Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals

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Skeletal muscle insulin resistance has been implicated in the pathogenesis of nonalcoholic fatty liver disease (NAFLD) and atherogenic dyslipidemia associated with the metabolic syndrome by altering the distribution pattern of postprandial energy storage. We conducted a study to examine this hypothesis by reversing muscle insulin resistance with a single bout of exercise and measuring hepatic de novo lipogenesis and hepatic triglyceride synthesis after a carbohydrate-rich meal. We studied 12 healthy, young, lean, insulin resistant individuals in an interventional, randomized cross-over trial. The response to the ingestion of a carbohydrate-rich meal was studied at rest and after one 45-min bout of exercise on an elliptical trainer. Hepatic de novo lipogenesis was assessed by using ^2H_2O, and changes in glycogen and fat content in liver and muscle were measured by ^{13}C and ^1H magnetic resonance spectroscopy, respectively. Exercise resulted in a greater than threefold increase in postprandial net muscle glycogen synthesis ($P < 0.001$), reflecting improved muscle insulin responsiveness, and a ≈40% reduction ($P < 0.05$) in net hepatic triglyceride synthesis. These changes in whole body energy storage were accompanied by a ≈30% decrease in hepatic de novo lipogenesis ($P < 0.01$) and were independent of changes in fasting or postprandial plasma glucose and insulin concentrations. These data demonstrate that skeletal muscle insulin resistance is an early therapeutic target for the treatment and prevention of atherogenic dyslipidemia and NAFLD in young insulin resistant individuals who are prone to develop the metabolic syndrome and type 2 diabetes.

liver triglyceride | nuclear magnetic resonance spectroscopy

The metabolic syndrome is characterized by a clustering of risk factors for cardiovascular disease that include abdominal obesity, atherogenic dyslipidemia, hypertension, hyperuricemia, a prothrombotic state, a proinflammatory state, nonalcoholic fatty liver disease (NAFLD), and insulin resistance (1, 2). The metabolic syndrome is estimated to afflict 50 million Americans, and approximately half of all Americans are predisposed to it (1). Individuals with the metabolic syndrome are at increased risk for the development of coronary heart disease and other diseases related to plaque buildup in artery walls, such as stroke and peripheral vascular disease, as well as type 2 diabetes. However, the underlying mechanism(s) responsible for these cardiovascular risk factors associated with the metabolic syndrome are not fully understood and appear to be complex.

In this regard, insulin resistance, localized to skeletal muscle, has been hypothesized to cause atherogenic dyslipidemia and NAFLD by changing the pattern of storage of ingested carbohydrate away from skeletal muscle glycogen synthesis into hepatic de novo lipogenesis, resulting in an increase in plasma triglyceride concentrations, reduction in plasma high-density lipoprotein concentrations and increased liver triglyceride synthesis in healthy, young, lean insulin resistant individuals (3). This hypothesis has important implications for the treatment of hyperlipidemia and NAFLD associated with the metabolic syndrome in

that it implicates skeletal muscle insulin resistance as a primary therapeutic target.

To directly examine this hypothesis, we examined whether this altered pattern of postprandial energy storage could be reversed in a similar group of young lean insulin-resistant individuals after one bout of exercise. Previous studies have shown that a single bout of exercise can normalize insulin-stimulated muscle glycogen synthesis by increasing insulin-stimulated glucose transport activity in muscle (4).

We assessed liver and skeletal muscle triglyceride synthesis by ^1H magnetic resonance spectroscopy (MRS) and liver and muscle glycogen synthesis by ^{13}C MRS after ingestion of a carbohydrate-rich meal before and after one 45-min bout of exercise on an elliptical trainer. Additionally, we measured hepatic de novo lipogenesis by monitoring the incorporation of deuterium from deuterium-labeled water into plasma very-low-density lipoprotein (VLDL) triglycerides (5, 6). The advantage of studying this group of healthy, young, lean insulin resistant subjects is that they do not have the confounding factors typically associated with the metabolic syndrome (e.g., obesity, hypertension, and medications) and the earliest factors responsible for the pathogenesis of atherogenic dyslipidemia and NAFLD associated with the metabolic syndrome can be examined.

