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Amino acid levels in nascent metabolic syndrome: A contributor to the pro-inflammatory burden

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ABSTRACT

Aims: Metabolic Syndrome (MetS) is a cluster of cardio-metabolic risk factors characterized by low-grade inflammation which confers an increased risk for type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Prior studies have linked elevated branched chain amino acids (BCAA) and aromatic amino acids (AAA) with T2DM and CVD. Due to the paucity of data in MetS, the aim of this study was to investigate the status of amino acids as early biomarkers of nascent MetS patients without T2DM and CVD or smoking.
Research design and methods: Healthy controls ($n = 20$) and MetS ($n = 29$) patients were recruited for the study. MetS was defined by criteria of National Cholesterol Education Program Adult Treatment Panel III of having at least 3 risk factors. Urinary amino acids were quantified by gas chromatography time-of-flight mass spectrometry at the Western NIH Metabolomics Center as expressed to urinary creatinine.
Results: Tyrosine and Isoleucine levels were significantly elevated in MetS patients. Isoleucine positively correlated with salient cardio-metabolic features and inflammatory biomarkers. Lysine and Methionine levels were decreased in MetS patients. Lysine correlated negatively with cardio-metabolic features and inflammatory bimarkers. Methionine also correlated negatively with blood pressure and certain inflammatory biomarkers.
Conclusion: Our novel results suggest that with regards to the cardio-metabolic risk factors and pro-inflammatory features of MetS, isoleucine (BCAA) demonstrated a positive correlation while lysine demonstrated a negative correlation. Thus, increased levels of isoleucine and decreased levels of lysine could be potential early biomarkers of MetS.

1. Introduction

Nearly 34% of the adult U.S. population has the Metabolic Syndrome (MetS), with prevalence increasing with age and in certain racial minorities.[1] The cardio-metabolic risk factors which characterize MetS include abdominal obesity, elevated fasting plasma glucose, hypertension, high serum triglycerides and low HDL-cholesterol levels.[1] We have also previously shown that MetS is a pro-inflammatory state and is associated with adipose tissue dysfunction.[2,4,5]

Studies have demonstrated that elevated plasma levels of BCAAs (valine, leucine, isoleucine) and AAAs have been associated with increased pro-inflammatory mediators and worsened overall metabolic health.[3] In a cross sectional study by Ntzouvani et al., BCAAs and AAAs displayed a positive association with the established cardiometabolic risk factors of MetS in a subset of patients of a male Mediterranean population which included smokers, diabetics, and dyslipidemic patients on therapy.[6] Similarly, Allam-Ndoul et al. discovered a direct association between leucine, isoleucine and fasting glucose levels in a population of obese and overweight subjects already diagnosed with MetS.[7]

The aim of our project was to investigate the role of amino acids as potential early biomarkers in nascent MetS before progression to CVD and T2DM. Nascent MetS is defined as MetS without the confounding of diabetes, CVD, and smoking.[5] We compared amino acid levels of nascent MetS and control patients and correlated them with the cardio-metabolic features of MetS and biomarkers of inflammation, oxidative stress and adipokine dysregulation. While the link between BCAAs and the incidence, progression and remission of insulin resistance, T2DM and CVD has been reasonably well documented, the role of other amino acids as early predictors in nascent MetS without confounding diabetes or CVD remains to be determined.[3]

1.1. Subjects and methods

We recruited participants (aged 24–72 years) from Sacramento county using procedures as described previously.[2,4,5] MetS subjects ($n = 29$) and healthy controls ($n = 20$) were defined by criteria of National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III).[1,2] Briefly, MetS subjects had at least 3 risk factors: central

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obesity defined by waist circumference, hypertension, fasting triglycerides >150 mg/dL, fasting HDL-cholesterol <40 mg/dL (men) or <50 mg/dL (women), fasting blood glucose levels 100–125 mg/dL. Control subjects had ≤2 of the risk factors and were not on anti-hypertensives.

