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Branched-chain amino acids are associated with cardiometabolic risk profiles found already in lean, overweight and obese young☆

Harald Mangge[a,*], Sieglinde Zelzer[a], Florian Prüller[a], Wolfgang J. Schnedl[b], Daniel Weghuber[c,d], Dietmar Enko[e], Peter Bergsten[f], Johannes Haybaeck[g], Andreas Meinitzer[a]

[a]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria
[b]General Practice for Internal Medicine, Bruck an der Mur, Austria
[c]Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria
[d]Obesity Research Unit, Paracelsus Medical University, Salzburg, Austria
[e]Institute of Laboratory Medicine, General Hospital Steyr, Steyr, Austria
[f]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
[g]Department of Pathology, Medical University of Graz, Graz, Austria

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Abstract

Cardiovascular risk is increased in obese subjects. Nevertheless, some overweight and obese remain cardiometabolically healthy (CMH), and normal-weight persons develop cardiovascular disease (CVD). Herein, we investigate the potential of branched-chain amino acids (BCAAs) to identify an increased CVD risk in a cross-sectional study of 666 adults and juveniles (age 25.3 ± 12.8 years), classified as lean, overweight or obese. Cardiometabolic groups were defined by cutoffs of systolic blood pressure<130 mmHg, diastolic blood pressure<85 mmHg, glucose<125 mg/dl, triglycerides<150 mg/dl, HDL-cholesterol>40 mg/dl (males), HDL-cholesterol>50 mg/dl (females) and HOMA-IR<5. CMH had ≤1 cutoff, and cardiometabolically abnormal (CMA) had ≥2 cutoffs. Amino acids were measured by high-pressure lipid chromatography after precipitation of serum with perchloric acid and derivatization with *o*-phthalaldehyde. Valine correlated with 5, leucine correlated with 3 and isoleucine correlated with 5 of the cardiac risk classification factors. Valine and leucine were significantly higher in the obese ($P<.001$, $P=.015$, respectively), overweight ($P<.001$, $P=.015$, respectively) and lean ($P=.024$, $P=.012$, respectively) CMA compared to CMH subjects. Isoleucine showed except of the lean group the same results. Taken together, BCAAs, especially valine and leucine, are proposed as a cardiometabolic risk marker independent of body mass index (BMI) category.

Keywords: Branched-chain amino acids; Cardiometabolic risk; Body mass index

1. Introduction

Individuals who are overweight or obese are at increased risk for developing type 2 diabetes, cardiovascular and cerebrovascular disease [cardiovascular disease (CVD)]. However, not all overweight/obese individuals will develop these diseases. This has been shown by Wildman *et al.* [1] in a cross-sectional sample of the National Health and Nutrition Examination Survey 1999–2004 cohort. Moreover, Badoud *et al.* [2] analyzed serum and adipose tissue amino acid (AA) homeostasis in obese compared to lean individuals. Using subcutaneous adipose tissue gene expression profiling, genes related to branched-chain amino acid (BCAA) catabolism and the tricarboxylic acid cycle were down-regulated in metabolically unhealthy compared to metabolically healthy overweight/obese (MHO) individuals. Thus, altered AA homeostasis in metabolically unhealthy obese was assumed [2]. Magnusson *et al.* investigated the BCAA, isoleucine and the aromatic AAs tyrosine and phenylalanine in a matched case–control study derived from the population-based Malmö Diet and Cancer Cardiovascular Cohort [3]. They proposed a potential of these mixed spectrum of AAs as novel risk pattern for CVD development, possibly linking diabetes and CVD susceptibility. Nevertheless, although all three of these AAs had consistent associations with incident CVD, no individual AA reached statistically significance as expected for a robust predictor [3].

When serum biomarkers were investigated in overweight and obese women with and without metabolic syndrome (MetS), specific BCAAs, aromatic AA and orosomucoid profiles were found to be associated with MetS and clinical risk factors, independently of body mass index (BMI), fat mass, waist circumference and physical activity/fitness [4]. BCAA utilization as fuel has been connected with high oxidative capacity and metabolic "fitness" [5] and may contribute to

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* Corresponding author. Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Auenbruggerplatz 15, A-8036, Graz, Austria. Tel.: +43-316-385-83340; fax: +43-316-385-13430.
E-mail address: harald.mangge@klinikum-graz.at (H. Mangge).

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explain these associations. Circulating BCAAs, leucine, isoleucine and valine, seem to play a role in the control of energy balance and food intake [6–9]. Insulin secretion [5], protein synthesis [10] and degradation [11] are directly and/or indirectly influenced by BCAAs. Leucine activates the signaling factor of mammalian target of rapamycin (mTOR) to promote protein synthesis in skeletal muscle and in adipose tissue [11]. Further, leucine regulates blood glucose levels by promoting gluconeogenesis and aids to retain lean mass in a hypocaloric state [12]. In other studies, BCAA and factors composed of AAs involved in the urea cycle pathway were independently associated with the development of diabetes [13], nonalcoholic fatty liver disease [14], exacerbations of cardiovascular burden after adjusting for known clinical risk factors [15] and particularly after adjusting of diabetes and measures of insulin resistance [16]. Nevertheless, it must be noted that the physiological regulatory mechanisms that control circulating BCAA levels remain to be clarified.

The aim of this study is to investigate whether peripheral blood AA profiles are associated with well-defined cardiovascular risk indicators already present as early as in young individuals irrespective of gender and BMI.

