subgroup analyses revealed significant effect modification [Figure 3 and Supplementary Table 26]. Associations between plasma BCAAs and various CMH markers were generally stronger in older adults, individuals with a higher BMI, and participants with hypertension (P for interaction < 0.05). Furthermore,

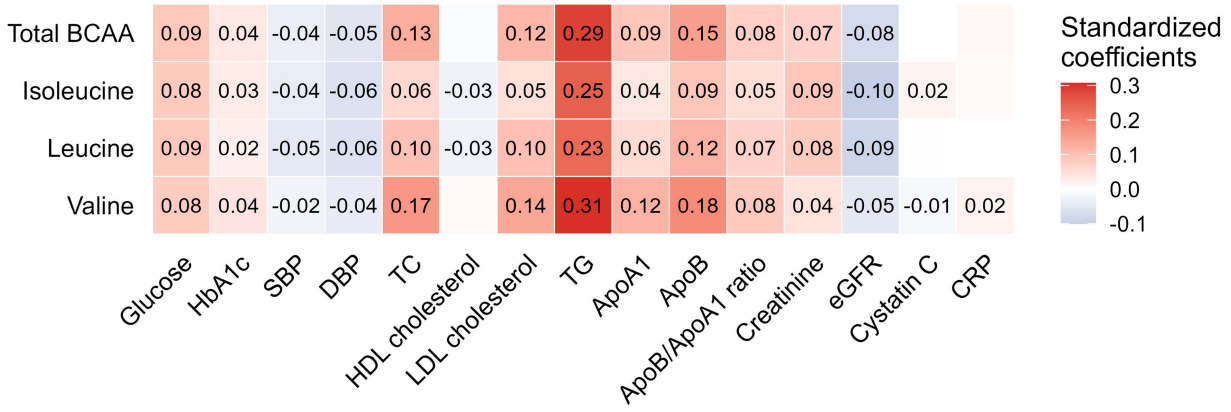

Figure 2. Associations between plasma BCAAs and cardiometabolic biomarkers. The heatmap displays standardized regression coefficients derived from fully adjusted multivariable linear regression models. Both plasma BCAAs and cardiometabolic biomarkers were log-transformed and Z-score standardized prior to analysis. Cells with standardized coefficient values indicate FDR < 0.05. BCAAs: Branched-chain amino acids; ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; CRP: C-reactive protein; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; FDR: false discovery rate; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SBP: systolic blood pressure; TC: total cholesterol; TG: triglycerides.