Additional exclusion criteria for controls were: fasting plasma glucose >100 mg/dL and triglycerides >200 mg/dL. For both groups, other exclusion criteria include: diabetes (fasting glucose >125 mg/dL and HbA_{1C} > 6.4%), clinical atherosclerosis (CAD, PAD, CVD) smoking, hypo- or hyperthyroidism, malabsorption, anticoagulant therapy, steroid therapy, anti-inflammatory drugs, statin and other hypolipidemic therapy, hypoglycemic agents, ARBs, TG > 400 mg/dL (for MetS subjects), oral contraceptives, use of antioxidant supplements in the past 6 months; pregnancy, abnormal complete blood count and alcohol consumption >1 oz./day; consumption of N-3 PUFA, postmenopausal women on estrogen replacement therapy, recent surgery, inflammatory or malignant disease. CRP >10 mg/L, chronic high intensity exercise (>100 min/week). None of the volunteers had renal or hepatic disease defined by an elevated creatinine or albuminuria >30 mg/g creatinine or abnormal liver function tests.

Informed consent was obtained from participants in the study, which was approved by the Institutional Review Board at the University of California Davis. After history and physical examination, fasting blood was obtained. A complete blood count, plasma lipid and lipoprotein profile, urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, glucose and TSH were assayed by standard laboratory techniques in the Clinical Pathology Laboratory. Insulin levels, IL-6, IL-8, LBP, nitrotyrosine, and leptin were assayed by ELISA (Linco Biosystems) and HOMA-IR was calculated from glucose and insulin levels as described previously.[2,4,5] Endotoxin levels were quantitated using reagents from Lonza.[4] Surface expression of TLR2 and TLR4 were quantified by flow cytometry as previously described.[3]

Gas chromatography time-of-flight mass spectrometry (GC-TOF MS) was employed for metabolomics analysis of urine amino acids at the Western NIH Metabolomics Center using internal standards. Briefly, metabolites were extracted from 5 to 30 mL samples by protein precipitation using cold isopropanol/acetonitrile/water. Extracts were dried and derivatized by trimethylsilylation to increase volatility for GC-TOF MS data acquisition. Data were deconvoluted by ChromaTOF vs 4.6 software and then calculated by the BinBase database for compound annotations. Data are expressed as peak height to creatinine peak height ratio.

For metabolomic variables, results are expressed as mean and standard deviation (SD) for parametric or as median and interquartile ranges for skewed variables. Log transformations were applied to variables with skewed distributions prior to parametric analyses. Comparisons between the control and metabolic syndrome groups were made with the Wilcoxon Rank Sum test or two-sample *t*-tests. Analysis of covariance was employed to adjust for age, BMI, and waist circumference. The association between continuous variables was assessed with Spearman rank correlation coefficients. General statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Significance was defined as a p value <0.05.

2. Results

Table 1 shows the salient cardio-metabolic features of the 2 groups. There were no statistically significant differences in age or gender distribution between the control and MetS group. Significant differences were present in waist circumference, BMI, BP-systolic, glucose, triglycerides, HDL-cholesterol levels. Also, both HOMA-IR and hsCRP were significantly increased as reported by us previously in the larger cohort.

Fig. 1 depicts the studied amino acid, isoleucine (Ile), tyrosine, lysine and methionine in MetS patients and control patients. Isoleucine levels were significantly elevated in MetS patients compared to the controls ($p < 0.0001$). Tyrosine levels were also elevated in MetS patients compared to the controls ($p = 0.001$). Both lysine ($p < 0.001$) and methionine ($p < 0.01$) levels were significantly lower in MetS patients.

Table 1
Salient Characteristics between MetS and Controls.

