Effects of Visceral Adiposity on Glycerol Pathways in Gluconeogenesis

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Introduction

Abdominal obesity and excess visceral fat, termed "visceral adiposity", has strong associations with insulin resistance, hyperglycemia and type 2 diabetes. 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%. 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. 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. Glycerol contributes about 10% of total glucose production after an overnight fast in healthy non-obese participants , 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. Glycerol enters gluconeogenesis/glycolysis after phosphorylation via glycerol kinase to generate glycerol 3-phosphate which rapidly exchanges with the trioses, DHAP and 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 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.

The purpose of this 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.

Methods and Results:

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 . 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.

Study Procedures

Participants arrived in the morning after an overnight fast. Vital signs and anthropometric data were acquired. A peripheral intravenous catheter was inserted and 0.5 ml of blood was drawn for glucose analysis using an YSI 2300 Biochemistry Analyzer. Participants in the high VAT-refed arm were then provided a standardized mixed meal as detailed above. [U-$^{13}C_3$] glycerol was then prepared in ½ cup of filtered water using the following formula: Weight (kg) x 0.05 g/kg x 1 ml/1.302 g = [U-$^{13}C_3$] glycerol (ml). Participants drank the glycerol followed by 1 cup of filtered water to rinse the bottle. Blood samples were subsequently drawn at +15, +30, +60, +90, +120, +150, and +180 minutes for analysis. For each sample 0.5 ml of blood was used for blood glucose analysis and 40 ml of each sample was immediately centrifuged and stored on ice to be used for nuclear magnetic resonance (NMR) analysis as described below. After completion of the study procedures, the participants were discharged. All participants provided written informed consent, and the protocol was approved by the Institutional Review Board.

Low Visceral Fat versus High Visceral Fat

There was a statistically significant 21% decrease in ^{13}C enrichment in glucose in the high VAT-fasting group compared with the low VAT-fasting group (p=0.03, **Figure 3, Panel A**). The high VAT-fasting group had a lower and longer duration to enrichment compared with the low VAT-fasting group, although enrichment became equilibrated at time +180 minutes. In all participants, there was a small amount of plasma glucose (<1% of total glucose) that underwent rearrangement due to the PPP (**Figure 3, Panel B**). Furthermore, a significant amount of glycerol (up to 20%) passed through the TCA cycle prior to gluconeogenesis in all participants and we observed a trend toward less enrichment of [5,6-$^{13}C_2$] glucose (**Figure 3, Panel C**).

Fasting versus Refeeding

There was a statistically significant 37% decrease in ^{13}C enrichment in glucose in the high VAT-refed group compared with the high VAT-fasting group (p=0.02, **Figure 4, Panel A**). The high VAT-refed group also had a lower and longer duration to enrichment compared with the high VAT-fasting group and enrichment remained lower in the high VAT-refed group throughout the study period.

Table. Characteristics of the Study Population

Group	Age (yrs)	Sex	Race	Weight (kg)	Body mass index (kg/m^2)	Waist Circumference (cm)	Visceral Fat (kg)	Blood Pressure (mmHg)	Plasma Glucose (mg/dl)
Low Visceral Fat									
Subject #1	56	F	White	101.6	42.3	124	1.8	117/77	110
Subject #2	44	F	White	105.2	35.3	101	1.4	130/89	73
Subject #3	47	F	White	87.6	31.2	108	1.2	107/68	86
Subject #4	43	F	White	96.1	40.1	103	2.1	135/85	103
High Visceral Fat									
Fasting									
Subject #1	45	F	White	115.7	45.2	121	3.3	135/90	100
Subject #2	41	M	Black	144.24	37.7	130	2.5	131/88	96
Subject #3	58	M	Black	141.7	38.5	128	3.2	131/63	101
Refed									
Subject #1	58	F	White	138.5	47.8	135	2.6	117/72	113
Subject #2	48	F	White	130.2	42.4	118	3.4	117/77	103

Conclusion

These studies demonstrate that a recently described method to detect gluconeogenesis from glycerol was easily extended to obese humans. Since the study involves only oral administration of a stable isotope tracer and venous blood sampling, the method is highly acceptable to patients. Using this novel method, we quantified the effects of excess visceral adiposity on the fraction of glucose derived from glycerol ("glycerol-gluconeogenesis") in multiple pathways in hepatic gluconeogenesis. Up to 20% of the labeled glycerol contributing to glucose passed through the TCA cycle prior to gluconeogenesis and bidirectional metabolism of glycerol was detected in all participants. Glucose produced undergoes carbon rearrangements due to flux through the oxidative branch of the PPP, but the fraction of plasma glucose involved in this pathway was small in all participants. Our study demonstrates that orally-administered [U-^{13}C] glycerol coupled with venous blood sampling and NMR analysis is a simple tool to detect biomarkers of mitochondrial metabolism, gluconeogenesis, and the PPP in obese persons.