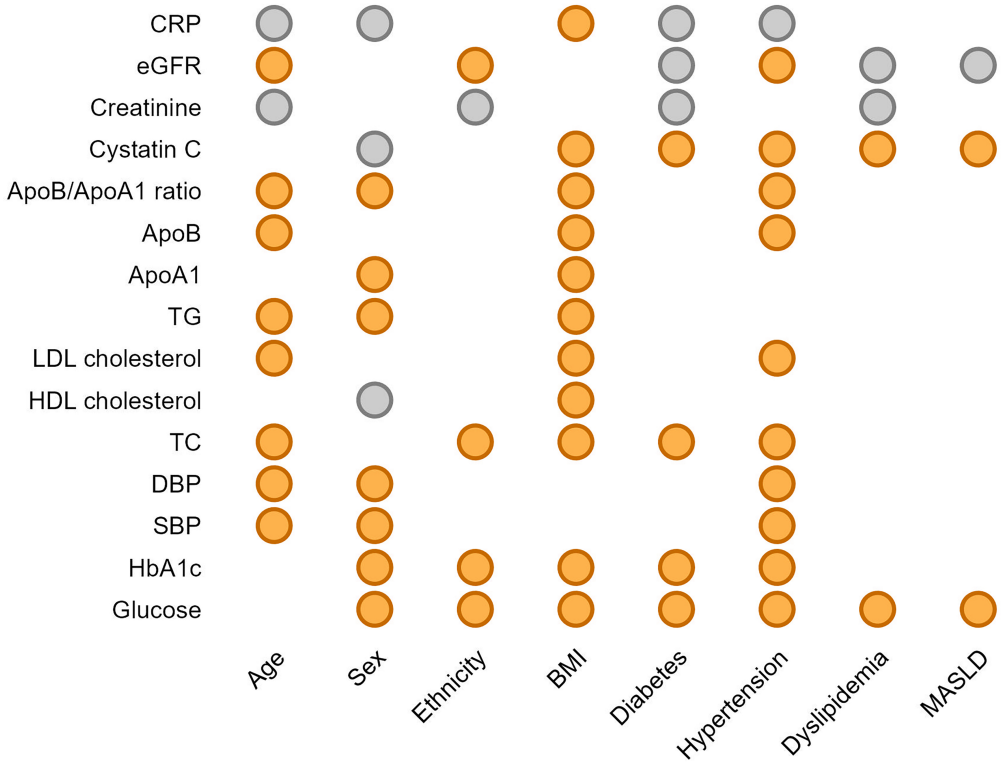

Figure 3. Effect modification by demographic characteristics and disease history in associations between total BCAA and cardiometabolic biomarkers. Bubbles denote significant interactions (FDR < 0.05). Bubble colors indicate whether the modifying factor amplifies (orange) or attenuates (gray) the main association between total BCAA and cardiometabolic biomarkers. For categorical modifiers, the reference groups are ≤ 60 for age, female for sex, other for ethnicity, normal for BMI, and the absence of disease for all clinical conditions. BCAA: Branched-chain amino acid; ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; BMI: body mass index; CRP: C-reactive protein; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; FDR: false discovery rate; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MASLD: metabolic dysfunction-associated steatotic liver disease; SBP: systolic blood pressure; TC: total cholesterol; TG: triglycerides.

the relationships between plasma BCAAs and biomarkers of glucose metabolism, renal function, and inflammation were more pronounced in participants with diabetes than those without (*P* for interaction <

