

Adding fat calories to meals after exercise does not alter glucose tolerance

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Fox, Amanda K., Amy E. Kaufman, and Jeffrey F. Horowitz. Adding fat calories to meals after exercise does not alter glucose tolerance. *J Appl Physiol* 97: 11–16, 2004. First published February 20, 2004; 10.1152/jappphysiol.01398.2003.—A single session of exercise increases insulin sensitivity for hours and even days, and dietary carbohydrate ingested after exercise alters the magnitude and duration of this effect. Although increasing systemic fatty acid availability is associated with insulin resistance, it is uncertain whether increasing dietary fat availability after exercise alters the exercise-induced increase in insulin sensitivity. The purpose of this study was to determine whether adding fat calories to meals after exercise alters glucose tolerance the next day. Seven healthy men cycled 90 min at $66 \pm 2\%$ peak oxygen uptake followed by a maximum of five high-intensity intervals. During the hours after exercise, subjects ingested three meals containing either low-fat (5% energy from fat) or high-fat (45% energy from fat) foods (Low-Fat and High-Fat groups, respectively). Each diet contained the same amount of carbohydrate and protein. An oral glucose tolerance test was performed the next morning. Muscle glycogen and intramuscular triglyceride (IMTG) concentrations were measured in muscle biopsy samples obtained immediately before exercise and the next morning. The day after exercise, muscle glycogen concentration was identical in High-Fat and Low-Fat (393 ± 70 and 379 ± 38 mmol/kg dry wt). At the same time, IMTG concentration was $\sim 20\%$ greater during High-Fat compared with Low-Fat (42.5 ± 3.4 and 36.3 ± 3.3 mmol/kg dry wt; $P < 0.05$). Despite the addition of ~ 165 g of fat to meals after exercise ($\sim 1,500$ kcal) and a resultant elevation in IMTG concentration, glucose tolerance was identical in High-Fat and Low-Fat (composite index: 8.7 ± 1.0 and 8.4 ± 1.0). In summary, as long as meals ingested in the hours after exercise contain the same carbohydrate content, the addition of $\sim 1,500$ kcal from fat to these meals did not alter muscle glycogen resynthesis or glucose tolerance the next day.

diabetes; insulin sensitivity; intramuscular triglycerides; muscle glycogen

A SINGLE SESSION OF EXERCISE can increase insulin sensitivity for hours or even days, and the meals ingested after exercise are known to have a potent influence on the magnitude and duration of this response (5, 35). Muscle glycogen concentration dictates much of this acute increase in insulin sensitivity after exercise (12, 24, 25). Therefore, an increased availability of dietary carbohydrate in the hours after exercise and the resultant increase in muscle glycogen resynthesis reverses the exercise-induced increase in insulin sensitivity (5, 25). However, it is not known whether increasing the amount of dietary fat in the hours after exercise also alters resting insulin sensitivity.

Elevated fatty acid availability and intracellular accumulation of fatty acid derivatives decrease glucose transport (13, 22, 43), storage, and oxidation (4, 36). These inhibitory effects of fat on glucose metabolism have been reported in response to

elevated plasma fatty acid concentrations via exogenous triglyceride and heparin infusion. It is not clear whether a large amount of dietary fat can inhibit glucose metabolism similarly. Additionally, accumulation of triglyceride in muscle [intramuscular triglycerides (IMTG)] has been found to be associated with impaired insulin sensitivity (20, 30, 31). Although IMTG are likely inert and do not directly influence insulin sensitivity, it is hypothesized that accumulation of by-products from IMTG hydrolysis may interfere with the insulin-signaling pathway (15, 23). Resynthesis of IMTG after exercise is largely determined by the fat content in the meals ingested after exercise (11, 27), and it is unknown whether an augmented IMTG resynthesis after exercise alters the exercise-induced increase in insulin sensitivity.

Previous studies examining the effect of high-fat meals on glucose metabolism and insulin sensitivity typically compare isocaloric diets and, therefore, reduce dietary carbohydrate content to compensate for the increase in dietary fat (11, 18). However, because a change in carbohydrate availability and the resultant change in muscle glycogen resynthesis are major regulators of insulin sensitivity, the strategy of reducing dietary carbohydrate to maintain caloric balance does not distinguish between the effects of the added dietary fat from the effects of ingesting less carbohydrate. The primary purpose of this study was to determine whether adding fat calories to meals after exercise impairs glucose tolerance the next day.

