Nutrition, Metabolism & Cardiovascular Diseases (2015) **25**, 937–942

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

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

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

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Received 17 February 2015; received in revised form 25 May 2015; accepted 5 June 2015
Available online 14 June 2015

KEYWORDS
Branched-chain amino acids;
Case–control study;
Coronary artery disease;
Odds ratio;
Risk factor

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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http://dx.doi.org/10.1016/j.numecd.2015.06.003
0939-4753/

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*-