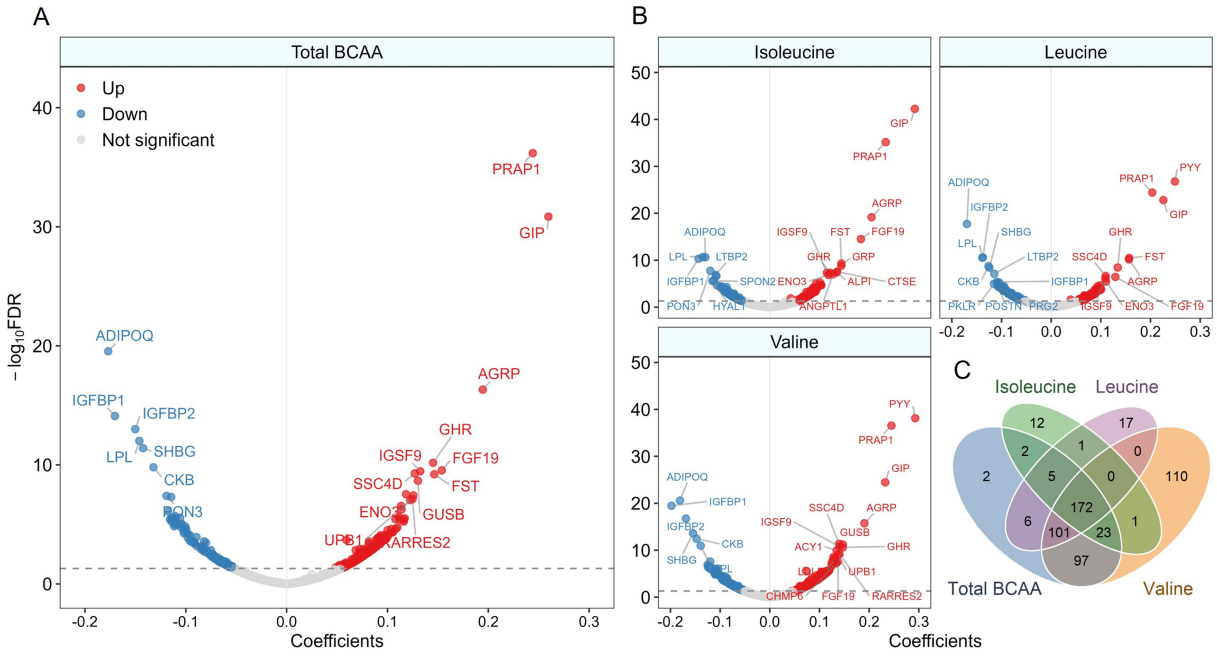

Figure 4. Associations of plasma BCAAs with the circulating proteome. (A) Associations between total BCAA and 1,459 proteins; (B) Associations between individual BCAA (isoleucine, leucine, and valine) and proteins. All models were fully adjusted for covariates. The horizontal dashed line indicates the significance threshold (FDR = 0.05); (C) Overlap of proteins significantly associated with total BCAA and all three individual BCAA species. BCAA: Branched-chain amino acid; FDR: false discovery rate.

0.05). Associations of plasma BCAAs with glucose and renal biomarkers were also stronger among participants with hyperlipidemia or MASLD (*P* for interaction < 0.05). Sex also emerged as a consistent effect modifier in the associations between plasma BCAAs and CMH markers (*P* for interaction < 0.05). Specifically, positive associations of plasma BCAAs with glycemic markers (blood glucose and HbA1c) and lipid markers (TG and ApoA1), as well as inverse associations with blood pressure, were more pronounced in males.

We further examined association patterns between plasma BCAAs and CMH-related circulating proteins. Overall, the protein profiles associated with individual BCAA species were highly concordant [Figure 4A and B, Supplementary Table 27]. Higher plasma BCAA levels were associated with increased abundance of proteins involved in energy balance and metabolic signaling, including GIP, PRAP1, AGRP, PYY, FST, GHR, and FGF19. In contrast, plasma BCAAs were inversely associated with proteins implicated in lipid metabolism and insulin sensitivity regulation, including ADIPOQ, LPL, IGFBP1, IGFBP2, SHBG, and CKB. Although the number of significant proteins differed across individual BCAAs, we identified 172 proteins consistently associated with both total BCAA and all three individual species, which were defined as consensus proteins. In addition, 14 proteins were uniquely associated with isoleucine, 23 with leucine, and 207 with valine [Figure 4C].

This shared, species-specific proteomic signature indicates the coexistence of common and distinct biological functions and pathways that are involved in the interplay between BCAA homeostasis and CMH. GO enrichment analysis based on the consensus proteins yielded 301 significant terms, including 246 biological processes (BP), 13 cellular components (CC), and 42 molecular functions (MF) [Figure 5A and Supplementary Table 28]. The most prominently enriched BP terms highlighted processes related to immune and inflammatory responses [e.g., positive regulation of chemokine (C-X-C motif) ligand 2 production; FDR < 0.01, fold enrichment = 28.67] and lipid metabolic homeostasis (e.g., high-density lipoprotein particle