MATERIALS AND METHODS

Subjects

Seven healthy, active men volunteered to participate in this study (Table 1). Subjects were screened with an aerobic fitness test [peak oxygen uptake ($\dot{V}O_{2\text{ peak}}$)], fasting blood glucose test, and body composition analysis using hydrostatic weighing. No subject had any evidence of metabolic or cardiovascular disorders nor were any of them taking prescription medications. All subjects regularly performed aerobic exercise (i.e., 30–60 min of cycling or jogging at 60–80% of their estimated maximal heart rate at least three times per week). All subjects were fully informed of the possible risks associated with the study and signed an informed consent form approved by the University of Michigan Institutional Review Board.

Preliminary Testing

Body fat percentages for all subjects were measured via the two-compartment method by hydrostatic weighing (1). $\dot{V}O_{2\text{ peak}}$ was measured (PhysioDyne Technologies, Quogue, NY) during upright cycle ergometer exercise to assess cardiorespiratory fitness. This $\dot{V}O_{2\text{ peak}}$ protocol consisted of a 4-min warm-up, after which the workload was progressively increased every minute until volitional fatigue. $\dot{V}O_{2\text{ peak}}$ was affirmed by at least two of the following criteria: 1) a leveling off of the rate of oxygen consumption ($\dot{V}O_2$), despite

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Table 1. *Subject characteristics*

Age	30.5 ± 3.3
Height, in.	72.0 ± 0.9
Weight, kg	76.4 ± 1.3
Body mass index, kg/m ²	22.9 ± 0.4
Body fat, %	14.8 ± 1.3
Fasting plasma glucose, mmol/l	4.9 ± 0.1
$\dot{V}O_{2\text{peak}}$, ml · kg ⁻¹ · min ⁻¹	55.5 ± 1.6

Values are means ± SE. $\dot{V}O_{2\text{peak}}$, peak oxygen consumption.

increases in workload, 2) respiratory exchange ratio >1.15, and 3) attainment of age-predicted maximal heart rate.

Experimental Protocol

All subjects performed two experimental trials lasting ~2 days each. These two trials differed only by the content of the meals ingested after exercise (see *Experimental Diets*). For 2 days before each trial, subjects were instructed to eat a standard diet (~60% carbohydrate, 30% fat, and 10% protein). They recorded their food intake during these days and ate the same diets for 2 days before their second trial. The day before each trial, subjects were provided with an evening meal (0.50 g fat/kg, 2.0 g carbohydrate/kg, 0.31 g protein/kg), which was eaten at home and completed at 1900. After this meal, subjects refrained from consuming anything except water. The next morning (*day 1*), subjects arrived at the laboratory at 0700 after an overnight fast (12 h). After subjects rested supine for 30 min, we measured resting $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) using a metabolic cart (PhysioDyne Technologies, Quogue, NY) to calculate resting energy expenditure (i.e., indirect calorimetry). These indirect calorimetry data were collected for at least 20 min while the subjects were lying down, awake but undisturbed, in a dark, quiet room. After this measurement, a blood sample was taken to measure basal plasma glucose and insulin concentrations. Then an initial muscle biopsy was obtained from the vastus lateralis muscle of the thigh using the percutaneous biopsy technique (3). After the biopsy incision was dressed and wrapped, subjects exercised on a stationary bicycle (Lode ergometer, Gronigen, Netherlands) for 90 min at ~65% $\dot{V}O_{2\text{peak}}$, followed by a maximum of five high-intensity intervals (4 min at ~85% $\dot{V}O_{2\text{peak}}$, interspersed by 2 min at ~50% $\dot{V}O_{2\text{peak}}$) lasting a maximum of 30 min (or to exhaustion) in attempt to deplete muscle glycogen and IMTG stores. Exhaustion criterion was established as a subject's inability to maintain the power output necessary to elicit 85% $\dot{V}O_{2\text{peak}}$. Indirect calorimetry data were collected intermittently throughout exercise to ensure that subjects were exercising at the appropriate intensity and to calculate energy expenditure during exercise. Subjects received 200 ml of water every 20 min during exercise trials. After exercise, subjects were fed a low-fat or high-fat meal in accordance with the specific trial (see *Experimental Diets* below). For both trials, equal portions of the diet were ingested at 1045, 1230, and 1900.

