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Changes in glucose-elicited blood metabolite responses following weight loss and long term weight maintenance in obese individuals with impaired glucose tolerance

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ABSTRACT

Aims: Weight loss improves insulin sensitivity and glucose tolerance in obese subjects with impaired glucose tolerance (IGT), but the long term dynamic effects on blood metabolites other than glucose during an oral glucose tolerance test (OGTT), are largely unknown. Here, we studied changes in OGTT-elicited metabolite patterns in obese subjects during a diet-induced weight loss study.

Methods: Blood samples from 14 obese individuals with IGT were collected at 0, 30 and 120 min during a standard 75 g OGTT at baseline (BMI 44 ± 2 kg/m^2), after weight loss (BMI 36 ± 2 kg/m^2) and after weight maintenance (BMI 35 ± 2 kg/m^2). Serum metabolite levels were analyzed by gas chromatography/mass spectrometry and compared to a lean glucose tolerant group.

Results: Changes in the OGTT-elicited metabolite patterns occurred differentially during weight loss and weight maintenance. Enhanced suppression of aromatic amino acids were associated with decreased insulinogenic index observed after weight loss (tyrosine: $r = 0.72$, $p = 0.013$; phenylalanine: $r = 0.63$, $p = 0.039$). The OGTT-elicited suppression and/or lack of increase in levels of glutamate, glutamine, isoleucine, leucine, and the fatty acids laurate, oleate and palmitate, improved towards the lean profile after weight maintenance, paralleling an improvement in glucose tolerance. The greater heterogeneity in the response before and after weight loss in the obese, compared to lean subjects, was markedly reduced after weight maintenance.

Conclusions: Diet-induced weight loss followed by weight maintenance results in changes in metabolite profiles associated with either hepatic insulin sensitivity or peripheral glucose tolerance. Our results highlight the importance of evaluating the effects of weight loss and weight maintenance separately.

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1. Introduction

Weight loss achieved by a combination of restricted calorie intake, increased physical activity and behavioral support has been reported to reduce the incidence of impaired glucose tolerance (IGT) and the risk of developing type 2 diabetes (T2D) in obese subjects [1–4]. Whereas improvements of discrete blood metabolites and hormones with weight loss, in particular glucose and insulin, are well described, less is known about changes in the global blood metabolite profile. Investigations on circulating metabolite levels in both children [5,6] and adults [7–9] have highlighted several differences in fasting metabolite levels between obese and lean individuals. These differences have mainly been found in levels of amino acids (AAs) and lipid metabolism intermediates [5–9]. Recent studies on weight loss elicited changes in metabolite levels have revealed abnormal fasting levels of several metabolites in obese subjects to improve after both diet- and gastric bypass surgery [10–15]. Specifically, elevated levels of aromatic AAs (tyrosine and phenylalanine) and branched chain AAs (BCAAs; isoleucine, leucine and valine), which have been associated with insulin resistance and an increased risk of developing T2D, have been shown to decrease after weight loss in obese individuals [16–20]. Moreover, reduction in BCAA levels was associated with improved insulin sensitivity [11]. However, investigations on fasting steady-state metabolism do not generally yield information on dynamic changes elicited by *e.g.* a meal. As a complement, profiling of metabolites during an oral glucose tolerance test (OGTT) has been used to assess perturbations in glucose homeostasis in insulin resistant obese subjects [21–24]. So far, there has been no report investigating the effect of weight loss followed by weight maintenance on these dynamics.

Here we investigated changes in 2 h OGTT metabolite profiles after weight loss and after a subsequent period of weight maintenance in subjects who were obese and glucose-intolerant at baseline.

2. Methods

2.1. Study population and design

Fourteen obese individuals from the outpatient unit at the Department of Endocrinology at Skåne University Hospital, Malmö, Sweden, were enrolled in a weight loss study as previously described [25]. All participants gave their written informed consent, and the study was approved by the ethics committee at Lund University, Sweden. None of the subjects were diagnosed with T2D, cardiovascular disease, or taking any medications related to metabolic disease and all participants were of European descent. The weight loss program consisted of two parts: a three month (102 ± 28 days [mean ± SD]) weight loss regime supported by low-calorie diet (LCD; 800-1 200 kcal/day), followed by a five month weight maintenance phase (158 ± 35 days [mean ± SD]) including a group-based program lead by a dietitian (Fig. 1). Inclusion criteria were weight loss greater than 10%, followed by weight stability (±5%) during the maintenance phase. All 14 participants were weight stable at inclusion (body mass index [BMI] 43.7 ± 1.5 kg/m^2 [mean ± SEM]), lost weight during the weight loss phase (−17.4 ± 1.4% of baseline total body weight; resulting BMI 36.2 ± 1.7 kg/m^2), and were weight stable during the maintenance phase (−20.0 ± 2.5% compared to baseline; resulting BMI 34.9 ± 1.8 kg/m^2), as detailed in Table 1. Standard OGTTs (75 g glucose) were performed after an overnight fast at three occasions: at baseline, after weight loss, and after weight maintenance (Fig. 1A). Blood samples were collected at 0, 30 and 120 min during the OGTT.

2.2. Plasma glucose, hormones and lipids

Plasma glucose was measured using the Infinity Glucose Oxidase^{TM} Liquid Stable Reagent (Thermo Electron Corporation, Victoria, Australia) and insulin was measured with the Coat-A Count Insulin RIA (DPC, Los Angeles, CA). Total-, LDL- and HDL-cholesterol, and fasting plasma triglycerides were measured at the Department of Clinical Chemistry at Skåne University Hospital, Malmö, Sweden.

2.3. Targeted metabolite profiling

Metabolites were extracted and analyzed by gas chromatography/time-of-flight mass spectrometry (GC/TOF-MS) as previously described [23,26]. Data was collected using Leco ChromaTof 3.34 (Leco Corp., St. Joseph, MI), exported as NetCDF-files and processed by hierarchical multivariate curve resolution [27] in MATLAB R2012b (Mathworks, Natick, MA). Two samples were missing; one at baseline (30 min of OGTT) and another after weight loss (120 min of OGTT). In total, 124 samples were included in this study and analyzed in random order. Comparative data from a previous study in lean healthy individuals (BMI 22.4 ± 2.4 kg/m^2) were included for comparison of metabolite profiles and have been reported in detail previously [23,26]. Metabolite profiling in the lean group was performed on the same platform as the one used in the present study.

2.4. Calculations and statistical analysis

Metabolite, hormone and lipid data were combined into a single data set, normalized [28], double-centered [29], scaled to unit variance and analyzed in SIMCA 13 (Umetrics, Umeå, Sweden). Skewed parameters were log-transformed. Metabolites with levels differing between different OGTT time points at baseline, weight loss and weight maintenance were identified using orthogonal projections to latent structures-discriminant analysis (OPLS-DA, Supplementary Fig. 1A, B and C) [30]. Metabolites differing in levels between two time points in the OGTT were identified from the loadings of nine OPLS-DA models calculated between different time points (0 vs. 30 min, 30 vs. 120 min, 0 vs. 120 min) at a given study phase (at baseline, after weight loss, and after weight maintenance). Significant changes were estimated from jack-knifed confidence intervals [31]. All models were cross-validated [32]. Subsequently, metabolite profiles were created for each of the study phases from metabolite levels acquired at three time points (0, 30 and 120 min) during the OGTT.