remodeling; FDR < 0.001, fold enrichment = 27.30). MF enrichment primarily involved binding functions, notably transforming growth factor-beta (TGF-β) receptor binding (type I and type II), and apolipoprotein binding, as well as structural and enzymatic functions, such as extracellular matrix structural constituents conferring compression resistance, and carbonate dehydratase activity (all FDR < 0.05). CC enrichment localized these proteins to extracellular structures and intracellular compartments, including the plasma lipoprotein particle and the lysosomal lumen (FDR < 0.05). Further analysis of BCAA species-specific proteins revealed distinct functional profiles. Isoleucine-specific proteins were primarily enriched in MF terms, including hormone activity and integrin binding (all FDR < 0.01) [Supplementary Figure 4 and Supplementary Table 29]. Leucine-specific proteins predominantly highlighted chemotaxis and chemokine receptor binding, and were primarily localized to structures like platelet alpha granules and the lysosomal lumen (all FDR < 0.05) [Supplementary Figure 5A and Supplementary Table 29]. Valine-specific proteins were strongly enriched in pathways related to coagulation and wound healing (e.g., platelet activation and fibrinolysis), as well as amino sugar metabolism, characteristically localizing to platelet dense granules and the CD40 receptor complex (all FDR < 0.05) [Supplementary Figure 6A and Supplementary Table 29]. KEGG pathway enrichment analysis of the 172 consensus proteins identified five significantly enriched pathways, comprising 28 proteins: nitrogen metabolism, pantothenate and CoA biosynthesis, fatty acid degradation, cytokine-cytokine receptor interaction, and hormone signaling (all FDR < 0.1) [Figure 5B and Supplementary Table 30]. PPI network analysis of these 28 proteins delineated three major functional modules centered on the positive regulation of the receptor signaling pathway via STAT, nitrogen metabolism, and branched-chain amino acid catabolism. Notably, LEP emerged as the primary hub, occupying central positions within the network [Figure 5C]. KEGG enrichment analysis of isoleucine-specific proteins did not identify any significantly enriched pathways. Conversely, leucine-specific proteins were enriched in 14 pathways related to immune responses, inflammation, and cell migration, such as the IL-17 signaling pathway and integrin signaling (all FDR < 0.05) [Supplementary Figure 5B and Supplementary Table 30]. Valine-specific proteins were significantly enriched in a single pathway, the complement and coagulation cascades (FDR < 0.01) [Supplementary Figure 6B and Supplementary Table 30]. Subsequent PPI network analyses of these pathway-enriched, BCAA species-specific proteins revealed CXCL8 and SDC1 as key hubs for leucine, and F10 and PLAT as hubs for valine [Supplementary Figures 5C and 6C]. Overall, among the 49 proteins mapped to significant KEGG pathways across these analyses, 29 proteins showed a positive correlation with plasma BCAAs, while 20 proteins showed a negative correlation [Supplementary Figure 7 and Supplementary Table 27].

DISCUSSION

In this large-scale study, we demonstrated that plasma BCAA concentrations were potentially shaped by dietary intake, particularly diets rich in animal protein and low in carbohydrates, alongside key non-dietary factors such as adiposity and male sex. Elevated BCAAs were robustly associated with adverse cardiometabolic profiles. Beyond clinical markers, we identified core proteomic signatures that were either shared across all BCAA species or specific to individual species. Pathway and network analyses indicated that consensus proteins converge on diverse metabolic and endocrine pathways, with LEP acting as a central hub. Collectively, these findings underscore that BCAAs function as systemic biomarkers associated with diet and lifestyle factors, as well as CMH.

Our study identified distinct dietary determinants of plasma BCAAs, with higher levels strongly associated with the consumption of animal-based foods (e.g., red meat and dairy products) and total animal protein. Conversely, we observed inverse associations with plant-based dietary components encompassing fruits, carbohydrates, plant protein, and dietary fiber. These findings indicate that the source of dietary protein plays a fundamental role in shaping BCAA homeostasis, consistent with previous work[30]. This divergence likely reflects the inherently higher BCAA content of animal proteins compared with plant sources[31].