Table 2. *Experimental diets and 24-h energy balance*

	Low-Fat			High-Fat		
	g	kcal	%kcal	g	kcal	%kcal
Carbohydrate	378 ± 6.1	1,513 ± 25	77%	384 ± 6.4	1,537 ± 26	44%
Fat	12 ± 0.2	105 ± 1.6	5%	177 ± 3.0	1,590 ± 27	46%
Protein	87 ± 1.4	346 ± 5.7	18%	86 ± 1.4	345 ± 5.6	10%
Total kcal ingested		1,965 ± 32*			3,472 ± 58	
Estimated kcal expended		3,493 ± 65			3,463 ± 78	
Energy balance, kcal		-1,527 ± 51*			9 ± 52	

Values are means ± SE. All values represent energy intake, expenditure, and energy balance during the 24-h experiments trials. *Significantly different from High-Fat, $P < 0.05$.

On *day 2* of each trial, subjects arrived at the laboratory at 0700 after an overnight fast. After subjects were allowed 30 min of supine rest, $\dot{V}O_2$ and $\dot{V}CO_2$ were measured for 20 min to calculate resting energy expenditure while the subjects rested quietly. After this measurement, an intravenous catheter was inserted into an antecubital vein and an initial blood sample was obtained. Then another muscle biopsy was obtained from the vastus lateralis of the leg not biopsied the day before. After this biopsy, a 3-h oral glucose tolerance test (OGTT) was administered to provide an index of insulin sensitivity. During this procedure, subjects ingested 75 g of glucose in a 25% solution (Sun Dex, Fisherbrand), and blood samples were withdrawn every 15 min for 2 h and every 30 min for an additional hour.

During each trial, subjects performed no additional activity other than the exercise performed in the laboratory on *day 1*. Between trials, subjects were instructed to continue their regular exercise regimen. They performed identical exercise bouts 3 days before each experimental trial and did not exercise 2 days before each trial. Trial order was counterbalanced and trials were separated by at least 1 wk.

Experimental Diets

The low-fat diet (Low-Fat) contained 77% carbohydrate, 5% fat, and 18% protein, whereas the high-fat diet (High-Fat) contained 44% carbohydrate, 46% fat, and 10% protein. High-Fat provided 2.3 g fat · kg body wt⁻¹ · day⁻¹, whereas Low-Fat provided 0.15 g fat · kg body wt⁻¹ · day⁻¹. Both diets contained exactly the same amount of carbohydrate (5 g fat · kg body wt⁻¹ · day⁻¹) and protein (1.1 g fat · kg body wt⁻¹ · day⁻¹). The only difference between the trials was that ~55 g of fat were added to each of the three meals during High-Fat (i.e., total of ~165 g more fat ingested during High-Fat compared with Low-Fat). Data describing each of the diets are presented in Table 2. Because the exercise-induced increase in insulin sensitivity is known to be greatly influenced by the amount of carbohydrate ingested after exercise (5), it was critical to match the diets for carbohydrate availability to determine the effect of dietary fat availability on the exercise-induced increase in insulin sensitivity. Therefore, the total caloric content was different between trials. Caloric content of High-Fat was designed to match the estimated total energy expenditure during the ~24-h trial, whereas the Low-Fat was designed to create an ~1,500 kcal energy deficit.

Blood and Muscle Sample Preparation

Blood samples were transferred from the syringe to chilled test tubes containing 0.03 mmol EDTA and 0.5 trypsin inhibitory units/ml aprotinin for analysis of glucose and insulin concentration. All samples were kept on ice and then centrifuged (1,600 g for 10 min at 4°C) within 30 min of collection. After centrifugation, the plasma from each sample was transferred into 12 × 75 mm plastic culture tubes and immediately frozen and stored at -80°C for later analysis. Muscle biopsy samples were frozen in liquid nitrogen within ~30 s of removal and stored at -80°C until biochemical analysis.

Analytical Procedures

Plasma insulin and glucose concentrations. Plasma insulin concentration was measured by radioimmunoassay kit (Linco Research, St. Charles, MO). Plasma glucose concentration was measured with a glucose autoanalyzer (Analox Instruments; Lunenburg, MA).

