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Branched-chain amino acid catabolism rather than amino acids plasma concentrations is associated with diet-induced changes in insulin resistance in overweight to obese individuals

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Abstract *Background & aims:* 3-Hydroxyisobutyrate (3-HIB), a catabolic intermediate of the BCAA valine, which stimulates muscle fatty acid uptake, has been implicated in the pathogenesis of insulin resistance. We tested the hypothesis that circulating 3-HIB herald insulin resistance and that metabolic improvement with weight loss are related to changes in BCAAs and 3-HIB.

Methods and results: We analyzed plasma and urine in 109 overweight to obese individuals before and after six months on hypocaloric diets reduced in either carbohydrates or fat. We calculated the homeostasis model assessment index (HOMA-IR) and whole body insulin sensitivity from oral glucose tolerance tests and measured intramyocellular fat by magnetic resonance spectroscopy. BCAAs and 3-HIB plasma concentrations were inversely related to insulin sensitivity but not to intramyocellular fat content at baseline. With 7.4 ± 4.5% weight loss mean BCAA and 3-HIB plasma concentrations did not change, irrespective of dietary macronutrient content. Individual changes in 3-HIB with 6-month diet but not BCAAs were correlated to the change in whole body insulin sensitivity and HOMA-IR independently of BMI changes.

Conclusions: 3-HIB relates to insulin sensitivity but is not associated with intramyocellular fat content in overweight to obese individuals. Moreover, changes in 3-HIB rather than changes in BCAAs are associated with metabolic improvements with weight loss.

Registration number for clinical trials: ClinicalTrials.gov Identifier: NCT00956566.

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Introduction

Mechanistic and epidemiological studies implicate branched-chain amino acids (BCAAs) in the pathogenesis of insulin resistance. Indeed, circulating BCAA concentrations predict future diabetes mellitus independently of age, sex, body-mass index, fasting glucose, and family history [1]. Moreover, in some studies BCAA supplementation in high fat-fed animals [2] or BCAA infusion in human subjects during euglycemic-hyperinsulinemic clamp tests [3] promoted insulin resistance. In other studies, however, BCAA supplementation did not changed or improved insulin resistance in patients with chronic liver disease [4] or with type 2 diabetes [5]. Impaired BCAA metabolism, β-cell toxicity, and disrupted insulin signaling through phosphorylation of mammalian target-of-rapamycin (mTOR), and insulin receptor substrate-1 (IRS1) serine residues in skeletal muscle may be involved [2,6–8]. Yet, insulin resistance itself could also augment circulating BCAAs [8]. Recently, the valine catabolite 3-hydroxyisobutyrate (3-HIB) has been identified as paracrine regulator of trans-endothelial fatty acid flux. In mice, 3-HIB stimulated muscular fatty acid uptake, thus, promoting muscular lipid accumulation, and insulin resistance [9]. A recent study in older obese women also suggests a potential role of 3-HIB on insulin-stimulated glucose disposal [10]. In addition, β-aminoisobutyric acid (BAIBA), a downstream product of 3-HIB increases hepatic fatty acid oxidation through PPARα and improves glucose tolerance in mice [11]. We performed metabolic profiling to test the hypothesis that increased circulating 3-HIB concentrations are related to insulin resistance and intramyocellular fat, and that improvements in glucose metabolism with dietary weight loss are associated with reductions in plasma levels of BCAAs and 3-HIB in overweight to obese individuals.

Methods

Patients

We recruited 170 overweight and obese otherwise healthy subjects (BMI $\geq$ 27 kg/m^2) on no medications (135 women and 35 men). All subjects completed a comprehensive medical evaluation including a dietary record for seven consecutive days before inclusion. Subjects reporting more than 2 h of physical activity per week assessed with a physical activity record over seven consecutive days were excluded. We also excluded subjects consuming >20 g/day of alcohol, with type 2 diabetes, acute or chronic infections, any diseases requiring treatment, and pregnant or nursing women. This study was carried out in accordance with the Declaration of Helsinki. The institutional review board of the Charité University Medical School approved the study and written informed consent was obtained before entry.

Study design

This was a prospective, randomized study conducted in an academic clinical research center between March 2007 and June 2010. The data were generated as part of the B-SMART study (ClinicalTrials.gov Identifier: NCT00956566), which compared weight loss and associated metabolic and cardiovascular markers with a hypocaloric diet [12]. Subjects underwent anthropometric, nutritional, cardiovascular and metabolic assessments before and after 6 months on a hypocaloric diet with either reduced carbohydrate or reduced fat content.

After randomization, subjects provided a baseline 7-day food protocol, which was analyzed for macro- and micronutrient content including BCAAs using Optidiet analysis software (V3.1.0.004, GOE, Linden, Germany). Energy content was reduced by 30% of the baseline energy intake to a minimum of 1200 kcal/d. In addition, nutrition counseling aimed at achieving a daily macronutrient content $\leq$90 g carbohydrates and a minimum of 30% fat in the reduced carbohydrate group, and a fat content of $\leq$20% of total energy intake and the remaining energy content provided by carbohydrates in the reduced fat group with 0.8 g protein intake per kg body weight for both groups. All participants attended either reduced carbohydrate or reduced fat weekly group sessions run by nutritionists throughout the 6-month weight reduction program. In addition, individual nutritional counseling by a nutritionist including analysis of a 7-day food protocol took place every 2 months during the 6-month intervention, to address individual questions, and to monitor adherence to the prescribed diet. Subjects were advised to continue their current physical activity level throughout the study.

Skeletal and myocardial fat quantification

Intramuscular lipids were quantified by ^{1}H single voxel spectroscopy of the tibialis anterior. In brief, a spin-echo single-voxel spectroscopy sequence (TR 3000, TE 30, voxel size 11 $\times$ 11 $\times$ 20 mm, number of averages 64) with frequency-selective water suppression was applied. After baseline and constant phase correction, a standard line-fitting procedure was performed for quantification. IMCL values were calculated as the area under the curve of the IMCL methylene line normalized to the creatine–CH3 signal and corrected for differences in T1 and T2, resulting in a dimensionless value. To quantify myocardial triglyceride content a 6–8 $\times$ 20 $\times$ 25 mm^3 voxel was positioned in the interventricular septum. We applied a cardiac and respiratory gated ^{1}H single voxel spectroscopy (SVS) sequence (Spin-Echo: TR according to respiratory cycle (>5 s), TE 30 ms) to acquire spectra at end-systole and in end-expiration. Lipid signals were taken from water-suppressed spectra (96 averages and water signals from unsuppressed spectra (4 averages)). Areas under water and lipid peaks were quantified using standard line fitting procedures (Siemens Syngo Spectroscopy) and myocardial triglyceride content was expressed as fat-to-water ratio (%) For all measurements, a clinical 1.5 T MR scanner (Sonata and Avanto, Siemens Medical Solutions AG, Erlangen, Germany) was used.