Atherosclerosis 286 (2019) 7–13

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

# Association between plasma essential amino acids and atherogenic lipid profile in a Chinese population: A cross-sectional study

Feng-Hua Wang[a], Jing Liu[a], Qiu-Ju Deng[a], Yue Qi[a], Miao Wang[a], Ying Wang[b], Xing-Guang Zhang[a], Dong Zhao[a,*]

[a] Department of Epidemiology, Beijing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart, Lung and Blood Vessel Diseases, The Key Laboratory of Remodelling-Related Cardiovascular Diseases, Ministry of Education, China
[b] Research Institute of Information Technology, Tsinghua University, Beijing, China

## HIGHLIGHTS

- Associations between essential amino acids level and lipid components were examined.
- Elevated branched-chain amino acids (BCAAs) positively correlated with sdLDL-C, RLP-C and TG but inversely correlated with HDL-C.
- Elevated threonine level was inversely correlated with sdLDL-C, RLP-C and TG.
- Higher level of BCAAs had increased risk of lipid triad while the higher level of threonine had lower that risk.

## ARTICLE INFO

*Keywords:*
Small dense low-density lipoprotein cholesterol
Remnant-like particle cholesterol
Triglycerides
Atherogenic lipid triad
Essential amino acids

## ABSTRACT

*Background and aims:* The association between amino acids and small dense low-density lipoprotein cholesterol (sdLDL-C) and remnant-like particle cholesterol (RLP-C) remains poorly understood. This study aims to investigate the association between plasma essential amino acids (EAAs) and atherogenic lipid profiles.
*Methods:* Plasma amino acid levels of 475 individuals were measured using liquid chromatography-mass spectrometry. SdLDL-C, RLP-C, and other lipid components were evaluated. Associations between EAAs and lipid components or dyslipidemia were determined using correlation analysis and multivariate logistic regression.
*Results:* Concentrations of plasma branched-chain amino acid (BCAA) were positively correlated with sdLDL-C, RLP-C, and triglycerides (TG) levels, but inversely correlated with high-density lipoprotein cholesterol (HDL-C). In contrast, threonine concentration was inversely correlated with sdLDL-C, RLP-C, and TG. Compared with the lowest tertile, individuals in the highest tertile of plasma total BCAAs level had an odds ratio (OR) of 2.33 (95% confidence interval [CI]: 1.35, 4.03) for the risk of high sdLDL-C, 3.63 (95%CI: 1.69, 7.80) for the risk of high RLP-C, 3.10 (95%CI: 1.66, 5.80) for the risk of high TG, and 3.67 (95%CI: 2.00, 6.73) for atherogenic lipid triad (all $p < 0.01$). In contrast, compared with the lowest tertile, individuals in the highest plasma threonine tertile had a 43% lower OR for high sdLDL-C, 56% lower OR for high TG, and 55% lower OR for lipid triad risk (all $p < 0.05$).
*Conclusions:* Among the EAAs evaluated, elevated plasma BCAAs were significantly associated with increased risk of atherogenic lipid profile. In contrast, elevated threonine was associated with reduced risk of atherogenic lipid profile.

## 1. Introduction

Proteins, composed of combinations of 20 amino acids, are the most important structural and functional components of the human body. Nine of the 20 amino acids are acquired from the diet as they cannot be synthesized endogenously or in sufficient amounts, and are thus termed the essential amino acids (EAAs), comprising valine, leucine, isoleucine, threonine, lysine, histidine, phenylalanine, tryptophan, and methionine [1]. With the recent development of increasingly accurate laboratory technology for the measurement of blood amino acids [2–4], increasing

* Corresponding author. Department of Epidemiology, Beijing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart, Lung and Blood Vessel Diseases, 2 Anzhen Rd, Chaoyang District, Beijing, 100029, China.
*E-mail address:* deezhao@vip.sina.com (D. Zhao).

https://doi.org/10.1016/j.atherosclerosis.2019.04.225
Received 23 August 2018; Received in revised form 9 April 2019; Accepted 24 April 2019
Available online 25 April 2019
0021-9150/ 

studies on plasma amino acids have found that EAAs are associated with common metabolic disorders. Several large studies consistently reported that branched-chain amino acid (valine, leucine, and isoleucine) levels are associated with the risk of developing type 2 diabetes, prediabetes, and insulin resistance [5–8]. Some studies also reported an association between EAAs and dyslipidemia [9,10], especially elevated triglycerides (TG) and low levels of high-density lipoprotein cholesterol (HDL-C) [3,11–13]. Previous studies have shown that individuals with high TG and low HDL-C typically have elevated levels of small dense low-density lipoprotein cholesterol (sdLDL-C) and remnant-like particle cholesterol (RLP-C) [14–16]. Experimental and clinical evidence has suggested that sdLDL-C [17–20] and RLP-C [21–23] levels are closely associated with atherosclerosis and cardiovascular disease (CVD). However, no studies to date have evaluated the independent association between EAAs and atherogenic sdLDL-C and RLP-C levels and atherogenic lipid triad, which is characterized by elevated TG, low levels of HDL-C, and high levels of sdLDL-C [24].

Furthermore, some studies have reported one of the EAAs, threonine, may exert a protective effect in metabolic disorders, and that threonine supplementation might decrease amino acid catabolism and prevent the conversion of amino acids to lipid [25,26]. However, it has not yet been reported whether plasma threonine level is associated with reduced risk of atherogenic lipid profile in humans. An exploration of the association between EAAs and atherogenic lipid profile might therefore provide an insight into potential associations between amino acids and atherogenic disease and establish a basis for a potential dietary intervention. Therefore, the present study was designed to determine whether plasma EAAs are associated with an atherogenic lipid profile, including high sdLDL-C, high RLP-C, high TG, and an atherogenic lipid triad, based on a cross-sectional population study design.

