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Association of branched-chain amino acids with coronary artery disease: A matched-pair case–control study

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Abstract *Background and aim:* Several recent studies have found an independent relationship between levels of plasma branched-chain amino acids (BCAAs) and risk factors for coronary artery disease (CAD); however, few studies have investigated the associations of BCAAs with CAD and the risk of cardiovascular events. Therefore, the aim of this study was to investigate the relationship between BCAAs and CAD.
Methods and results: We studied 143 patients with CAD diagnosed by coronary angiography at Beijing Hospital (Beijing, China) during 2008–2011. Apparently healthy control individuals (n = 286) and the patients with CAD were matched (2:1 ratio) by age and gender. The healthy control individuals were selected at random from a set of subjects who attended an annual physical examination at the same hospital in 2011. Conditional logistic regression models were used to evaluate the associations between measured variables and CAD. After multivariate adjustment for traditional CAD risk factors, each one-standard-deviation increase in BCAA concentration was associated with an approximately twofold increase in the risk of CAD (odds ratio = 1.63, 95% confidence interval (CI): 1.21–2.20, P = 0.001). As compared with subjects in the lowest quartile of BCAA levels, the odds ratios (95% CIs) for CAD risk in subjects belonging to quartiles 2, 3, and 4 were 1.65 (0.75–3.61), 2.04 (0.92–4.53), and 3.86 (1.71–8.69), respectively (P trend = 0.01).
Conclusion: Our results demonstrate that BCAAs are significantly related to CAD development. This relationship is independent of diabetes, hypertension, dyslipidemia, and body mass index.

Introduction

Coronary artery disease (CAD) is a complex disease that is commonly known as one of the primary causes of death worldwide. The early identification of individuals at a risk of CAD is particularly crucial [1]. The use of advanced technologies to evaluate individuals may improve risk stratification and enhance our knowledge of the disease process. Our ability to identify cases of CAD and our understanding of CAD development could be substantially improved by metabolomics, which is the study of small-

Abbreviations: AIP, atherosclerosis index of plasma; BCAAs, branched-chain amino acids; CAD, coronary artery disease; DBP, diastolic blood pressure; FBG, fasting blood glucose; SBP, systolic blood pressure.

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molecule metabolites that are the end products of cellular processes [1,2].

Leucine (Leu), isoleucine (Ile), and valine (Val) constitute the branched-chain amino acids (BCAAs), which are a subgroup of the essential amino acids in humans (i.e., the amino acids that cannot be synthesized de novo by the organism). In addition to their roles as key building blocks for protein synthesis, BCAAs are also significant sources for the biosynthesis of sterol, ketone bodies, and glucose [3]. Various factors can contribute to the elevation of BCAAs in circulation, including dietary intake, synthesis via gut microbiota, and catabolic defects [4]. The accumulation of BCAAs and related metabolites may produce adverse effects ranging from neurological distress to cardiomyopathy [5].

Recently, some metabolomic studies indicated that BCAAs may be both markers and effectors of insulin resistance [6–8], can be used to predict the future development of diabetes [9,10], and are highly responsive to therapeutic interventions [11–13]. The underlying cellular mechanisms may include activation of mTOR (mammalian target of rapamycin), JNK (c-Jun N-terminal kinase), and insulin receptor substrate-1 signaling pathways [7,9]. Other investigations, including our previous studies, demonstrated that incremental circulating levels of BCAAs are independently associated with several CAD risk factors, in addition to impaired glucose tolerance. Indeed, independent associations were found with elevated ambulatory blood pressure [14]; atherogenic dyslipidemia [15], which is characterized by an increase in serum triglycerides (TG), a decrease in high-density lipoprotein cholesterol (HDL-C), and the prevalence of small, dense low-density lipoprotein (LDL); and increased carotid intima–media thickness in subclinical atherosclerosis [16]. However, few studies have investigated the associations of BCAAs with the development of atherosclerosis and CAD. Therefore, the aim of this study was to investigate the relationship between BCAAs and CAD.

