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Effects of visceral adiposity on glycerol pathways in gluconeogenesis☆

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ABSTRACT

Objective. To determine the feasibility of using oral ^{13}C labeled glycerol to assess effects of visceral adiposity on gluconeogenic pathways in obese humans.

Research Design and Methods. Obese (BMI ≥30 kg/m^2) participants without type 2 diabetes underwent visceral adipose tissue (VAT) assessment and stratification by median VAT into high VAT-fasting (n = 3), low VAT-fasting (n = 4), and high VAT-refed (n = 2) groups. Participants ingested [U-$^{13}C_3$] glycerol and blood samples were subsequently analyzed at multiple time points over 3 h by NMR spectroscopy. The fractions of plasma glucose (enrichment) derived from [U-$^{13}C_3$] glycerol via hepatic gluconeogenesis, pentose phosphate pathway (PPP), and tricarboxylic acid (TCA) cycle were assessed using ^{13}C NMR analysis of glucose. Mixed linear models were used to compare ^{13}C enrichment in glucose between groups.

Results. Mean age, BMI, and baseline glucose were 49 years, 40.1 kg/m^2, and 98 mg/dl, respectively. Up to 20% of glycerol was metabolized in the TCA cycle prior to gluconeogenesis and PPP activity was minor (<1% of total glucose) in all participants. There was a 21% decrease in ^{13}C enrichment in plasma glucose in the high VAT-fasting compared with low VAT-fasting group (p = 0.03), suggesting dilution by endogenous glycerol. High VAT-refed participants had 37% less ^{13}C enrichment in glucose compared with high VAT-fasting (p = 0.02). There was a trend toward lower [1,2-$^{13}C_2$] (via PPP) and [5,6-$^{13}C_2$]/[4,5,6-$^{13}C_3$] (via TCA cycle) glucose in high VAT versus low VAT groups.

Conclusions. We applied a simple method to detect gluconeogenesis from glycerol in obese humans. Our findings provide preliminary evidence that excess visceral fat disrupts multiple pathways in hepatic gluconeogenesis from glycerol.

Abbreviations: DHS, Dallas Heart Study; DXA, dual x-ray absorptiometry; MAG, monoacetone glucose; MRI, magnetic resonance imaging; NMR, nuclear magnetic resonance; PPP, pentose phosphate pathway; TCA, tricarboxylic acid; TPI, triose phosphate isomerase; VAT, visceral adipose tissue.

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1. Introduction

Abdominal obesity and excess visceral fat, termed "visceral adiposity", have strong associations with insulin resistance, hyperglycemia and type 2 diabetes [1–5]. However the amount of visceral adipose tissue (VAT) in most individuals represents only a relatively small fraction of body fat burden, generally less than 15% [6]. The disproportionate influence of visceral fat on systemic metabolism has been attributed to resistance of mesenteric fat cells to the anti-lipolytic effects of insulin [3]. Consequently, persistent turnover of mesenteric triglycerides in spite of hyperinsulinemia delivers glycerol and fatty acids directly into the portal circulation, providing both a gluconeogenic substrate and energy for gluconeogenesis in the liver [7,8]. Glycerol contributes about 10% of total glucose production after an overnight fast in healthy non-obese participants [9–11], but little is known about the contribution of glycerol to glucose production in participants with visceral adiposity.

