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## Postexercise insulin sensitivity is not impaired after an overnight lipid infusion

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**Schenk, Simon, Jill N. Cook, Amy E. Kaufman, and Jeffrey F. Horowitz.** Postexercise insulin sensitivity is not impaired after an overnight lipid infusion. *Am J Physiol Endocrinol Metab* 288: E519–E525, 2005. First published November 2, 2004; doi:10.1152/ajpendo.00401.2004.—High plasma fatty acid availability and a positive energy balance in sedentary individuals reduce insulin sensitivity. This study's purpose was to determine whether high plasma fatty acid availability and systemic caloric excess after exercise also impair insulin sensitivity. On two separate occasions, seven nonobese women performed 90 min of exercise at ~65% peak oxygen uptake. In one trial, a lipid + heparin emulsion (Lipid) was infused overnight to increase plasma fatty acid availability. In the other trial, saline was infused as control. The next morning, a muscle biopsy was taken to measure muscle glycogen and intramuscular triglyceride (IMTG) concentrations. Three hours after the overnight infusion was stopped, insulin sensitivity was assessed with an intravenous glucose tolerance test, using minimal model analysis ( $S_i$ ). During the overnight infusions, plasma fatty acid concentration was approximately fourfold higher [means (SD): 0.84 (0.36) vs. 0.22 (0.09) mmol/l;  $P = 0.003$ ], and the next morning IMTG concentration was ~30% greater [49.2 (6.6) vs. 38.3 (7.7) mmol/kg dry wt;  $P = 0.036$ ] in Lipid compared with saline. However, muscle glycogen concentration was not different between trials ( $P = 0.82$ ). Lipid caused a 24-h surplus of ~1100 kcal above energy balance ( $P = 0.00001$ ), whereas energy balance was maintained in saline. Despite these differences in fatty acid and energy availability,  $S_i$  the morning after exercise was not different between trials ( $P = 0.72$ ). Thus insulin sensitivity the morning after a single exercise session was not reduced despite overnight exposure to a fourfold increase in plasma fatty acid concentration, elevated IMTG concentration, and systemic delivery of ~1,100-kcal excess.

fatty acids; insulin resistance; intramuscular triglyceride; energy balance

OBESSE INDIVIDUALS have excessive fatty acid availability in the circulation (22) and uptake by skeletal muscle (26), which have been causally linked to the etiology of skeletal muscle insulin resistance (43). This link is substantiated by the fact that increasing plasma fatty acid availability in lean, healthy individuals causes insulin resistance (11). In the muscle cell, fatty acids have two major routes of disposal: oxidation in the mitochondria or storage as intramuscular triglyceride (IMTG). Insulin resistance in obesity has been associated with a decreased muscle oxidative capacity and a corresponding reduction in the capacity to oxidize fatty acids (26, 38, 44). Moreover, it has been hypothesized that the accumulation of IMTG is associated with the development of insulin resistance (19, 37), although recent research has questioned a causal role for IMTG (6, 15, 18). Instead, it appears that the accumulation of

intracellular fatty acid intermediates (e.g., diacylglycerol, ceramide, long-chain acyl-CoA) is responsible for the fatty acid-induced insulin resistance because of their ability to impair insulin signaling (49) through activation of the novel and/or conventional protein kinase C, and possibly the I $\kappa$ B kinase/nuclear factor- $\kappa$ B, pathway (24, 27). To date, however, all studies investigating the effects of increased systemic fatty acid availability on insulin sensitivity have been performed in sedentary subjects.

A single session of exercise increases insulin sensitivity for hours and even days (20). In parallel with these improvements in insulin sensitivity, a single session of exercise increases the ability of the muscle cell to partition fatty acids toward oxidation (28, 46) and IMTG storage (9, 23), which could ultimately limit the accumulation of fatty acid intermediates inside the cell and thereby limit their harmful effects on insulin signaling. In a recent study from our laboratory (15), despite the addition of ~160 extra grams of dietary fat to the meals after exercise, and a 20% increase in IMTG concentration, glucose tolerance was not impaired. These data suggest that exercise may mediate the relationship between excessive fat availability and insulin sensitivity.

