Associations among circulating branched-chain amino acids and tyrosine with muscle volume and glucose metabolism in individuals without diabetes

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Abstract

Background and Aims: Amino acid metabolites including branched chain amino acids (BCAA) and tyrosine (Tyr) affect glucose metabolism. The effects of BCAA on insulin resistance in patients with diabetes seem to conflict with mechanisms determined in animal models and cultured cells. We investigated the physiological effects of BCAA and Tyr on glucose metabolism among healthy community dwellers to clarify the controversy surrounding the effects of BCAA.

Participant and methods: We investigated associations among BCAA and Tyr and metabolic parameters in 78 residents (median age, 52 y) of Mie, Japan who did not have pre-diabetes, diabetes, or a BMI > 30 kg/m^2.

Results: Muscle volume, serum BCAA and Tyr levels were higher in men than in women (n = 32 and 46, respectively; all $p < 0.0001$). Stepwise multiple regression analysis associated BCAA positively with muscle volume (regression coefficient/t/p/ 95%CI = 281.8/ 3.7/ 0.0004/ 129.7 – 433.8), fasting blood glucose (FBG) (12699.4/ 3.22/ 0.0020/ 4830.9 – 20567.8), fasting immunoreactive insulin (IRI) (8505.1/ 2.75/ 0.0078/ 2322.5 – 14687.6) and homeostasis model assessment of β cell function (HOMA-β) (893.6/ 2.58/ 0.0122/ 201.8 – 1585.5), and negatively with the HOMA-insulin resistance (HOMA-IR) (-9294.1/ -2.89/ 0.0052/ -15711.0 – -2877.1). Tyr positively correlated with fasting IRI (26.0/ 2.77/ 0.0072/ 7.3 – 44.7).

Conclusions: Insulin sensitivity and muscle volume are positively associated with BCAA in individuals without diabetes. In turn, BCAA correlate with increased FBG and fasting IRI levels. Tyr correlated with fasting IRI, but not with insulin sensitivity.

Key Words: insulin resistance; β cell function; fasting blood glucose, Spearman's test, stepwise multiple regression analysis

Running head: Effects of BCAA and Tyr on glucose metabolism

Abbreviations: AAA, aromatic amino acids; ADA, American Diabetes Association; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCAA, branched chain amino acids; BCAT, branched-chain aminotransferase; BG, blood glucose; BMI, body mass index; BTR, molar ratio of branched chain amino acids to tyrosine; BUN, blood urea nitrogen; CI, confidence interval; Cr, creatinine; DBP, diastolic blood pressure; FBG, fasting blood glucose; γ-GTP, γ-glutamyl transpeptidase; GLUT, glucose transporter; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-β; homeostasis model assessment-beta cell function; HOMA-IR, homeostasis model assessment-insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; LXR, liver X receptor α; mTOR, mammalian target of rapamycin; NAFLD, non-alcoholic fatty liver disease; OGTT, oral glucose tolerance test; %FAT, ratio of body fat; PI3K, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferator-activated receptor; SBP, systolic blood pressure; SREBP1-c, sterol regulatory element binding protein-1c; T2DM, type-2 diabetes mellitus; T-Chol, total cholesterol; TG, triglycerides; UCP,

uncoupling proteins; WHR, waist-hip ratio.

Introduction

Branched chain amino acids (BCAA), comprising the essential amino acids valine, leucine and isoleucine, affect glucose metabolism in adipose tissue, skeletal muscle and the liver [1]. Many essential amino acids are mainly catabolized in the liver, but the activity of branched chain aminotransferase (BCAT), a key enzyme in BCAA catabolism, is not significant in the liver [2]. Of the essential amino acids in skeletal muscle proteins, about 35% are BCAA [3] and these are actively oxidized in skeletal muscle [4].