## 2. Patients and methods

### 2.1. Study population

Study participants were recruited from the Shougang community cohort in the Chinese Multi-provincial Cohort Study (CMCS)-Beijing Project, a community-based cohort study [27]. The flow chart of participant selection is presented in Supplementary Fig.1. Briefly, in 2007, a total of 1324 participants were surveyed for demographic information and cardiovascular risk factors, and blood samples were collected. After excluding participants who had cardiovascular disease or took antihypertensive drugs, 475 participants (189 males and 286 females) were finally included, and their plasma amino acids level and concentrations of sdLDL-C, RLP-C, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), TG, HDL-C, and fasting blood glucose (FBG) were measured.

All participants gave written informed consent, and this study was approved by the Ethics Committee of Beijing Anzhen Hospital, Capital Medical University and was conducted in accordance with standards set forth in the Declaration of Helsinki.

### 2.2. Data collection and laboratory assays

Demographic information was collected using a standard questionnaire. Blood pressure (BP) was measured three times consecutively and the mean value was used for analysis. Waist circumference (WC) was measured at the midpoint of the line between the lower margin of the rib arch and the anterior superior iliac ridge, and measurements were accurate to 0.5 cm.

Fasting venous blood samples (more than 8 h) were collected from an antecubital vein into tubes containing EDTA, and TC, LDL-C, TG, HDL-C, and FBG concentrations were measured on the same day in 2007. Serum TC, TG, and FBG levels were determined by enzymatic methods. HDL-C and LDL-C levels were measured using a homogeneous assay (HUMAN, Wiesbaden, Germany), while sdLDL-C and RLP-C concentrations were measured in 2015 using previously frozen (−80 °C) serum samples obtained in 2007 and not subject to repeated freeze-thaw cycles. sdLDL-C was assayed using the previously described precipitation method of filtration [28] (Denka Seiken Co., Ltd., Tokyo, Japan) on a Hitachi 7180 automatic analyzer with a coefficient of variation of 3.25% for low-range controls and 4.18% for high-range controls. RLP-C levels were determined using direct measurement methods (Denka Seiken Co., Ltd, Tokyo, Japan).

### 2.3. Measurement of plasma essential amino acid concentrations

Plasma EAA concentrations were measured in 2015 using liquid chromatography coupled to tandem mass spectrometry in the fasting blood samples obtained in 2007. Plasma samples were stored at −80 °C, placed at −20 °C for 30 min, and thawed at 4 °C. A 40 μL aliquot of each plasma sample was used for the analysis. Samples were extracted with 20 μL of sulfosalicylic acid, mixed for 1 min, and centrifuged for 15 min at 20,000*g*, after which 5 μL of supernatant was transferred to a sample vial. Auto sampler temperatures were maintained at 8 °C. The column temperature was held at 50 °C and the sample injection volume was 1 μL. The chromatographic separation of compounds was achieved using a Phenomenex Luna C18 column (150 mm × 4.6 mm, 5 μm particle size) at a flow rate of 0.8 mL/min. A binary gradient of water (mobile phase A) and acetonitrile (mobile phase B), both of which contained 0.1% formic acid and 0.01% heptafluorobutyric acid, was delivered according to the program shown in Supplementary Table 1. The compound detection and identification were performed using a Sciex QTRAP 5500 tandem mass spectrometer operated in multiple reaction monitoring (MRM) mode. The acquisition and processing of all of data were conducted using Analyst 1.6.1 software (AB Sciex). Prior to sample quantification, detection method verification, including analytical specifications for limit of detection, evaluated quantification ranges, specificity, potential interferences, linearity, precision and accuracy, reproducibility, and stability, was performed.

### 2.4. Definitions

High sdLDL-C was defined by a serum sdLDL-C level > 0.99 mmol/L (> the median). High RLP-C was defined by a serum RLP-C level > 0.12 mmol/L (> the median). Atherogenic lipid triad was defined as the presence of all of the following: (1) high TG: serum TG ≥ 1.70 mmol/L (150 mg/dL); (2) low HDL-C: serum HDL-C < 1.04 mmol/L (40 mg/dL) for men or < 1.30 mmol/L (50 mg/dL) for women; and (3) high sdLDL-C: serum sdLDL-C > 0.99 mmol/L (> the median).

### 2.5. Statistical analysis

Baseline characteristics of participants are described using mean ± standard deviation (SD) for continuous variables with a normal distribution or approximate normal distribution, median (interquartile range, IQR) for continuous variables with a skewed distribution, and number (percent) for categorical variables. To examine the correlation between EAAs and serum lipid component level, simple correlation and partial correlation adjusted for confounding factors were used, including age, gender, WC, FBG, systolic blood pressure (SBP), and use of lipid-lowing or glucose-lowering medications, during the previous 2 weeks of the survey. One-way analysis of variance (ANOVA) or Wilcoxon rank sum test was used to compare median/mean changes in lipid parameters in groups defined by tertiles of amino acids.

Multivariate logistic regression analysis was used to study the relationship between EAAs and the risk of high sdLDL-C, high RLP-C, high TG, and atherogenic lipid triad after adjustment for age, gender, WC, SBP, FBG, and use of lipid-lowing or glucose-lowering medications

**Table 1**
Baseline characteristics of the study population.

| Subject characteristics | Total (n = 475) |
|---|---|
| Age, years | 58.67 ± 6.30 |
| Women, n (%) | 286(60.21) |
| BMI, kg/m$^2$ | 24.80 ± 3.20 |
| WC, cm | 82.06 ± 10.22 |
| FBG, mmol/L | 5.68 ± 1.34 |
| SBP, mmHg | 130.15 ± 15.63 |
| DBP, mmHg | 79.91 ± 9.03 |
| Glucose-lowering medication, n (%) | 120(14.05) |
| Lipid-lowering medication, n (%) | 96(11.24) |
| **Blood lipid levels** | |
| TG, mmol/L | 1.35(1.00–2.02) |
| TC, mmol/L | 5.42 ± 0.91 |
| HDL-C, mmol/L | 1.38 ± 0.31 |
| LDL-C, mmol/L | 3.51 ± 0.79 |
| sdLDL-C, mmol/L | 0.99(0.73–1.34) |
| RLP-C, mmol/L | 0.12(0.07–0.22) |
| **EAAs, (μmol/L)** | |
| Valine | 247.66 ± 43.09 |
| Leucine | 151.32 ± 26.77 |
| Isoleucine | 68.43 ± 13.30 |
| Threonine | 134.88 ± 27.81 |
| Lysine | 159.32 ± 20.89 |
| Histidine | 70.35 ± 8.13 |
| Phenylalanine | 66.87 ± 8.30 |
| Tryptophan | 55.91 ± 8.96 |
| Methionine | 29.94 ± 5.77 |