Methods

Study design and subjects

We studied 143 hospitalized patients (102 males and 41 females, 30–84 years of age) who underwent coronary angiography at the Beijing Hospital (Beijing, China) during 2008–2011 and who were diagnosed with CAD based on angiograms with >50% stenosis in one or more arteries. Patients with the following characteristics were excluded from the study: both unstable angina and myocardial infarction within the preceding 2 months, or receiving lipid-regulating therapies in the preceding 6 months. Apparently healthy control individuals ($n = 286$) and the patients with CAD were matched (2:1 ratio) by age interval (±3 years) and gender. The healthy controls were selected at random from a group of Beijing residents who attended an annual physical examination at the Beijing Hospital in 2011. The controls had no history of angina pectoris, previous coronary angiography, myocardial infarction, or other known cardiovascular diseases. The smoking status of all subjects was recorded using a list of questionnaire. Height, weight, and sitting blood pressure were measured at the same time. Fasting blood samples were taken from the subjects, and the sera were isolated and stored at −80 °C until analysis. This study was reviewed and approved by the Ethics Committee of the Beijing Hospital. All enrolled individuals received written notice of the intended use of their blood samples and provided written consent.

Measurements of serum BCAAs and other parameters

The serum BCAA levels (Val, Ile, and Leu) were measured using our previously reported isotope dilution liquid chromatography tandem mass spectrometry (LC/MS/MS) method [15]. Briefly, 0.05-mL aliquots of calibrators or serum samples were mixed with 0.05 mL of the isotopically labeled internal standard solution. The amino acids were extracted with 0.4 mL of acetonitrile containing 0.1% formic acid and analyzed using LC/MS/MS with positive electronic spray ionization in the multiple reaction monitoring mode. The serum samples were also tested for the levels of fasting blood glucose (FBG), total cholesterol (TC), TG, HDL-C, and LDL cholesterol (LDL-C) using assay kits from Sekisui Medical Technologies (Osaka, Japan) on a Hitachi 7180 chemistry analyzer (Hitachi, Tokyo, Japan). The atherosclerosis index of plasma (AIP) was calculated as log (TG/HDL-C), with TG and HDL-C being expressed in molar concentrations [17].

Statistical analyses

Categorical variables are presented as frequencies and percentages. Continuous variables are summarized in terms of means and standard deviations (SD), or medians and interquartile ranges (25th–75th percentile) for variables with skewed distributions. Hypertension was defined as a systolic blood pressure (SBP) ≥ 140 mm Hg or a diastolic blood pressure (DBP) ≥90 mm Hg. Diabetes mellitus was defined as a fasting glucose concentration > 7.0 mmol/L. Dyslipidemia was defined as a serum TC > 6.21 mmol/L, LDL-C > 4.14 mmol/L, TG > 1.70 mmol/L, or HDL-C < 1.04 mmol/L. We used generalized linear mixed models to compare continuous variables and categorical variables by case/control status, respectively, accounting for clustering by matching status. Correlations were assessed using partial correlation coefficients after adjusting for matched pair. The associations between measured variables and the presence of CAD were evaluated in various multivariate conditional logistic regression models. The conditional logistic regression analyses were performed using the COXREG command in SPSS according to previously reported methods [18]. Odds ratios (ORs) for CAD(+) vs. CAD(−) were estimated with the corresponding 95% confidence intervals (CIs). The ORs were adjusted for body mass index (BMI); smoking status; and the presence or absence of diabetes, hypertension, and dyslipidemia. All reported *P*-values are two-tailed, and a *P*-

value < 0.05 was considered to indicate statistical significance. Analyses were performed using SPSS software, version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the study population

The characteristics of patients with CAD and the age- and gender-matched controls are compared in Table 1. Overall, the prevalences of current smoking, diabetes mellitus, hypertriglyceridemia, and hypo-HDL cholesterolemia were significantly higher among patients with CAD than among controls (48% vs. 10%, 12% vs. 4%, 45% vs. 23%, and 59% vs. 19%, respectively). Compared with controls, CAD patients had significantly higher BMI, TG, AIP, Val, Ile, and Leu. Further, the sum of the three BCAA concentrations was significantly higher in the CAD(+) group than in the control group. By contrast, patients with CAD had markedly lower TC, HDL-C, and LDL-C values.

Because we found significantly higher serum BCAA levels in men than in women ($P < 0.001$), we also performed a stratified analysis, in which the differences between the serum BCAA levels in the CAD (+) and control groups were analyzed separately for men and women (Fig. 1). The sum of the three BCAA concentrations was significantly higher for men with CAD ($n = 102$) than for men in the control group ($n = 204$) ($Z = -4.627$, $P < 0.001$). Similarly, the sum of three BCAA concentrations was significantly higher for women with CAD ($n = 41$) than for women in the control group ($n = 82$) ($Z = -4.947$, $P < 0.001$).