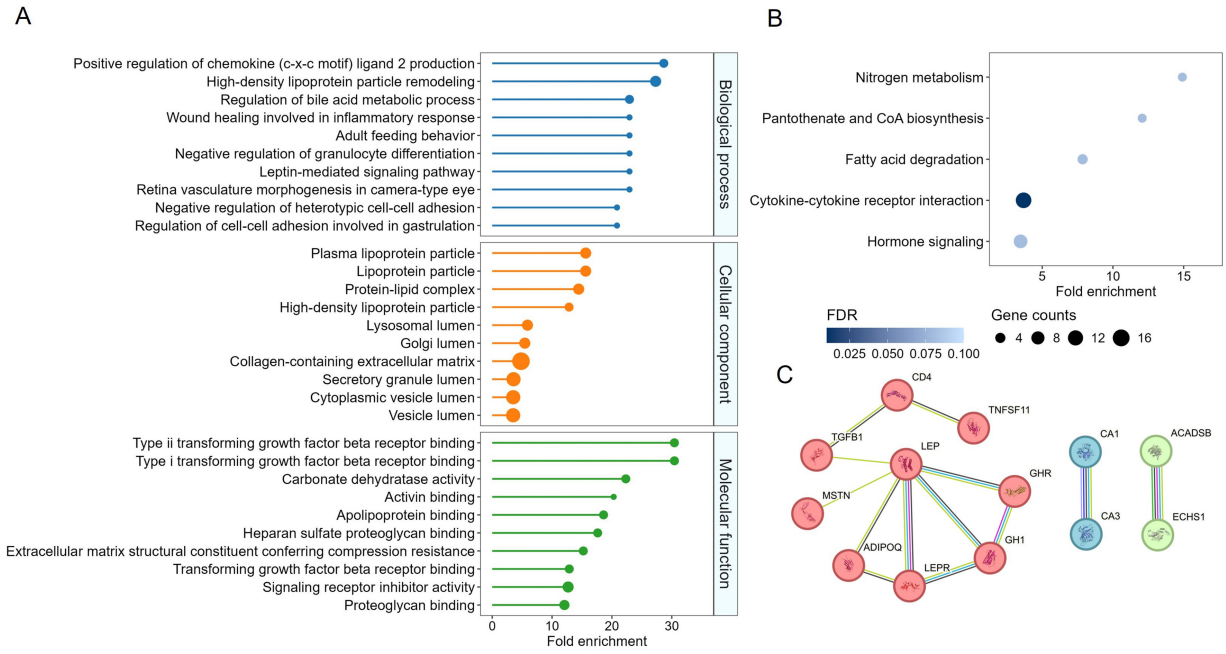

Figure 5. Functional enrichment and PPI network analyses of BCAA-related proteins. (A) GO enrichment analysis of the consensus proteins. For visualization purposes, only the top 10 significantly enriched terms with the highest fold enrichment are displayed across biological process, cellular component, and molecular function categories; (B) KEGG pathway enrichment analysis of the consensus proteins; (C) PPI network constructed from 28 proteins mapped to the significantly enriched KEGG pathway. Proteins are clustered (from left to right, top to bottom) into three functional modules: positive regulation of receptor signaling pathway via STAT, nitrogen metabolism, and branched-chain amino acid catabolism. Unconnected nodes were excluded. BCAA: Branched-chain amino acid; CoA: coenzyme A; FDR: false discovery rate; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; STAT: signal transducer and activator of transcription.

However, from a food metabolomics perspective, circulating BCAAs should be interpreted not merely as direct dietary components, but as dynamic indicators of dietary quality and post-consumption metabolism. Owing to their limited hepatic extraction following intestinal absorption[32], circulating BCAA concentrations are highly responsive to the dietary matrix, which governs nutrient bioavailability[33]. Accordingly, beyond differences in amino acid composition, plant-based diets may further modulate BCAA homeostasis through multiple secondary metabolites. For instance, higher fiber intake has been shown to downregulate microbial genes responsible for BCAA biosynthesis in animal models, thereby improving host metabolic profiles[34]. Plant-derived bioactive compounds, including flavonoids and polyphenols, undergo extensive biotransformation by host and microbial enzymes. The resulting metabolites may indirectly participate in the regulation of BCAA metabolism by modulating gut microbiota function, influencing microbiota-derived metabolites, and activating the AMPKα pathway[35,36]. Accumulating evidence indicates that replacing animal-based diets with plant-based dietary patterns reduces mortality from cardiovascular and cerebrovascular diseases[37]. Our findings suggest that lower plasma BCAAs are potentially involved in these cardioprotective effects. Accordingly, adherence to plant-based or Mediterranean dietary patterns may represent a practical strategy to improve CMH through modulation of BCAA homeostasis.