Data are presented as mean ± standard deviation (SD) for continuous variables with a normal distribution or approximate normal distribution; median (interquartile range) for continuous variables with a skewed distribution; and number (percent) for categorical variables.
BMI, body mass index; WC, waist circumference; FBG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL-C, small and low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol.

during the previous 2 weeks of the survey. To examine the possibility of a quantitative relationship between BCAA, threonine, and risk of dyslipidemia, study participants were classified into three groups according to the concentrations of each BCAA, threonine and by total BCAAs, with the lowest tertile used as references.

Statistical analyses were conducted using Stata software (version 14.0; Stata Corporation, College Station, TX, USA). Two-tailed *p*-values < 0.05 were considered to represent statistical significance.

## 3. Results

### 3.1. Population characteristics

A total of 475 participants (189 men and 286 women) with a mean age of 58.67 ± 6.30 years (60.96 ± 7.21 years for men and 57.15 ± 5.09 years for women) were included in this study. As shown in Table 1, the median sdLDL-C, RLP-C, and TG concentrations were 0.99 (0.73–1.34) mmol/L, 0.12 (0.07–0.22) mmol/L, and 1.35 (1.00–1.02) mmol/L, respectively. Mean TC, LDL-C, and HDL-C concentrations were 5.42 ± 0.91 mmol/L, 3.51 ± 0.79 mmol/L, and 1.38 ± 0.31 mmol/L, respectively. Among the nine examined EAAs, valine, a branched-chain amino acids (BCAAs), having the highest mean concentration (247.66 ± 43.09 μmol/L) and methionine, having the lowest (29.94 ± 5.77 μmol/L).

### 3.2. Correlations between EAAs and lipid components

Correlation analyses of each EAAs with each lipid components are shown in Table 2. Each BCAA (valine, leucine, and isoleucine) and the total concentration of BCAAs, defined as the sum concentrations of valine, leucine, and isoleucine, were positively correlated with level of sdLDL-C (all $p < 0.01$), RLP-C (all $p < 0.05$), and TG (all $p < 0.01$), but negatively correlated with HDL-C (all $p < 0.01$), even after adjustment for age, gender, WC, SBP, FBG, and use of lipid-lowing or glucose-lowering medications during the previous 2 weeks of the survey. In contrast, threonine was negatively correlated with sdLDL-C, RLP-C, and TG (all $p < 0.05$), even after adjusting for the above-mentioned potential confounding factors and the total concentration of BCAAs. No significant correlations were observed between the remaining amino acids (lysine, histidine, phenylalanine, tryptophan, and methionine) and lipid components after adjusting for potential confounding factors. After additional adjustment for TG and HDL-C, the positive correlation between concentrations of BCAA and sdLDL-C levels and the negative correlation between threonine and sdLDL-C levels remained statistically significant (Supplementary Table 2). No significant correlations were observed between plasma EAA concentration and TC or LDL-C (Supplementary Table 3).

Median levels of sdLDL-C, RLP-C, TG, and mean HDL-C level were significantly different between the tertile levels of BCAAs or threonine (Supplementary Table 4**)**. The highest tertile of BCAA level had the highest mean concentrations of sdLDL-C, RLP-C, and TG (all $p < 0.001$), while the highest tertile of threonine level had the lowest mean concentrations of sdLDL-C, RLP-C, and TG (all $p < 0.001$). Further analysis showed that participants in the category with high BCAAs and low threonine had the highest concentrations of sdLDL-C, RLP-C, or TG compared with other participants. In contrast, participants in the category with low BCAAs and high threonine concentrations had the lowest concentrations of sdLDL-C, RLP-C, or TG (Fig. 1).

### 3.3. Association between EAAs and prevalent risk of atherogenic lipid profile

The association between BCAAs, threonine, and high sdLDL-C, high RLP-C, high TG, and atherogenic lipid triad was further investigated using logistic regression models. Odds ratios (ORs) and 95% confidence intervals (CIs) after adjustment for potential confounding factors for atherogenic lipid profile are shown in Table 3 and Fig. 2**.** The highest tertile of BCAA concentration (each BCAA and the total concentration of BCAAs) was significantly associated with increased risk of high sdLDL-C ($OR_{BCAAs}$: 2.33, 95%CI: 1.35–4.03, $p = 0.002$), high RLP-C ($OR_{BCAAs}$: 3.63, 95%CI: 1.69–7.80, $p = 0.001$), and high TG ($OR_{BCAAs}$: 3.10, 95%CI: 1.66–5.80, $p < 0.001$). Among BCAAs, elevated leucine was associated with the highest risk of high sdLDL-C; we identified a 2.63-fold increased risk of leucine level for high sdLDL-C (OR: 2.63, 95%CI: 1.42–4.88, $p = 0.002$). Furthermore, elevated valine was associated with the highest risk of high RLP-C and high TG, as we identified a 3.48-fold increased risk of valine level for high RLP-C (OR: 3.48, 95%CI: 1.66–7.28, $p = 0.001$), and a 3.13-fold increased risk for high TG (OR: 3.13, 95%CI: 1.69–5.78, $p < 0.001$), respectively (Table 3).

The highest tertile of threonine concentration was significantly associated with reduced risk of high sdLDL-C and high TG, even after adjustment for conventional metabolic risk factors and the total concentration of BCAAs (Table 3). Individuals in the highest threonine tertile had a 43% decreased risk for high sdLDL-C ($p = 0.038$) and a 56% decreased risk for high TG ($p = 0.004$) compared with those in the lowest tertile.