Although increasing plasma fatty acid availability in sedentary humans causes insulin resistance (11), it is not known whether increasing plasma fatty acid availability after exercise also decreases insulin sensitivity. This is an important question, because obese individuals have very high rates of plasma fatty acid turnover (22, 26), and the ramifications of elevated fatty acid availability in the hours after exercise are poorly understood. Therefore, the purpose of this study was to determine whether increasing plasma fatty acid availability in lean subjects in the hours after exercise, to levels typically found in obesity, reduces insulin sensitivity. Because the carbohydrate content of meals ingested after exercise and muscle glycogen concentration can have a potent effect on insulin sensitivity (8), similar to our previous study (15), we fed subjects the same amount of carbohydrate after exercise. By doing so, we were able to investigate the effect of increased plasma fatty acid availability on insulin sensitivity independently of the potentially confounding influence of carbohydrate availability and muscle glycogen concentration.

### METHODS

#### Subjects

Seven women who were considered to be in good health after a comprehensive medical examination, which included a history and physical examination, a 12-lead electrocardiogram, and standard

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blood and urine tests, volunteered to participate in this study. No subject was taking regular medications (except birth control) or smoked tobacco. Subjects underwent an incremental peak oxygen uptake ( $\dot{V}O_{2\text{ peak}}$ ) test on a stationary bicycle ergometer to assess aerobic fitness, and hydrostatic weighing was used to assess body composition. All subjects had a stable body weight (i.e.,  $\pm 2$  kg) and had been sedentary (regular exercise  $< 2$  h/wk) for at least 6 mo before the study. Subjects were studied during the first 2 wk of the follicular phase of their menstrual cycle. 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. Subject characteristics are presented in Table 1.

#### Experimental Protocol

All subjects performed two experimental trials that were separated by at least 7 days. The order of the trials was randomized, and the two trials differed only by the contents of the overnight infusion. A timeline of events is presented in Fig. 1. The day before each trial, subjects received from the General Clinical Research Center (GCRC) a standardized evening meal [2.25 g carbohydrate (CHO)/kg, 0.5 g fat/kg, 0.375 g protein/kg] that was eaten at home and completed at 2130 (24-h clock). The next morning (*day 1*), subjects were admitted to the GCRC at 0830 after an overnight fast. After resting supine for 30 min, resting oxygen consumption ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) were measured using a metabolic cart (DeltaTrac; SensorMedics, Yorba Linda, CA) to assess resting energy expenditure. Starting at 1000, subjects began 90 min of exercise at  $\sim 65\%$   $\dot{V}O_{2\text{ peak}}$ . Exercise consisted of 45 min of treadmill exercise, immediately followed by 45 min of exercise on a cycle ergometer (Lode Excalibur, Groningen, The Netherlands). Subjects were allowed free access to water during the exercise. To ensure subjects were exercising at the appropriate intensity and to quantify energy expenditure during exercise, indirect calorimetry (PhysioDyne Technologies, Quogue, NY) measurements were taken from 0 to 5 min and from 35 to 40 min during both the treadmill and ergometer exercise, and changes in the exercise intensity were made if necessary. After the 90-min exercise session, subjects showered and were then provided with a low-fat meal (4 g CHO/kg, 0.15 g fat/kg, 0.55 g protein/kg) at 1200. A second low-fat meal was provided at 1400 (2 g CHO/kg, 0.075 g fat/kg, 0.275 g protein/kg). At  $\sim 1415$ , two intravenous catheters were placed, one in an antecubital vein for the overnight infusion and the other in a hand vein in the contralateral arm for blood sampling. The overnight infusions began at 1500 and were continued until 0700 the next morning. The content of these infusions was the only difference between the two experimental trials. On one occasion (Lipid), subjects were infused overnight with a 20% lipid emulsion (Abbott Laboratories, North Chicago, IL;  $0.55 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and heparin (Elkins-Sinn, Cherry Hill, NJ;  $5 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ), with the goal to increase overnight plasma fatty acid concentration to a high physiological level ( $\sim 1.0$  mmol/l). During the other trial (Saline), subjects were infused overnight with normal saline ( $0.55 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). Subjects ingested their final meal of the day at 2100 (2 g CHO/kg, 0.075 g fat/kg, 0.275 g protein/kg), after which they were allowed only water. The next morning, the overnight infusion was stopped at 0700. At 0900 a muscle biopsy was obtained from the

vastus lateralis muscle of the thigh, using the percutaneous biopsy technique. Beginning at 1000, an intravenous glucose tolerance test (IVGTT) was conducted to assess insulin sensitivity, using the minimal model technique (48). Briefly, subjects were injected with a bolus of glucose (300 mg/kg) at 1000, and 20 min later a bolus of insulin ( $0.02 \mu\text{U}/\text{kg}$ ) was provided intravenously. Blood was sampled frequently for 3 h after the glucose injection.

