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

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ABSTRACT

Objectives: 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. The aim of this study was to clarify the controversy surrounding the effects of BCAA by investigating the physiological effects of BCAA and Tyr on glucose metabolism in healthy community dwellers.

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 prediabetes, diabetes, or a body mass index >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% confidence interval, 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 to −2877.1). Tyr positively correlated with fasting IRI (26/2.77/0.0072/7.3–44.7).

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.

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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 BCAAs [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 (IR) induced by BCAA have been identified in insulin target organs such as adipose tissue, skeletal muscle, and the liver. Briefly, BCAAs increase glucose uptake through the phosphorylation of Akt and mammalian target of rapamycin (mTOR) in adipose tissue [8]. BCAAs 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]. BCAAs activate the liver X receptor-α (LXR)/sterol regulatory element binding protein-1c (SREBP1-c) pathway to upregulate liver-type glucokinase and GLUT2 in the liver. The activation of LXR/SREBP-1 reduces gluconeogenesis through down-regulating glucose-6-phosphatase expression [11]. Additionally, BCAAs 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 IR 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 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 fat mass (%fat) and can improve glucose metabolism [16–18].

However, the effects of BCAAs on IR in obese individuals, patients with diabetes, or both 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) showed that amelioration of the diabetic state induced by either insulin or antidiabetic drugs reduces serum BCAA levels [19]. Serum levels of BCAA-related metabolites positively correlate with IR in obese humans [20]. Moreover, serum levels of BCAA-related metabolites positively correlate with the waist-to-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 A_{1c} (HbA_{1c}) values and decreases serum BCAA levels [21]. These findings suggest that BCAA can lead to impaired glucose metabolism in obese individuals, patients with T2DM, or both. 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 IR. Therefore, the aim of the present study was 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 [Tyr], 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 Tyr (BTR) is rapidly determined enzymatically [23] and can be substituted for the ratio of BCAA to AAA [24]. Tyr is an 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 IR [25]. However, serum levels and the metabolic effects of Tyr have not been directly evaluated in humans. A second aim of this study was to determine the effects of Tyr on metabolic parameters, including obesity, muscle volume, and glucose metabolism in community dwellers without diabetes.

Materials and methods

In 2014, 110 residents (men, n = 46; women, 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. All residents underwent a 75-g oral glucose tolerance test (OGTT) after an overnight fast of ≥12 h. Nine individuals who had diabetes, as defined according to the American Diabetes Association (ADA) [26], with FBG ≥126 mg/dL (7 mmol/L) and/or a blood glucose (BG) concentration ≥200 mg/dL (11.1 mmol/L) at 2 h after a 75 g OGTT and/or $HbA_{1c} \geq 6.5\%$, 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 2 h after 75 g OGTT, 140–199 mg/dL, 7.8–11.1 mmol/L) or both, respectively. IFG and IGT are collectively referred to as prediabetes 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 (n = 32 men; n = 46 women; 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 (TC), high-density lipoprotein cholesterol (HDL-C), triacylglycerol (TG) and creatinine (Cr), blood urea nitrogen (BUN), HbA_{1c}, 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 nicotinamide adenine dinucleotide (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 min. Individuals with BMI ≤18.5, 25 to 30, and >30 kg/m^2 were defined as underweight (BMI ≤18.5 kg/m^2), overweight, and obese (BMI >30 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 2 men and 11 women were underweight (BMI ≤18.5 kg/m^2). Regardless, all were physically well and free from significant health problems.

We calculated HOMA-IR as [28] fasting serum insulin level [(IRI_0; μU/mL) × FBG (mg/dL)]/405. HOMA-β cell function (HOMA-β) was calculated as IRI_0 (μU/mL) × 360/FBG (mg/dL) − 63 [27].