Results

All subjects were young, lean, and insulin resistant as defined by an insulin sensitivity index (ISI) below 3.9×10^4 dL/min per microunit/mL, which is in the lower quartile of the ISI distribution for healthy, young, lean, sedentary individuals (7) (Table 1). Fasting plasma glucose (5.1 ± 0.1 vs. 5.0 ± 0.2 mmol/L) and fasting plasma insulin (9.2 ± 0.9 vs. 9.3 ± 1.0 microunits/mL) as well as postprandial plasma glucose, insulin, and C-peptide concentrations were similar at both visits (Fig. 1 A–C). Similarly, fasting and postprandial plasma triglyceride and plasma fatty acid concentration did not differ between study days (Fig. 1 D and E). Plasma triglyceride concentrations increased as a result of the meals and decreased during the night, whereas plasma fatty acid concentrations were suppressed by food intake and increased during the night (Fig. 1 D and E). Mean liver and muscle glycogen concentrations were 105 ± 9 mmol/L and 168 ± 12 mmol/L, respectively, on the morning of the resting studies and 78 ± 6 mmol/L and 152 ± 13 mmol/L, respectively, on the morning after the exercise studies. Mean intramyocellular lipid (IMCL) and intrahepatic lipid content (IHL) were $1.29 \pm 0.11\%$ and $0.81 \pm 0.21\%$, respectively, on the morning of the resting

Table 1. Subject baseline characteristics

	Baseline characteristics
No. of subjects	12
Age, yr	24 ± 1
Body weight, kg	77.8 ± 2.5
Body Mass Index, kg/m^2	23.8 ± 0.7
Body fat, %	16.5 ± 1.6
Lean body mass, kg	64.8 ± 1.8
Insulin Sensitivity Index, dL/min per microunits/mL	2.7 ± 0.1
Activity, miles per day	2.3 ± 0.2

studies and 1.10 ± 0.07% and 0.76 ± 0.21%, respectively, on the morning after the exercise studies.

The respiratory quotient (RQ) measured by indirect calorimetry increased similarly after the meals during the resting visit (from 0.81 ± 0.02 to 0.93 ± 0.01; $P < 0.001$) and during the exercise visit (from 0.82 ± 0.01 to 0.92 ± 0.01; $P < 0.001$). There were no differences in the change in RQ between the baseline visit and the exercise visit (0.11 ± 0.02 vs. 0.10 ± 0.02; $P =$ not significant). Basal and postprandial rates of oxygen consumption (V_{O2}) and rates of carbon dioxide expiration (V_{CO2}) did not differ between study days.

The single bout of exercise resulted in a more than threefold increase in postprandial muscle glycogen synthesis (from 6.2 ± 2.3 to 28.5 ± 2.9 mmol/L muscle; $P < 0.001$) (Fig. 2) and a 40% reduction in hepatic triglyceride synthesis (Fig. 3). Postprandial hepatic glycogen synthesis did not differ between the resting and the exercise study days (Fig. 3). The single bout of exercise also resulted in a 27% reduction in hepatic de novo lipogenesis after carbohydrate ingestion (from 13.2 ± 1.0 to 9.7 ± 0.5%; $P < 0.01$) (Fig. 3). Intramyocellular lipid content did not differ between baseline and exercise measurements, and did not change as a result of the meals (Fig. 2).

Discussion

We found that reversal of muscle insulin resistance, with one bout of exercise, increased postprandial muscle glycogen synthesis and decreased postprandial hepatic de novo lipogenesis and hepatic triglyceride synthesis in young, lean, insulin resistant individuals. Taken together, these data demonstrate that a single bout of exercise can have a profound effect on the distribution and metabolic fate of ingested carbohydrate by diverting ingested carbohydrate away from the liver and into the muscle, thereby reducing hepatic de novo lipogenesis and net hepatic triglyceride synthesis. This study offers strong evidence in support of the hypothesis that muscle insulin resistance promotes the early development of atherogenic dyslipidemia and NAFLD and that it is an early factor in the pathogenesis of the metabolic syndrome (3).