Correlation analyses

The relationships between BCAAs and the traditional risk factors for CAD in the total study population are shown in Table 2. In the partial correlation analyses, Val, Ile, Leu, and the total BCAA concentrations were significantly and positively correlated with BMI ($P < 0.001$), FBG ($P < 0.001$), TG ($P < 0.001$), and AIP ($P < 0.001$) after adjusting for matched pair. Furthermore, the concentration of each BCAA concentration was significantly and inversely associated with HDL-C ($P < 0.001$). Marked correlations were also observed between each pair of BCAAs ($P < 0.001$ for each comparison).

Conditional logistic regression model

We performed conditional logistic regression analyses to further quantify association between BCAA and CAD in this matched-pair case–control study (Fig. 2). Multivariate adjustment was used to control for traditional CAD risk factors, including BMI, smoking, diabetes mellitus, hypertension, hypertriglyceridemia, hypercholesterolemia, hyper-LDL cholesterolemia, and hypo-HDL cholesterolemia. After this multivariate adjustment, each one-SD increase in BCAA concentration was associated with an

Table 1 Demographic, clinical, and laboratory characteristics of patients with CAD and matched controls.

Variable	CAD(−) ($n = 286$)	CAD(+) ($n = 143$)	*P*-value[a]
Age, years	64.7 ± 10.7	64.5 ± 10.8	0.979
Men, %	71.3	71.3	1.000
Smoker, %	9.6	48.3	<0.001
Hypertension, %	29.0	35.9	0.187
Diabetes, %	4.0	11.9	0.012
Hypertriglyceridemia, %	23.1	44.8	<0.001
Hypercholesterolemia, %	5.9	6.3	0.471
Hyper-LDL cholesterolemia, %	4.3	2.8	0.349
Hypo-HDL cholesterolemia, %	18.5	59.4	<0.001
BMI, kg/m^2	24.4 ± 5.9	25.7 ± 3.2	<0.001
SBP, mmHg	128.0 (119.0–140.0)	130.0 (120.0–140.0)	0.512
DBP, mmHg	78.0 (71.0–82.0)	73.0 (70.0–80.0)	0.111
FBG, mmol/L	5.4 (5.1–5.8)	5.4 (4.9–6.2)	0.532
TC, mmol/L	4.9 ± 0.9	4.6 ± 1.0	0.005
TG, mmol/L	1.3 (0.9–1.7)	1.6 (1.2–2.2)	<0.001
HDL-C, mmol/L	1.3 (1.1–1.6)	1.0 (0.8–1.2)	<0.001
LDL-C, mmol/L	2.8 ± 0.8	2.5 ± 0.8	<0.001
AIP	−0.02 (−0.2 to 0.2)	0.2 (0.04–0.4)	<0.001
Val, μmol/L	243.3 (220.5–269.5)	269.7 (242.3–293.9)	<0.001
Ile, μmol/L	68.2 (59.2–79.0)	82.0 (71.5–91.6)	<0.001
Leu, μmol/L	132.3 (117.2–149.0)	146.8 (129.2–159.6)	<0.001
BCAA, μmol/L[b]	442.6 (395.3–495.3)	502.7 (446.4–544.0)	<0.001

Data are mean ± SD, median (interquartile range) for continuous variables, or percentage for categorical variables. Abbreviations: CAD, coronary artery disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; AIP, atherosclerosis index of plasma; BCAA, branched-chain amino acid.

[a] *P*-values are derived from generalized linear mixed models for continuous and categorical variables controlling for matched sets. Age and gender were matching variables.

[b] The sum of the concentrations of Val, Ile, and Leu.

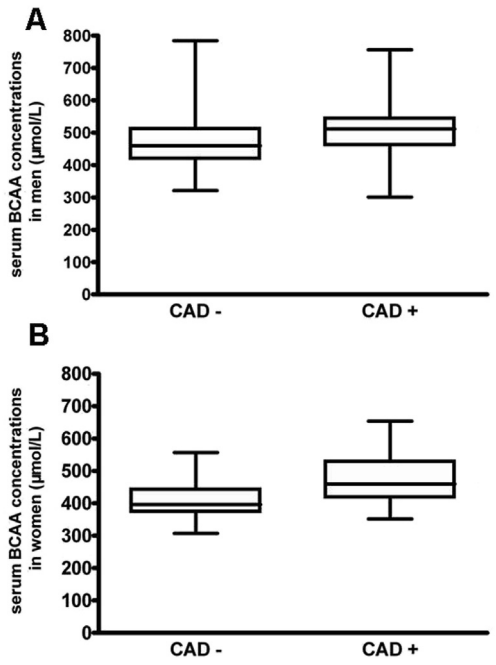

Figure 1 Differences between serum BCAA levels in the CAD(+) and control groups, as analyzed separately for men (A) ($n = 306$) and women (B) ($n = 123$). The box plots show the median and 25th and 75th percentiles. Whiskers in the plots represent the highest and lowest values. Abbreviations: BCAA, branched-chain amino acids; CAD, coronary artery disease.

approximately twofold increase in the risk of CAD (OR = 1.63, 95% CI: 1.21–2.20, $P = 0.001$). As compared with subjects in the lowest quartile of BCAA levels, the OR (95% CIs) for CAD risk in subjects belonging to quartiles 2, 3, and 4 were 1.65 (0.75–3.61), 2.04 (0.92–4.53), and 3.86 (1.71–8.69), respectively (P trend = 0.01).

