Effects of fatty acids on exercise plus insulin-induced glucose utilization in trained and sedentary subjects

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Matzinger, Oscar, Philippe Schneiter, and Luc Tappy. Effects of fatty acids on exercise plus insulin-induced glucose utilization in trained and sedentary subjects. Am J Physiol Endocrinol Metab 282: E125–E131, 2002; 10.1152/ajpendo.00177.2001.—Fatty acids are known to decrease insulin-mediated glucose utilization in humans, both at rest and during exercise. To evaluate the effect of endurance training in this process, we infused lipids or saline in groups of sedentary and highly trained subjects. Whole body glucose utilization and substrate oxidation were monitored during a 2.5-h hyperinsulinemic clamp. During the last 30 min, a cycling exercise was superimposed. During hyperinsulinemia at rest, whole body glucose utilization and glucose oxidation were higher in trained subjects than in sedentary subjects. Compared with the control experiments with the antilipolytic agent acipimox, lipid infusion stimulated lipid oxidation to the same extent in trained as in sedentary subjects. It reduced whole body glucose utilization by 37% in trained and by 41% in sedentary subjects. During exercise, lipid infusion increased more lipid oxidation in trained than in sedentary subjects and reduced whole body glucose utilization by 43 ± 4% in trained and by 22 ± 4% in sedentary subjects (P < 0.01). The present data indicate that lipid infusion has similar effects on lipid oxidation and whole body glucose utilization during hyperinsulinemia at rest in trained and sedentary subjects. During exercise, however, it increases more lipid oxidation and produces a more important reduction in glucose utilization in trained than in sedentary subjects. These results suggest that endurance training enhances the inhibitory effect of lipids on whole body glucose metabolism during exercise.

Insulin; exercise; whole body glucose utilization; lipid oxidation

It has been known for a long time that infusion of a lipid emulsion impairs glucose tolerance (15) and decreases insulin-mediated glucose disposal (12) in healthy humans. Lipids inhibit both glucose oxidation and storage (33) and exert this effect primarily in skeletal muscle (24). These effects are observed not only at rest but also during exercise (16, 28) and during the recovery period after an exercise (6).

Endurance training has profound effects on skeletal muscle substrate utilization. It results in an increased lipid utilization by working muscles at low to moderate exercise intensity (17). This increased contribution of lipid oxidation to total energy expenditure in endurance-trained vs. sedentary individuals is observed at both the same relative and same absolute workloads (1, 17). Endurance training may affect several key steps in lipid metabolism to attain such an increase in lipid oxidation by skeletal muscle: an enhanced lipolysis of triglyceride stored in adipose tissue and/or within the muscle fibers, an enhanced uptake of circulating lipids by working muscle, and an enhanced β-oxidation of fatty acids may all possibly be involved (19). How these metabolic adaptations to endurance training affect the interactions between glucose and lipid metabolism remains, however, unknown. The aim of this study was therefore to assess the effects of endurance training on the inhibition of whole body glucose metabolism by lipids at rest and during exercise.

METHODS

Subjects

Nine endurance-trained cyclists (group T) and seven healthy sedentary volunteers (group S) were selected to take part in this study. All subjects underwent a medical evaluation including history, physical examination, and electrocardiogram. They were all in good physical health, were not taking any medication, were nonsmokers, and had no familial history of diabetes or obesity (Table 1).

The endurance-trained cyclists were selected on the basis of having biked >4,000 km/yr for the previous 2 yr. The sedentary volunteers did not perform any kind of sport (including physical activity at work) for the previous 2 yr.

The experimental protocol was approved by the ethics committee of the Lausanne University Medical School, and all participants provided informed written consent. Percent body fat was estimated from skinfold thickness measurements with the use of standard formulas (10).

Maximal oxygen uptake was measured on an electronically braked cycle ergometer during an incremental exhaustive exercise test. For the trained subjects, exercise started at a power output of 150 W, which was increased by 30 W every minute until exhaustion. The same procedure was used for sedentary subjects, except that exercise was started at a power output of 50 W. Respiratory gases were analyzed via open-circuit indirect calorimetry as described (20) and were recorded on line.

