Dietary Fat and Carbohydrates Differentially Alter Insulin Sensitivity During Caloric Restriction

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Background & Aims: **We determined the effects of acute and chronic calorie restriction with either a low-fat, high-carbohydrate (HC) diet or a low-carbohydrate (LC) diet on hepatic and skeletal muscle insulin sensitivity.** ***Methods:*** **Twenty-two obese subjects (body mass index, 36.5 ± 0.8 kg/m^2) were randomized to an HC (>180 g/day) or LC (<50 g/day) energy-deficit diet. A euglycemic-hyperinsulinemic clamp, muscle biopsy specimens, and magnetic resonance spectroscopy were used to determine insulin action, cellular insulin signaling, and intrahepatic triglyceride (IHTG) content before, after 48 hours, and after ~11 weeks (7% weight loss) of diet therapy.** ***Results:*** **At 48 hours, IHTG content decreased more in the LC than the HC diet group (29.6% ± 4.8% vs 8.9% ± 1.4%; $P < .05$) but was similar in both groups after 7% weight loss (LC diet, 38.0% ± 4.5%; HC diet, 44.5% ± 13.5%). Basal glucose production rate decreased more in the LC than the HC diet group at 48 hours (23.4% ± 2.2% vs 7.2% ± 1.4%; $P < .05$) and after 7% weight loss (20.0% ± 2.4% vs 7.9% ± 1.2%; $P < .05$). Insulin-mediated glucose uptake did not change at 48 hours but increased similarly in both groups after 7% weight loss (48.4% ± 14.3%; $P < .05$). In both groups, insulin-stimulated phosphorylation of c-Jun-N-terminal kinase decreased by 29% ± 13% and phosphorylation of Akt and insulin receptor substrate 1 increased by 35% ± 9% and 36% ± 9%, respectively, after 7% weight loss (all $P < .05$).** ***Conclusions:*** **Moderate calorie restriction causes temporal changes in liver and skeletal muscle metabolism; 48 hours of calorie restriction affects the liver (IHTG content, hepatic insulin sensitivity, and glucose production), whereas moderate weight loss affects muscle (insulin-mediated glucose uptake and insulin signaling).**

Insulin resistance is the most common metabolic complication associated with obesity and is associated with an increased risk of developing nonalcoholic fatty liver disease and type 2 diabetes mellitus.[1,2] A reduced calorie diet is a primary therapy for insulin-resistant obese persons, because even moderate diet-induced weight loss (5%–10% of body weight) decreases intrahepatic triglyceride (IHTG) content and improves hepatic and skeletal muscle insulin sensitivity.[3–9] However, the effect of brief calorie restriction (CR) (≤3 days) is confusing because short-term therapy with a very low calorie diet (≤800 kcal/day) improves insulin action,[10,11] whereas short-term fasting induces insulin resistance.[12,13]

The mechanism responsible for the apparent discrepancy between severe and complete CR on insulin action is not clear, but it is possible that differences in total carbohydrate intake could be responsible. Data from studies that used the hyperinsulinemic-euglycemic clamp technique to assess insulin action found that short-term CR with low-carbohydrate (LC) intake (0–50 g/day) is associated with a decline in hepatic and skeletal muscle insulin sensitivity,[14,15] whereas short-term CR with adequate carbohydrate intake (100 g/day) is associated with an increase in both hepatic and skeletal muscle insulin sensitivity.[4] We previously found that carbohydrate restriction, not total energy restriction, is responsible for initiating the lipolytic response to fasting; providing daily energy requirements by infusing a lipid emulsion (carbohydrate restriction) resulted in the same increase in lipolytic rate that occurred after complete fasting.[16] The summation of these data suggests that short-term CR with an LC diet could have adverse effects on insulin sensitivity because of increased free fatty acid release into the circulation, which can cause both hepatic[17,18] and skeletal muscle[19] insulin resistance.

The current recommended dietary guidelines for treating obesity are to reduce daily energy intake by 500–1000 kcal.[20] Although both LC and high-carbohydrate (HC), low-fat diets are frequently used to lose weight, it is not known whether the short-term and long-term effects of CR on IHTG content and insulin action in liver and

Abbreviations used in this paper: CR, calorie restriction; GCRC, General Clinical Research Center; HC, high-carbohydrate; HOMA-IR, homeostasis model assessment of insulin resistance; IHTG, intrahepatic triglyceride; JNK, c-Jun-N-terminal kinase; LC, low-carbohydrate; PKB, protein kinase B; Ra, rate of appearance; Rd, rate of disappearance.

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muscle differ between diets. Therefore, the purpose of the present study was to evaluate the short-term and long-term metabolic effects of a 1000-kcal/day deficit HC (≥180 g/day) or LC (≤50 g/day) diet in obese insulin-resistant subjects. A euglycemic-hyperinsulinemic clamp procedure, in conjunction with stable isotope tracer infusion, was performed to assess hepatic and muscle insulin sensitivity, vastus lateralis muscle samples were obtained to determine the concentration of key factors that regulate skeletal muscle insulin sensitivity, and magnetic resonance spectroscopy was used to determine IHTG content after short-term CR (48 hours) and moderate (7%) weight loss. We hypothesized that, compared with an energy-deficit HC diet, consuming an energy-deficit LC diet has adverse effects on insulin action.

