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Branched-chain and aromatic amino acids, insulin resistance and liver specific ectopic fat storage in overweight to obese subjects

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Abstract *Background & aims:* Amino acids may interfere with insulin action, particularly in obese individuals. We hypothesized that increased circulating branched-chain and aromatic amino acids herald insulin resistance and ectopic fat storage, particularly hepatic fat accumulation.
Methods and results: We measured fasting branched-chain and aromatic amino acids (tryptophan, tyrosine, and phenylalanine) by mass spectrometry in 111 overweight to obese subjects. We applied abdominal magnetic resonance imaging and spectroscopy to assess adipose tissue distribution and ectopic fat storage, respectively. Plasma branched-chain amino acids concentrations were related to insulin sensitivity and intrahepatic fat independent from adiposity, age and gender, but not to abdominal adipose tissue or intramyocellular fat.
Conclusions: In weight stable overweight and obese individuals, branched-chain amino acid concentrations are specifically associated with hepatic fat storage and insulin resistance.

Introduction

In overweight and obese persons, the distribution of excess fat determines metabolic disease risk [1]. Intrahepatic lipid accumulation (IHL) is particularly important in this regard [2]. Moreover, excessive hepatic fat accumulation predisposes to nonalcoholic steatohepatitis, which may progress to cirrhosis and hepatic cancer [3]. Identification of early risk markers may beget new targets for more specific prevention of obesity-associated metabolic and liver disease. A previous epidemiological study analyzed plasma metabolite profiles among normoglycemic individuals in three independent cohorts with more than 12-years follow up [4]. The branched-chain amino acids (BCAAs) isoleucine, leucine, and valine and the aromatic amino acids (AAs), tyrosine and phenylalanine predicted future diabetes mellitus independent of age, sex, body mass index, fasting glucose, and family history. Moreover, BCAAs promote insulin resistance in animals [5] and in human subjects [6], this metabolic trait is closely linked with hepatic steatosis [2,7]. In obesity, key BCAA catabolic enzymes appear to be down-regulated in a tissue-specific manner with reductions in liver and adipose tissue but not in skeletal muscle [8]. Therefore, we employed metabolite profiling to test the hypothesis that

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increased circulating BCAA and aromatic AA concentrations are markers for insulin resistance and organ-specific fat storage in abdominal subcutaneous and visceral adipose tissue, liver, and skeletal muscle.

Methods

Subjects

This is a cross-sectional analysis of baseline data from 111 overweight/obese otherwise healthy volunteers who subsequently participated in a 6-month dietary intervention (ClinicalTrials.gov Identifier: NCT00956566) [9]. All subjects completed a comprehensive medical evaluation including dietary records for 7 consecutive days before study participation. Volunteers reported <2 h of physical activity per week and were not taking medications that could affect metabolism or liver function. Subjects consuming >20 g/day of alcohol, with diagnosis of type 2 diabetes, with acute or chronic infections, with any disease requiring treatment, or with known drug abuse were excluded. The study was carried out in accordance with the Declaration of Helsinki. Our institutional review board approved the study, and written informed consent was obtained before entry.

Anthropometric and metabolic evaluation

After an overnight fast, we determined weight, waist circumference and height in a standardized fashion. During an oral glucose load (OGTT, 75 g glucose/300 ml), we obtained blood samples at baseline and 15, 30, 45, 60, 90 and 120 min after glucose ingestion to measure glucose and insulin. After another overnight fast, subjects also underwent imaging studies. Subjects also provided a baseline 7-day food protocol, which was analyzed for macro- and micronutrient content using Optidiet (V3.1.0.004, GOE, Linden, Germany), a professional analysis software, based on nutritional content of food as provided by the German National Food Key.

Abdominal and ectopic fat quantification

We applied a T1-weighted, water suppressed gradient echo technique (TR 80 ms, TE 6.11 ms, 512 × 512 matrix, FOV 500 × 500 mm, slice thickness 10 mm, interslice gap 10 mm) to image abdominal fat during repetitive breathholds as recently published [10]. We quantified visceral and subcutaneous adipose tissue by semi-automated image segmentation software employing a contour-following algorithm (Vitom, Germany). In addition, we measured intrahepatic lipid content expressed as fat/(fat + water) (%) by respiratory gated ^{1}H SVS (Spin-Echo: TR according to respiratory cycle (>5 s), TE 30 ms) as described previously [9]. Furthermore, intramyocellular lipids were quantified by ^{1}H single voxel spectroscopy of the tibialis anterior muscle. All measurements were performed in a clinical 1.5 T MR scanner (Sonata and Avanto, Siemens Medical Solution AG, Erlangen, Germany).

Biochemical measurements and calculations

Glucose (mmol/l), insulin (μU/ml), and lipoproteins were determined by standard methods in a certified clinical chemistry laboratory. Insulin resistance was estimated by homeostasis model assessment index (HOMA-IR) from fasting insulin and glucose by (insulin [μU/ml] × glucose [mmol/l])/22.5). Whole body insulin sensitivity was calculated by the composite insulin-sensitivity index (C-ISI) [11]. C-ISI = $10{,}000/\sqrt{[(FPG \times FPI) \times (G \times I)]}$, where FPG and FPI are fasting plasma glucose (mg/dl) and fasting plasma insulin ([μU/ml), respectively, and G (mg/dl) and I ([μU/ml) are the mean glucose and mean insulin concentration during the 2-h OGTT. The hepatic insulin resistance index was calculated as the product of the area under the curves for glucose and insulin during the first 30 min of the OGTT. The approach has been validated in non-diabetic subjects against euglycemic insulin clamp testing in combination with tritiated glucose [12].

Metabolic profiling

The BCAAs isoleucine, leucine and valine, as well as the aromatic AAs tyrosine, phenylalanine, and tryptophan were measured from venous blood samples collected after an overnight fast. Metabolite profiling of all plasma samples was performed by metanomics GmbH (Berlin, Germany). For relative quantification of metabolites two types of mass spectrometry analyses, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), were used for MxP® Broad Profiling as described elsewhere [13]. Fractionation and derivatization of plasma samples (60 μl) and detection technologies have been previously described [13–15]. Briefly, proteins were removed from plasma samples by precipitation. Subsequently polar and non-polar fractions were separated by adding water and a mixture of ethanol and dichloromethane followed by centrifugation. For GC-MS analysis, the non-polar fraction was treated with methanol under acidic conditions to yield the fatty acid methyl esters derived from both free fatty acids and hydrolyzed complex lipids. The non-polar and polar fractions were further derivatized with O-methyl-hydroxylamine hydrochloride and pyridine to convert oxo-groups to O-methyl-oximes and subsequently with a silylating agent before analysis [14]. For LC-MS/MS analysis, both fractions were reconstituted in appropriate solvent mixtures. High-performance liquid chromatography (HPLC) was performed by gradient elution on reversed phase separation columns. Mass spectrometric detection technology was applied which allows target and high-sensitivity MRM (multiple reaction monitoring) profiling in parallel to a full scan analysis [16]. Metabolite data normalization - Metabolite profiling generated semi-quantitative measurements of metabolite concentrations, that is, the concentration of a metabolite in each study sample relative to the concentration of the metabolite in a reference sample. All six amino acids were detected with GC-polar. Following comprehensive analytical validation