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Table 1. Subjects' characteristics

<table>
<thead>
<tr>
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<th>Sedentary Group</th>
<th>Trained Group</th>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>9</td>
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<tr>
<td>Age, yr</td>
<td>23.4 ± 3.2</td>
<td>24.3 ± 3.3</td>
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<tr>
<td>Body mass, kg</td>
<td>61.5 ± 9.6</td>
<td>68.2 ± 5.1</td>
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<td>Height, cm</td>
<td>174.6 ± 5.9</td>
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<tr>
<td>BMl, kg/m²</td>
<td>20.3 ± 2.1</td>
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<td>Body fat, %</td>
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<td>10.4 ± 2.3</td>
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<tr>
<td>FFM, kg</td>
<td>52.4 ± 8.4</td>
<td>61.1 ± 4.7</td>
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<tr>
<td>VO₂max, ml/min</td>
<td>2541 ± 217</td>
<td>4591 ± 170</td>
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Values are means ± SD. BMI, body mass index; FFM, fat-free mass; VO₂max, maximal oxygen uptake.

Experimental Protocol

Each subject took part in two different protocols separated by ≥2 wk. The order in which the two protocols were performed was randomly assigned. During the 3 days preceding each protocol, subjects were requested not to perform heavy physical activity. They were also asked not to consume any drinks containing caffeine or alcohol during the 24 h preceding each protocol.

In both protocols, subjects were studied in the morning after an overnight fast. After having voided, subjects lay quietly supine in bed. Intravenous catheters were placed percutaneously in an antecubital vein of each arm. One catheter was used for infusion of [6,6-2H₂]glucose, insulin, dextrose, and a soybean lipid emulsion (20% Lipovenos, Fresenius, Stans, Switzerland). The contralateral catheter was used for periodic collection of blood samples. The heated hand technique was used, with a thermostabilized box heated at 50°C, to obtain partially arterialized blood samples. Throughout the experiments, respiratory gas exchanges were monitored by open-circuit indirect calorimetry by means of a transparent hood ventilated with ~40 l/min (16). A primed (4 mg/kg bolus), continuous infusion of [6,6-2H₂]glucose, (40 μg·kg⁻¹·min⁻¹; MassTrace, Woburn, MA) was started immediately after the insertion of the venous cannula. Sixty minutes later, a hyperinsulinemic euglycemic (0.4 mU·kg⁻¹·min⁻¹) clamp (glucose = 5 mmol/l) was started (time 0). Exogenous glucose was labeled with 1.25% [6,6-2H₂]glucose (hot infusate technique) to calculate whole body glucose rate of disappearance (14).

Two hours after the beginning of the clamp, the subjects were transferred to an ergometric bicycle (Schiller ergometer CE 0.124; Baar, Switzerland) where they remained seated for the next 35 min. A hood was fitted over their heads to allow collection of expiratory gases and was ventilated with ambient air at a rate of 250 l/min. After 5 min of preparation and adaptation of the subject, a cycling exercise at a power output of 100 W was performed during 30 consecutive minutes. Because there was a significant degree of hyperventilation during the initial period of exercise, only the last 10 min of exercise were recorded. Plasma bicarbonate concentrations remained steady during this period.

Protocol 1: lipid infusion. This test was performed to assess insulin-mediated glucose disposal at rest and during exercise in presence of elevated plasma free fatty acid (FFA) concentrations (condition L). A continuous infusion of 20% Lipovenos (1 ml/min) was started 60 min before the beginning of insulin infusion and was continued throughout the test to maintain elevated plasma FFA concentrations.

Protocol 2: administration of acipimox. This test (condition Acx) was performed to assess insulin sensitivity at rest and during exercise in the presence of very low FFA plasma levels. All subjects received 250 mg of the antilipolytic agent acipimox (Olbetam; Pharmacia & Upjohn, Dübendorf, Switzerland) at the beginning of the test and again 2 h later to efficiently suppress plasma FFA concentrations.