We also observed several non-dietary host factors that contributed to plasma BCAAs. Consistent with prior evidence[38], we observed elevated plasma BCAAs in individuals with insufficient physical activity. Skeletal muscle serves as the primary site for BCAA storage and catabolism, meaning that physical inactivity directly diminishes catabolic efficiency and contributes to elevated circulating levels[39]. The reduced plasma BCAAs observed in smokers and alcohol consumers likely reflect underlying physiological impairment rather than a protective adaptation. These reductions may result from tobacco-induced disruption of insulin signaling and

mitochondrial function[40] and alcohol-related hepatic injury[41], both of which also contribute to cardiometabolic disturbances. Lower BCAA levels in older adults and in females, particularly premenopausal females, were observed. These patterns may reflect sex-specific hormonal differences and dynamic changes in hormonal and metabolic levels across physiological phases[42]. Postmenopausal females experience a decline in estrogen, which is often accompanied by an imbalance in energy and metabolic homeostasis; hormone therapy has been shown to improve the relevant metabolic markers[43]. Furthermore, males typically have greater skeletal muscle mass[44], which may contribute to higher BCAA levels. The liver, as another major metabolic organ in BCAA metabolism, likely contributes to these sex differences through hormone-regulated metabolic processes and cross-talk with gut, muscle, and adipose tissues[45]. Plasma BCAAs were also higher in individuals with obesity in our analyses. Obesity may elevate plasma BCAAs through a combination of excessive dietary BCAA intake and impaired BCAA catabolism within adipose tissue[46]. The resulting accumulation of circulating BCAAs persistently activates the mTOR pathway, thereby inhibiting insulin signaling and reducing peripheral insulin sensitivity[39,46]. This exacerbates dyslipidemia and broader metabolic dysfunction, perpetuating obesity in a vicious cycle.

Our observation that elevated BCAAs are associated with adverse CMH biomarker profiles aligns with their established role in cardiometabolic pathology[47]. By systematically evaluating a comprehensive panel of biomarkers, we extended existing evidence beyond glucose and lipid homeostasis. Notably, the worsened renal function associated with BCAAs in our study suggests that plasma BCAAs may impair renal function by activating mTOR signaling and promoting fibrosis-related epithelial-mesenchymal transition[48]. Although linear models in our study indicated an overall inverse association between plasma BCAAs and blood pressure, RCS analyses revealed a concentration-dependent, inverted U-shaped relationship. Mechanistically, our KEGG enrichment analyses of differential proteins offer a potential explanation. Among the 49 identified network proteins, both positively and negatively BCAA-associated proteins included blood pressure-protective and risk-associated factors, suggesting that multiple, potentially competing pathways may underlie the observed association[49]. In addition, the overall negative association may be partly attributable to reverse causality or residual confounding inherent to the cross-sectional study design. We also observed significant sexual heterogeneity in the associations between plasma BCAAs and CMH biomarkers, potentially reflecting hormone-related differential regulation of BCAA metabolism and downstream pathways. Due to the absence of the protective effects of estrogen, men may be more susceptible to glucose and lipid metabolism disorders at elevated BCAA levels[50].

Subsequent proteomic analyses revealed a distinct circulating signature associated with BCAAs. Elevated levels of BCAAs were positively correlated with proteins associated with atherosclerotic or cardiometabolic risk, including AGRP, FGF19, and PRAP1[51]. Conversely, they were inversely associated with proteins involved in metabolic regulation and the maintenance of vascular function, including LPL, ADIPOQ, IGFBP1, IGFBP2, and SHBG[51]. The observed upregulation of cardioprotective proteins like PYY, GIP, and FST may reflect compensatory endocrine and metabolic responses to early systemic stress, potentially capturing a subclinical stage of cardiometabolic dysregulation[52-54]. Consistent with this interpretation, pathway enrichment analysis revealed that the mechanism underlying the association between plasma BCAAs and CMH may involve a multilevel molecular network. The shared pathways, including nitrogen metabolism, pantothenate and CoA biosynthesis, fatty acid degradation, cytokine-cytokine receptor interaction, and hormone signaling, are anchored by the consensus hub protein LEP (i.e., leptin), which is a key regulator of energy and metabolic homeostasis[55]. Previous studies suggest that high BCAA levels often coexist with elevated leptin levels[56]. Pathologically, aberrant leptin signaling induces insulin resistance, dyslipidemia, and elevated inflammatory markers, accelerating atherosclerosis[57]. It also impairs renal hemodynamics and glomerular structure, leading to reduced eGFR[58]. Collectively, these effects contribute to the onset and progression of cardiometabolic diseases. Beyond these shared mechanisms, we identified