Our findings show that participants with high VAT had lower ^{13}C enrichment in glucose compared with participants with low VAT, likely reflecting greater VAT contribution of endogenous glycerol to hepatic gluconeogenesis, independent of fasting glucose level and BMI. We also showed that viscerally obese individuals had a trend toward lower ^{13}C-labeled glucose isotopomers produced through the PPP or TCA cycle suggesting a possible link between excess VAT, inflammation, PPP dysregulation, and mitochondrial failure. Furthermore, we observed that refeeding results in lower ^{13}C enrichment in glucose through direct gluconeogenesis from [U-$^{13}C_3$] glycerol, through PPP activity or through the TCA cycle prior to gluconeogenesis, compared with the fasting state, consistent with prior observations in non-obese refed rodents .

Overall, our findings provide intriguing evidence that excess VAT may act as a "constitutively fed state" and result in increased risk for hyperglycemia and type 2 diabetes through overstimulation of hepatic gluconeogenesis by chronic delivery of glycerol arising from mesenteric triglyceride turnover directly into the portal circulation and to the liver. These findings should stimulate further study with larger numbers of participants to more comprehensively elucidate the effects of visceral adiposity, independent of obesity, on gluconeogenic pathways. In conclusion, for the first time in obese humans, we used a simple stable isotope technique to investigate *in vivo* the mechanisms underlying the effects of excess visceral adiposity on gluconeogenesis from glycerol.

Figure 1.

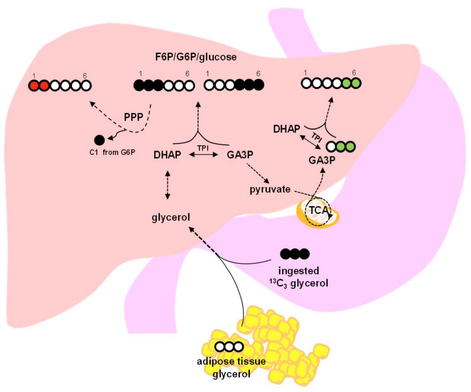

Figure 1. Glycerol-gluconeogenesis is directly interrogated by determining the fraction of $^{13}C_3$ enrichment in blood glucose using NMR spectroscopic quantification of ^{13}C-labeled glucose isotopomers. Total ^{13}C enrichment in plasma glucose is measured by the sum of all glucose isotopomers with excess ^{13}C. Additional information about specific pathways is derived from specific glucose isotopomers.

Figure 2.

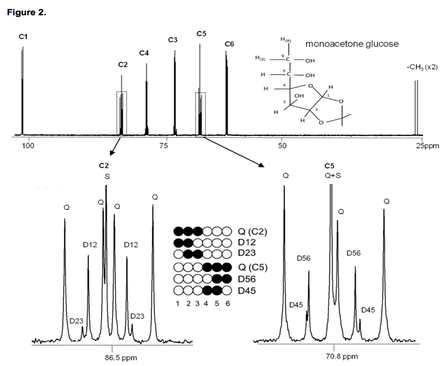

Figure 2. **^{13}C** An overnight fasted participant with high VAT ingested [U-$^{13}C_3$] glycerol and blood was drawn at multiple time points. Glucose was converted to MAG for ^{13}C NMR analysis and the spectrum is from blood drawn at 180 min after the oral load of [U-$^{13}C_3$] glycerol. [1,2,3-$^{13}C_3$]- and [4,5,6-$^{13}C_3$] glucose were produced through gluconeogenesis directly from [U-$^{13}C_3$] glycerol. Gluconeogenesis via the TCA cycle produced double-labeled ([1,2-$^{13}C_2$], [2,3-$^{13}C_2$], [4,5-$^{13}C_2$] and [5,6-$^{13}C_2$]) glucose. Hepatic PPP activity produced additional [1,2-$^{12}C_2$] glucose.

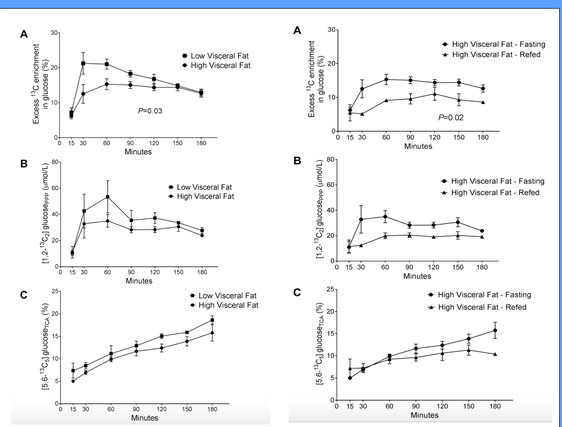

Figure 3. Contribution of [U-$^{13}C_3$] Glycerol to Glucose Production via Multiple Pathways in Low Visceral Fat vs. High Visceral Fat Participants

Figure 4. Contribution of [U-$^{13}C_3$] Glycerol to Glucose Production via Multiple Pathways in Fasting vs. Refed High Visceral Fat Participants

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