This knowledge gap is due to the complexity of glycerol metabolism and the limited applicability of arterial and hepatic vein cannulation for clinical research [8,12,13]. Glycerol enters gluconeogenesis/glycolysis after phosphorylation via glycerol kinase to generate glycerol 3-phosphate which rapidly exchanges with the trioses, dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GA3P). Subsequent metabolism yields pyruvate, if flux through glycolysis is active, or glucose, if gluconeogenesis is dominant. Studies of ^{13}C–labeled glycerol in humans have assumed that glycerol is converted directly to glucose and that the products of glycerol metabolism do not pass through the pentose phosphate pathway (PPP) or tricarboxylic acid (TCA) cycle prior to glucose export [14]. However, other studies demonstrated appearance of $^{14}CO_2$ from ^{14}C–enriched glycerol which would not be expected from the direct conversion of glycerol to glucose [15]. Previs and colleagues found that a significant fraction of glucose derived from [U-^{13}C] glycerol in rodents and non-human primates passed through the oxaloacetate pool in the TCA cycle prior to gluconeogenesis [16]. More recent studies in rodents [17] and healthy humans [18] demonstrated that most glycerol is metabolized directly to glucose but that a modest fraction enters the TCA cycle prior to re-synthesis to glucose and a small fraction of glycerol carbons are redistributed in the oxidative arm of the PPP. These observations are consistent with reports of bidirectional metabolism in the gluconeogenic and glycolytic pathways [19,20]. Since glycerol biochemistry in vivo is complex, analytical methods must be sensitive to these pathways. Compared to either mass spectrometry or radiotracer methods, the use of ^{13}C, a nonradioactive tracer detected by NMR, offers more detail about the pathways involved in glucose production. This advantage arises from information about site-specific enrichment and ^{13}C-^{13}C spin–spin coupling. [U-$^{13}C_3$] glycerol can be applied to quantify the fraction of glucose derived from glycerol in these pathways from a single ^{13}C NMR spectrum [10,21].

The purpose of this pilot study was to evaluate the feasibility of using orally-administered [U-$^{13}C_3$] glycerol in obese participants to probe the effects of visceral adiposity on various glucose production pathways *in vivo*. We hypothesized that participants with high VAT would have lower ^{13}C enrichment in glucose, signifying greater endogenous adipose contribution of glycerol to hepatic gluconeogenesis, compared with participants with low VAT, independent of fasting blood glucose level or body mass index (BMI). We also examined the effects of refeeding on these gluconeogenic pathways in participants with high VAT.

2. Materials and Methods

2.1. Study Population and Variable Ascertainment

Participants were recruited through the Dallas Heart Study (DHS), a multiethnic, probability-based, population cohort study of Dallas County adults, as well as through patient referrals from community physicians. Detailed methods of the DHS have been described previously [22]. For inclusion in the study, participants had to be age ≥ 18 years, obese (defined as a BMI ≥ 30 kg/m^2 at both the time of visceral fat imaging and at enrollment), without a diagnosis of type 2 diabetes mellitus (both by self-reported medical history and fasting plasma glucose <126 mg/dl measured at enrollment), and have an assessment of VAT by either magnetic resonance imaging (MRI) or dual x-ray absorptiometry (DXA). Participants were excluded if they were pregnant (by urine pregnancy test at the time of enrollment) or breastfeeding, incarcerated, non-obese at the time of visceral fat imaging or enrollment, had a > 10% change in body weight between the time of visceral fat imaging and enrollment, or had donated blood within 6 weeks of enrollment.

Participants were stratified by median VAT level into high VAT and low VAT groups. Among those with high VAT, participants were randomly assigned to fasting (no food or drink for ≥8 h prior to [U-$^{13}C_3$] glycerol ingestion) or re-fed: given a standardized mixed meal containing 880 kcal with 80 g carbohydrates (45%), 37 g fat (21%), and 59 g protein (34%) within 60 min of [U-$^{13}C_3$] glycerol ingestion. All low VAT participants were fasting. Age, sex, and race/ethnicity were self-reported. Weight and height were measured using standard scales and BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured 1 cm above the iliac crest and hip circumference at the widest circumference of the buttocks at the area of the greater trochanters. A total of 10 participants were enrolled but one participant withdrew consent from the study before any study procedures were performed so the present analysis includes a final sample size of 9 participants. All participants provided written informed consent, and the protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center.

2.2. Visceral Fat Measurements

Seven participants underwent whole body composition analysis with a GE Lunar Prodigy Advance iDXA scanner (GE Healthcare, Madison, WI) and images were analyzed with CoreScan enCORE software version 14.10.022. This software

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