The influence of BCAA on glucose metabolism has been intensively investigated in animal models and cultured cells. Serum levels of BCAA are significantly increased in transgenic mitochondrial BCAT gene-knockout ($BCAT^{-/-}$) mice compared with wild-type mice, whereas fasting blood glucose (FBG), fasting serum insulin and the homeostasis model assessment-insulin resistance (HOMA-IR) are significantly reduced [5]. Other groups have shown that leucine and isoleucine improve insulin sensitivity in mice [6] [7]. Distinctive molecular pathways for improved insulin resistance induced by BCAA have been identified in insulin target organs such as adipose tissue, skeletal muscle and the liver. Briefly, BCAA increase glucose uptake through the phosphorylation of Akt and mammalian target of rapamycin (mTOR) in adipose tissue [8]. BCAA activate phosphatidylinositol 3-kinase (PI3K) and protein kinase C that translocate glucose transporter 1 (GLUT1) and GLUT4 to the plasma membrane where they promote glucose uptake from the bloodstream into skeletal muscle [9] [10]. BCAA

activate the liver X receptor α (LXR)/sterol regulatory element binding protein-1c (SREBP1-c) pathway to up-regulate liver-type glucokinase and GLUT2 in the liver. The activation of LXR/SREBP-1 reduces gluconeogenesis through down-regulating glucose-6-phosphatase expression [11]. In addition, BCAA increase the expression of peroxisome proliferator-activated receptor (PPAR) α, subsequent uncoupling protein 2 (UCP2) in the liver and UCP3 in muscle. Both UCP2 and 3 improve insulin resistance by promoting the oxidation of free fatty acid [12] [13].

Leucine is involved in protein synthesis through activating mTOR and subsequently upregulating downstream molecules [14]. Leucine can also increase satiety by stimulating postprandial leptin secretion [15]. These facts are consistent with the finding that dietary supplementation with leucine or BCAA decreases body weight and that fat mass (%FAT) and can improve glucose metabolism [16-18].

However, the effects of BCAA on insulin resistance in obese individuals and/or in patients with diabetes seem to contradict these mechanisms, which have mainly been determined in animal models and cultured cells. One study of patients with type 1 or type 2 diabetes mellitus (T2DM) has shown that amelioration of the diabetic state induced by either insulin or anti-diabetic drugs reduces serum BCAA levels [19]. Serum levels of BCAA-related metabolites positively correlate with insulin resistance in obese humans [20]. Moreover, serum levels of BCAA-related metabolites positively correlate with the waist-hip ratio (WHR) and serum levels of alanine aminotransferase (ALT) in patients with T2DM [21]. Treating such patients with pioglitazone and alogliptin improves glycated hemoglobin A1c (HbA1c) values and decreases serum BCAA levels

[21]. These findings suggest that BCAA can lead to impaired glucose metabolism in obese individuals and/or patients with T2DM. That is, the effects of BCAA on glucose metabolism including insulin sensitivity have not been established in humans. Both T2DM and obesity might interfere with the effects of BCAA on insulin resistance. We therefore aimed to determine the physiological effects of BCAA on glucose metabolism among healthy community dwellers to clarify the controversy surrounding the effects of BCAA.

The molar ratios of serum BCAA to aromatic amino acids (AAA; phenylalanine, tyrosine, and tryptophan) are commonly reduced in patients with liver cirrhosis [1]. It has been reported that BCAA catabolism is enhanced in cirrhotic rats and in patients with liver cirrhosis [22]. Therefore, the decrease in the ratio of BCAA to AAA may be used as a good index of liver impairment and derangements of amino acid metabolism in patients with liver cirrhosis [22]. The molar ratio of BCAAs to tyrosine (Tyr) (BTR) is rapidly determined enzymatically [23] and can be substituted for the ratio of BCAA to AAA [24]. Tyr is an aromatic amino acid (AAA) that is essential for the thyroid hormones thyroxin and triiodothyronine, melanin pigment and neurotransmitters including dopamine, norepinephrine and epinephrine. Metabolites of amino acids including Tyr can be associated with insulin resistance [25]. However, serum levels and the metabolic effects of Tyr have not been directly evaluated in humans. We also aimed to determine the effects of Tyr on metabolic parameters, including obesity, muscle volume and glucose metabolism among community dwellers without diabetes.