#### Blood Sampling and Muscle Sample Preparation

Before exercise on *day 1*, a fasting blood sample (5 ml) was taken (0930) for measurement of plasma glucose and insulin. Additional blood (5 ml) was sampled immediately before the overnight infusion was started (1500) and every hour thereafter until the infusion was stopped at 0700 on the morning of *day 2*. After the infusion was stopped, another blood sample was taken at 0900. All of these blood samples were analyzed for plasma fatty acid, glucose, and insulin concentrations. Before the IVGTT, three blood samples (2 ml) were taken at 5-min intervals before the glucose injection at 1000. During the IVGTT, additional blood samples (2 ml) were collected at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 70, 80, 90, 100, 120, 140, 160, and 180 min after the glucose bolus for analysis of plasma glucose and insulin concentrations. Blood samples were transferred from the syringe to chilled test tubes containing 0.03 mmol EDTA and 0.5 trypsin inhibitor unit/ml aprotinin. Samples were kept on ice and then centrifuged (3,000 rpm for 20 min at  $4^\circ\text{C}$ ) within 30 min of collection. After centrifugation, plasma was transferred into plastic culture tubes and immediately frozen and stored at  $-80^\circ\text{C}$  for later analysis. For the muscle biopsy samples, one sample was immediately frozen in liquid nitrogen for later analysis. A second sample was dissected free of adipose and connective tissue, rinsed in saline, blotted dry, and then frozen in liquid nitrogen. Muscle samples were stored at  $-80^\circ\text{C}$  until biochemical analysis.

#### Analytical Procedures and Calculations

**Plasma substrate and insulin concentrations.** Plasma glucose (Sigma, St. Louis, MO) and fatty acid (Waco Chemicals, Neuss, Germany) concentrations were measured by colorimetric assay. Plasma insulin concentration was measured by radioimmunoassay (Linco Research, St. Charles, MO).

**IMTG and muscle glycogen analysis.** Muscle biopsies were lyophilized at  $-60^\circ\text{C}$  for 48 h, and aliquots were weighed to the nearest 0.1 mg. IMTG was measured from the liberation of free glycerol (16). Briefly, triglycerides were extracted from the dried muscle sample with 2:1 chloroform-methanol and saponified in 4% ethanolic KOH. Free glycerol concentration in these samples was then determined fluorometrically. Muscle glycogen was determined from the measurement of glucose after acid hydrolysis (36). Briefly, samples were homogenized and then hydrolyzed in 2 N HCl and heated at  $100^\circ\text{C}$  for 2 h. Samples were then neutralized using 1 N NaOH to pH 6.5–7.5, and the free glucose concentration was determined by colorimetric assay.

**Energy expenditure.** Resting metabolic rate (RMR) was calculated from resting  $\dot{V}O_2$  and  $\dot{V}CO_2$  measurements, using the Weir equation (47). RMR was multiplied by 1.2 to account for energy expenditure due to activities of daily living for sedentary subjects confined to their hospital floor (42). Exercise energy expenditure was calculated from the indirect calorimetry measurements made during exercise. Twenty-four-hour energy expenditure was estimated as the weighted sum of resting energy expenditure (22.5 h) and exercise energy expenditure (1.5 h).

**Insulin sensitivity.** Plasma glucose and insulin samples from the IVGTT were used to calculate the insulin sensitivity index ( $S_i$ ), using the MINMOD Millennium (version 6.02, MinMod) computer analysis software. It has been found that the minimal model is sufficiently sensitive to detect fatty acid-induced reductions in insulin sensitivity (25). We also assessed insulin sensitivity before exercise vs. the day

Table 1. *Subject characteristics*

Age, yr	26.6 (9.4)
Weight, kg	62.6 (9.8)
Height, cm	165.4 (8.4)
Body mass index, $\text{kg}/\text{m}^2$	22.9 (2.7)
Body fat, %	28.7 (3.9)
$\dot{V}O_{2\text{ peak}}$ , $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	40.9 (6.4)
Fasting plasma glucose, mmol/l	4.7 (0.5)

Values are means (SD).  $\dot{V}O_{2\text{ peak}}$ , peak  $O_2$  consumption.

