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Hepatic glucose production pathways after three days of a high-fat diet

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ABSTRACT

Objective. A three-day high-fat diet induces hepatic steatosis and hepatic insulin resistance in rats without altering fasting plasma glucose concentration or the rate of glucose production. However, as the nutrient profile available to the liver is substantially altered by a high-fat diet, we hypothesized that the relative fluxes supporting hepatic glucose production would be altered.

Materials/Methods. To test this hypothesis, we used multiple tracers ([3,4-$^{13}C_2$]glucose, ^2H_2O, and [U-$^{13}C_3$]propionate) followed by NMR analysis of blood glucose to quantify net glucose production and the contributions of glycogen and key gluconeogenesis precursors in 4–5-h fasted rats.

Results. NMR analysis demonstrated that the majority of blood glucose was derived from glycogen and the citric acid cycle, while a smaller fraction of glucose was derived from glycerol in both controls and high-fat-fed animals. High-fat feeding was associated with a two-fold increase in plasma glycerol concentration and an increase in the contribution (both fractional and absolute) of glycerol-gluconeogenesis. The increase in gluconeogenesis from glycerol tended to be balanced by a decrease in glycogenolysis. The absolute fluxes associated with the citric acid cycle including gluconeogenesis from the cycle intermediates, pyruvate cycling and the citric acid cycle flux itself, were not altered by this short high-fat diet.

Conclusions. A short term high-fat diet altered the specific pathways for hepatic glucose production without influencing the overall rate of glucose production or flux in the citric acid cycle.

Abbreviations: CS, citrate synthase; DHAP, dihydroxyacetone phosphate; EGP, endogenous glucose production; FBP, fructose 1,6-bisphosphate; FUM, fumarate; F6P, fructose 6-phosphate; GA3P, glyceraldehyde 3-phosphate; GNG, gluconeogenesis; G6P, glucose 6-phosphate; G6Pase, Glucose 6-phosphatase; HFD, high-fat diet; MAG, monoacetone glucose; MAL, malate; ME, malic enzyme; NEFAs, non-esterified fatty acids; NMR, nuclear magnetic resonance; OAA, oxaloacetate; PCR, polymerase chain reaction; PEP, phospho*enol*pyruvate; PEPCK, phospho*enol*pyruvate carboxykinase; PK, pyruvate kinase; PKC, protein kinase C; ppm, parts/million; PPP, pentose phosphate pathway; SUCC, succinyl-CoA.

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1. Introduction

Hepatic glucose production is supported by the sum of flux through glycogenolysis, gluconeogenesis from glycerol, and gluconeogenesis from intermediates of the citric acid cycle. Fluxes through these individual pathways are sensitive to multiple factors such as dietary fat content or diseases such as type 2 diabetes or nonalcoholic fatty liver disease. For example, fasting hyperglycemia among patients with type 2 diabetes has been attributed to hepatic glucose over-production, but the contribution from glycerol, glycogen or the citric acid cycle remains controversial. Several studies reported elevated glycogenolysis and increased liver glycogen contents in patients with diabetes and in obese subjects [1–3] while other studies using ^{13}C magnetic resonance spectroscopic analysis reported the opposite [4–6]. In animal models, Zucker diabetic fatty rats had dramatically higher hepatic glycogen after a 24-h fast compared to controls and they had substantially increased glycogenolysis contributing to fasting hyperglycemia [7]. Mouse models of diabetes, ob/ob and db/db mice, were also reported to have increased hepatic glycogen [8–10].