Participants and methods

One hundred and ten residents (male, n = 46; female, n = 64; median age, 59 y) of Mie Town, Japan, who had never been diagnosed with diabetes, attended health checks at a medical center that included diabetes testing during 2014. All residents underwent a 75-g oral glucose tolerance test (OGTT) after an overnight fast of at least 12 hours. Nine of them who had diabetes, as defined according to the American Diabetes Association (ADA) [26], with FBG ≥ 126 mg/dL (7.0 mmol/L) and/or a blood glucose (BG) concentration ≥ 200 mg/dL (11.1 mmol/L) at two hours after a 75 g OGTT and/or HbA1c ≥ 6.5%, were excluded from the study. Eleven, three and nine had impaired fasting glucose (IFG; FBG levels 100 – 125 mg/dL, 5.6 – 6.9 mmol/L), impaired glucose tolerance (IGT; BG values at two hours after 75 g OGTT, 140 – 199 mg/dL, 7.8 – 11.1 mmol/L) and both, respectively. IFG and IGT are collectively referred to as pre-diabetes and thus individuals with IFG or IGT were excluded from the analysis. We defined IFG and IGT according to the ADA [26]. Thereafter, data from 78 residents were analyzed (male, n = 32; female, n = 46; median age, 52 y). Two, one and two of the included participants were under medication for hypertension, dyslipidemia and both, respectively.

We evaluated nutritional and metabolic status by measuring body weight, body mass index (BMI), %FAT, WHR, systolic blood pressure (SBP), diastolic blood pressure (DBP) and serum levels of aspartate aminotransferase (AST), ALT, γ-glutamyl transpeptidase (γ-GTP), total cholesterol (T-Chol), HDL-C, TG and creatinine (Cr),

blood urea nitrogen (BUN), HbA1c, FBG and fasting immunoreactive insulin (IRI). Values for BCAA, Tyr and BTR were determined using Daiyacolor-BTR kits® (Toyobo, Osaka, Japan) according to the manufacturer's protocol. In brief, leucine dehydrogenase converts BCAA to branched chain α-keto acids with the formation of NADH from NAD^+. The NADH is coupled with a redox system and the absorbance of the generated formazan is measured at 600 nm. Tyrosine is decarboxylated to tyramine by carboxylase. Tyramine is then oxidized by tyramine oxidase to generate 4-hydroxyphenyl acetaldehyde and hydrogen peroxide. Peroxidase acts on hydrogen peroxide, 4-amino antipyrine and N-Ethyl-N-(2-hydroxy-3-sulfopropyl) to produce a quinone dye, the absorbance of which is measured at 546 nm [23].

Muscle volume and %Fat were measured by multiple-frequency bioimpedance technology using an MC-190® body composition analyzer (TANITA Corporation, Tokyo, Japan). Blood pressure was measured in the right upper arm using an HEM-907® digital manometer (Omron Healthcare Co. Ltd., Kyoto Japan) after resting for > 10 minutes. Individuals with BMI ≤ 18.5, 25.0 – 30.0, and > 30.0 kg/m^2 were defined as underweight (BMI ≤ 18.5 kg/m^2), overweight and obese (BMI > 30.0 kg/m^2), respectively. None of the participants in the present study were obese according to these standards. The BMI of 13 participants was > 25 kg/m^2, which the Japan Society for the Study of Obesity defines as obese [27], and two men and 11 women were underweight (BMI ≤ 18.5 kg/m^2). Regardless, all were physically well and free of significant health problems.

We calculated HOMA-IR as [28]: fasting serum insulin level (IRI_0; μU/mL) ×

FBG (mg/dL)]/405. The homeostasis model assessment-beta cell function (HOMA-β) was calculated as IRI_0 (μU/mL) × 360 / FBG (mg/dL) − 63 [27].

Data are expressed as medians with ranges. Before analyzing correlations among parameters, we assessed whether the continuous variables were normally distributed. The Shapiro-Wilk test revealed the non-normal distribution of muscle volume, WHR, AST, ALT, γ-GTP, T-Chol, TG, CRE, BUN, FBG, HbA1c, Fasting IRI, HOMA-IR, and HOMA-β. Correlation coefficients between variables in univariate analyses were calculated using Spearman's rho (ρ) corrected for ties. Abnormally distributed values were log-transformed and were analyzed using Pearson's correlation coefficient. Multivariate analysis comprised the stepwise selection of multiple linear regression models. Log transformations can transform variables in the regression model to improve the linearity. They can be valuable both for helping to meet the assumptions of inferential statistics and for making the data more interpretable. Values from two groups were compared using the Mann-Whitney U test. All tests were two-tailed, and values with $p < 0.05$ were considered statistically significant. Data were statistically analyzed using JMP 10.0.2 software (SAS Institute Japan Co. Ltd., Tokyo, Japan).