In addition, we also found that elevation of each BCAA and total concentration of BCAAs were associated with increased risk of atherogenic lipid triad and threonine was associated with reduced risk of atherogenic lipid triad (Fig. 2). The highest tertile of total BCAAs showed a 3.67-fold increased risk of lipid triad (OR: 3.67, 95%CI: 2.00–6.73, $p < 0.001$), while threonine showed a 55% lower prevalent risk of lipid triad (OR: 0.45, 95%CI: 0.26–0.76, $p = 0.003$) compared with the lowest tertile.

**Table 2**
Correlation (r) between levels of essential amino acids and serum lipid concentrations.

| EAAs (μmol/L) | sdLDL-C | | RLP-C | | TG | | HDL-C | |
|---|---|---|---|---|---|---|---|---|
| | Model 1 | Model 2 | Model 1 | Model 2 | Model 1 | Model 2 | Model 1 | Model 2 |
| Valine | 0.31** | 0.23** | 0.35** | 0.13* | 0.35** | 0.18** | −0.38** | −0.25** |
| Leucine | 0.31** | 0.20** | 0.32** | 0.11* | 0.34** | 0.17** | −0.40** | −0.25** |
| Isoleucine | 0.28** | 0.20** | 0.29** | 0.12* | 0.31** | 0.18** | −0.41** | −0.27** |
| Threonine | −0.18** | −0.18** | −0.19** | −0.12* | −0.17** | −0.14* | < 0.01 | 0.06 |
| Lysine | 0.01 | −0.01 | 0.10 | 0.04 | 0.09 | 0.05 | −0.12 | −0.08 |
| Histidine | 0.06 | 0.03 | 0.10 | 0.01 | 0.09 | 0.02 | −0.10 | −0.05 |
| Phenylalanine | 0.03 | 0.01 | 0.08 | 0.07 | 0.07 | 0.06 | −0.17** | −0.08 |
| Tryptophan | 0.16* | 0.09 | 0.21** | 0.07 | 0.20** | 0.09 | −0.14* | −0.06 |
| Methionine | < 0.01 | 0.03 | 0.07 | 0.02 | 0.04 | −0.01 | −0.10 | −0.01 |
| BCAAs | 0.32** | 0.24** | 0.36** | 0.14* | 0.36** | 0.20** | −0.42** | −0.28** |

Model 1: Simple correlations between essential amino acids and serum lipid concentration.
Model 2: Partial correlation adjusted for age, gender, waist circumference, FBG, SBP, use of glucose-lowering medications during the past 2 weeks, use of lipid-lowering medications during the past 2 weeks.
EAAs, essential amino acids; sdLDL-C, small and low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol. Correlation is significant at $*p < 0.05$, $**p < 0.01$.

## 4. Discussion

In this cross-sectional study, our data indicate that, first, among the nine EAAs examined, plasma BCAAs were positively correlated with levels of sdLDL-C, RLP-C, and TG but inversely correlated with HDL-C. In contrast, threonine level was inversely correlated with sdLDL-C, RLP-C, and TG. Second, elevation of plasma BCAAs was significantly associated with increased risk of high sdLDL-C, high RLP-C, high TG, and atherogenic lipid triad, while elevated threonine level was associated with a reduced risk of high sdLDL-C, high TG, and atherogenic lipid triad, independent of FBG level. Finally, participants who were simultaneous carriers for low BCAAs and high threonine concentrations had the low concentrations of sdLDL-C, RLP-C, or TG. Our data provide novel insights into the potential relationship between levels of amino acids and atherogenic lipid profile.

Recently, an increasing number of studies on the association between free amino acid profiles and metabolic diseases have been reported, although studies focusing on the relationship between EAAs and high sdLDL-C, high RLP-C, and atherogenic lipid triad are lacking. Yamamoto et al. measured plasma amino acid levels of 1890

