Metabolic response to high-carbohydrate and low-carbohydrate meals in a nonhuman primate model

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Fabbrini E, Higgins PB, Magkos F, Bastarrachea RA, Voruganti VS, Comuzzie AG, Shade RE, Gastaldelli A, Horton JD, Omodei D, Patterson BW, Klein S. Metabolic response to high-carbohydrate and low-carbohydrate meals in a nonhuman primate model. Am J Physiol Endocrinol Metab 304: E444–E451, 2013. First published December 26, 2012; doi:10.1152/ajpendo.00347.2012.—We established a model of chronic portal vein catheterization in an awake nonhuman primate to provide a comprehensive evaluation of the metabolic response to low-carbohydrate/high-fat (LCHF; 20% carbohydrate and 65% fat) and high-carbohydrate/low-fat (HCLF; 65% carbohydrate and 20% fat) meal ingestion. Each meal was given 1 wk apart to five young adult (7.8 ± 1.3 yr old) male baboons. A [U−13C]glucose tracer was added to the meal, and a [6,6-2H2]glucose tracer was infused systemically to assess glucose kinetics. Plasma areas under the curve (AUCs) of insulin, C-peptide in the femoral artery and of glucose in the portal vein were higher (P < 0.05) after ingestion of the HCLF compared with the LCHF meal. Compared with the LCHF meal, the rate of appearance of ingested glucose into the portal vein and the systemic circulation was greater after the HCLF meal (P < 0.05). Endogenous glucose production decreased by ~40% after ingestion of the HCLF meal but was not affected by the LCHF meal (P < 0.05). Portal vein blood flow increased (P < 0.001) to a similar extent after consumption of either meal. In conclusion, a LCHF diet causes minimal changes in the rate of glucose appearance in both portal and systemic circulations, does not affect the rate of endogenous glucose production, and causes minimal stimulation of C-peptide and insulin. These observations demonstrate that LCHF diets cause minimal perturbations in glucose homeostasis and pancreatic β-cell activity.

stable isotope tracers; portal vein catheterization; β-cell function

THE TYPICAL DIET IN THE US provides ~50% of ingested calories as carbohydrate, 35% as fat, and 15% as protein (4). However, data from several studies have shown that a low-carbohydrate diet containing 35–40% of calories as carbohydrate can have therapeutic effects in patients with type 2 diabetes by lowering plasma glucose, triglyceride, and very low-density lipoprotein (VLDL) cholesterol, by increasing plasma high-density lipoprotein (HDL) cholesterol concentrations, and by decreasing insulin requirements (17–19). In addition, low-carbohydrate diets are often used to help induce weight loss in obese people (15). Although the use of low-carbohydrate diets continues to be a popular dietary therapy, the metabolic response to high- and low-carbohydrate meals has not been carefully evaluated.

The metabolic response to a meal is carefully coordinated among several organs systems to prevent large excursions in plasma glucose concentration and to deliver ingested nutrients to appropriate tissues for storage or utilization. The normal regulation of postprandial plasma glucose involves an increase in insulin secretion from pancreatic β-cells into the portal vein, insulin-mediated suppression of endogenous (primarily hepatic) glucose production, and insulin-mediated stimulation of glucose uptake by peripheral tissues (primarily skeletal muscle). In fact, abnormalities in plasma glucose concentration after an oral glucose load is ingested are used as a clinical tool to diagnose impaired glucose tolerance and type 2 diabetes (3).

After meal ingestion, glucose consumed in the meal and insulin secreted from the pancreas are delivered directly into the portal vein for transport to the liver. Accordingly, the ability to sample portal vein blood is important for understanding postprandial metabolic regulation. Several studies conducted in animal models have used portal vein catheterization to evaluate the effect of intraportal nutrient delivery on hepatic and whole body metabolism (11, 43, 49). However, we are not aware of any studies that have used portal vein catheterization to evaluate the effect of differences in dietary macronutrient composition on the metabolic response to a meal.

The purpose of the present study was to establish a chronic portal vein catheterization model in an awake nonhuman primate to assess the metabolic responses to a high-carbohydrate/low-fat (HCLF) and low-carbohydrate/high-fat (LCHF) meal. These experiments were performed in baboons because of their similarity to humans, thereby reducing the chance of a confounding effect of interspecies differences in our assessment of metabolic outcomes (6, 7, 33, 34). The metabolic response to each meal was evaluated by using stable, isotopically labeled tracer methods in conjunction with mathematical modeling to assess glucose kinetics and insulin secretion.