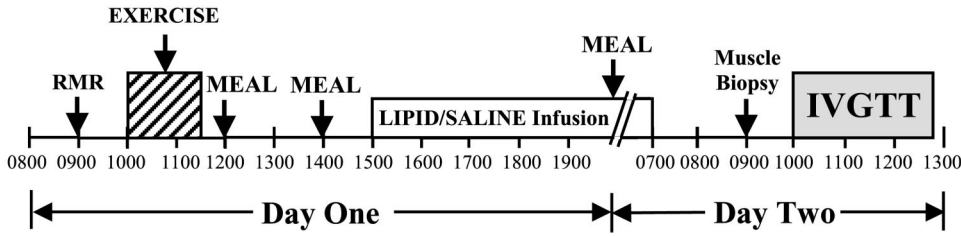


Fig. 1. Timeline of events. Subjects performed 2 trials in a randomized order. Trials were identical with the exception that, in one trial, subjects received an infusion of lipid + heparin (Lipid), and in the other, they received saline (Saline), which served as the control. RMR, resting metabolic rate; IVGTT, intravenous glucose tolerance test.

after exercise using homeostatic model assessment (HOMA) (32). HOMA provides a reasonable assessment of insulin sensitivity compared with the IVGTT method (3). Fasting blood samples taken before exercise on the morning of *day 1* and before the IVGTT on *day 2* were used for HOMA analysis, which was calculated as fasting plasma glucose (mmol/l) × fasting plasma insulin (μU/ml)/22.5.

**Statistical Analysis**

A one-way ANOVA was used to test for significant differences in IMTG, muscle glycogen, and  $S_i$  between trials. A two-way ANOVA (treatment × time) for repeated measures with Bonferroni post hoc analysis was used to test for significant differences in overnight plasma fatty acid, glucose, and insulin concentrations, and HOMA. Statistical analyses were performed using SigmaStat for Windows (version 3.0.1a; Systat Software, Point Richmond, CA). Statistical significance was defined as  $P < 0.05$ . All results are presented as means (SD).

**RESULTS**

*Overnight Plasma Substrates and Insulin Concentrations*

Lipid significantly increased plasma fatty acid concentration above Saline throughout the infusion period ( $P < 0.05$ ; Fig. 2A), and the mean (SD) plasma fatty acid concentration during the infusion was approximately fourfold higher during Lipid compared with Saline [0.84 (0.36) vs. 0.22 (0.09) mmol/l,  $P = 0.003$ ]. Plasma glucose concentration was not different between trials at any time point during the infusion (Fig. 2B). Despite a difference in plasma insulin concentration before the infusions, plasma insulin levels were nearly identical in Lipid and Saline throughout the infusion period (Fig. 2C). Plasma insulin concentration was slightly but significantly greater in Lipid than in Saline at 7 h ( $P < 0.001$ ) and 8 h ( $P < 0.001$ ) of the infusion. However, the average plasma insulin concentration during the infusion was not different between Lipid and Saline [26.2 (11.6) vs. 23.3 (9.2) μU/ml,  $P = 0.29$ ].

*IMTG and Muscle Glycogen Concentration*

The overnight lipid infusion increased IMTG concentration, and, the morning after the overnight infusion, IMTG concentration was ~30% higher in Lipid compared with Saline [49.2 (6.6) vs. 38.3 (7.7) mmol/kg dry wt,  $P = 0.036$ ; Fig. 3A]. Conversely, the lipid infusion did not affect muscle glycogen concentration, which was not different between Lipid and Saline [523 (148) vs. 507 (46) mmol/kg dry wt,  $P = 0.82$ ; Fig. 3B].

*Insulin Sensitivity*

Immediately before the IVGTT was started (i.e., 3 h after stopping the overnight infusion), there were no differences between trials in the plasma concentrations of fatty acids ( $P = 0.08$ ), glucose ( $P = 0.25$ ), or insulin ( $P = 0.92$ ) (Table 2).