Analytical Procedures

Plasma glucose was determined by the oxidase method, using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma FFA concentrations were determined by a colorimetric assay kit (Wako, Freiburg, Germany). Plasma lactate concentrations were measured enzymatically (18). Plasma insulin concentrations were measured by radioimmunoassay (kit from Biodata Guidonia, Montecello, Italy, with <14% cross-reactivity with proinsulin). Urinary urea concentration was analyzed enzymatically using a Beckman urea analyzer (Beckman Instruments). Plasma [6,6-2H₂]glucose was measured by gas chromatography-mass spectrometry, as described (32).

The rates of glucose appearance (Ra) and disappearance (Rd) were calculated from plasma [6,6-2H₂]glucose enrichment using hot infusate equations (14).

Endogenous glucose production was calculated as glucose Rd – the rate of exogenous dextrose infusion.

The percent reduction in glucose Rd induced by lipids between protocols 1 (lipids, L) and 2 (acipimox, Acx) was calculated as

\[
\% \text{ reduction} = \frac{R_{dL} - R_{dAcx}}{R_{dAcx}} \times 100
\]

Statistical Analysis

All results are expressed as means ± SE unless stated otherwise. Comparisons between groups were performed by multiple way analysis of variance. Post hoc comparisons were made using Fisher’s protected least significant difference. Statistical significance was set at α = 0.05.

RESULTS

Plasma Hormones and Substrate Concentrations

During the initial 2 h at rest, plasma glucose was clamped at the following values: trained subjects during lipid infusion (TL), 5.54 ± 0.29 mmol/l; trained subjects after acipimox (TAcx), 5.44 ± 0.35 mmol/l; sedentary subjects during lipid infusion (SL), 5.55 ± 0.23 mmol/l; and sedentary subjects after acipimox (SAcx), 5.57 ± 0.63 mmol/l. During the next 30 min, with a cycling exercise at a power output of 100 W, the following plasma glucose concentrations were maintained: TL, 5.29 ± 0.54 mmol/l; TAcx, 5.32 ± 0.62 mmol/l; SL, 5.6 ± 0.52 mmol/l; and SAcx, 5.58 ± 0.4 mmol/l. Steady-state plasma insulin concentrations at rest were similar under all four conditions (TL, 5.54 ± 0.29 mmol/l; TAcx, 5.44 ± 0.35 mmol/l; SL, 5.55 ± 0.23 mmol/l; SAcx, 5.57 ± 0.63 mmol/l). They were minimally affected by exercise (Fig. 1). Steady-state plasma FFA concentrations were similarly increased to high physiological values in TL (0.395 ± 0.12 mmol/l) and SL (0.415 ± 0.28 mmol/l); they were suppressed to the same extent in both groups with acipimox (TAcx, 0.033 ± 0.004 mmol/l; SAcx, 0.035 ± 0.003 mmol/l; P < 0.0001 vs. TL and SL; Fig. 2).
Plasma lactate concentrations were similar in the two groups under both Lipoveno and acipimox at rest: TL, 1.319 ± 0.035 mmol/l; TAcx, 1.358 ± 0.055 mmol/l; SL, 1.147 ± 0.042 mmol/l; and SAcx, 1.212 ± 0.039 mmol/l. During exercise, plasma lactate concentrations markedly increased in sedentary subjects with both Lipoveno and acipimox (P < 0.001 under both conditions) but remained unchanged in trained individuals (Fig. 3).