METHODS

Animals. Five young adult (7.8 ± 1.3 yr old) male baboons (Papio hamadryas Sp.) were studied. Prior to the study, animals were housed in groups in outdoor enclosures in the Southwest National Primate Research Center (SNPRC; San Antonio, TX) according to established National Research Council guidelines. A licensed veterinarian performed a standard health assessment, including a physical examination and serum chemistry and hematology profiling in all animals.
before their participation in this study. All animals were considered to be healthy, and none had any history of medical illness.

For this study, all animals were housed individually in indoor facilities at the SNPRC. In order for animals to acclimate to the indoor laboratory conditions, they were housed in large individual aluminum cages inside the facility, which was kept at constant room temperature and humidity in a 12:12-h light-dark cycle for 7 days before the experimental protocol began. During this period, the animals were also habituated to the presence of the tether jacket and coil system (9). Each animal was fitted to a cotton tether jacket connected to a stainless-steel coil via a molded plastic box attached to the rear of the jacket. The stainless-steel coil provided a conduit through which blood sampling lines, infusion lines, and flow probe monitor wiring were later passed from the animal to the sampling, infusion, and recording equipment at the top of the cage. Animals were fed a standard nonhuman primate diet (SL60 solid feed, LabDiet; PMI, St. Louis, MO) ad libitum and had continuous access to water. All study procedures were submitted to and approved by the Institutional Animal Care and Use Committee of the Texas Biomedical Research Institute, San Antonio, TX.

Catheters and flow probe placement. One week after indoor housing and tether jacket acclimation, laparotomy was performed under general anesthesia to implant a polyurethane catheter (5 Fr Hydrocoat; Access Technologies, Skokie, IL) in the portal vein and to place a 6-mm-diameter perivascular flow probe (MC6PSS ultrasound transit time chronic flow probe; Transonic Systems, Ithaca, NY) on the portal vein. Animals were sedated with ketamine HCl (10 mg/kg im), and gas anesthesia was induced and maintained by using isoflurane (1.5%). During surgery, animals were given a prophylactic antibiotic (25 mg/kg cephalozin). The probe was fitted around the vessel so that the vessel would lie in the space between the probe body and the reflector piece without impeding blood flow through the portal vein. The location of the flow probe and integrity of the portal vasculature and surrounding tissue was confirmed by fluoroscopy immediately after implantation. The catheters and flow probe were routed subcutaneously to the midscapular region of the back, where they exited the skin into a molded plastic box (backpack) attached to a tether jacket. Catheters were then implanted in the femoral artery and vein (5 Fr Hydrocoat; Access Technologies) via a skin incision over the femoral vessels in the femoral triangle of the inner thigh. These catheters were also routed subcutaneously to the midscapular region of the back to exit the skin at the location of the tether jacket backpack box.

After surgery, analgesic (0.01 mg/kg qid buprinex for 3 days) and prophylactic antibiotic treatments (25 mg/kg bid cephalozin for 7 days) were provided. In addition, a single dose of midazolam (0.1 mg/kg) was administered intravenously after the animals regained consciousness after surgery. Parenteral nutrition support was also provided after the surgery and was continued for up to 6h until the animals began to eat solid foods. Catheters were kept patent by using continuous heparinized saline (5 U/ml) infusion from volumetric infusion pumps maintained at the top of the cage, as described previously (9).

Acclimation of the animals to the presence of a technician was conducted at regular intervals for 4 wk after surgery. In addition, food and water intake were monitored daily, and animals were sedated weekly (10 mg/kg ketamine) to perform health assessments, which included a physical examination, inspection of catheter and flow probe exit sites, body weight measurement, and routine blood tests. The flow probe cabling was extended c. 12 m from the cage top to an adjacent monitoring room to connect to a three-channel transit time flow meter (TS403 Console with TS420 Perivascular Flow Meter Modules; Transonic Systems). The flow probes were precalibrated with a specified calibration gain programmed into a calibration key sent directly from the manufacturer. Manufacturer-recommended performance testing of the received signal was undertaken weekly by using the flow meter’s test mode. The received signal testing was conducted across the range of measured voltage. Chronic portal flow measurements were recorded and logged using the Ponemah Physiology Platform with P3 Plus Software for Windows (LDS Test Measurement and Gould Instrument Systems, Valley View, OH). All animals adapted well to the tether system, had normal food and water intakes, and were weight stable before initiation of the mixed-meal metabolic studies. Body composition. Three weeks after placement of catheters and flow probe, animals were sedated with ketamine HCl (10 mg/kg) to assess body composition. Body weight was measured by using an electronic scale (GSE 665; Texas Scales, Cibolo, TX). Total body fat mass and fat-free mass were determined by using dual-energy X-ray absorptiometry ( Lunar Prodigy densitometer; GE Healthcare, Madison, WI). Animals were placed in the supine position on the dual-energy X-ray absorptiometry bed, and extremities were positioned within the scanning region. Scans were analyzed by using encore2007 software version 11.40.004 (GE Healthcare).