Discussion

Recently, there has been mounting evidence that BCAAs are associated with insulin resistance and causally involved in the development of metabolic dysfunction.

Table 2 Partial correlations (r) of BCAA with other CAD risk factors, as adjusted for matched pair.

	Val	Ile	Leu	BCAA[a]
BMI	0.199***	0.199***	0.194***	0.207***
SBP	0.082	0.074	0.013	0.062
DBP	0.001	0.026	0.012	0.009
FBG	0.210***	0.200***	0.202***	0.216***
TC	0.021	−0.066	0.011	0.001
TG	0.327***	0.312***	0.255***	0.317***
HDL-C	−0.358***	−0.445***	−0.324***	−0.382***
LDL-C	0.041	−0.045	0.002	0.014
AIP	0.415***	0.435***	0.363***	0.423***

*$P < 0.05$; **, $P < 0.01$; ***$P < 0.001$. Abbreviations: CAD, coronary artery disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; AIP, atherosclerosis index of plasma; BCAA, branched-chain amino acid.

[a] The sum of the concentrations of Val, Ile, and Leu.

Although some studies have found independent relationships between BCAAs and CAD risk factors, few studies have investigated the associations of BCAAs with CAD and the risk of cardiovascular events. By using targeted metabolomics, Shah and colleagues demonstrated that BCAAs were positively and independently associated with the presence of CAD in both a small patient cohort (OR = 1.36, $P = 0.02$) [19] and a larger patient cohort (OR = 1.20, $P = 0.005$) [20]. All of the subjects included in these studies were undergoing cardiac catheterization because of suspected CAD. Accordingly, the studies were limited to individuals with high burdens of cardiovascular risk factors. The results of the present study have confirmed that individuals with elevated levels of BCAAs are at a higher risk of CAD. Further, our study has expanded upon the previous findings by including a more general control group, which was composed of apparently healthy individuals. Our study sample appears to differ from the cohorts in previous studies in terms of diet, ethnicity, BMI, and probably other culture-related characteristics, such as lifestyle factors. Nonetheless, the current results support the hypothesis that BCAA accumulation may mediate CAD development independently of the relationships of CAD with diabetes, hypertension, dyslipidemia, and BMI.

BCAAs are essential amino acids, and they must be acquired from food. Increased dietary intake of protein, 15–25% of which consists of BCAAs [21], can contribute significantly to the elevation of BCAAs in circulation. However, in general, observed blood amino acid patterns are probably not a direct reflection of diet-derived amino acids. McCormack et al. [22] found that the plasma BCAA level, but not dietary BCAA intake, was associated with obesity and insulin resistance. Wang et al. [9] also indicated that the associations of BCAAs with insulin resistance and risk of diabetes were not influenced by protein consumption. By contrast, some large-scale population studies showed that high intake of BCAAs was significantly associated with a lower prevalence of being overweight [23] and a decreased risk of diabetes [24], thereby contradicting the observations for plasma BCAA levels. The effects of dietary BCAA intake and BCAA circulation levels continue to contradict each other, and the reasons remain unknown. However, we assumed that the involvement of elevated plasma BCAA levels in cardiometabolic disease development is mostly caused by the obstacle of BCAA catabolism, rather than by increased dietary intake.

