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Clinical Science

Effect of Insulin Sensitizer Therapy on Amino Acids and Their Metabolites☆

Brian A. Irving[a,*], Rickey E. Carter[b], Mattias Soop[a], Audrey Weymiller[c], Husnain Syed[d], Helen Karakelides[a], Sumit Bhagra[a], Kevin R. Short[a], Laura Tatpati[e], Rocco Barazzoni[a], K. Sreekumaran Nair[a,**]

[a] Division of Endocrinology, Endocrinology Research Unit, Mayo Clinic College of Medicine, Rochester, MN
[b] Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN
[c] Department of Nursing, Mayo Clinic College of Medicine, Rochester, MN
[d] Department of Family Medicine, Mayo Clinic College of Medicine, Rochester, MN
[e] Division of Reproductive Endocrinology, Mayo Clinic College of Medicine, Rochester, MN

ARTICLE INFO

Article history:
Received 13 August 2014
Accepted 16 January 2015

Keywords:
Insulin resistance
Biomarkers
Metabolomics
Obesity
Diabetes

ABSTRACT

Aims. Prior studies have reported that elevated concentrations of several plasma amino acids (AA), particularly branched chain (BCAA) and aromatic AA predict the onset of type 2 diabetes. We sought to test the hypothesis that circulating BCAA, aromatic AA and related AA metabolites decline in response to the use of insulin sensitizing agents in overweight/obese adults with impaired fasting glucose or untreated diabetes.

Methods. We performed a secondary analysis of a randomized, double-blind, placebo, controlled study conducted in twenty five overweight/obese (BMI ~30 kg/m^2) adults with impaired fasting glucose or untreated diabetes. Participants were randomized to three months of pioglitazone (45 mg per day) plus metformin (1000 mg twice per day, N = 12 participants) or placebo (N = 13). We measured insulin sensitivity by the euglycemic-hyperinsulinemic clamp and fasting concentrations of AA and AA metabolites using ultra-pressure liquid chromatography tandem mass spectrometry before and after the three-month intervention.

Results. Insulin sensitizer therapy that significantly enhanced insulin sensitivity reduced 9 out of 33 AA and AA metabolites measured compared to placebo treatment. Moreover, insulin sensitizer therapy significantly reduced three functionally clustered AA and metabolite pairs: i) phenylalanine/tyrosine, ii) citrulline/arginine, and iii) lysine/α-aminoadipic acid.

Conclusions. Reductions in plasma concentrations of several AA and AA metabolites in response to three months of insulin sensitizer therapy support the concept that reduced insulin sensitivity alters AA and AA metabolites.

☆ Trial Registration: clinicaltrials.gov identifier: NCT00443755.

* Correspondence to: B.A. Irving, Geisinger Obesity Institute, 100 N. Academy Ave, Geisinger Health System, Danville, PA 17868. Tel.: +1 271 5555x55234.

** Correspondence to: K.S. Nair, Endocrinology Division, 200 First Street SW, Joseph 5–193, Mayo Clinic, Rochester, MN 55905. Tel.: +1 507 255 2415; fax: +1 507 255 4828.

E-mail addresses: bairving@geisinger.edu (B.A. Irving), nair@mayo.edu (K.S. Nair).

http://dx.doi.org/10.1016/j.metabol.2015.01.008
0026-0495/

1. Introduction

In people with insulin resistance plasma concentrations of branched chain amino acids (BCAA; leucine, isoleucine, and valine), aromatic amino acids (AAA; phenylalanine and tyrosine), and amino acid (AA) metabolites are elevated [1–4]. Moreover, BCAA were recently reported to be elevated in metabolically well compared to metabolically unwell adults, independent of obesity [5,6]. Importantly, elevations in BCAA and AAA occur approximately a decade prior to the development of type 2 diabetes (T2D), which has led to the proposal that these AA are useful predictors for future T2D [7]. *In vitro*, supraphysiological doses of AA impair insulin signaling at several steps critical for glucose uptake [8] and glycogen synthesis [9]. *In vivo*, the infusion of AA, especially BCAA, reduces insulin sensitivity [10–12]. BCAA deprivation in rodents also improves insulin sensitivity in insulin resistant mice [13]. On the other hand, the rapid increase in insulin sensitivity and improvement in glucose homeostasis following weight loss surgery coincides with reductions in circulating BCAA, AAA, and several AA metabolites [14–16]. In addition, three months of metformin monotherapy has been reported to reduce AAA in patients with T2D [17]. Therefore, monitoring changes in BCAA, AAA, and AA metabolites may provide insight into the early responses to T2D therapies [18]. The close association between elevations AA, especially BCAA and AAA, and insulin resistance has led to the hypothesis that elevations in BCAA and AAA cause insulin resistance [1]. However, another equally plausible hypothesis is that reductions in the activity of branched chain ketoacid dehydrogenase (BCKD) and tyrosine aminotransferase (TAT) in states of insulin resistance lead to increased tissue and circulating BCAA and AAA concentrations, respectively [19].