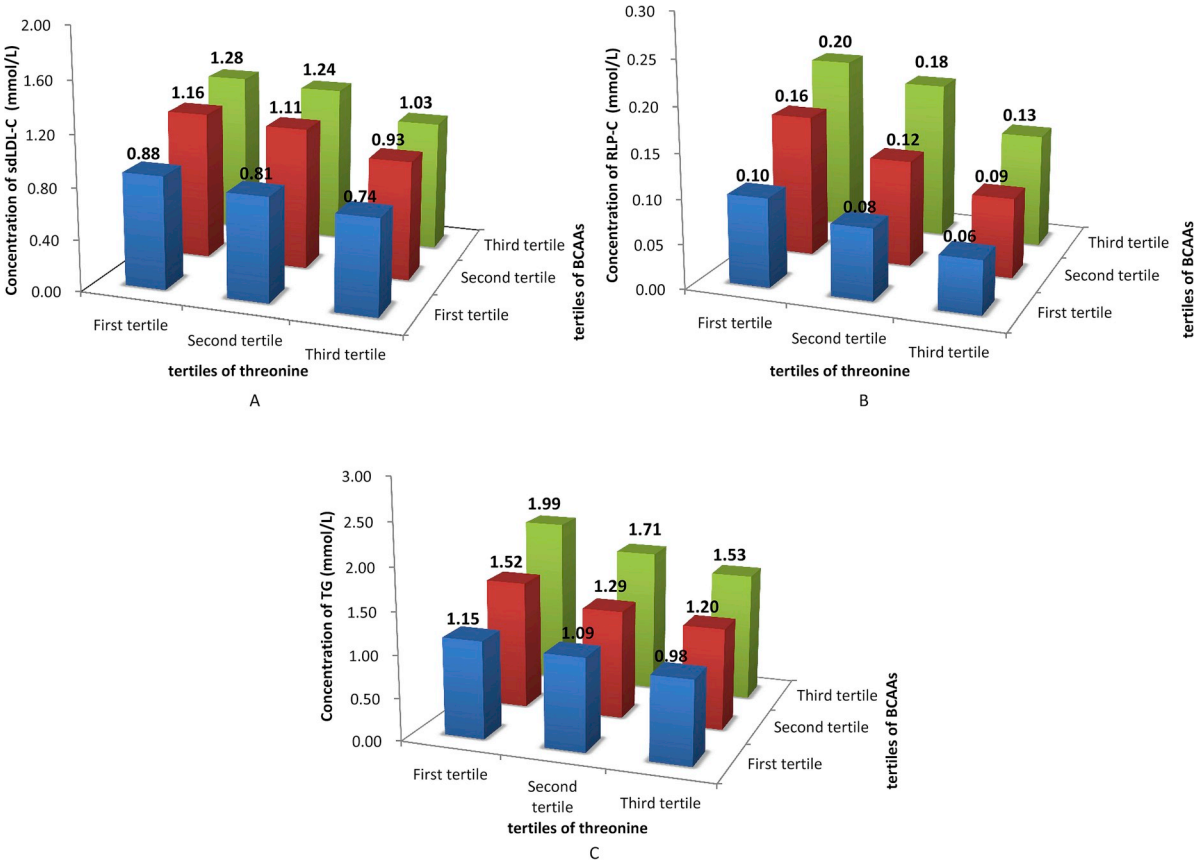


**Fig. 1.** Concentrations of sdLDL-C, RLP-C, and TG among subgroups defined by tertiles of BCAAs and threonine.
(A) sdLDL-C, (B) RLP-C, and (C) TG. sdLDL-C, small dense low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol; TG, triglycerides; BCAA: branched-chain amino acid.

**Table 3**
Associations between amino acids and high sdLDL-C, high RLP-C, and high TG.

| EAAs(μmol/L) | n[c] | High sdLDL-C[a] OR (95%CI) | p | n[c] | High RLP-C[a] OR (95%CI) | p | n[c] | High TG[b] OR (95%CI) | p |
|---|---|---|---|---|---|---|---|---|---|
| **Valine** | | | | | | | | | |
| First tertile | 54 | 1.0 (reference) | | 51 | 1.0 (reference) | | 31 | 1.0 (reference) | |
| Second tertile | 83 | 1.56 (0.92,2.63) | 0.098 | 82 | 2.09 (1.06,4.10) | 0.033 | 56 | 2.12 (1.21,3.72) | 0.008 |
| Third tertile | 101 | 1.91 (1.06,3.44) | 0.030 | 105 | 3.48 (1.66,7.28) | 0.001 | 78 | 3.13 (1.69,5.78) | < 0.001 |
| **Leucine** | | | | | | | | | |
| First tertile | 51 | 1.0 (reference) | | 54 | 1.0 (reference) | | 34 | 1.0 (reference) | |
| Second tertile | 84 | 1.93 (1.12,3.30) | 0.017 | 86 | 2.41 (1.25,4.66) | 0.009 | 55 | 2.28 (1.30,4.00) | 0.004 |
| Third tertile | 103 | 2.63 (1.42,4.88) | 0.002 | 98 | 2.39 (1.11,5.14) | 0.026 | 76 | 2.95 (1.57,5.55) | 0.001 |
| **Isoleucine** | | | | | | | | | |
| First tertile | 58 | 1.0 (reference) | | 54 | 1.0 (reference) | | 33 | 1.0 (reference) | |
| Second tertile | 80 | 1.34 (0.80,2.27) | 0.269 | 85 | 2.55 (1.31,4.97) | 0.006 | 56 | 2.15 (1.23,3.77) | 0.007 |
| Third tertile | 100 | 1.81 (1.01,3.30) | 0.048 | 99 | 2.65 (1.23,5.71) | 0.013 | 76 | 3.00 (1.61,5.59) | 0.001 |
| **BCAAs** | | | | | | | | | |
| First tertile | 51 | 1.0 (reference) | | 54 | 1.0 (reference) | | 34 | 1.0 (reference) | |
| Second tertile | 87 | 2.20 (1.15,3.94) | 0.016 | 78 | 2.18 (1.01,4.30) | 0.025 | 51 | 1.94 (1.11,3.42) | 0.021 |
| Third tertile | 100 | 2.33 (1.35,4.03) | 0.002 | 106 | 3.63 (1.69,7.80) | 0.001 | 80 | 3.10 (1.66,5.80) | < 0.001 |
| **Threonine** | | | | | | | | | |
| First tertile | 88 | 1.0 (reference) | | 90 | 1.0 (reference) | | 71 | 1.0 (reference) | |
| Second tertile | 85 | 0.99 (0.60,1.69) | 0.983 | 81 | 0.92 (0.51,1.64) | 0.774 | 51 | 0.58 (0.34,0.97) | 0.039 |
| Third tertile | 65 | 0.57 (0.33,0.97) | 0.038 | 67 | 0.63 (0.35,1.14) | 0.125 | 43 | 0.44 (0.26,0.77) | 0.004 |

[a] Logistic regression was adjusted for age, gender, waist circumference, SBP, FBG, high TG status, low HDL-C status, use of glucose-lowering medications during the previous 2 weeks of the survey, and use of lipid-lowering medications during the previous 2 weeks of the survey.
[b] Logistic regression was adjusted for age, gender, waist circumference, SBP, use of lipid-lowering medication during the previous 2 weeks of the survey, and use of lipid-lowering medications during the previous 2 weeks of the survey EAAs, essential amino acids; sdLDL-C, small and low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol.
[c] n: Number of events.

individuals, for the reference intervals (RIs) in Japanese individuals [29]. Compared with the levels of plasma EAAs in Japanese, there were no large differences in the levels of plasma EAAs between our study and theirs. Several previous studies have reported that amino acid levels are associated with a perturbed lipid metabolism [3,10–12,30]. Consistent with the data obtained in previous studies, we found that BCAA concentrations were significantly positively correlated with TG and negatively correlated with HDL-C, with no significant correlations found between these amino acids and TC or LDL-C concentrations. Interestingly, we found that BCAAs were also positively correlated with sdLDL-C and RLP-C. We compared the average levels of sdLDL-C and RLP-C in our study with the Multi-Ethnic Study of Atherosclerosis

