Effect of gender on lipid kinetics during endurance exercise of moderate intensity in untrained subjects

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Mittendorfer, Bettina, Jeffrey F. Horowitz, and Samuel Klein. Effect of gender on lipid kinetics during endurance exercise of moderate intensity in untrained subjects. Am J Physiol Endocrinol Metab 283: E58–E65, 2002; 10.1152/ajpendo.00504.2001.—We evaluated lipid metabolism during 90 min of moderate-intensity (50% VO2 peak) cycle ergometer exercise in five men and five women who were matched on adiposity (24 ± 2 and 25 ± 1% body fat, respectively) and aerobic fitness (VO2 peak: 49 ± 2 and 47 ± 1 ml·kg fat-free mass·min−1, respectively). Substrate oxidation and lipid kinetics were measured by indirect calorimetry and [13C]palmitate and [3H2]glycerol tracer infusion. The total increase in glycerol and free fatty acid (FFA) rate of appearance (Ra) in plasma during exercise (area under the curve above baseline) was 65% greater in women than in men (glycerol Ra: 317 ± 40 and 195 ± 33 μmol/kg, respectively; FFA Ra: 652 ± 46 and 453 ± 70 μmol/kg, respectively; both P < 0.05). Total fatty acid oxidation was similar in men and women, but the relative contribution of plasma FFA to total fatty acid oxidation was higher in women (76 ± 5%) than in men (46 ± 5%; P < 0.05). We conclude that lipolysis of adipose tissue triglycerides during moderate-intensity exercise is greater in women than in men, who are matched on adiposity and fitness. The increase in plasma fatty acid availability leads to a greater rate of plasma FFA tissue uptake and oxidation in women than in men. However, total fat oxidation is the same in both groups because of a reciprocal decrease in the oxidation rate of fatty acids derived from nonplasma sources, presumably intramuscular and possibly plasma triglycerides, in women.

gender; fatty acids; lipolysis; exercise; stable isotopes

ENDOGENOUS TRIGLYCERIDES are an important source of fuel for working muscles during exercise endurance (24). Increased lipolysis of adipose tissue triglycerides during exercise, which is mediated primarily by an increase in β-adrenergic receptor stimulation (1, 20), releases fatty acids into the systemic circulation for delivery to skeletal muscle for oxidation. In addition, exercise stimulates lipolysis of intramuscular triglycerides, which release fatty acids that are directly oxidized by local mitochondria.

The effect of gender on the mobilization and oxidation of endogenous triglycerides during exercise is unclear because of conflicting results from different studies. Most studies that evaluated regional and whole body lipolytic rates during moderate-intensity endurance exercise, by using either microdialysis probes or isotope tracers, have reported that lipolytic rates in women are greater than in men (1, 6, 14, 23). Others, however, found that the lipolytic response to exercise is the same in men and women (5). Similarly, studies that evaluated substrate oxidation during exercise have reported that women use more fat and less carbohydrate than men (3, 6, 15, 26, 49), whereas other studies found that relative fuel use was similar in men and women (5, 8, 14, 30, 41). The reason(s) for the discrepancies between studies is not clear but may be related to differences in body composition and aerobic fitness between men and women who participated in those studies; both body composition and fitness can independently influence the rate of lipolysis and fat oxidation during endurance exercise (24, 25).

The purpose of the present study was to determine the effect of gender on lipid metabolism during endurance exercise, independent of the potential confounding effects of body composition and fitness. Stable isotope-labeled tracers and indirect calorimetry were used to determine whole body lipid kinetics at rest and during moderate-intensity cycle ergometer exercise in untrained women and men who were matched on age, aerobic fitness, and body composition. We hypothesized that fat oxidation is greater in women than in men because of increased availability and utilization of plasma fatty acids.