Variable	Controls ($n = 20$)	MetS ($n = 29$)	p-value Controls vs MetS**
Age (yrs)	48 ± 13	53 ± 9	0.16
Sex, F/M	17/3	22/7	0.50
Waist (cm)	92 ± 14	109 ± 14	0.0002*
BMI (kg/m^2)	30.2 ± 5.6	35.1 ± 6.3	0.01*
BP-systolic (mm Hg)	117 ± 12	132 ± 11	0.0006*
BP-diastolic (mm Hg)	74 ± 9	80 ± 9	0.10
Glucose (mg/dL)	86 ± 8	98 ± 13	0.003*
Triglycerides (mg/dL)	63 (48–92)	147 (103,177)	0.0002*
HDL cholesterol (mg/dL)	52 ± 12	40 ± 11	0.002*
LDL cholesterol (mg/dL)	126 ± 20	123 ± 23	0.85
HOMA-IR	1.6 (1.1, 2.8)	2.8 (1.9, 5.8)	0.003*
hsCRP (mg/L)	1.3 (0.4, 3.2)	4.0 (2.2, 5.8)	0.005*

Results are presented as Mean ± standard deviation or Median (25th percentile, 75th percentile).

* Indicates significance p-value <0.05.

** p-value for sex from Fisher's Exact test, p-values for continuous variable from the Wilcoxon Rank Sum test.

As shown in Table 2, isoleucine levels correlated positively with cardio-metabolic features including BMI, waist circumference, SBP (systolic blood pressure), DBP (diastolic blood pressure) and inversely with HDL-cholesterol. Additionally, isoleucine correlated with biomarkers including IL-6, LBP, endotoxin, nitrotyrosine ($r = 0.37$, $p = 0.041$) and leptin levels ($r = 0.38$,$p = 0.022$). There was a trend to significance between Ile and hsCRP, ($r = 0.27$, $p = 0.07$). Elevated tyrosine levels did not correlate with any of the inflammatory markers. Interestingly, lysine correlated negatively with certain features of MetS including SBP, DBP and blood glucose and positively with HDL-cholesterol levels. Lysine also correlated negatively with IL-6, TLR4 and endotoxin. There was a trend to significance between lysine and triglycerides ($r = -0.29$, $p = 0.027$) and oxidized LDL ($r = -0.35$, $p = 0.07$). Methionine correlated negatively with SBP and TLR4.

3. Discussion

The purpose of our study was to gain insight into the role of amino acids as potential early biomarkers in nascent MetS before progression to CVD and T2DM. In our study, we found that while isoleucine and tyrosine levels were significantly elevated in nascent MetS patients compared to controls, lysine and methionine levels were decreased. It is important to emphasize that none of our patients were smokers or had diabetes or CVD. Additionally, our data also shows a positive correlation between isoleucine, and cardio-metabolic features of MetS and certain biomarkers. On the other hand, lysine and methionine were negatively correlated with inflammatory biomarkers and cardio-metabolic parameters.

Dysregulation of BCAA metabolism resulting in increased plasma concentrations of isoleucine, leucine and valine have been described by studies to be an early predictor of insulin resistance. As early as 1969, in a cohort study of 20 obese and non-obese patients, Felig et al. discovered a negative correlation between levels of isoleucine and insulin sensitivity, establishing the link between elevated isoleucine and insulin resistance.[8] BCAAs have also been associated with the pathogenesis of obesity. Zeng et al., found elevated BCAAs in obese children and proposed that they could be used as early biomarkers of childhood obesity.[9] In a more recent cross-sectional study examining the relationship between BCAAs, MetS and cardiovascular risk profile in a Chinese population, Hu et al. found that the percentage of subjects with 3 ~ 5 MetS features increased stepwise with levels of BCAAs.[10]

Evidence from such studies demonstrates that the metabolism of branched chain amino acids such as isoleucine may be a significant factor in the pathophysiology of multifactorial diseases such as MetS.[11] Newgard proposed a pathway by which dysregulated BCAA metabolism leading to elevated BCAA levels contributes to the development of

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