Muscle substrates. Muscle biopsy samples were lyophilized at -60°C for 48 h. Aliquots of dried muscle were weighed (~ 10 mg) and separated for analysis of muscle glycogen and IMTG concentrations. For muscle glycogen, the samples were mechanically homogenized in a glycerol- Na_2HPO_4 buffer, hydrolyzed in 2 N HCl for 2 h, and neutralized with NaOH, and then glucose concentration was determined enzymatically (32). For IMTG concentration, triglycerides were extracted from the dried muscle sample by use of a 2:1 chloroform-methanol solution and saponified in ethanolic KOH (16). Free glycerol concentration in these samples was determined fluorometrically (14).

Calculations

Area under the curve. Area under the curve for glucose vs. time and insulin vs. time were calculated during the 3-h OGTT using the trapezoidal rule.

Indexes of glucose tolerance and insulin sensitivity. The following calculations were used to assess insulin sensitivity:

	Reference
Composite index: $10,000 \times \sqrt{(\text{FPG} \times \text{FPI}) \times (\text{G} \times \text{I})}$	29
Cederholm index: $\text{Glucose uptake}/(\text{G} \times \log \text{I})$	7
Belfiore index: $2/[(\text{G} \times \text{I})/(\text{C} + 1)]$	2
HOMA: $k/(\text{FPI} \times \text{FPG})$	39

where FPG and FPI are fasting plasma glucose and insulin concentrations, respectively; G and I are mean plasma glucose and insulin concentrations during the 3-h OGTT, respectively; HOMA is homeostatic model assessment (39); $C = 1,000$; and $k = 22.5 \times 18$.

Energy expenditure. Resting metabolic rate was calculated from resting $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ measurements using the Weir equation (41). To account for energy expenditure due to activities of daily living during the inactive period of the day, multiplying resting metabolic rate by 1.2 has been found to provide a reasonable estimation of daily energy expenditure when inactive (37). Exercise energy expenditure was calculated from the $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ measured during exercise. Twenty-four-hour energy expenditure was calculated as the weighted sum of resting energy expenditure (~ 22 h) and exercise energy expenditure (~ 2 h).

Statistical analysis. A two-way ANOVA for repeated measures (treatment \times day) with Tukey's post hoc analysis was used to determine differences among trials and between days for muscle glycogen and IMTG concentrations and plasma concentrations of glucose and insulin. A one-way ANOVA for repeated measures with Tukey post hoc analysis was used to assess differences among trials for indexes of insulin sensitivity and glucose tolerance. A P value of ≤ 0.05 was considered statistically significant.

RESULTS

Responses During Exercise

Subjects cycled continuously for 90 min at $66 \pm 2\%$ $\dot{V}\text{O}_{2\text{peak}}$ during both trials. The exercise intensity during this 90-min period was identical in the Low-Fat and High-Fat trials, as evidenced by the same $\dot{V}\text{O}_2$ (2.8 ± 0.1 and 2.8 ± 0.1 l/min), power output (202 ± 10 and 202 ± 10 W), and heart rate responses (151 ± 6 and 149 ± 5 beats/min). Immediately after this 90-min steady-state exercise bout, subjects performed a maximum of five high-intensity intervals (255 ± 9 W) for 4 min with 2 min of low-intensity (140 ± 3 W) recovery periods

between intervals. Three of the seven subjects completed all five of the high-intensity intervals during both trials. The remaining four subjects completed two to four intervals before exhaustion during their first trial, and the same number of intervals was repeated during their second trial. Therefore, in all cases, subjects performed identical exercise bouts during their two trials.

Estimated Energy Expenditure and Energy Intake

Because the exercise protocol was the same in both trials, estimated energy expenditure during the exercise bouts was identical ($1,336 \pm 59$ kcal). One-day estimated resting energy expenditure was also the same in Low-Fat and High-Fat ($2,157 \pm 68$ and $2,127 \pm 55$ kcal, respectively) and the different diets did not affect measurements of resting energy expenditure the next morning. Therefore, estimated total daily energy expenditure was identical between trials (Table 2). As designed, caloric intake was markedly higher during High-Fat compared with Low-Fat (Table 2) because of the added fat calories to the meals during High-Fat (carbohydrate and protein contents were identical between trials). As a result, during High-Fat subjects ingested approximately the same number of kilocalories that they expended (i.e., energy balance), whereas during Low-Fat they were $\sim 1,500$ kcal below energy balance (i.e., $\sim 1,500$ kcal energy deficit).