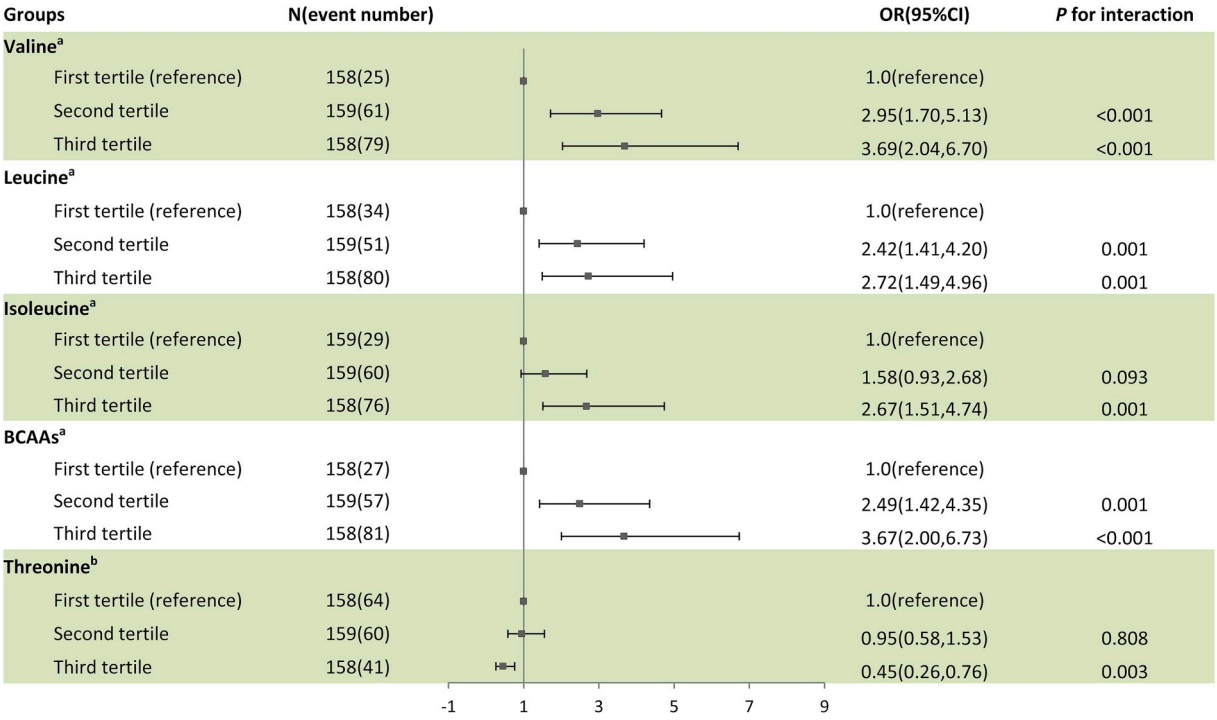


**Fig. 2.** Association between branched-chain amino acids and threonine concentrations and the risk of atherogenic lipid triad.
[a] Logistic regression was adjusted for age, gender, waist circumference, SBP, FBG, high TG status, high HDL-C status, use of glucose-lowering medications during the previous 2 weeks of the survey, and use of lipid-lowering medications during the previous 2 weeks of the survey. [b] Logistic regression was adjusted for age, gender, waist circumference, SBP, FBG, high TG status, high HDL-C status, use of glucose-lowering medications during the previous 2 weeks of the survey, use of lipid-lowering medications during the previous 2 weeks of the survey and total concentration of BCAAs.

[23,31] and the Atherosclerosis Risk in Communities (ARIC) Study [17]. We found that the levels of sdLDL-C and RLP-C in our study population were very close to the levels of those two studies. To our knowledge, this is the first study to evaluate the association between plasma EAAs levels and high sdLDL-C or high RLP-C. The sdLDL subfraction is a major contributor to the risk for incident cardiovascular disease associated with LDL-C [17], and individuals with a predominance of small LDL particles have a larger atherogenic burden but may have optimal LDL-C levels because of the lower cholesterol-carrying capacity of smaller lipoprotein particles [32]. RLP-C represents the cholesterol content of triglyceride-rich lipoproteins [33], and elevated RLP-C has been reported as a more likely causal factor of cardiovascular disease than increased TG and reduced HDL cholesterol [34–36]. sdLDL-C and RLP-C are thus closely related to atherogenic diseases. In our study, we found that BCAAs were associated with high sdLDL-C and RLP-C levels, even after adjustment for age, gender, WC, SBP, FBG, high TG status, low HDL-C status, and use of lipid-lowing or glucose-lowering medications during the previous 2 weeks of the survey. These results indicate that elevated levels of BCAAs may be associated directly with atherogenic diseases rather than via an increase in TG levels or decrease in HDL-C. In addition, the catabolic pathway of BCAAs has been shown to be associated with coronary artery disease [37]. This is consistent with thr hypothesis we have described above. However, the mechanism of the association between amino acids and lipid concentrations remains unclear.

In addition, in the present study, a significant negative correlation was observed between threonine and levels of sdLDL-C and TG, and elevated threonine was associated with a reduced risk of high sdLDL-C, high TG, and atherogenic lipid triad. Our findings are similar to the Framingham Heart Study, which showed a negative correlation between threonine and TG levels [30]. In addition, Yamaguchi et al. also showed that plasma threonine levels were higher in people without dyslipidemia than in those with dyslipidemia [38]. However, these studies failed to show statistical significant for these effects. Our results showed a significant association between plasma threonine and reduced risk of high sdLDL-C, high TG, and atherogenic lipid triad, which occurs in numerous clinical settings associated with high cardiovascular risk [39]. However, the mechanism of the relationship between threonine and lipids has not yet been fully elucidated, although threonine supplementation has been shown to reduce the concentrations of total lipid, TG, liver cholesterol, and plasma LDL-C in rats [40] and ducks [25]. Another study [26] reported that threonine dehydrogenase is inhibited by certain fatty acids and their derivatives. The irreversible degradation of threonine under the action of threonine dehydratase is the sole pathway of threonine catabolism in humans [41,42]. Therefore, an increase in lipid levels is accompanied by a decrease in threonine levels, which is consistent with our finding that threonine is negatively correlated with certain lipid components. However, further studies are necessary to elucidate the mechanisms linking threonine with these lipids.