During the IVGTT, plasma glucose and insulin concentrations were identical in Lipid and Saline (Fig. 4, A and B, respectively). Consequently,  $S_i$  was the same in Lipid and Saline [7.0 (2.7) vs. 6.7 (3.3) (mU/l)<sup>-1</sup>·min<sup>-1</sup>,  $P = 0.72$ ; Fig. 5]. No association existed between IMTG concentration and insulin sensitivity ( $r = 0.087$ ,  $P = 0.77$ ). In confirmation of these findings, HOMA was not different between Saline and Lipid on *day 2* [1.73 (0.79) vs. 1.66 (0.60),  $P = 0.90$ ]. However, HOMA on *day 2* was significantly lower (~35%) than that on *day 1* [*day 1* HOMA = 2.76 (1.73) vs. 2.49 (1.07), Saline vs. Lipid], reflecting a significant improvement in insulin sensitivity after exercise in both the Saline ( $P = 0.007$ ) and Lipid ( $P = 0.024$ ) trials.

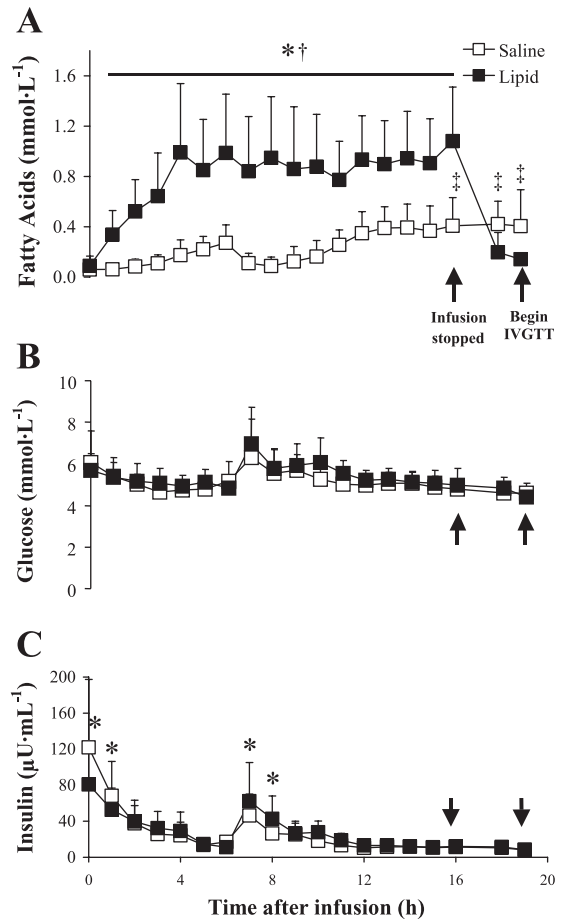


Fig. 2. Plasma fatty acid (A), glucose (B), and insulin (C) concentrations before (*time 0*), during (1–16 h), and after an overnight infusion of either Saline or Lipid. \*Lipid significantly different from Saline,  $P < 0.05$ . †Lipid significantly different from *time 0*,  $P < 0.05$ . ‡Saline significantly different from *time 0*,  $P < 0.05$ .

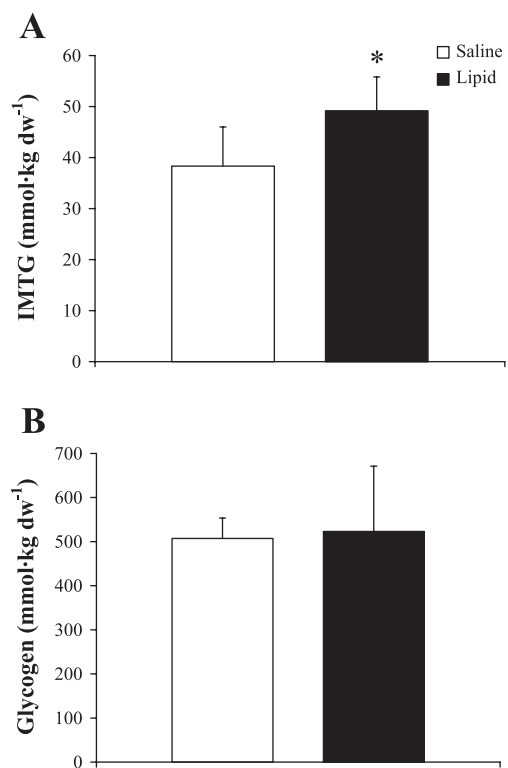


Fig. 3. Intramuscular triglyceride (IMTG; A) and muscle glycogen (B) concentrations in muscle biopsy samples from vastus lateralis the morning after an infusion of either Saline or Lipid. \*Lipid significantly different from Saline,  $P < 0.05$ .