The advantage of examining this question in healthy, young, lean, insulin-resistant individuals is that they have none of the other confounding factors that are typically associated with obesity and type 2 diabetes, which have been postulated to play a major role in causing the metabolic syndrome. Therefore, the role of skeletal muscle insulin resistance per se in the pathogenesis of atherogenic dyslipidemia can be examined at its earliest stages. Furthermore, previous studies have demonstrated that insulin resistance in these subjects can mostly be attributed to defects in insulin-stimulated muscle glycogen synthesis caused by defects in insulin-stimulated glucose transport activity in skeletal muscle (8). We found that one bout of exercise increased postprandial muscle glycogen synthesis more than threefold. These results are consistent with our prior studies that found that one bout of exercise increased insulin-stimulated muscle glycogen synthesis by ≈70% in young, lean, insulin resistant individuals during a hyperinsulinemic-hyperglycemic clamp (4). Using ^{31}P NMR to monitor intramyocellular glucose-6-phosphate concen-
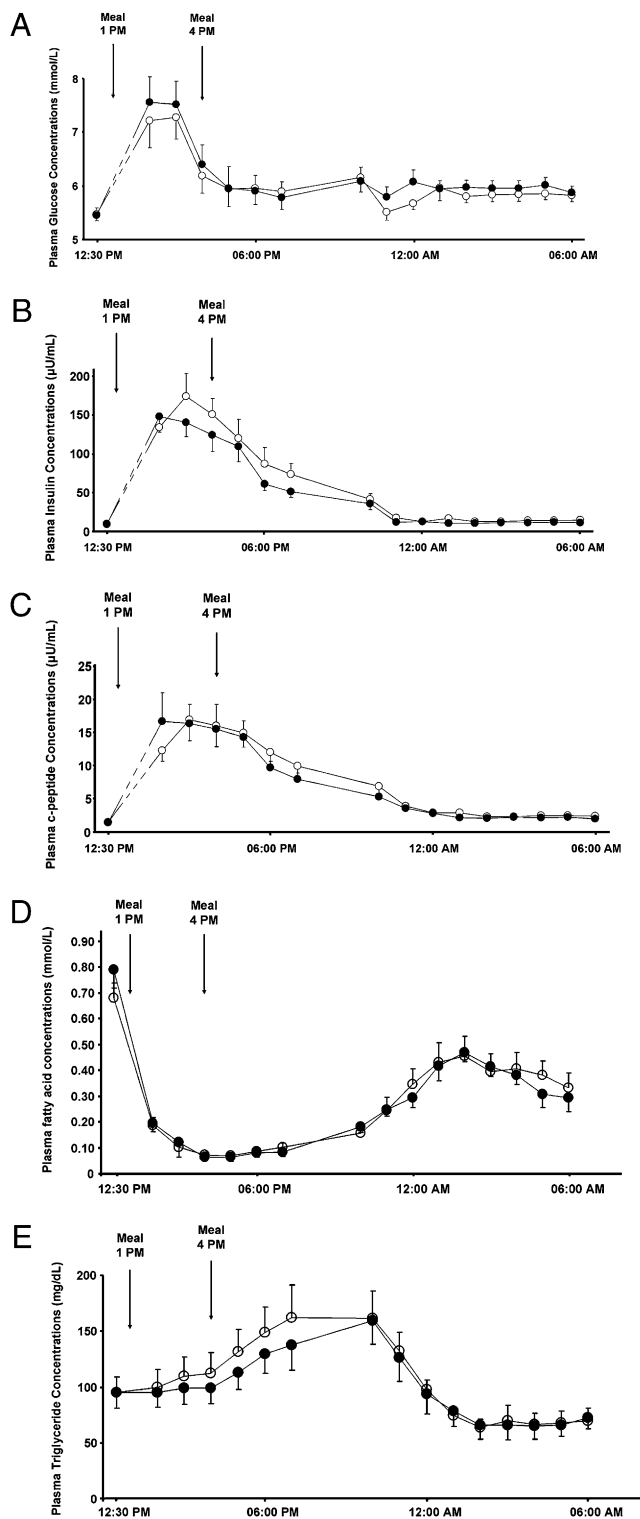
Fig. 1. (*A*) Plasma concentrations of glucose in response to two carbohydrate-rich mixed meals ingested at 1 PM and 4 PM resting study (○) and exercise study (●). (*B*) Plasma concentrations of insulin in response to two carbohydrate-rich mixed meals ingested at 1 PM and 4 PM resting study (○) and exercise study (●). (*C*) Plasma concentrations of c-peptide in response to two carbohydrate-rich mixed meals ingested at 1 PM and 4 PM resting study (○) and exercise study (●). (*D*) Plasma concentrations of fatty acids in response to two carbohydrate-rich mixed ingested served at 1 PM and 4 PM resting study (○) and exercise study (●). (*E*) Plasma concentrations of triglycerides in response to two carbohydrate-rich mixed meals ingested at 1 PM and 4 PM resting study (○) and exercise study (●).
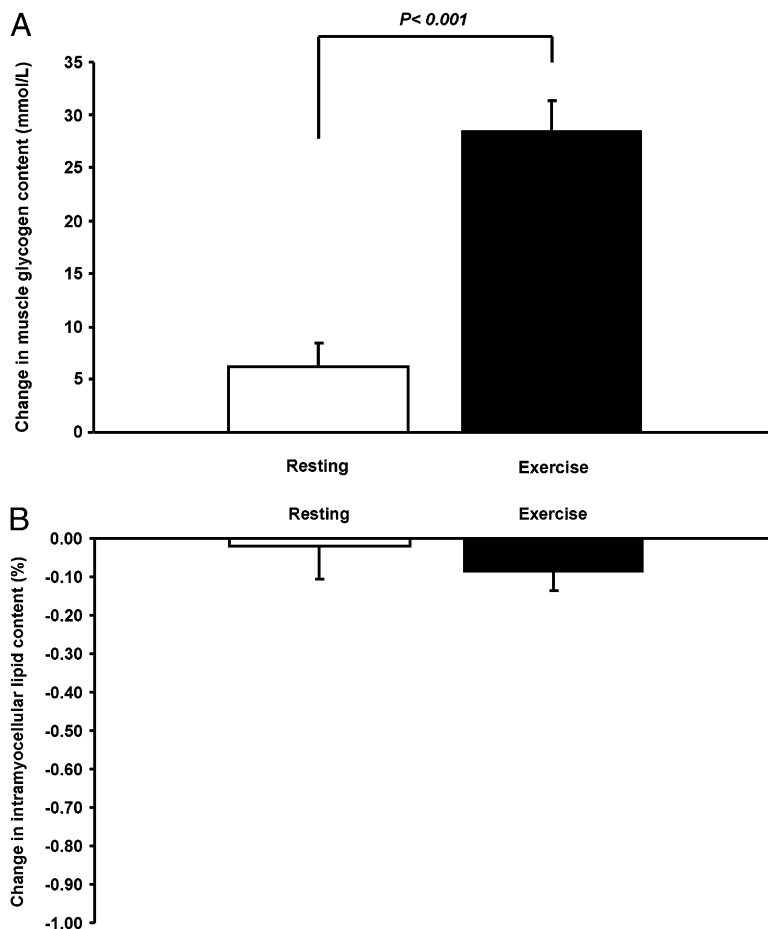
Fig. 2. (*A*) ^{13}C MRS measurements of changes in muscle glycogen concentrations for the resting and exercise study. (*B*) ^{1}H MRS measurements of changes in intramyocellular lipid concentration for the resting and exercise study.