Muscle Substrates

Muscle glycogen concentration. Subjects began exercise with identical muscle glycogen concentrations during Low-Fat and High-Fat (620 ± 55 and 629 ± 55 mmol/kg dry wt, respectively). Despite the difference in dietary fat content of the meals, ingesting the same carbohydrate content after exercise restored muscle glycogen concentrations to the same concentration in Low-Fat and High-Fat (379 ± 38 and 393 ± 70 mmol/kg dry wt, respectively). However, muscle glycogen concentrations were not replenished to basal concentrations in either trial (Fig. 1).

IMTG concentration. IMTG concentration was identical before exercise during Low-Fat and High-Fat (36.7 ± 1.8 and 37.5 ± 2.0 mmol/kg dry wt, respectively). High-Fat increased IMTG storage after exercise, and the next morning IMTG

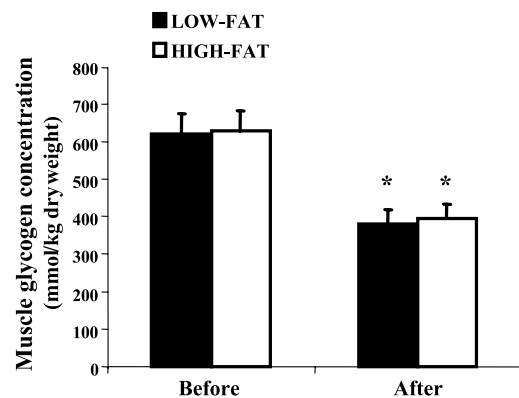


Fig. 1. Muscle glycogen concentration in muscle biopsy samples from the vastus lateralis before exercise on day 1 and in the morning of day 2, before the oral glucose tolerance test (OGTT). *Significantly different from day 1, $P < 0.05$.

concentration was $19 \pm 6\%$ greater during High-Fat compared with Low-Fat ($P < 0.05$) (Fig. 2).

Plasma Glucose and Insulin Concentrations

Plasma glucose and insulin. Plasma glucose and insulin concentrations during the OGTT were not affected by the preceding diet. The changes in plasma glucose and insulin concentrations after ingestion of the glucose load were remarkably similar during High-Fat and Low-Fat (Fig. 3).

Indexes of glucose tolerance and insulin sensitivity. Estimates of insulin sensitivity were calculated from plasma glucose and insulin concentrations measured before and during the OGTT (Table 3). These data confirm that there were no differences in glucose tolerance between Low-Fat and High-Fat.

DISCUSSION

The availability of dietary carbohydrate in the hours after exercise reduces the magnitude and duration of the exercise-induced increase in insulin sensitivity (5, 42). Although increased availability of fat in the circulation as well as inside the muscle cell has been linked to impaired insulin sensitivity (4, 13, 36), the major finding of the present study was that when dietary carbohydrate content was kept constant, the addition of ~ 165 g of fat to meals after exercise and a resultant 20% increase in intramuscular triglyceride concentration did not alter glucose tolerance the next day.

The increase in insulin sensitivity after exercise has been found to be directly proportional to the magnitude of muscle glycogen depletion during the exercise bout (5, 42). Alternatively, this increase in insulin sensitivity is reversed with the ingestion of carbohydrate after exercise and the subsequent increase in muscle glycogen concentration (5). Because the rate of muscle glycogen resynthesis is primarily dependent on dietary carbohydrate (8), we had our subjects ingest the same carbohydrate content during their low-fat and high-fat diets, and muscle glycogen concentration was identical between trials the next day. This was critical to the design of this study because it allowed us to determine the effect of adding fat to meals after exercise, without the confounding influence of differences in carbohydrate availability.

The relationship between increased systemic fatty acid concentration and impaired insulin sensitivity has been recognized for several decades (33). Increased fatty acid uptake has been found to interfere with the insulin signal responsible for

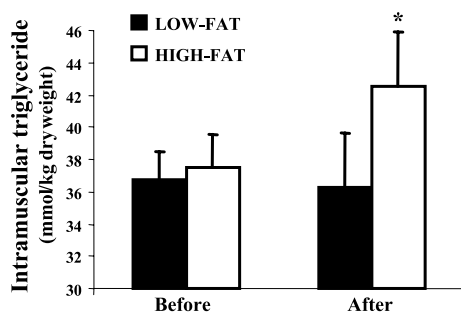


Fig. 2. Intramuscular triglyceride (IMTG) concentrations in muscle biopsy samples from the vastus lateralis before exercise of *day 1* and in the morning of *day 2* (before the OGTT). *Significantly different from Low-Fat, $P < 0.05$.