#### Twenty-Four-Hour Energy Balance

Neither energy intake nor estimated 24-h energy expenditure was different between the two trials (Table 3). During Saline, subjects were estimated to be in energy balance (Table 3). However, during Lipid, the overnight infusion significantly increased systemic caloric availability, and, as a result, subjects were in a positive energy balance of 1,060 (574) kcal, which was significantly greater than that for Saline ( $P = 0.00001$ ).

#### DISCUSSION

A single session of exercise is known to improve insulin sensitivity in both lean and obese individuals (10). The duration of this effect is strongly associated with glycogen repletion (8) and therefore is largely dependent on exogenous carbohydrate availability after exercise. However, little is understood about the effects of alterations in lipid and energy availability after exercise on insulin sensitivity, independent of changes in carbohydrate availability and glycogen concentration. We recently found that, when the carbohydrate content of meals

Table 2. Fasted plasma substrate and insulin concentrations

	Saline	Lipid
Fatty acids, mmol/l	0.41 (0.29)	0.14 (0.05)
Glucose, mmol/l	4.6 (0.5)	4.4 (0.5)
Insulin, $\mu\text{U/ml}$	8.2 (3.1)	8.3 (2.5)

Values are means (SD). Fasted plasma substrate and insulin concentrations the morning after the infusion and immediately before the intravenous glucose tolerance test.

ingested after exercise was identical, the addition of  $\sim 165$  g of dietary fat (i.e.,  $\sim 1,500$  kcal) did not affect glucose tolerance the next day (15). The present study expands on these findings by demonstrating that, despite providing an additional  $\sim 110$  g of lipid intravenously (i.e.,  $\sim 1,100$  kcal above energy balance), an approximately fourfold elevation in overnight plasma fatty acid concentration, and a  $\sim 30\%$  increase in IMTG concentration, insulin sensitivity was not impaired the day after exercise.

Increasing systemic (11) or dietary (31) fat availability has been found to impair insulin sensitivity. However, exercise can play an important role in mediating the effect of high fat availability on insulin sensitivity (29). Our current findings agree with previous work from our laboratory (15) and others' (9, 45) that demonstrated that increasing fat availability after exercise does not reduce insulin sensitivity. In rodents, a single session of exercise attenuates insulin resistance caused by high fat availability (33), as does acute pharmacological activation of AMP-activated protein kinase (AMPK) (35), a major regulator of muscle metabolism that is activated during exercise. Mechanistically, we hypothesize that acute exercise mediates the relationship between fat availability and insulin sensitivity by altering the partitioning of fatty acids toward storage as IMTG and/or oxidation within the mitochondria. This mechanism would limit the accumulation of fatty acid intermediates (e.g., diacylglycerol, ceramide, long-chain acyl-CoA) that have been demonstrated to impair insulin signaling and insulin sensitivity (49).

Accumulation of IMTG has been associated with impaired insulin sensitivity (19, 37). Interestingly, however, this relationship has only been demonstrated in sedentary subjects (19, 37). The present findings agree with previous work indicating

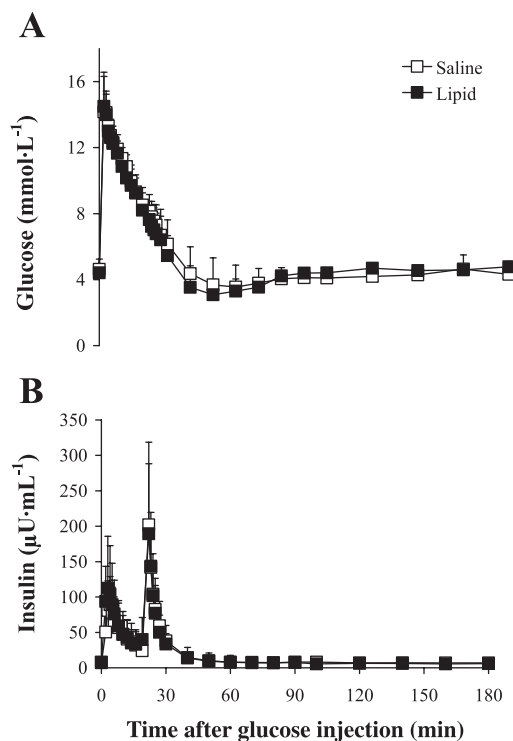


Fig. 4. Plasma glucose (A) and insulin (B) concentrations during the IVGTT, which was performed the morning after the overnight infusion of either Saline or Lipid.