trations, it could be demonstrated that this improvement in insulin-stimulated muscle glycogen synthesis could be attributed to reversal of defects in insulin-stimulated muscle glucose transport/phosphorylation activity (9).

Exercise did not induce changes in postprandial hepatic glycogen synthesis in our young, insulin resistant subjects. Taken together these findings support the hypothesis that skeletal muscle insulin resistance precedes hepatic insulin resistance in young, lean, insulin resistant individuals and that muscle insulin resistance leads to postprandial increases in net hepatic triglyceride synthesis, potentially leading to NAFLD. NAFLD is now the most common liver disease in both adults and children, and hepatic insulin resistance is strongly linked to NAFLD, which is a major factor responsible for the transition from normoglycemia to impaired glucose tolerance and type 2 diabetes (10–14).

It is also noteworthy that we, despite observing major differences in intra- and interorgan postprandial transfer of metabolic energy in the form of glucose and lipid after exercise, did not find any detectable differences in fasting or postprandial plasma glucose, fatty acids, triglycerides, insulin, and C-peptide concentrations. These observations demonstrate the importance of monitoring intracellular processes in an organ-dependent fashion for delineating the pathophysiology of the metabolic syndrome.

In conclusion, we have shown that one bout of exercise improved postprandial skeletal muscle glycogen synthesis and decreased postprandial de novo lipogenesis and hepatic triglyceride synthesis in young, lean, insulin resistant individuals (Fig. 4). These data demonstrate that muscle specific insulin resistance, due to reduced insulin-stimulated muscle glycogen synthesis, alters the distribution

pattern of postprandial energy storage resulting in increased hepatic de novo lipogenesis and increased net hepatic triglyceride synthesis. Chronically these processes will lead to hypertriglyceridemia, reductions in plasma HDL concentrations, and NAFLD. Taken together these data suggest muscle insulin resistance is one of the earliest factors in the pathogenesis of the metabolic syndrome and reversing muscle insulin resistance, by exercise or pharmacological intervention, may be an excellent therapeutic strategy for the treatment and prevention of atherogenic dyslipidemia and NAFLD in young insulin resistance individuals who are prone to develop the metabolic syndrome and type 2 diabetes.

Methods

Twelve young (age: 24 ± 1 y), lean (body mass index: 23.8 ± 0.7 kg/m^2), insulin resistant volunteers (10 Caucasian/2 African-American) were identified from a group of n = 88 healthy, lean, nonsmoking, male volunteers with normal birth weight, normal glucose tolerance, and a sedentary lifestyle. Insulin sensitivity was assessed by the ISI (15), and subjects were selected from the lower quartile of the ISI distribution (3).

All subjects completed a diet history and physical activity questionnaires (16), and their sedentary lifestyle was confirmed by activity monitoring for three consecutive days by using pedometers. Body composition was measured by bioelectrical impedance (Tanita BC-418; Tanita). All meals and snacks were provided for 3 d before each study to ensure that all subjects consumed a eucaloric diet containing 55% carbohydrate, 10% protein, 35% fat. These meals consisted of regular food and were prepared by the Metabolic Kitchen of the Yale Center for Clinical Investigation Hospital Research Unit (HRU) (17).