**Indirect Calorimetry**

Basal carbohydrate oxidations were lower during lipid infusion (TL, 12.16 ± 0.74 vs. SL, 13.72 ± 2.52 mmol·kg⁻¹·min⁻¹) than after acipimox (TAcx, 17.55 ± 2.46 vs. SAcx, 16.64 ± 1.54 mmol·kg⁻¹·min⁻¹, \( P < 0.05 \) in both groups) (Fig. 3). Carbohydrate oxidation rates during hyperinsulinemia at rest were also lower during lipid infusion (TL, 16.43 ± 1.24 vs. SL, 15.01 ± 1.16 mmol·kg⁻¹·min⁻¹) than after acipimox (TAcx, 25.05 ± 2.05 vs. SAcx, 23.36 ± 2.33 mmol·kg⁻¹·min⁻¹, \( P < 0.01 \) in both groups). During exercise, carbohydrate oxidation increased significantly in all protocols. It was, however, significantly lower in trained than in sedentary subjects with both lipid infusion (\( P < 0.0005 \)) and acipimox (\( P = 0.0071 \)). Compared with the values obtained after acipimox, lipid infusion reduced carbohydrate oxidation during exercise by 27.6% in trained subjects [not significant (NS)] and by 38.5% in sedentary subjects (NS) (Fig. 4).

Lipid oxidation at rest mirrored carbohydrate oxidation. Basal lipid oxidation rate was 67% higher in TL than in TAcx, and 73% higher in SL than in SAcx. During hyperinsulinemia at rest, lipid oxidation rate was 307% higher in TL than in TAcx, and 404% higher in SL than in SAcx. Lipid oxidation rates during this period were comparable in trained and sedentary subjects in both protocols. During exercise, lipid oxidation increased markedly in trained subjects, but only modestly in sedentary subjects. Lipid oxidation rates were 263% higher in TL than in SL (\( P < 0.0004 \)) and 283% higher in TAcx than in SAcx (NS) (Fig. 4).

**Glucose Ra**

During the last 15 min of hyperinsulinemia at rest, glucose Ra values were TL, 6.74 ± 0.51 mg·kg⁻¹·min⁻¹; TAcx, 10.71 ± 0.74 mg·kg⁻¹·min⁻¹ (\( P < 0.0001 \) vs. TL); SL, 4.09 ± 0.66 mg·kg⁻¹·min⁻¹ (\( P < 0.0001 \) vs. TL); and SAcx, 6.89 ± 0.34 mg·kg⁻¹·min⁻¹ (\( P = 0.0001 \) vs. TAcx, \( P = 0.0045 \) vs. SL) (Fig. 4). Glucose Ra values during the last 10 min of exercise were TL, 11.68 ± 1.01 mg·kg⁻¹·min⁻¹; TAcx, 20.93 ± 1.69 mg·kg⁻¹·min⁻¹ (\( P < 0.0001 \) vs. TL); SL, 9.56 ± 0.75 mg·kg⁻¹·min⁻¹; and SAcx, 18.23 ± 1.82 mg·kg⁻¹·min⁻¹ (\( P < 0.0001 \) vs. TL) (Fig. 4).
The percent reduction in glucose Rd induced by lipids at rest was 37.2 ± 2.1% in trained subjects and 38.9 ± 10.9% in sedentary subjects (NS). During exercise, it was 43.6 ± 3.7% in trained subjects and 21.9 ± 3.9% in sedentary subjects (P, 0.01) (Fig. 6).

**DISCUSSION**

Endurance training increases the utilization of lipids as an energetic substrate to the working skeletal muscle (1, 17). The aim of this study was to assess whether these metabolic adaptations to endurance training alter the inhibitory effects of lipids on whole body glucose utilization. For this purpose, we studied highly endurance-trained athletes and sedentary individuals. The important difference in physical fitness between the two groups of subjects tested was indeed documented by the markedly higher maximal oxygen uptake observed in trained athletes as well as by the increase in lactate concentrations during a moderate exercise in sedentary subjects. The rationale for studying these two groups of individuals lies in the increased capacity of endurance-trained subjects to oxidize lipids during an exercise performed at the same absolute workload (1, 17). We therefore expected that infusion of a lipid emulsion during an exercise performed at physiological high insulin concentrations would produce a similar increase in plasma FFA concentrations in trained and sedentary subjects but a more important stimulation of lipid uptake and oxidation in skeletal muscle of trained subjects. To avoid possible differences in the antilipolytic effects of insulin and in stimulation of lipolysis during exercise between the two groups of subjects, control experiments with pharmacological inhibition of lipolysis were performed. This allowed us to evaluate the effects of lipids on glucose disposal by calculating the relative reduction in glucose disposal between the lipid and antilipolytic (acipimox) protocols.