The Ethics Committee at Mie University approved the study (Approval No. 1415), for which all included individuals provided written informed consent to participate.

Results

1. Basic and physical characteristics

Table 1 summarizes the basic and physical characteristics of the participants. Age did not significantly differ between the men and the women. Muscle volume, BMI, WHR and DBP were significantly higher, whereas %FAT was significantly lower among the men.

2. Serum levels of BCAA and Tyr in men and women

Serum levels of BCAA and Tyr as well as the BTR were significantly higher in men than in women (Figure 1A, B and C).

3. Univariate analysis of correlations among serum levels of BCAA and Tyr and physical and laboratory parameters

Table 2A shows correlations among serum levels of BCAA and Tyr with physical and laboratory parameters determined using Spearman's test. Circulating levels of BCAA and Tyr significantly correlated with various physical and laboratory parameters for obesity, hypertension, liver and renal function, and lipid and glucose metabolism. Serum BCAA levels correlated positively with BMI, muscle volume, WHR, SBP, DBP, AST, ALT, γ-GTP, TG, Cr, BUN, FBG, fasting IRI and HOMA-IR, and negatively

with HDL-C. Serum Tyr levels correlated positively with BMI, muscle volume, SBP, DBP, AST, ALT, γ-GTP, Cr, FBG, fasting IRI, and HOMA-IR, and negatively with HDL-C.

In men, BCAA correlated positively with BMI, %FAT, TG, Cr, FBG, fasting IRI, and HOMA-IR, and negatively with HDL-C, whereas Tyr correlated positively with BMI, %FAT, SBP, TG, fasting IRI, and HOMA-IR, and negatively with HDL-C. In women, BCAA correlated positively with ALT, γ-GTP, BUN, FBG, fasting IRI, and HOMA-IR, whereas Tyr correlated positively with SBP, FBG, fasting IRI, and HOMA-IR.

Table 2B summarizes correlations among serum BCAA and Tyr levels, and physical and laboratory parameters using log-transformed abnormally distributed values and Pearson's correlation coefficient. The results were consistent with those of the Spearman tests. Among the many correlations among BCAA, Tyr and parameters for obesity, hypertension, liver and renal function as well as lipid and glucose metabolism, HOMA-IR associated positively with serum levels of BCAA and Tyr in both univariate analyses. The FBG values expressed as mmol/L were also calculated as [mg/dL]/18. Correlations (ρ and p) between BCAA and Tyr were the same regardless of whether FBG was expressed as mmol/L or mg/dL.

Serum BCAA and Tyr levels significantly correlated (all, $r = 0.5620$, $p < 0.0001$, 95%CI = 0.3880 – 0.6973; male, $r = 0.5108$, $p < 0.0028$, 95%CI = 0.1972 – 0.7295;

female, r = 0.4756, p = 0.0008, 95%CI = 0.2150 – 0.6730).

4. Multivariate analysis: correlations among serum BCAA and Tyr, and physical and laboratory parameters

We investigated correlations among serum BCAA (Fig. 2A, B and C) and Tyr (Fig. 2D, E and F), as well as physical and laboratory parameters using stepwise multiple regression analysis. Correlation coefficients were significant between predicted and measured values of BCAA (R^2 = 0.51 – 0.89, p < 0.0001 – 0.0041) and Tyr (R^2 = 0.36 – 0.76, p = 0.0003 – 0.0005).