On study day 1, the subjects were admitted to the HRU at 5 PM, served dinner at 6 PM (33% of their daily caloric intake), and remained fasting until the first meal the following day (Day 2). On Day 2 at 8:30 AM, the subjects
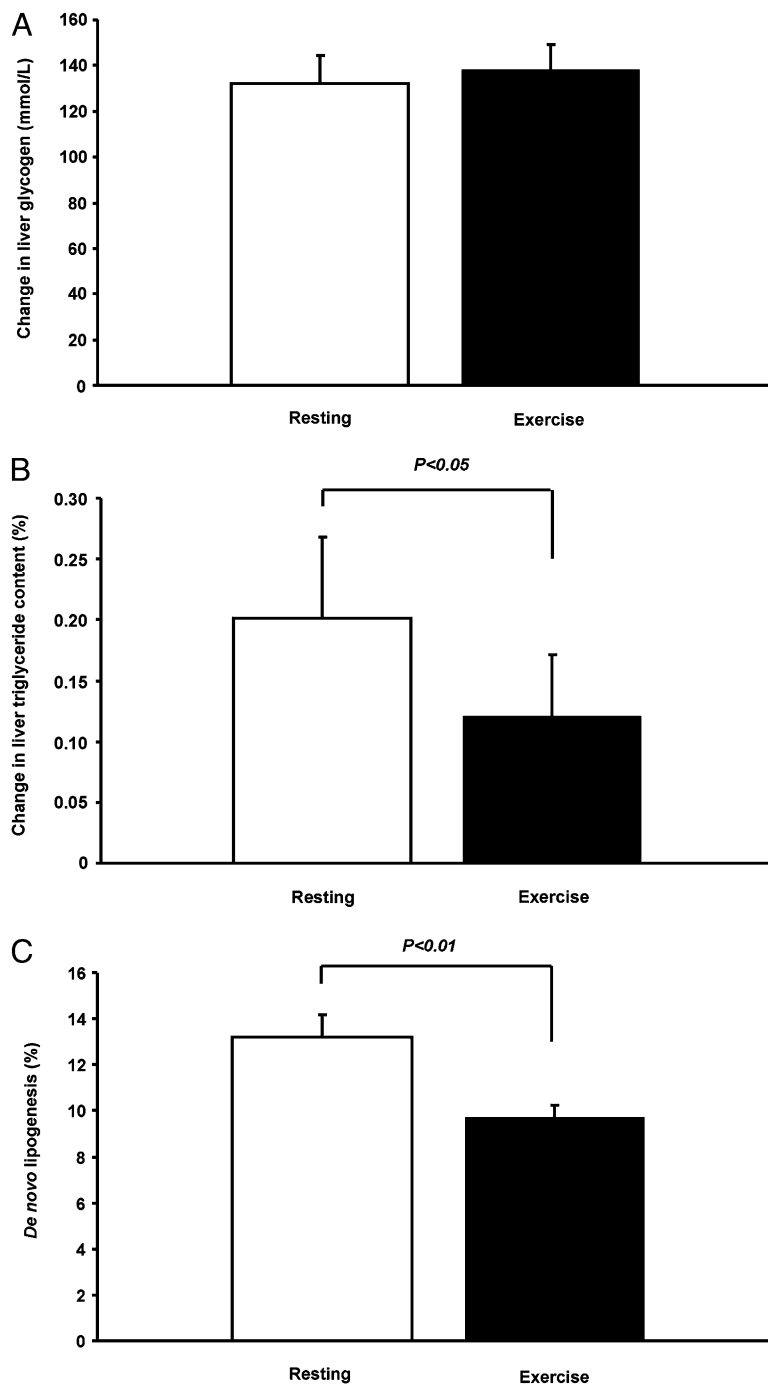
Fig. 3. (*A*) ^{13}C MRS measurements of changes in liver glycogen concentrations for the resting and exercise study. (*B*) ^{1}H MRS measurements of changes in liver triglyceride content for the resting and exercise study. (*C*) Hepatic de novo lipogenesis for the resting and exercise study.

were transported in a wheelchair to the Yale Magnetic Resonance Research Center (MRRC) for measurements of baseline muscle and liver lipid and glycogen content by using ^{1}H/^{13}C MRS. These measurements were begun at 9 AM and completed by 11:30 AM. The subjects were then returned to the HRU where an i.v. line was inserted into an antecubital vein and baseline blood samples were collected. The liquid high carbohydrate meal was served in two portions of equal size at 1 PM and 4 PM. The meals were prepared by the HRU Metabolic Kitchen and contained all of the required daily energy (35 kcal/kg body weight, 55% carbohydrate, 10% protein, 35% fat) with an additional 25% of the daily energy requirements added in the form of sucrose (3). At 2 PM and 2:30 PM, loading doses of deuterium-labeled water ([2]H$_2$O) were given orally (3 mL [2]H$_2$O per kg of body water; 99.8%, Cambridge Isotopes). Plasma deuterium enrichment was maintained by pro-

viding the subjects with deuterium-labeled water (0.45% [2]H$_2$O) for ad lib drinking during the remaining part of the study.

At 7:30 PM, the subjects were returned to the MRRC and, at 8 PM, postprandial muscle and liver lipid and glycogen contents were measured by using ^{1}H/^{13}C MRS. The subjects stayed in bed throughout the study (except for bathroom breaks), and all transport to and from the MRRC was done by wheelchair.