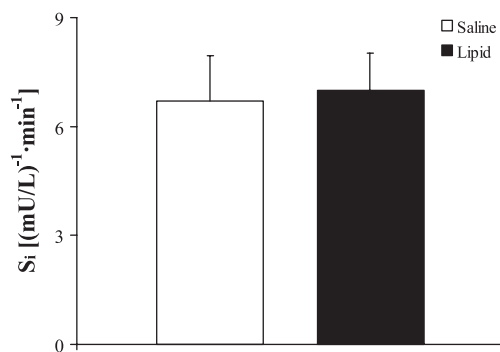


Fig. 5. Insulin sensitivity index ( $S_i$ ), as measured by the IVGTT, the morning after an overnight infusion of either Saline or Lipid.

that this relationship does not exist after a single session of exercise (9, 15, 33) or with endurance training (6, 18). The mechanisms underlying this disconnect between IMTG and insulin sensitivity in exercised individuals may be related to exercise-mediated changes in the ability of the muscle cell to partition fatty acids toward storage as IMTG. Short-term endurance training increases IMTG concentration (41), and a number of mechanisms could underlie this increase in IMTG synthesis after exercise. Fatty acid uptake is increased after exercise (5), as is the capacity to esterify fatty acids into IMTG (1). Moreover, exercise upregulates the expression of key genes involved in triglyceride synthesis (23). Accordingly, we propose that shuttling excess fatty acids toward storage as IMTG during Lipid would serve to limit the accumulation of fatty acid intermediates in the cytosol, thereby limiting their detrimental effects on insulin signaling and insulin sensitivity. Indeed, Bachmann et al. (4) demonstrated that, when fat availability was increased through either diet or a lipid + heparin infusion, subjects with the greatest capacity to partition fatty acids toward IMTG storage had the smallest impairment in insulin sensitivity.

The partitioning of fatty acids toward oxidation is also increased after an acute bout of exercise (28, 46), especially when dietary fat availability is increased (40). In particular, prior exercise preferentially increases the oxidation of unsaturated fatty acids (46), which account for ~90% of the fatty acids that were infused in Lipid. Although we did not measure overnight fat oxidation, increased partitioning of fatty acids toward oxidation during Lipid would also serve to limit the accumulation of fatty acid intermediates. Several cellular mechanisms could mediate enhanced partitioning of fatty acids toward oxidation after exercise. Acute exercise increases the abundance of the fatty acid transporter FAT/CD36 at the mitochondrial membrane, which is paralleled by increased fatty acid oxidation (7). Furthermore, the activity of pyruvate dehydrogenase, a key regulator of carbohydrate metabolism, is significantly reduced for at least 18 h after an acute session of exercise (28), resulting in a corresponding increase in fatty acid oxidation. The activity of AMPK, which when activated increases fatty acid oxidation, is also elevated in the hours after exercise (39). Therefore, increased postexercise AMPK activity and decreased pyruvate dehydrogenase activity, along with an enhanced capacity to transport fatty acids into the mitochondria, would thereby serve to enhance the partitioning of excess fatty acids toward oxidation.

Insulin resistance, particularly in obesity, has been associated with reduced skeletal muscle oxidative capacity (i.e., mitochondrial content or ATP synthesis rate) and a resultant lower capacity to oxidize fatty acids (26, 38, 44). Although an increase in muscle oxidative capacity may indeed lead to improvements in insulin sensitivity, a reduced capacity for oxidative fatty acid metabolism cannot fully account for the insulin resistance that is common in obesity. For example, a single session of moderate exercise clearly improves insulin sensitivity in obese individuals with insulin resistance (10), although such improvements occur despite the fact that a single session of exercise does not markedly increase the oxidative capacity of the muscle (13). Accordingly, we hypothesize that a single session of exercise is able to improve insulin sensitivity or, as in the present study, prevent a decrease in insulin sensitivity in the face of elevated lipid and energy availability, due to an improved ability of the muscle to partition fatty acids toward oxidation (and storage), independently of an increase in the oxidative capacity of the muscle. Therefore, performing endurance exercise at least 3–4 times/wk would provide the dual benefit of acutely enhancing the partitioning of fatty acids to more favorable disposal routes within the cell, as well as the well-known improvements in oxidative capacity seen after weeks of exercise training.