pathways specific to individual BCAA species. Notably, leucine-specific proteins localize to secretory granules and the extracellular matrix, where they regulate immune cell chemotaxis and migration through interactions involving chemokines, cytokines, receptors, and pattern recognition receptors, thereby contributing to tissue morphogenesis and repair. The leucine-specific network is centered on CXCL8 and SDC1. CXCL8 is a key chemokine that regulates the directed migration and activation of neutrophils and is involved in angiogenesis[59]. SDC1 is a key regulator of signal transduction and lipid transport[60] and may contribute to atherosclerosis. In contrast, valine is potentially involved in inflammatory injury and aberrant repair, concurrently with alterations in the immune-coagulation-fibrinolysis-apoptosis-remodeling pathway. Specifically, valine-specific protease F10 and PLAT are key components of coagulation and fibrinolysis, respectively, with antagonistic functions. Activated F10 serves as the core of the prothrombinase complex, catalyzing the conversion of prothrombin to thrombin and functioning as a critical convergence point of the intrinsic and extrinsic coagulation pathways[61]. PLAT, a physiological activator of fibrinolysis, catalyzes plasminogen conversion to plasmin, initiating fibrin clot degradation[62]. The coagulation-fibrinolysis balance is essential for vascular homeostasis, and valine may regulate this equilibrium, thereby influencing CMH-related pathophysiological processes, though the precise molecular mechanisms remain to be elucidated.

Several strengths support the robustness of our observations, including the large sample size, standardized metabolomic profiling, comprehensive dietary and biomarker assessment, and integration of proteomic data. Nevertheless, our findings require cautious interpretation. First, the cross-sectional design precludes causal inference. We cannot determine whether higher BCAAs are a cause, consequence, or correlate of cardiometabolic dysfunction. Second, diet was assessed using a baseline 24-h recall, which quantifies estimation of habitual intake but remains susceptible to dietary fluctuations and reporting bias. Third, given the consumption of mixed diets and the lack of external validation, associations between BCAAs and specific food categories should be interpreted with caution. Fourth, although we adjusted for major demographic, lifestyle, and dietary covariates, residual confounding due to food processing, cooking practices, underlying health conditions, and medication use cannot be ruled out.

In conclusion, this large-scale study demonstrates that plasma BCAAs are strongly influenced by modifiable host factors, particularly dietary factors, and are robustly associated with adverse cardiometabolic and proteomic profiles. These findings support a role for BCAAs as biomarkers associated with lifestyle factors and the development of cardiometabolic diseases. Future longitudinal studies and precision nutrition intervention trials are needed to establish causality and to determine whether dietary modulation of the BCAAs can mitigate cardiometabolic risk.

DECLARATIONS

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Authors' contributions

Conceptualization: Luo X, He X, Yang Y, Pan XF
Methodology: Luo X, He X, Jiao A, Xue Q, Yang Y, Pan XF
Software: Luo X, Wang Y, Zhang S, Yang Y
Validation: Luo X, Zhao Y, Li R
Formal analysis: Luo X, Pan XF
Investigation: Wang Y, He Q
Resources: Li F, Li S, Wen Y, Yang Y
Data curation: Luo X, Li Y
Writing - original draft preparation: Luo X, He X

Writing - review and editing: Yang Y, Pan XF
Visualization: Luo X, He Q, Zhao Y, Yang Y
Supervision: Wang T, Dong Y
Project administration: Pan XF
All authors read and approved the final manuscript.

Availability of data and materials

UK Biobank data can be requested by bona fide researchers for approved projects, including replication studies, through https://www.ukbiobank.ac.uk/.

AI and AI-assisted tools statement

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The UK Biobank study was approved by the North West Multi-center Research Ethics Committee (MREC; REC reference: 21/NW/0157; IRAS project ID: 299116), and all participants provided written informed consent prior to data collection. The present analysis was conducted under UK Biobank Application Number 103011 and required no additional institutional ethical approval because only de-identified data were used.

Consent for publication

Not applicable.

Supplementary Materials

Supplementary Materials

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