The exercise study included a total of 45 min of exercise on an elliptical trainer from 7 AM to 8 AM on Day 2 before the baseline measurements of liver and muscle glycogen and lipid content. The subjects performed three sets of 15 min of exercise on a Nautilus NE 2000 elliptical trainer (Nautilus) at 75–85% of their calculated maximal heart rate. The subjects were allowed 5 min of rest between sets. The order of the exercise study was randomized to control for a potential effect of adaptation to the study protocol.
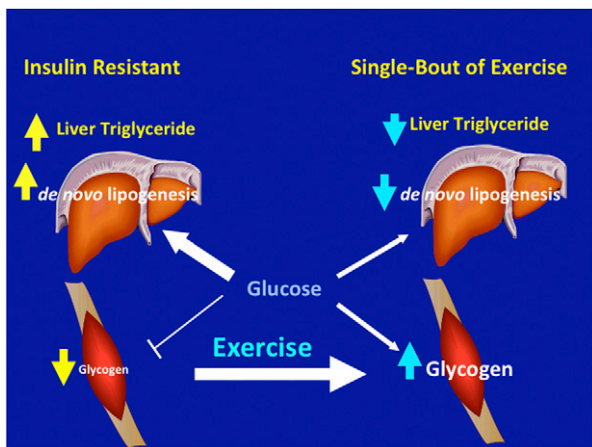
Fig. 4. Schematic of whole-body energy distribution after a carbohydrate-rich meal at rest and after one bout of exercise in young, lean, insulin resistant subjects.

On both the resting and the exercise study day, indirect calorimetry (Deltatrac; Datex-Ohmeda) was performed in the fasted state (at 6 AM on Day 2) and in the postprandial state (at 6 PM on Day 2).

Hepatic de Novo Lipogenesis. The incorporation of deuterium into plasma VLDL during administration of deuterium-labeled water was used to determine the fractional synthetic rate of fatty acids as described (5, 6). Blood was collected to assess de novo lipogenesis before the first dose of deuterium-labeled water and hourly from 10 PM until 6 AM (18). After centrifugation, plasma was split into two parts, one part was stored at −20 °C until analysis, and the other part was processed immediately by ultracentrifugation for purification and removal of chylomicrons and plasma triglyceride extraction (5, 6). Deuterium enrichment in palmitate and plasma water was measured by gas chromatography–mass spectrometry (GC-MS) (5971A Mass Selective Detector; Hewlett–Packard) as described (5, 6), and fractional rates of de novo lipogenesis were calculated as described (19).

^{1}H/^{13}C MRS Measurements of Triglyceride and Glycogen Content in Liver and Muscle. All MRS measurements were performed on a whole-body, 4.0-T Medspec (Bruker) system. Muscle lipid content was measured in the soleus muscle by using an 8.5-cm diameter, circular ^{13}C surface coil with twin, orthogonal circular 13-cm ^{1}H coils arranged in quadrature as previously described (7, 20–22). Then after repositioning the subject, ^{13}C MRS spectra were acquired in a $40 \times 80 \times 80$ mm^3 volume placed within the vastus lateralis by using a coil assembly composed of a 8-cm, circular ^{13}C coil and twin 11×14 cm elliptical ^{1}H coils arranged in quadrature. Protons were decoupled during the 25-ms acquisition time with a WALTZ-4, and localization of the volume was performed by using 3D adiabatic outer-volume suppression. Glycogen content was calculated by comparison to a glycogen solution of known concentration.

Intrahepatic lipid content was measured with a coil assembly composed of a 12-cm, circular ^{13}C coil and twin 13×19 cm elliptical ^{1}H coils arranged in quadrature as described (7). The liver triglyceride content was measured by ^{1}H respiratory-gated stimulated echo acquisition mode sequence spectroscopy in a $15 \times 15 \times 15$ mm^3 voxel (21, 22). Acquisition was synchronized to the respiratory cycle and triggered at the end of expiration. A water-suppressed lipid spectrum and a lipid-suppressed water spectrum were acquired and carried out in three different locations of the liver. The total lipid content was expressed as a percentage of the water content, averaged for the three locations and corrected for T1 and T2 relaxation. Liver glycogen content was acquired with the same coil assembly and method as the one used for the muscle glycogen content.

Analytical Methods. Plasma glucose concentrations were measured by using a YSI STAT 2700 Analyzer (Yellow Springs Instrument). Plasma concentrations of insulin and C-peptide were measured with double-antibody RIA kits (Linco). Plasma fatty acid and triglyceride concentrations were determined by using a microfluorimetric method (24).

Statistical Analysis. Statistical analyses were performed by using the StatView package (Abacus Concepts). Paired Student's t tests were performed where appropriate to detect statistical differences. All data are expressed as mean ± SEM.

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