METHODS

Subjects

Five premenopausal women and five men participated in this study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine in St. Louis, MO. Male and female subjects were matched on age, percent body fat, and peak aerobic capacity (Table 1). All subjects 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 blood and urine tests. No sub-

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Table 1. Characteristics of the study subjects

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<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>33 ± 3</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>78 ± 2</td>
<td>57 ± 2*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25 ± 1</td>
<td>21 ± 1*</td>
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<tr>
<td>Fat-free mass, kg</td>
<td>59 ± 2</td>
<td>43 ± 2*</td>
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<tr>
<td>Fat mass, kg</td>
<td>19 ± 2</td>
<td>14 ± 1*</td>
</tr>
<tr>
<td>Body fat, % body wt</td>
<td>24 ± 2</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>VO₂peak, ml·kg·body wt⁻¹·min⁻¹</td>
<td>37 ± 2</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>VO₂peak, ml·kg·FFM⁻¹·min⁻¹</td>
<td>49 ± 2</td>
<td>47 ± 1</td>
</tr>
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Values are means ± SE. FFM, fat-free mass; VO₂peak, whole body oxygen consumption. *Value significantly different from corresponding value in men, P < 0.05.

Each subject's peak aerobic capacity (VO₂peak) was measured during cycle ergometer exercise. After 4 min of warm-up at 50 W, the work rate was increased every minute by 25 W until a plateau in oxygen consumption (VO₂), despite increasing workload, and a respiratory exchange ratio (RER) ≥1.1 over ≥1 min was achieved (usually within 7–10 min).

Fat mass (FM) and fat-free mass (FFM) were determined by dual-energy X-ray absorptiometry (model QDR 1000/w; Hologic, Waltham, MA).

Experimental Protocol

The evening before the isotope infusion study, subjects were admitted to the Washington University School of Medicine GCRC. At 1900 on the day of admission, subjects were given a standard meal containing 12 kcal/kg body wt (55% of total energy carbohydrates, 30% fat, and 15% protein). At 2230, the subjects ingested a liquid formula (Ensure; Ross, Columbus, OH) containing 80 g carbohydrates, 12.2 g fat, and 17.6 g protein and then fasted until completion of the study the following day.

The following morning, a stable isotope infusion protocol was performed to evaluate lipid kinetics at rest and during moderate-intensity exercise. At 0600, catheters were inserted in a forearm vein for isotope infusion and in a radial artery for blood sampling. At 0800 (−75 min), while the subjects were sitting in a chair, a primed constant infusion of [1,2,3,3-²H₅]glycerol (priming dose: 1.5 µmol/kg; infusion rate: 0.1 µmol·kg⁻¹·min⁻¹; Cambridge Isotope Laboratories, Andover, MA) and a constant infusion of [1-¹³C]palmitate (Cambridge Isotope Laboratories) bound to human albumin were started and maintained for 165 min by using calibrated syringe pumps (Harvard Apparatus, Natick, MA). From 0915 (0 min) until 1045 (90 min), subjects exercised at 50% of their VO₂ peak on a cycle ergometer (Ergometrics model 800; Ergo-line) that was modified for recumbent cycling to enhance comfort and compliance.

The mobilization of endogenous fuels during exercise causes a decline in expired ¹³CO₂ enrichment (55). Therefore, each subject performed the exercise protocol without tracer infusion within 1 wk of the tracer infusion study to determine the normal change in expired ¹³CO₂ enrichment during exercise, which was used as background expired breath CO₂ enrichment to calculate the rate of plasma free fatty acid (FFA) oxidation during the tracer infusion experiments.

Sample Collection

Blood samples were collected before the start of the isotope infusion to determine background glycerol and palmitate tracer-to-tracer ratios (TTRs) every 5 min from −15 to 0 min during resting conditions and every 10 min during exercise (10–90 min). Blood samples were immediately transferred to 1) chilled tubes containing EDTA to determine plasma FFA and glycerol concentrations and TTRs; 2) chilled tubes containing EDTA and Trasylol to measure insulin and glucagon concentrations; and 3) chilled tubes containing reduced glutathione and EGTA to determine plasma catecholamine concentrations. Blood samples were placed in ice, and plasma was separated by centrifugation within 30 min of collection. Plasma was stored at −70°C until final analyses were performed.