The present study shows that BCAAs are positively correlated with sdLDL-C, RLP-C, and TG levels while threonine is negatively correlated with these parameters. Therefore, we further explored the association between the pattern of low BCAAs and high threonine level with sdLDL-C, RLP-C, and TG and found that participants in the category of low BCAAs and high threonine concentrations had the lowest concentrations of sdLDL-C, RLP-C, or TG compared with other participants. This result further suggests that the pattern of low concentration of BCAAs and high concentration of threonine may represent a protective factor to reduce the prevalent risk of metabolic lipid disorders humans.

Our study had some limitations. First, the study population comprised middle-aged and older adults; therefore, extrapolation of our results to individuals in other age groups requires caution. Second, the associations between BCAAs and atherogenic dyslipidemia were identified in a cross-sectional analysis that does not permit inferences regarding causality to be made.

In conclusion, our results suggest that elevated plasma BCAAs levels are associated with an atherogenic lipid profile and increased threonine is associated with a reduced risk of atherogenic lipid profile. These findings support a new hypothesis whereby plasma amino acids may represent a risk factor or protector for atherogenic disease. However, further studies are necessary to clarify whether plasma BCAAs and threonine levels are associated with the development of dyslipidemia and the risk of atherosclerotic cardiovascular disease, and to elucidate the mechanisms linking these factors.

## Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

## Financial support

Participant recruitment and data collection were supported by the National Science & Technology Pillar Program during the Eleventh Five-Year Plan Period (2006BAI01A01 and 2006BAI01A02). The study design, data analysis, data interpretation, and other pertinent activities were funded by the National Program on Key Basic Research Project (973 Program) of China (2012CB517806).

## Author contributions

Feng-Hua Wang analyzed the data and drafted the manuscript. Dong Zhao contributed to the initial concepts and study design. All authors contributed to the acquisition or interpretation of data. Dong Zhao and Qiu-Ju Deng contributed to manuscript revision. All authors approved the final version of the manuscript.

## Acknowledgments

We wish to acknowledge the support of the Key Laboratory of Remodeling-Related Cardiovascular Diseases, Beijing AnZhen Hospital, Capital Medical University, Ministry of Education, Beijing, China.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.04.225.

## References

[1] S.M. Hutson, A.J. Sweatt, K.F. Lanoue, Branched-chain [corrected] amino acid metabolism: implications for establishing safe intakes, J. Nutr. 135 (6 Suppl) (2005) 1557s–1564s.
[2] J. Kim, T.T.H. Tran, S.P. Hong, J.S. Jeong, A reference measurement procedure for amino acids in blood using isotope dilution ultra-performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1055–1056 (2017) 72–80.
[3] A.C. Gill, R. Yang, J. Dong, et al., Rapid and precise measurement of serum branched-chain and aromatic amino acids by isotope dilution liquid chromatography tandem mass spectrometry, PLoS One 8 (12) (2013) e81144.
[4] S.B. Narayan, G. Ditewig-Meyers, K.S. Graham, R. Scott, M.J. Bennett, Measurement of plasma amino acids by ultraperformance(R) liquid chromatography, Clin. Chem. Lab. Med. 49 (7) (2011) 1177–1185.
[5] T. Tillin, A.D. Hughes, Q. Wang, et al., Diabetes risk and amino acid profiles: cross-sectional and prospective analyses of ethnicity, amino acids and diabetes in a South Asian and European cohort from the SABRE (Southall and Brent REvisited) Study, Diabetologia 58 (5) (2015) 968–979.
[6] Y. Lu, Y. Wang, C.N. Ong, et al., Metabolic signatures and risk of type 2 diabetes in a Chinese population: an untargeted metabolomics study using both LC-MS and GC-MS, Diabetologia 59 (11) (2016) 2349–2359.
[7] R. Wang-Sattler, Z. Yu, C. Herder, et al., Novel biomarkers for pre-diabetes identified by metabolomics, Mol. Syst. Biol. 8 (2012) 615.
[8] E. Ferrannini, A. Natali, S. Camastra, et al., Early metabolic markers of the development of dysglycemia and type 2 diabetes and their physiological significance, Diabetes 62 (5) (2013) 1730–1737.
[9] D.O. Mook-Kanamori, W. Romisch-Margl, G. Kastenmuller, et al., Increased amino acids levels and the risk of developing of hypertriglyceridemia in a 7-year follow-