Stepwise multivariate regression models found that serum levels of BCAA correlated positively with muscle volume, BUN, AST, FBG, fasting IRI, and HOMA-β, and negatively with HOMA-IR and HDL-C (Table 3, upper panel), and that those of Tyr correlated positively with fasting IRI and AST (Table 3, lower panel). In men, BCAA correlated positively with HOMA-β, FBG, γ-GTP, BUN, and muscle volume, and negatively with HOMA-IR and DBP (Table 4, upper panel), whereas Tyr correlated positively with BMI, SBP, AST, and HbA1c and negatively with ALT and muscle volume (Table 4, lower). In women, BCAA correlated positively with BUN, AST, fasting IRI, and SBP, and negatively with DBP (Table 5, upper), but not at all with HOMA-IR, and Tyr correlated positively with BUN, AST, HDL-C, fasting IRI, Cr, and muscle volume, and negatively with γ-GTP, ALT, and HOMA-β (Table 5, lower panel).

Discussion

Stepwise multivariate analysis revealed that circulating BCAA levels correlated negatively with HOMA-IR, supporting the notion that BCAA improves insulin sensitivity in humans. The present study excluded individuals with IFG, IGT and diabetes based on the results of the 75-g OGTT and no-one had a BMI > 30 kg/m^2. Only 13 (12.9 %) of the 101 study participants were obese according to the guidelines of the Japan Society for the Study of Obesity (> 25 kg/m^2). The exclusion of individuals with pre-diabetes or diabetes and the low rate of obesity might reveal a favorable effect of BCAA on insulin sensitivity.

The positive correlations between BCAA and insulin sensitivity were consistent with the results in animal models and cultured cells. In turn, BCAA also correlated positively with FBG and fasting IRI. These results seemed to contradict previous finding in animal models [5,9,11], but were in line with human studies in which elevated BCAA levels were associated with T2DM, non-alcoholic fatty liver disease (NAFLD), obesity, the intima-media thickness of the carotid artery, and the risk of coronary artery disease [3] [29-32]. Model $BCAT^{-/-}$ mice with elevated serum BCAA levels are resistant to developing diabetes [5], but the simultaneous intake of a high-fat diet and BCAA can also cause insulin resistance [20]. The effects of BCAA on glucose and lipid metabolism appeared to be both favorable and adverse, and thus cross-talk among them was likely. We found that the correlation between BCAA and HOMA-IR became weaker when analyzed together with pre-diabetes (regression coefficient/t/p/

95%CI = -7712.88/ -2.53/ 0.0133/ -13777.98 – -1647.80). The ability of BCAA to increase FBG and fasting IRI might modulate insulin resistance under the pathophysiological conditions associated with T2DM and/or obesity, which might have caused the conflicting findings regarding insulin resistance among healthy or obese individuals, and/or patients with diabetes.

The present findings of the univariate and multivariate analyses were notably paradoxical. After adjustment for various anthropometric parameters such as BMI, %FAT, WHR and blood pressure, increased serum levels of BCAA were associated with insulin sensitivity in individuals without diabetes, suggesting that these parameters might be confounding factors for HOMA-IR. The results of the univariate analysis should be interpreted with caution.

Gender bias should be taken into consideration when analyzing metabolic parameters [33] because the development of diet-induced IR and obesity differs according to gender [34]. For example, the sex-related hormone, estrogen, regulates the activity and expression of the key enzymes that are involved in glucose transport, glycolysis, the citric acid cycle, fatty acid oxidation, energy balance, and body composition [35]. Physical characteristics significantly differed between men and women in the present study; for example, men had a significantly higher BMI, muscle volume, higher DBP and lower %FAT. Separate analyses of data from men and women revealed higher muscle volume and serum levels of both BCAA and Tyr in men. Multivariate analysis uncovered significant correlations between BCAA and muscle

volume and between BCAA and HOMA-IR in men, but not in women. In addition, serum BCAA were significantly associated with reduced TG and DBP only in men. Sex-related hormones can also influence the correlation of BCAA with various metabolic parameters through modulating glucose and lipid metabolism and body composition.