2. Methods

2.1. Study population

Study participants are from the prospective, observational study STYJOBS/EDECTA (STYrian Juvenile Obesity Study/Early DEteCTion of Atherosclerosis; ClinicalTrials.gov Identifier NCT00482924), which investigates metabolic/cardiovascular parameters in obese individuals who were free of chronic health conditions except MetS. We included individuals aged 21.3 ± 12.8 years. The analysis of normal-weight controls was included in the design of this study to provide a reference for the MHO. Participants were excluded if they suffered from acute or chronic diseases and/or received medications. Written consent was obtained for all adult participants, and parental consent was obtained for youth. Procedures were as follows: standard anthropometric data (height, weight, waist and hip circumference, waist-to-hip and waist-to-height ratios) were obtained from each subject as described elsewhere [17].

2.2. Ethics

The study was approved by the ethics committee of the Medical University of Graz (EK number 20-029 ex 08/09) and conducted in compliance with guidelines for human studies as described in the Helsinki Declaration of 1975, revised in 1996.

2.3. Classifying of patients

Individuals included in this analysis were pooled and were classified into three categories: lean, overweight and obese — lean juveniles <18 years old with BMI between 5th and 84.9th percentile and lean adults with BMI between 18.5 and 24.9 kg/m^2; overweight juveniles with BMI between 85th and 94th percentile and overweight adults with BMI greater than 25 kg/m^2 but less than 30.0 kg/m^2; and obese juveniles with BMI >95th percentile and obese adults with BMI>30 kg/m^2. Cardiometabolic abnormalities were defined as follows: (1) impaired fasting glucose≥126 mg/dl; (2) systolic blood pressure (SBP)≥130 mmHg or diastolic blood pressure (DBP)≥85 mmHg; (3) triglycerides≥150 mg/dl; (4) HDL-cholesterol <40 mg/dl in men or HDL-cholesterol<50 mg/dl in women; and (5) insulin resistance (calculated by the Homeostatic Model Assessment Index HOMA-IR>5) [18] (see Table 1). The threshold was chosen to be consistent with previous reports of the relationship between metabolic wellness and BMI [1] although HOMA-IR>2.6 is generally accepted as the clinical definition of insulin resistance [19]. Cardiometabolically healthy (CMH) individuals were defined as having zero or one of these cardiometabolic abnormalities, and cardiometabolically abnormal (CMA) individuals were defined as having two or more cardiometabolic abnormalities. Out of the total number of 1189 patients, 46 patients collected from 2006 to 2014 were excluded because of missing laboratory parameters to classify the cardiometabolic status. Based on their BMI, the remaining 1143 participants were divided in three groups: 638 lean, 257 overweight and 248 obese. Further cardiometabolic classification led to 517 CMH and 121 CMA subjects in the lean group, 149 CMH and 108 CMA subjects in the overweight group and 103 CMH and 145 CMA in the obese group. From the largest CMH/lean group, 121 subjects were age and sex selected compared to the CMA/lean group. From these six groups, further 81 subjects were excluded because of less sample material. A total of 666 patients left: 112 in the lean/CMH group, 112 in the lean/CMA group, 128 in the overweight/CMH group, 92 in the overweight/CMA group, 93 in the obese/CMH group and 129 in the obese/CMA group.

Table 1
Baseline characteristics of the study subjects (n = 666, age 21.3 ± 12.8 SD years) by BMI categories and CMH and CMA categories.

	BMI≤25		BMI=25.1–29.9		BMI≥30	
	CMH	CMA	CMH	CMA	CMH	CMA
	N[a]	N	N	N	N	N
	112	112	128	92	93	129
SBP[b]	14	77	23	64	19	92
DBP[c]	8	64	8	30	5	45
Glucose[d]	—	27	3	21	5	37
Triglycerides[e]	32	61	9	34	7	66
HDL-cholesterol[f]	17	56	25	43	31	97
HOMA-IR[g]	—	27	5	25	3	68

[a] 48, without any cardiometabolic criteria.
[b] SBP>130 mmHg.
[c] DBP>85 mmHg.
[d] Glucose>100 and <125 mg/dl.
[e] Triglycerides>150 mg/dl.
[f] HDL-C<40 mg/dl (males), HDL-C<50 mg/dl (females).
[g] HOMA-IR> 5.

2.4. Laboratory methods

Fasting blood samples were collected from 08:00 to 10:30 am. Ultrasensitive C-reactive protein (US-CRP) was analyzed with a Tina-quant C-reactive protein latex ultrasensitive assay (Roche Diagnostics, Germany). Cholesterol, HDL-cholesterol and triglycerides were measured by enzymatic photometric methods (Roche Diagnostics, Germany); LDL-cholesterol was calculated by the Friedewald formula [20]. Plasma insulin was measured by ELISA (Mercodia, Uppsala, Sweden); plasma glucose was measured by the glucose hexokinase method. HOMA-IR was calculated as defined by Matthews *et al.* [18]. AAs were measured with modifications of previous described chromatographic methods [21]. Briefly, after precipitation of serum with perchloric acid following neutralization of the supernatant with sodium carbonate, the extracted AAs were derivatized with *o*-phthalaldehyde and separated on a reversed-phase column with gradient elution. Quantification was performed with ratios of fluorescence signals of the relevant AAs to the internal standard or valine in comparison to the appropriated calibration curves. Intraassay and interassay CVs were all below 10%.

2.5. Statistical analysis

Statistical analyses were carried out using PASW Statistics 22.0 for Windows 8. Normal distribution of all clinical and biochemical measures was controlled by the Kolmogorov–Smirnov test. In cases