Energy balance can also have a profound effect on insulin sensitivity. For example, during diet-induced weight loss, a large proportion of the improvement in insulin sensitivity is due to being in a negative energy balance, rather than weight loss, per se (2). Conversely, being in positive energy balance is associated with a reduction in insulin sensitivity (4). Even short-term exposure to excessive calories, without a change in body weight, has been found to reduce insulin sensitivity (4). Bachmann et al. (4) found that insulin sensitivity was significantly impaired after overfeeding subjects (~750 kcal/day) for 3 days. Conversely, in the present study, we found that insulin sensitivity the morning after exercise was not decreased, despite a >1,000-kcal surplus from the overnight lipid infusion. The reasons for this discrepancy may be due differences in methodology, in that we provided 1,000 additional extra calories in a 16-h period, whereas, in the study by Bachmann et al., the subjects were overfed ~750 kcal for 3 days. Alternatively, it is possible that acute exercise is able to overcome the deleterious effects of positive energy balance and weight gain. In fact, an increase in body weight, which is known to be associated with impaired insulin sensitivity (14), failed to decrease insulin sensitivity in physically active individuals (34).

The major goal of our study was to assess the effect of excessive fatty acid availability in the hours after exercise on insulin sensitivity. By stopping the lipid infusion 3 h before insulin sensitivity was measured, plasma glucose and insulin concentrations were not different between trials at the time of

Table 3. Twenty-four-hour energy balance

	Saline	Lipid
Energy intake	2,224 (496)	2,233 (513)
Estimated energy expenditure	2,238 (194)	2,165 (265)
Energy from lipid infusion		992 (155)
Energy balance	-14 (399)	+1,060 (571)*

Values are means (SD), in kcal. \* $P = 0.0001$ , Lipid different from Saline.



insulin sensitivity measurement. Although plasma fatty acid concentrations were not significantly different between trials immediately before the IVGTT, we did observe a trend for a lower plasma fatty acid concentration immediately before the IVGTT during Lipid. This trend was likely due to fatty acid-mediated inhibition of endogenous adipose tissue lipolysis (12). Stopping the infusion before starting the IVGTT allowed us to more directly evaluate the impact of excess lipid availability after exercise on insulin sensitivity without the confounding influence of the well-described reduction in insulin sensitivity seen during an acute elevation in fatty acids (i.e., elevated fatty acid concentration while measuring insulin sensitivity) (11). In addition, because muscle glycogen concentration can strongly influence insulin sensitivity (8), we fed our subjects the same carbohydrate content during Lipid and Saline, resulting in identical muscle glycogen concentrations. Therefore, in this study, we were able to investigate the effects of increased lipid and energy availability on insulin sensitivity independently of differences in plasma substrates, insulin, and muscle glycogen concentrations at the time of insulin sensitivity measurement. We must acknowledge that, although several studies indicate that both men and women demonstrate impaired insulin sensitivity with lipid infusion (21, 30), this finding is not universal (17). Therefore, one must use caution when applying these findings to men.

Because we performed an IVGTT the day after exercise in both of our experimental trials (and not the day after physical inactivity), we cannot determine directly that exercise protected against a fatty acid-induced impairment in  $S_i$  when subjects were sedentary. However, using HOMA, which has been found to provide a reasonable assessment of insulin sensitivity compared with the IVGTT (3), we did find that exercise resulted in a similarly significant reduction in HOMA in Saline and Lipid. This suggests that our exercise intervention was sufficient to improve insulin sensitivity and provides preliminary evidence that a single session of exercise can protect against insulin resistance that is caused by increased fatty acid availability.

In conclusion, there is considerable evidence in sedentary humans that acutely increasing systemic fat availability and IMTG concentration and a positive energy balance induce insulin resistance. However, we found that an approximately fourfold increase in overnight plasma fatty acid availability, an ~30% increase in IMTG concentration, and a provision of >1,000 kcal above energy balance did not impair insulin sensitivity the morning after exercise. These findings suggest that exercise plays an important role in mediating the relationship between increased fatty acid availability and insulin sensitivity. Mechanisms responsible for this effect are most likely related to exercise-induced changes in the partitioning of fatty acids toward IMTG storage and/or oxidation within the mitochondria.

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