Our results indicate that our experimental design was indeed adequate for our purpose. Insulin infusion resulted in similar plasma insulin concentrations in trained and sedentary subjects both during lipid infusion and after acipimox. Lipid infusion allowed the maintenance of plasma FFA concentrations in the normal postabsorptive range throughout the hyperinsulinemic and exercise procedures. Moreover, absolute plasma FFA concentrations were quite comparable between trained and sedentary subjects. Acipimox administration nearly completely suppressed adipose tissue lipolysis throughout the procedure, as indicated by steady, low plasma FFA concentrations during the 220 min of the experiments. Finally, plasma glucose concentrations were adequately clamped at comparable values in all protocols.
During the initial 2-h period of hyperinsulinemia, while subjects were resting quietly in the supine position, insulin-mediated glucose disposal was 65% higher in trained vs. sedentary subjects during lipid infusion and 55% higher after acipimox. This observation was expected and reflects the well-known effect of physical fitness and endurance training to increase insulin sensitivity (4, 7). Infusion of lipids, which produced similar increases in plasma FFA concentrations and in lipid oxidation rates in trained and sedentary subjects, also produced the same relative inhibition of insulin-mediated glucose disposal in both groups of subjects. This indicates that elevated plasma FFA concentrations are equally effective in impairing insulin actions in trained and sedentary subjects at rest.

The mechanisms by which elevated FFAs produce insulin resistance are still controversial. It was proposed by Randle et al. (26) that an elevated concentration of FFA stimulates FFA oxidation in skeletal and cardiac muscle. According to the hypothesis enunciated by these investigators, fat oxidation gives rise to increased intracellular concentrations of acetyl-CoA, ATP, and citrate, which in turn inhibit glycolysis and the entry of pyruvate into the tricarboxylic acid cycle. It results in an increase in intracellular glucose 6-phosphate, which in turn inhibits hexokinase and cellular glucose uptake. Several observations, however, suggest that FFA may decrease insulin sensitivity through other mechanisms unrelated with lipid oxidation. Boden et al. (2) observed that an infusion of lipids in healthy humans rapidly increased glucose oxidation at the expense of lipid oxidation; inhibition of nonoxidative glucose disposal, however, was delayed by several hours, suggesting that this effect was not directly related to lipid oxidation. Furthermore, glucose 6-phosphate concentrations were not decreased in skeletal muscle biopsies obtained 6 h after the beginning of lipid infusions. Roden et al. (27) studied skeletal muscle metabolism with nuclear magnetic resonance spectroscopy in healthy subjects during lipid infusion. They observed that lipids suppressed glycogen synthesis and decreased the intracellular concentrations of glucose 6-phosphate. Interestingly, it was also reported that skeletal muscle glucose 6-phosphate concentrations are not increased in humans or Rhesus monkeys with type 2 diabetes (25). In view of these observations, it is proposed that FFAs exert a direct inhibitory effect in the insulin resistance induced by lipids. Inhibition of insulin receptor substrate 1 associated with phosphatidylinositol 3-kinase activity may be involved in this effect (9).

During exercise, lipid infusion resulted in similar plasma FFA concentrations in trained and sedentary subjects, but in markedly higher lipid oxidation rates in trained subjects. This reflects the well-known effect of training on muscle fuel oxidation (17). The major novel observation of this study was that, despite similar plasma FFA concentrations, the reduction by lipids of whole body glucose disposal during exercise was about twice as important in trained as in sedentary subjects.