Stepwise multivariate regression models revealed that serum levels of BCAA correlated positively with muscle volume. Shimomura Y et al. reported that BCAA supplementation before exercise can reduce delayed-onset myalgia [36], suggesting that BCAA can repress exercise-induced muscle breakdown. In addition, leucine-rich BCAA can rescue an impaired mTOR1-signaling pathway and repress autophagic proteolysis in the skeletal muscle of patients with sarcopenia [37]. These findings suggest that BCAA, especially leucine, can promote protein synthesis and reduce proteolysis in skeletal muscle. Such repressed muscle breakdown and enhanced muscle protein synthesis can contribute to increased muscle volume. In addition, BCAA promote glucose uptake by enhancing GLUT1 and GLUT4 expression in the plasma membrane [9], [10]. Furthermore, BCAA facilitate free fatty acid oxidation by up-regulating PPARα and subsequent UCP3 expression in skeletal muscle [12], [13]. Increased muscle volume can help BCAA to promote glucose uptake and free fatty acid oxidation. A recent study showed that sarcopenia was highly prevalent in patients with liver cirrhosis, and was significantly associated with their outcome [38]. The positive associations among BCAA, muscle volume and insulin sensitivity can be in line with

their results that BCAA supplementation improved the survival of sarcopenic patients with liver cirrhosis.

Serum Tyr levels correlated positively with fasting IRI but not with HOMA-IR, suggesting that Tyr does not influence insulin sensitivity and could increase the incidence of T2DM. Metabolomics profiling of three distinct cohorts uncovered significantly higher levels of BCAA-related amino acids including Tyr among individuals with metabolic diseases [31]. Direct correlations between serum Tyr and metabolic parameters were not investigated in that study, but the findings of that and the present study are nevertheless partly in line.

Serum BCAA and Tyr levels significantly correlated in the present study. Serum BCAA and Tyr levels were significantly associated with BUN and/or AST, but not with Cr and ALT (Table 3). These results suggest that protein catabolism and skeletal muscle degradation affect serum levels of BUN and AST rather than renal and hepatic dysfunction. We did not neglect these parameters when constructing reliable multiple regression models for predicting BCAA and Tyr levels. Muscle breakdown can be taken into consideration as an elevating factor for serum BCAA and Tyr levels.

To determine which BCAA is involved in increasing muscle volume and in reducing insulin resistance is important. However, BTR cannot determine separate amino acid levels, which was a limitation of the present study.

In conclusion, BCAA are positively associated with insulin sensitivity in individuals without diabetes. Muscle volume is an important factor associated with

circulating levels of BCAA. In turn, BCAA correlates with increased values of FBG and fasting IRI, which might interfere with insulin resistance under the pathophysiological conditions associate with T2DM and/or obesity. Tyr is positively associated with fasting IRI, but does not affect insulin sensitivity. The optimal intake of BCAA probably confers favorable effects on insulin sensitivity among healthy individuals, but a long-term prospective follow up study is needed to confirm this notion.

Conflicts of interest

None of the authors have any potential conflicts of interest that could inappropriately influence this work.

Statement of authorship

This original article is not currently under consideration for publication or in press elsewhere.

The authors' contributions are as follows:

TH: study concept and design, data acquisition, sample analyses, statistical analysis, data interpretation and drafting the article.

YK, KT, MI: study concept and design, data acquisition, statistical analysis, data analysis, data interpretation and critically revising the article for important intellectual content.

HH: study concept and design, data interpretation and critically revising the article for important intellectual content.

OT, YT: study concept, design and coordination, data interpretation and critically revising the article for important intellectual content.

YS: study concept, design and coordination, data acquisition, data interpretation, and critically revising the article for important intellectual content.

All authors have read and approved this version for submission.

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Figure legends

Figure 1. Comparison of serum BCAA, tyrosine levels and BTR between male (n = 41) and female (n = 60) community dwellers.

Box plots show BCAA (A) Tyr (B) BTR (C) in males and females. BCAA, branched chain amino acids; BTR, ratio of branched chain amino acids to tyrosine; Tyr, tyrosine.

Figure 2. Predictors determined by stepwise multiple regression analysis in all (n = 101) participants and in males (n = 41) and females (n = 60).

Relationships between predicted and measured serum BCAA levels in all participants (A), in men (B) and women (C). Relationships between predicted and measured Tyr levels in all participants (D), in men (E) and in women (F). BCAA, branched chain amino acids; Tyr, tyrosine.