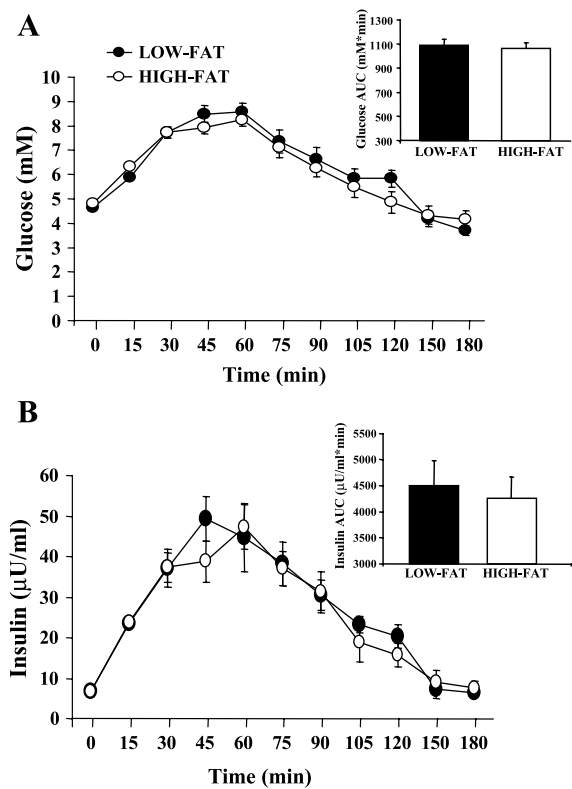


Fig. 3. Plasma glucose (A) and plasma insulin concentrations (B) during the OGTT the morning after exercise. Inset bar graphs display the integrated area under the curve (AUC) for each trial.

GLUT-4 translocation (4, 26, 43). Recently, it has been demonstrated that the accumulation of fatty acid intermediates (e.g., long-chain fatty acyl-CoA, diacylglyceride) within the cytosol of the muscle cell can disrupt the insulin signal within the cell (26, 43). Several studies have also associated insulin resistance with an accumulation of triglycerides within the muscle cell (20, 30, 31), but the direct effect of IMTG concentration on insulin sensitivity has been questioned (17, 23). Although our findings agree with other recent reports demonstrating that alterations in dietary fat can influence the resynthesis of IMTG in the hours and days after exercise (10, 11, 27), the increase in IMTG storage in our study did not impair glucose tolerance. The inverse relationship between IMTG concentration and insulin sensitivity has only been demonstrated in sedentary subjects (20, 31). Our findings agree with evidence from endurance-trained athletes (21), suggesting that a relationship between IMTG and insulin resistance is not causal. It is more likely that IMTG deposits themselves are inert

Table 3. Indexes of glucose tolerance during an OGTT the day after exercise

	Low-Fat	High-Fat
Composite index	8.4 ± 1.0	8.7 ± 1.0
Cederholm index	31.6 ± 1.2	32.0 ± 1.2
Belfiore index	0.98 ± 0.1	1.02 ± 0.1
HOMA	0.84 ± 0.2	0.79 ± 0.1

Values are means \pm SE. Composite index from Ref. 29; Cederholm index from Ref. 7; Belfiore index from Ref. 2; HOMA (Homeostatic model assessment) from Ref. 39. OGTT, oral glucose tolerance test.

and the accumulation of IMTG may simply serve as a marker for a high rate of fatty acid flux into the cell.

Our finding that muscle glycogen concentration was identical the morning after exercise whether the subjects ingested meals containing ~10 or ~175 g of dietary fat provides additional evidence that the ingestion of high-fat meals after exercise did not affect glucose tolerance. Skeletal muscle lipoprotein lipase activity, which catalyzes the hydrolysis of triglyceride-rich lipoproteins, is upregulated after exercise (19). An increase in lipoprotein lipase activity together with ~180 g of triglycerides provided in the high-fat diet would likely augment the availability and uptake of the fatty acids liberated from the exogenous triglycerides (as evidenced by an elevated IMTG concentration during High-Fat). Therefore, our findings suggest that, despite a probable increased availability and uptake of fatty acids due to a nearly 20-fold increase in dietary fat content, this increase in fat availability did not alter glycogen resynthesis after exercise. This may be explained by a repartitioning of the fatty acids taken up by the cell to oxidation and storage in IMTG. This exercise-induced repartitioning of the fatty acids may prevent the accumulation of fatty acid intermediates within the cytosol of the cell and thereby prevent interference of intracellular lipid availability on glucose uptake and glycogen synthesis. We hypothesize that, if exercise had not been performed before ingesting the high-fat diet, the augmented availability of exogenous fat may have impaired glucose metabolism. Therefore, exercise may override some of the negative effects of a high-fat diet.