Several explanations can be proposed for the enhanced effect of lipids on glucose metabolism in endurance-trained individuals. Infused lipids initially combine with circulating apolipoproteins to form lipoproteins similar to very-low-density lipoproteins in the circulation (5). The triglycerides present in these particles are subsequently hydrolyzed to fatty acids by lipoprotein lipase in the capillaries of skeletal muscle and adipose tissue. Endurance training significantly increases the synthesis of lipoprotein lipase in skeletal muscle (21, 30) and, hence, is expected to enhance the proportion of infused triglycerides, which are taken up as FFA by skeletal muscle. A role of lipoprotein lipase in insulin sensitivity is indeed supported by the recent observation of transgenic mice overexpressing lipoprotein lipase being markedly insulin resistant (13).

It is recognized that FFAs enter the skeletal muscle cell by a carrier-mediated process (3). Three putative fatty acid transporters have been identified so far [fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (FABP\textsubscript{pm}), fatty acid transport protein]. If cellular FFA uptake was increased as a consequence of endurance training, it might result in an increase in intracellular long-chain fatty acyl-CoA concentrations, the putative mediator of glucose transport inhibition. An upregulation of fatty acid transporters by exercise training has indeed been suggested. Several reports indicate that chronic muscle stimulation increases FABP (24) and FAT/CD36 (3) or FABP and FAT/CD36 (26) in rats. Other studies, however, failed to observe an effect of training on these parameters (11, 34). Our present observation indicates that similar FFA concentrations are equally effective in reducing insulin-mediated glucose metabolism in trained and sedentary subjects at rest and exert an enhanced inhibition only during exercise. If the effects of FFA were primarily exerted through an increase in intracellular long-chain fatty acyl-CoA, this would imply an acute increase in skeletal muscle FFA transport during muscle contraction. Such an effect of muscle contraction on FFA uptake has indeed been suggested (11) but remains to be demonstrated.

Lipid oxidation was significantly higher in endurance-trained individuals. This may be secondary to an increased uptake of FFA in skeletal muscle. An enhanced activity of the enzyme carnitine palmitoyltransferase I may also be involved (31). Interestingly, lipid oxidation was higher in endurance-trained individuals during exercise even when acipimox was used to inhibit adipose tissue lipolysis. This observation is consistent with the report that endurance training increases oxidation of intramuscular triglycerides (29).

Altogether, our results indicate that the inhibitory effects of lipids on whole body glucose utilization are enhanced in endurance-trained subjects during exercise plus hyperinsulinemia but not during hyperinsulinemia alone. As discussed earlier, an increased uptake of FFA in skeletal muscle due to stimulation of lipoprotein lipase and/or FABP appears the most likely explanation for this effect. The hypothesis that an increased lipid oxidation per se inhibits glucose uptake
has never been actually disproved; it appears, however, unlikely in regard to the observation that chronic inhibition of lipid oxidation causes accumulation of intramyocellular triglycerides and insulin resistance in rats (8). In the present study, the inhibitory effects of lipids being more important in trained subjects only during exercise but not at rest suggests that muscle contraction per se may stimulate transfer of lipids from lipoprotein particles into the muscle fiber and its conversion into long-chain acyl-CoA in trained individuals. It remains, however, possible that our observation was merely due to the fact that endurance training per se has profound effects on skeletal muscle. It increases muscle capillary density (22) and GLUT-4 content (23) and, hence, can be expected to facilitate glucose utilization. This increased insulin sensitivity is readily observed in the endurance-trained subjects of our study. Because lipid suppressed glucose utilization to the same absolute values in both trained and sedentary subjects, it is also possible that the dose of lipids administered exerted a maximal inhibition of glucose utilization in both groups but that the higher percentage of reduction observed in trained individuals merely reflected a large initial glucose utilization.

In conclusion, these results indicate that the inhibitory effects of lipids on whole body glucose utilization are increased in endurance-trained subjects during exercise plus hyperinsulinemia but not during hyperinsulinemia at rest. This suggests that endurance training and muscle contraction upregulate regulatory steps in muscle lipid uptake and metabolism, which are operative in reducing glucose transport and oxidation.

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REFERENCES


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