Mixed-meal metabolic study. Two mixed-meal metabolic studies (LCHF and HCLF) were performed ~1 wk and again ~2 wk after body composition was determined. Animals were randomized to receive either a 350-kcal HCLF (65% of calories as carbohydrates, 15% as protein, and 20% as fat) or a 350-kcal LCHF (20% of calories as carbohydrate, 15% as protein, and 65% as fat) meal at each study. The calorie content and macronutrient composition of each of the two test meals were chosen to 1) provide isocaloric meals that contained about one-third of the animals’ daily energy requirements and 2) provide a large range in carbohydrate loads, representing the macronutrient content of a low-carbohydrate diet (e.g., the Atkins diet) and a conventional low-fat diet (1, 2) used to treat obesity to maximize the differences in β-cell response and glucose metabolism elicited by each meal. The HCLF meal was prepared by using crushed brown rice krispies, water, glucose powder, butter, and whey protein isolate. Glucose powder (53 g) was dissolved in heated water to form a syrup, and [U-13C]glucose tracer (2% of total glucose content) was added and dissolved in this syrup. The solution was mixed by using a magnetic stirring rod and sonicated to ensure homogeneity. Butter (9.9 g), whey protein (14 g), and crushed brown rice krispies (8.5 g) were then added to the solution (56.9 g total carbohydrate), and the ingredients were mixed by using a spatula. The mixture was then allowed to solidify and refrigerated at 4°C until used the following day. The LCHF meal was prepared by using butter, smooth peanut butter, honey crackers, whey protein, and dextrose. Glucose powder (9.4 g) was dissolved in heated water to form a syrup, and [U-13C]glucose tracer (10% of total glucose) was added and dissolved in this syrup. This solution was mixed by using a magnetic stirring rod and sonicated to ensure homogeneity. Butter (17.5 g), crushed honey crackers (5 g), whey protein (8.1 g), and smooth peanut butter (22 g) were added to the syrup solution (17.5 g total carbohydrate), and the ingredients were mixed by using a spatula and refrigerated at 4°C until they were used for the study the following day.

At 0700 on the day before the mixed meal, the heparinized saline infusion was discontinued, and 0.45% saline was infused at 20 ml/h to keep catheters patent. The following day, the mixed-meal metabolic study was performed. Low-dose midazolam was infused intravenously as needed to ensure that the animals were awake but resting quietly in the study cage. At 0800, a primed continuous infusion of [6,6,13C2]glucose (priming dose: 22 μmol/kg; infusion rate: 0.22 μmol/kg·min–1) was administered through the femoral vein catheter and continued for 9 h. After 3 h of isotope tracer infusion (basal period), animals were randomized to receive either the HCLF or the LCHF test meal, which was always completely consumed within 20 min. Approximately 1 wk later, the mixed-meal metabolic study was repeated using the alternate meal.

Blood samples during the mixed-meal metabolic studies were obtained through the tether catheter lines, which had a dead space volume of 1.5 ml. Thirty seconds before each blood sample was taken, the dead space was cleared by withdrawing blood into a 3-mI syringe
METABOLIC RESPONSE TO DIFFERENT MEALS IN BABOONS

Alanine transaminase, U/l  30  28  28  20  32  28
HDL cholesterol, mmol/l  1.7  1.9  1.7  1.0  1.0  1.4
Total cholesterol, mmol/l  2.2  3.0  2.4  2.9  2.6  2.6
Triglycerides, mmol/l  0.5  0.7  0.7  0.3  0.3  0.5