We proposed that pharmacologically improving insulin sensitivity would result in reductions in plasma concentrations of BCAA, AAA, or other AA metabolites in insulin-resistant adults. Insulin is a potent antiproteolytic hormone that results in hypoaminoacidemia when systemically administered [20–22] due to a concomitant reduction in skeletal muscle protein breakdown [23] and increased AA transport into the muscle [24]. Previous studies have also shown that insulin deprivation in c-peptide negative adults with type 1 diabetes increases protein turnover (i.e., synthesis and breakdown) [25], AA oxidation [25], and transamination rates of leucine [26], and is accompanied by large increases in circulating AA concentrations, especially BCAA [26]. Moreover, protein degradation in multiple tissues is increased in people with T2D compared to non-diabetic controls under hyperglycemic conditions [27]. As stated previously, reductions in BCKD activity, TAT activity, and amino acid oxidation in states of insulin resistance lead to increased tissue and circulating BCAA and AAA concentrations [19]. Reductions in the degradation of BCAA also could lead to reductions in branched chain α-ketoacids concentrations and the synthesis of monomethyl branched-chain fatty acids with adipose tissue and perhaps muscle [28]. Monomethyl branched-chain fatty acids have recently been demonstrated to positively correlate with insulin sensitivity [28]. Together these data support the premise that a reduction in insulin action contributes to elevations in tissue and circulating BCAA, AAA, and AA metabolite concentrations. Since it has been reported that BCAA, especially leucine, can inhibit insulin action [1] there may be a type of feed-forward relationship between insulin resistance and the concentration of circulating AA.

The current study was designed to determine whether chronically enhancing insulin sensitivity reduces plasma BCAA, AAA, and AA metabolites in insulin resistant adults. Specifically, we investigated the impact of three months of dual insulin sensitizer therapy (45 mg pioglitazone per day plus 1000 mg metformin twice per day) in overweight/obese adults who had impaired fasting glucose or untreated diabetes.

2. Methods

The Mayo Clinic's Intuitional Review Board approved the study protocol in accordance to the principles of the Declaration of Helsinki. All participants provided written and informed consent prior to participation.

2.1. Study Design and Participants

We previously reported the overall study design for the parent study [29]. The current report primarily examines the effect of three months of insulin sensitizer therapy on plasma concentrations of BCAA, AAA, and AA metabolites in overweight/obese adults with fasting hyperglycemia, defined as either impaired fasting glucose or untreated diabetes [29]. Briefly, 25 drug naïve, Northern European American participants with fasting blood glucose concentrations of 108–180 mg/dL were randomized to receive either 45 mg of pioglitazone per day plus 1 g of metformin twice per day (n = 12) or placebo (n = 13) for 12 weeks. We chose metformin based on its proven effect on hepatic insulin sensitivity and pioglitazone based on its effect on peripheral insulin sensitivity. Current use of hypoglycemic medications excluded participants from the present study.

Insulin sensitivity was measured using a hyperinsulinemic-euglycemic clamp as previously described [29]. In brief, participants received a continuous infusion of insulin (1.5 mU/kg-FFM/min) for 8 hours. Participants also received a continuous infusion of AA (5.4% NephrAmine, B. Braun Medical Inc.) to prevent insulin-induced hypoaminoacidemia. The AA analyses in the current report were performed on fasting blood samples that were acquired before any infusions began. Arterialized-venous blood was used to measure plasma glucose concentrations every 10 min using an automated glucose analyzer (GM9, Analox Instruments, London, UK). The glucose infusion rate (GIR) (40% dextrose) was adjusted to maintain euglycemia [~5.0 mmol/L (90 mg/dL)] during the 8 h insulin infusion. Insulin sensitivity was defined as the steady-state glucose infusion rate (GIR, μmol/kg-FFM/min) achieved during the last 2 h of the 8 h hyperinsulinemic-euglycemic clamp [30]. Fasting glucose and insulin concentrations were used to calculate the Quantitative Insulin Sensitivity Check Index (QUICKI = 1/log[glucose] + log[insulin]) [31]. Fasting plasma AA and AA metabolite concentrations were measured using ultra-pressure liquid chromatography tandem mass spectrometry (UPLC-MS/MS)