The effect of diet on glucose production has also been examined. In rodents, a short term high-fat diet causes hepatic steatosis and hepatic insulin resistance, without the confounding effects of peripheral insulin resistance [11,12]. Using this model, we have shown that hepatic insulin resistance is associated with accumulation of hepatic diacylglycerol and PKCε activation, which in turn impairs insulin signaling [13,14]. Though this short term diet clearly induced hepatic insulin resistance, basal, fasting plasma glucose concentration and the rate of endogenous glucose production (EGP) were unchanged [13]. However, since glucose production is supported by multiple sources, the contribution of these fluxes may be altered following this dietary intervention. It is also worth emphasizing that flux through specific pathways may not correlate closely with expression of the putative controlling enzymes [15]. For example, up-regulation of phospho*enol*pyruvate carboxykinase (PEPCK) expression after a 12-week high-fat diet [16] may not mean the same degree of PEPCK flux or not even glucose production. Burgess et al. reported that substantial reduction of hepatic PEPCK contents caused only small changes in the flux through PEPCK [17]. As another example, Zucker diabetic fatty rats have increased expression of PEPCK and increased glucose production [7,18]. Yet when probing the metabolic network that supports glucose production, we observed that the increased flux from oxaloacetate (OAA) to phospho*enol*pyruvate (PEP) was balanced by an increase in flux from PEP back to pyruvate. Together these changes prevented a net contribution of PEP to glucose [7]. In general, the gene expression profile is a poor indicator of metabolic fluxes; these must be measured directly.

High-fat feeding will increase lipid turnover and may lead to increased rates of lipolysis and subsequently increased concentrations of non-esterified fatty acids (NEFAs) and glycerol [19–22]. Glycerol appearance was increased in rats fed on a high-fat diet for 8 weeks [22], and glycerol was suggested to be the most important precursor in adding new carbons for glucose pool in type 2 diabetes [23,24]. Since the rate of gluconeogenesis from glycerol was reported to be proportional to plasma glycerol [23], increased gluconeogenesis would be expected after a brief high-fat diet. It is possible that a three-day high-fat diet may increase the contribution in some fluxes that are balanced by decreases in others, thus preserving normal basal glucose production.

The goal of the present study was to assess the effect of a brief high-fat diet on all fluxes in the pathways involved in glucose production. Metabolic fluxes were estimated using three stable isotope tracers: [3,4-$^{13}C_2$]glucose for glucose turnover measurement [25], ^2H_2O for the measurement of fractional contributions of glycogenolysis, gluconeogenesis from glycerol, and gluconeogenesis from the citric acid cycle to EGP [26,27], and [U-$^{13}C_3$]propionate for the measurement of fluxes related to the citric acid cycle [28]. Three-day High-fat feeding increased both plasma glycerol and hepatic triglycerides in rats without change in plasma glucose, hepatic glucose production, or the citric acid cycle flux in the liver. However, flux through glycerol gluconeogenesis was increased significantly by this brief diet.

2. Methods

2.1. Materials

[3,4-$^{13}C_2$]glucose (99%) was purchased from Omicron Biochemicals (South Bend, IN). [U-$^{13}C_3$]propionate (99%), ^2H_2O (99.9%) and deuterated acetonitrile (99.8%) were obtained from Cambridge Isotopes (Andover, MA). Other common chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Pathways supporting glucose production

The study of glucose production pathways was approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center. Male Sprague–Dawley rats were purchased from Charles River Laboratories. The rats were placed on a 12-h day/night cycle and provided ad-libitum access to food and water, except when specified by experimental protocol. One week before the study day, catheters were inserted into the right internal jugular vein, extending to the right atrium under general anesthesia and the animals were allowed to recover for seven days. Upon recovery from the surgery, one group of rats (n=9) was switched to a high-fat diet with 60% calories provided from fat, 20% from carbohydrate and 20% from protein (5.24 kcal/g; cat. no. D12492; Research Diets Inc., New Brunswick, NJ) for three days before the study day. Lard and soybean were the fat sources (lard:soybean=9:1 in kcal) for the high-fat diet. The other group (n=9) continued a standard chow diet with 12% calories provided from fat, 66% from carbohydrate and 22% from protein (3.0 kcal/g; cat. no. 2016; Teklad FG rodent diet, Harlan Teklad). Soybean was the main fat source for the standard diet. The three-day high-fat diet did not induce body weight difference between two groups (324±4 g in controls vs. 338±8 g in high-fat diet; 12-week-old rats). On the study day, rats were fasted for 4–5 h beginning at 8:00 a.m. with free access to water. At t=−20 min, rats received an intravenous bolus injection of ^2H_2O-[U-$^{13}C_3$]propionate