Subjects and Methods

Subjects

Twenty-two obese subjects (4 men and 18 women; 43.6 ± 2.5 years of age; body mass index, 36.5 ± 0.8 kg/m^2) participated in this study. All subjects completed a medical evaluation, which included a history and physical examination, standard blood and urine tests, an electrocardiogram, and a 2-hour oral glucose tolerance test. All subjects were considered insulin resistant, defined as a homeostasis model assessment of insulin resistance (HOMA-IR) value >3.0.[21] In addition, 63% of subjects had impaired glucose tolerance based on a plasma glucose concentration between 140 and 199 mg/dL at 2 hours after a 75-g oral glucose load.[22] Subjects who had diabetes, a history of excessive alcohol consumption, liver disease, or evidence of other serious illnesses or organ dysfunction as well as subjects who smoked tobacco products or took medications that are known to alter glucose metabolism were excluded from the study. All subjects were weight stable (≤2% change in body weight) and had been sedentary (<1 hour of exercise per week) for at least 3 months before being enrolled in the study.

The study was approved by the Human Studies Committee of Washington University School of Medicine (St Louis, MO). Written informed consent was obtained from each subject before participation in this study.

Experimental Design

Body composition assessments. Total body fat mass and fat-free mass were determined by using dual-energy x-ray absorptiometry (QDR 4500; Hologic, Waltham, MA).[23] Total abdominal, subcutaneous abdominal, and intra-abdominal fat volumes were quantified by using magnetic resonance imaging (Siemens Vision 1.5 Tesla imager; Siemens, Erlanger, Germany). IHTG content was determined by using proton magnetic resonance spectroscopy with a 1.5T scanner (Magneton Vision Scanner; Siemens)[24]; three 2 × 2 × 2 voxels were analyzed for each subject, and the values were averaged for data analyses. These body composition assessments were made at baseline (before diet intervention), after 48 hours of CR with either an HC or LC diet, and after subjects lost 7% of their initial body weight and were weight stable for 4 weeks.

Euglycemic-hyperinsulinemic clamp procedure. Subjects were admitted to the inpatient unit of the General Clinical Research Center (GCRC) on 2 separate occasions. A euglycemic-hyperinsulinemic clamp procedure, in conjunction with stable isotopically labeled tracer infusion, was performed at baseline (before diet intervention), after 48 hours of CR with either an HC or LC diet, and after subjects lost 7% of their initial body weight and were weight stable for 4 weeks. Subjects were instructed to abstain from exercise and to maintain their regular diet for at least 3 days and to abstain from consumption of caffeine and alcohol for at least 24 hours before each admission. Female subjects were studied during the follicular phase of their menstrual cycle.

During the first GCRC admission, subjects were admitted for 4 days. In the evening on the day of admission, subjects consumed a standard meal containing 15 kcal/kg fat-free mass and 55% of total energy as carbohydrates, 30% as fat, and 15% as protein at ~6:00 PM and then fasted (except for water) and rested in bed until completion of the clamp procedure the next day. The following morning, at 6:00 AM, a catheter was inserted into an antecubital vein of one arm to infuse stable isotopically labeled glucose, insulin, and dextrose; another catheter was inserted in a contralateral hand vein, which was placed in a thermostatically controlled (65°C) box to obtain arterialized blood.[25] At 6:30 AM, resting energy expenditure was determined by using a metabolic measuring cart (Delta Trac; SensorMedics, Yorba Linda, CA). At ~7:00 AM, after a blood sample was obtained to determine the background glucose enrichment, a primed, continuous infusion of [6,6-$^2H_2$]glucose was started and maintained for 7 hours. At 210 minutes after starting the tracer infusion, insulin was infused at a rate of 40 mU · m^2 body surface area^{-1} · min^{-1} for 210 minutes (initiated with a 2-step priming dose of 160 mU · m^2 body surface area^{-1} · min^{-1} for 5 minutes followed by 80 mU · m^2 body surface area^{-1} · min^{-1} for 5 minutes). Dextrose (20%), enriched with [6,6-$^2H_2$]glucose to ~2.5% to minimize changes in plasma glucose enrichment,[26] was infused at a variable rate to maintain euglycemia (plasma glucose concentration of 5.6 mmol/L). The infusion rate of [6,6-$^2H_2$]glucose was decreased by 75% during the clamp procedure to account for the expected decline in hepatic glucose production. Blood samples were taken every 10 minutes during the last 30 minutes of the basal period and the clamp procedure to determine plasma glucose tracer-to-tracee ratio and concentration and plasma insulin concentration during basal conditions and insulin infusion. A muscle biopsy specimen from the vastus lateralis was taken at 240 minutes (ie, 30 minutes after