up, J. Endocrinol. Investig. 37 (4) (2014) 369–374.
[10] M. Yamakado, K. Nagao, A. Imaizumi, et al., Plasma free amino acid profiles predict four-year risk of developing diabetes, metabolic syndrome, dyslipidemia, and hypertension in Japanese population, Sci. Rep. 5 (2015) 11918.
[11] P. Yang, W. Hu, Z. Fu, et al., The positive association of branched-chain amino acids and metabolic dyslipidemia in Chinese Han population, Lipids Health Dis. 15 (2016) 120.
[12] W. Hu, L. Sun, Y. Gong, et al., Relationship between branched-chain amino acids, metabolic syndrome, and cardiovascular risk profile in a Chinese population: a cross-sectional study, Int. J. Endocrinol. 2016 (2016) 1–10.
[13] X. Bi, S.L. Tey, Y.T. Loo, C.J. Henry, Central adiposity-induced plasma-free amino acid alterations are associated with increased insulin resistance in healthy Singaporean adults, Eur. J. Clin. Nutr. 71 (9) (2017) 1080–1087.
[14] H. Ohnishi, S. Saitoh, S. Takagi, et al., Relationship between insulin-resistance and remnant-like particle cholesterol, Atherosclerosis 164 (1) (2002) 167–170.
[15] T. Hirano, Y. Ito, S. Koba, et al., Clinical significance of small dense low-density lipoprotein cholesterol levels determined by the simple precipitation method, Arterioscler. Thromb. Vasc. Biol. 24 (3) (2004) 558–563.
[16] M.J. Stampfer, R.M. Krauss, J. Ma, et al., A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction, Jama 276 (11) (1996) 882–888.
[17] R.C. Hoogeveen, J.W. Gaubatz, W. Sun, et al., Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study, Arterioscler. Thromb. Vasc. Biol. 34 (5) (2014) 1069–1077.
[18] C.D. Gardner, S.P. Fortmann, R.M. Krauss, Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women, Jama 276 (11) (1996) 875–881.
[19] T. Nishikura, S. Koba, Y. Yokota, et al., Elevated small dense low-density lipoprotein cholesterol as a predictor for future cardiovascular events in patients with stable coronary artery disease, J. Atheroscler. Thromb. 21 (8) (2014) 755–767.
[20] B. Lamarche, A. Tchernof, S. Moorjani, et al., Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study, Circulation 95 (1) (1997) 69–75.
[21] J. Pitha, J. Kovar, Z. Skodova, et al., Association of intima-media thickness of carotid arteries with remnant lipoproteins in men and women, Physiol. Res. 64 (Suppl 3) (2015) S377–S384.
[22] J.R. McNamara, P.K. Shah, K. Nakajima, et al., Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham Heart Study, Atherosclerosis 154 (1) (2001) 229–236.
[23] A. Saeed, E.V. Feofanova, B. Yu, et al., Remnant-like particle cholesterol, low-density lipoprotein triglycerides, and incident cardiovascular disease, J. Am. Coll. Cardiol. 72 (2) (2018) 156–169.
[24] R.W. Nesto, Beyond low-density lipoprotein: addressing the atherogenic lipid triad in type 2 diabetes mellitus and the metabolic syndrome, Am. J. Cardiovasc. Drugs : Drugs Dev. Other Interv. 5 (6) (2005) 379–387.
[25] Y. Jiang, J. Tang, M. Xie, Z.G. Wen, S.Y. Qiao, S.S. Hou, Threonine supplementation reduces dietary protein and improves lipid metabolism in Pekin ducks, Br. Poult. Sci. 58 (6) (2017) 687–693.
[26] R. Guerranti, R. Pagani, S. Neri, S.V. Errico, R. Leoncini, E. Marinello, Inhibition and regulation of rat liver L-threonine dehydrogenase by different fatty acids and their derivatives, Biochim. Biophys. Acta 1568 (1) (2001) 45–52.
[27] J. Liu, Y. Hong, RB D'Agostino, et al., Predictive value for the Chinese population of the Framingham CHD risk assessment tool compared with the Chinese Multi-Provincial Cohort Study, Jama 291 (21) (2004) 2591–2599 Sr..
[28] Y. Ito, M. Fujimura, M. Ohta, T. Hirano, Development of a homogeneous assay for measurement of small dense LDL cholesterol, Clin. Chem. 57 (1) (2011) 57–65.
[29] H. Yamamoto, K. Kondo, T. Tanaka, et al., Reference intervals for plasma-free amino acid in a Japanese population, Ann. Clin. Biochem. 53 (Pt 3) (2016) 357–364.
[30] S. Cheng, E.P. Rhee, M.G. Larson, et al., Metabolite profiling identifies pathways associated with metabolic risk in humans, Circulation 125 (18) (2012) 2222–2231.
[31] M.Y. Tsai, B.T. Steffen, W. Guan, et al., New automated assay of small dense low-density lipoprotein cholesterol identifies risk of coronary heart disease: the Multi-ethnic Study of Atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 34 (1) (2014) 196–201.
[32] H. Sumino, K. Nakajima, M. Murakami, [Possibility of new circulating atherosclerosis-related lipid markers measurement in medical and complete medical checkups: small dense low-density lipoprotein cholesterol and lipoprotein lipase], Rinsho Byori Jpn. J. Clin. Pathol. 64 (3) (2016) 298–307.
[33] M.J. Chapman, H.N. Ginsberg, P. Amarenco, et al., Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management, Eur. Heart J. 32 (11) (2011) 1345–1361.
[34] C. Emerging Risk Factors, E. Di Angelantonio, N. Sarwar, et al., Major lipids, apolipoproteins, and risk of vascular disease, Jama 302 (18) (2009) 1993–2000.
[35] W.E. Boden, J.L. Probstfield, T. Anderson, et al., Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy, N. Engl. J. Med. 365 (24) (2011) 2255–2267.
[36] A. Varbo, M. Benn, B.G. Nordestgaard, Remnant cholesterol as a cause of ischemic heart disease: evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment, Pharmacol. Ther. 141 (3) (2014) 358–367.
[37] S. Bhattacharya, C.B. Granger, D. Craig, et al., Validation of the association between a branched chain amino acid metabolite profile and extremes of coronary artery disease in patients referred for cardiac catheterization, Atherosclerosis 232 (1) (2014) 191–196.
[38] N. Yamaguchi, M.H. Mahbub, H. Takahashi, et al., Plasma free amino acid profiles evaluate risk of metabolic syndrome, diabetes, dyslipidemia, and hypertension in a large Asian population, Environ. Health Prev. Med. 22 (1) (2017) 35.
[39] M.J. Chapman, J.S. Redfern, M.E. McGovern, P. Giral, Optimal pharmacotherapy to combat the atherogenic lipid triad, Curr. Opin. Cardiol. 26 (5) (2011) 403–411.
[40] A.E. Harper, W.J. Monson, G. Litwack, D.A. Benton, J.N. Williams Jr., C.A. Elvehjem, Effect of partial deficiency of threonine on enzyme activity and fat deposition in liver, Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY), 84 1953, pp. 414–417 84.
[41] C. Han, H. Gu, J. Wang, W. Lu, Y. Mei, M. Wu, Regulation of L-threonine dehydrogenase in somatic cell reprogramming, Stem cells (Dayton, Ohio) 31 (5) (2013) 953–965.
[42] A.V. Malinovsky, Reason for indispensability of threonine in humans and other mammals in comparative aspect, Biochemistry (Mosc.) 82 (9) (2017) 1055–1060.