An OGTT is widely used to provide a reasonable assessment of glucose tolerance and insulin sensitivity (28, 29, 38, 40). Several indexes have been derived to assess insulin sensitivity from an OGTT (2, 7, 29). Our findings demonstrate that the addition of ~165 g of fat to meals after exercise did not alter insulin sensitivity regardless of the calculation used. We recognize that more sensitive measures of insulin sensitivity (e.g., glucose clamp methods, minimal model method) may be able to detect very small differences in insulin sensitivity that may exist between High-Fat and Low-Fat. However, we have two independent outcomes (i.e., no differences in OGTT and identical muscle glycogen resynthesis) that concur to suggest that, as long as carbohydrate availability is the same, the content of fat in meals ingested after exercise does not alter glucose tolerance.

Although muscle glycogen concentrations were the same during Low-Fat and High-Fat, muscle glycogen was not restored to preexercise concentrations. To replenish muscle glycogen stores after exhaustive exercise, it has been recommended that ~500–600 g of carbohydrate should be ingested over the 24 h after exercise (9). To provide a vast difference in dietary fat between our trials while matching carbohydrate content, yet still maintain energy balance during High-Fat, the carbohydrate content in our study diets was about 25% less than the recommended amount for complete muscle glycogen repletion after exercise. Because a reduction in muscle glycogen concentration stimulates insulin sensitivity (12, 24), it is possible that the incomplete resynthesis of muscle glycogen on day 2 of our study may have eclipsed any inhibitory influence from the high-fat diet and the subsequent increase in IMTG concentration on glucose metabolism. As such, we cannot rule out the possibility that if muscle glycogen concentration was fully restored to basal concentrations on day 2 in both trials, the

excessive dietary fat may have indeed altered insulin sensitivity. Therefore, the magnitude of muscle glycogen resynthesis after exercise may impact the effect of dietary fat on the exercise-induced increase in insulin sensitivity. This alternative hypothesis further emphasizes the importance of endurance exercise and the subsequent reduction in muscle glycogen concentration that is primarily responsible for the exercise-induced increase in insulin sensitivity.

We recognize that our study design does not allow us to distinguish between the effect of added fat and simply the influence of added calories, and there was nearly a 1,500-kcal difference in caloric intake between High-Fat and Low-Fat. Because caloric deficit has been associated with enhanced insulin sensitivity (6, 34), our finding that glucose tolerance was not different between trials despite the relatively large energy deficit during Low-Fat underscores the importance of dietary carbohydrate availability rather than caloric balance in the regulation of glucose tolerance after exercise. We also acknowledge that our findings may only reflect responses to those individuals who are already reasonably insulin sensitive (i.e., healthy active young adults) and may not be representative of the population as a whole. However, we hypothesize that a reduction in insulin sensitivity under these conditions should be more pronounced in our nonobese volunteers than in abdominal obese persons with insulin resistance. First, the higher insulin sensitivity in healthy lean subjects makes it easier to detect subtle reductions in insulin action. Second, the addition of ~165 g of dietary fat to meals after exercise in our subjects represents a large increase in their systemic lipid availability. In contrast, persons with abdominal obesity already have excessive lipid availability (i.e., high lipolytic rates, high plasma triglyceride concentrations) and providing more exogenous dietary fat may not have the same potential to affect insulin sensitivity. However, it is certainly of great interest to determine whether acute exercise can also override some of the negative effects of a high-fat diet in persons with abdominal obesity and insulin resistance.

In summary, a single session of exercise is known to enhance insulin sensitivity for hours or even days. Although elevations in fat availability and IMTG concentration have been linked with insulin resistance, we found that a high-fat diet after exercise did not alter the exercise-induced increase in glucose tolerance, despite a ~20% increase in IMTG concentration. We conclude that when dietary carbohydrate and protein were kept constant, the addition of ~165 g of fat to meals after exercise did not alter the exercise-induced increase in glucose tolerance the next day.

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