Glucose, mmol/l  5.3  4.3  5.0  5.0  5.0  5.0
Fat-free mass, kg  1.1  1.3  2.55  2.55  2.55  2.55
Fat mass, %body weight  3.9  4.3  5.0  4.0  4.7  4.4
Fat mass, kg  1.1  1.3  1.4  1.4  1.4  1.4
Body weight, kg  28.1  28.9  28.9  27.5  27.5  28.6 ± 0.8

Average values are means ± SD.
Glucose kinetics in response to the meal. There was a significant diet-by-time interaction for the $R_a$ of ingested glucose into the systemic circulation and into the portal vein, endogenous glucose $R_a$, and total (both ingested and endogenous) glucose $R_a$ (all $P < 0.001$), indicating that the HCLF and the LCHF meals had a different effect on these kinetic outcome measurements (Fig. 4). Compared with the LCHF meal, the $R_a$ of ingested glucose into the systemic circulation and into the portal vein was significantly greater within 10 min after the HCLF meal consumption was initiated and remained higher throughout the entire study ($P < 0.05$; Fig. 4). Based on the difference in glucose appearance in the portal venous and arterial circulation, we calculated that hepatic extraction of glucose was 20 ± 5% after the HCLF meal and 7 ± 3% after the LCHF meal ($P < 0.05$). Ingestion of the HCLF meal decreased endogenous glucose $R_a$ by ~40% for several hours after meal ingestion ($P < 0.05$; Fig. 4), whereas the LCHF meal did not affect endogenous glucose production (Fig. 4). Total glucose $R_a$ into the systemic circulation, which is comprised of both ingested and endogenously produced glucose, was higher after the ingestion of the HCLF than the LCHF meal ($P < 0.05$; Fig. 4).

The relative contribution (calculated as the average of the individual time points over 6 h after the meals) of endogenous glucose production (EGP) to total glucose $R_a$ into plasma was 42 ± 18% after ingestion of the HCLF meal and 83 ± 10% after ingestion of the LCHF meal ($P < 0.001$); conversely, the contribution of ingested glucose to total glucose $R_a$ was 58 ± 18% after the HCLF meal and 17 ± 10% after ingestion of the LCHF meal ($P < 0.001$).

Portal vein blood flow. Portal vein blood flow increased after both HCLF and LCHF meal ingestion ($P < 0.001$), but there was no difference between the HCLF and the LCHF meals (interaction between time and diet $P = 0.968$) (Fig. 5).

DISCUSSION

In the present study, we established, for the first time, a model of chronic portal vein catheterization in awake nonhuman primates, which we used to evaluate the dynamic response to LCHF and HCLF meal ingestion. In this model, portal vein sampling and blood flow measurement provide a comprehensive in vivo metabolic assessment of meal ingestion. Stable isotopically labeled glucose tracers were administered orally and intravenously, and both systemic arterial and portal vein blood samples were obtained to evaluate ingested and endogenous glucose metabolism and the β-cell and α-cell responses to feeding. Our data demonstrate that a LCHF meal causes
chronic consumption of a high-carbohydrate diet can have adverse effects on overall glucose and lipid metabolism by stimulating de novo lipogenesis and the accumulation of intrahepatic triglyceride (36), increasing plasma triglyceride concentration (20, 31), and decreasing insulin sensitivity (17). The results from our study suggest that a LCHF diet can have beneficial effects in both preventing and treating diabetes by reducing meal-induced plasma glucose excursions and requiring minimal β-cell action and insulin response to meal ingestion. The variability in the response to ingesting the same meal observed in our homogeneous baboon model suggests that much larger numbers of subjects will be needed when studies evaluating the metabolic response to food intake in people are conducted.

Most glucose ingested during a meal is delivered into the systemic circulation and taken up by peripheral tissues, whereas only a portion of ingested glucose is metabolized by the liver during its first pass from the portal vein. We found that ~20% of ingested glucose was extracted by the liver after a HCLF meal was ingested in our baboon model, which is the middle of the range reported in studies that assessed fractional hepatic glucose extraction after ingestion of an oral glucose load in human subjects (14, 24, 29, 37). Therefore, our results, which were derived from direct measurement of portal vein minimal changes in the Rₐ of glucose in both portal and systemic circulations, does not affect the rate of EGP, and causes minimal stimulation of C-peptide and insulin secretion and less suppression of plasma glucagon. In addition, we found that plasma glucose concentration is maintained primarily by the delivery of ingested glucose into the systemic circulation after a HCLF meal is consumed, whereas plasma glucose concentration is maintained primarily by EGP after a LCHF meal. These observations demonstrate that LCHF diets cause minimal perturbations in glucose homeostasis and pancreatic β-cell and α-cell activities and help explain why low-glycemic diets have therapeutic effects on glycemic control (28, 47) and reduce the risk of developing type 2 diabetes (39).