Disorders of BCAA homeostasis may result from BCAA catabolic defects, which themselves may primarily arise from the abnormal expression and activity of genes encoding certain enzymes, such as branched-chain aminotransferase (BCAT), branched-chain α-keto acid dehydrogenase (BCKD), and BCKD phosphatase [14,25,26]. Several studies found that the activity of the BCAA catabolic enzyme was reduced in obese and insulin-resistant rodents [27,28]. In obese humans, bariatric surgery was followed by markedly decreased BCAA levels and increased expression of the enzyme BCKD [11]. Furthermore, as effective activators of cell signaling, circulating

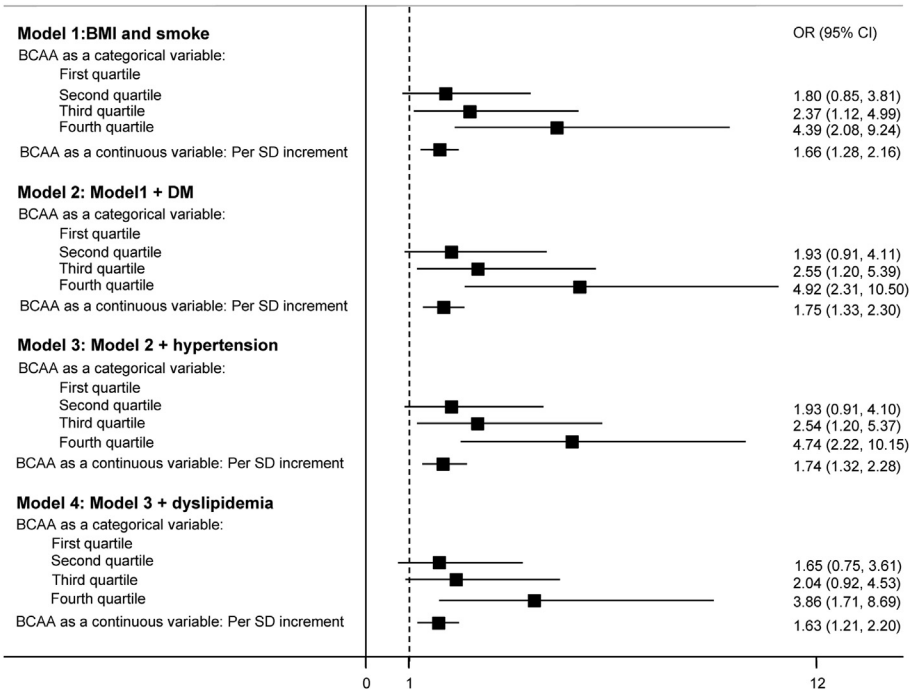

Figure 2 Association of serum BCAA levels with CAD under different conditional logistic regression models for matched-pair case–control study. Model 1 is adjusted for BMI and smoking. Model 2 is further adjusted for diabetes mellitus. Model 3 is further adjusted for hypertension. Model 4 is further adjusted for hypertriglyceridemia, hypercholesterolemia, hyper-LDL cholesterolemia, and hypo-HDL cholesterolemia. Abbreviations: BCAA, branched-chain amino acid; CAD coronary artery disease; DM, diabetes mellitus.

BCAAs may directly promote insulin resistance, primarily via chronic stimulation of the mTOR pathway, but possibly also via the activation of JNK and insulin receptor substrate-1 signaling pathways in rat skeletal muscles [7,29].

However, the nature of any mechanistic link that may explain the BCAA–CAD association is less clear. In both the current investigation and previous studies [19,20], the association of elevated BCAA levels with CAD risk remained intact even after adjusting for the presence of diabetes mellitus, suggesting that the association between BCAA and CAD may not be driven entirely by insulin resistance. Besides, Mels et al. [14] supposed that increased insulin secretion could lead to a signal promoting excessive growth, which would stimulate various proliferative and pro-atherogenic events in vascular smooth muscle and endothelial cells (these are the hallmarks of the pathogenic mechanisms involved in atherosclerosis) [30].

Several limitations to this study warrant discussion. First, we did not analyze the effects of individual BCAAs; instead, we analyzed the sum of BCAA concentrations because they were strongly intercorrelated, which may influence the accuracy of our explanation of the model. Second, insulin levels were not available for the participants in our study; therefore, insulin resistance could not be quantified. Third, although our results remained consistent after multiple adjustments, we cannot exclude the possibility of residual confounding because some information was not recorded, including a family history of CAD, dietary factors, sedentary lifestyle, stress, depression, and other possible risk factors for CAD. Finally, although the study was conducted as a case–control design, the temporal sequence of the association cannot be determined as the BCAAs were measured at the same time as subject recruitment. The findings need to be confirmed in future prospective studies.

In conclusion, our results add value by demonstrating an independent relationship between elevated BCAA levels and CAD risk, regardless of the nature of the observed mechanism behind the elevated BCAA levels. The relationship between BCAA and CAD risk remained significant after adjusting for traditional CAD risk factors. Further, the presence of this relationship was demonstrated in a population that is more general than previously investigated populations, which had high burdens of CAD risk. Future studies may elucidate the mechanisms that underlie the significant association between BCAAs and CAD.

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