Decreasing the glycemic load of meals by reducing the amount or composition of dietary carbohydrates has beneficial therapeutic effects on overall glycemic control and plasma glucose concentration. In subjects with type 2 diabetes, a low-glycemic index diet decreases fasting plasma glucose concentration, decreases 2-h glucose concentration during an oral glucose tolerance test, and improves glycemic control, as assessed by plasma Hb A₁c concentration (28, 47). A low-glycemic index diet also has beneficial effects in overweight and obese nondiabetic subjects by lowering postprandial glucose and insulin concentrations (13, 32). Even in healthy normal-weight subjects, reducing the carbohydrate load leads to a reduction in mean plasma glucose concentration over 24 h (5, 26). In contrast, a high-carbohydrate diet, particularly one that contains high-glycemic index foods, is associated with an increased risk of developing type 2 diabetes (39). We found that the HCLF meal had a prolonged suppressive effect on plasma FFA concentration, which lasted for 5 h after the meal. The increase in plasma insulin and greater reduction in plasma FFA availability after meal ingestion were likely responsible for the greater suppression of EGP observed after the HCLF than after the LCHF meal (25, 35). Although this effect on lipolysis and EGP has acute benefits on glucose control,
glucose concentration and glucose tracer/tracee ratios, support the estimates made from previous studies conducted in people, which used indirect assessments to determine hepatic fractional extraction.

Portal vein blood flow is involved in regulating glucose metabolism because it provides ~80% of the blood delivered to the liver (22). The uptake and metabolism of glucose by the liver after food intake is affected by the rate of glucose and hormone delivery, which in turn depends on portal vein blood flow during a meal (11). Portal venous blood flow increases by 20 to 80% 30 min after a meal, depending on the type of meal (liquid vs. solid), and remains elevated for 2 h after meal consumption (10, 12, 16, 44). The main determinant of the postprandial increase in portal vein flow is the gastrointestinal hyperemic response that follows food digestion and absorption, which leads to a marked increase in blood outflow from extrahepatic splanchnic tissues to the portal system (38). We found that both LCHF and HCLF meals caused similar increases in portal vein blood flow. This observation in our awake baboon model is consistent with data from studies conducted in an anesthetized dog model, which found that the increase in portal vein blood flow was similar after intrajejunal infusion of either fat or carbohydrate (8). In contrast, Hoost et al. (27) found that portal vein flow in healthy people was slightly greater after a high-fat meal compared with a high-carbohydrate meal. The reasons for the differences in their results and the other studies are not clear but could be related to the use of Doppler ultrasonography through the abdominal wall to assess portal vein blood flow in the study conducted in

![Ingested glucose Ra](chart1.png)

![Total glucose Ra into systemic circulation](chart2.png)

![Endogenous glucose Ra](chart3.png)

![Portal vein blood flow](chart4.png)

**Fig. 4.** The rate of appearance (Ra) of ingested glucose into the systemic circulation (top left) and into the portal vein (bottom left), total glucose Ra into the systemic circulation (top right), and endogenous glucose Ra (bottom right) after ingestion of either a HCLF or a LCHF meal. *Value different from HCLF meal value, P < 0.05; ‡P < 0.06.

**Fig. 5.** Portal vein blood flow before and for 6 h after a HCLF or a LCHF meal. Time point 0 is the average of the measurements carried out for 30 min before the meals were given. Portal vein blood flow increased (P < 0.001) after HCLF and LCHF meals in a similar fashion (no interaction between time and type of meal was observed). Values are presented as means ± SE.
people, rather than the direct assessment of portal vein blood flow, by using an intraportal flow probe in our study.

In summary, we developed a model of chronic portal vein catheterization in awake baboons to evaluate the integrated metabolic response to meal ingestion. Our data demonstrate that in adult male baboons a LCHF meal elicited a less robust insulin secretory response compared with a HCLF meal and support the importance of further studies to assess the potential for therapeutic benefit of a low-carbohydrate diet on glycemic control and prevention of type 2 diabetes.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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