Breath samples were collected in 20-ml Vacutainer tubes before the tracer infusion and at 60, 70, 80, and 90 min of exercise to determine the ¹³CO₂ enrichment in expired breath, as previously described (25). Whole body VO₂ and carbon dioxide production (VCO₂) were determined at 55–63, 65–73, and 75–90 min during exercise by using a metabolic cart (model 2900; SensorMedics, Yorba Linda, CA) that was recalibrated between each measurement period.

Sample Analyses

Plasma insulin concentrations were measured by RIA (19). Plasma epinephrine and norepinephrine concentrations were determined by a single isotope derivative radioenzymatic method (45). Plasma glycerol concentration was determined by gas chromatography-mass spectrometry (GC-MS) after adding [2-¹³C]glycerol to plasma as an internal standard. Plasma FFA concentrations were quantified by gas chromatography (model 5890-II; Hewlett-Packard, Palo Alto, CA) after adding heptadecanoic acid to plasma as an internal standard (36).

Plasma palmitate and glycerol TTRs were determined by GC-MS (MSD 5973 system with capillary column; Hewlett-Packard) as previously described (25, 40). Plasma proteins were precipitated with ice-cold acetone, and hexane was used to extract plasma lipids. FFAs were isolated by using solid-phase extraction columns and converted to their methyl esters with iodomethane. Ions at mass-to-charge ratio (/m/z) 270.2 and 271.2, produced by electron-impact (EI) ionization, were selectively monitored by GC-MS. The aqueous phase, containing glycerol, was dried by Speed-Vac centrifugation (Savant Instruments, Farmingdale, NY). Heptafluorobutyric (HFB) anhydride was used to form an HFB derivative of glycerol, and ions were produced by EI ionization. Glycerol concentration and TTR were determined by selectively monitoring ions at /m/z 253, 254, and 257. The ¹³CO₂-to-¹²CO₂ ratio in expired air was determined by isotope ratio mass spectrometry (Sira II; VG Fisons, Cheshire, UK) as previously described (25).

Calculations

Glycerol and palmitate kinetics. Glycerol rate of appearance (Ra) provides an index of the whole body lipolytic rate and measures glycerol released in the systemic circulation from hydrolysis of adipose tissue and intramuscular and plasma triglycerides. Palmitate Ra provides an index of...
plasma FFA availability and measures the release of fatty acids that are primarily derived from hydrolysis of adipose tissue triglycerides into plasma. Glycerol and palmitate that are released during lipolysis of intraperitoneal fat are cleared by the liver and are not detected by systemic tracer infusion. During resting conditions, the Ra of glycerol and palmitate in plasma were calculated by dividing the tracer infusion rate by the average arterial glycerol or palmitate TTR obtained between -15 and 0 min (Steele’s equation for steady-state conditions; see Ref. 47). The rate of disappearance (Rd) of palmitate (i.e., palmitate tissue uptake) was assumed to be equal to Ra palmitate during rest. During exercise, glycerol Ra, and palmitate Ra and Rd were calculated by using Steele’s equation for non-steady-state conditions (16, 47). The effective volume of distribution was assumed to be 60 ml/kg FFM for palmitate (29) and 300 ml/kg FFM for glycerol (2). However, even a 50% error in the estimated effective volume of distribution would cause a <5% change in calculated Ra and Rd because of the minimal changes in TTR between samples.

The total lipolytic response to exercise was calculated as the area under the glycerol Ra curve above baseline. Similarly, the total release of fatty acids into the systemic circulation during exercise was calculated as the area under the palmitate Ra curve above baseline divided by the proportional contribution of palmitate to total plasma FFA concentration.

Substrate oxidation. Whole body fat and carbohydrate oxidation rates during the last 30 min of exercise were calculated by using the Vo2 and Vco2, as previously described (13). Plasma palmitate oxidation rate was calculated by dividing the Rd of 13CO2 in expired breath (Vco2 times 13CO2 enrichment) by the plasma palmitate TTR. This value was corrected for incomplete 13CO2 recovery by using previously published values (44, 46). The rate of total plasma FFA oxidation was calculated by dividing the plasma palmitate oxidation rate by the proportional contribution of palmitate to total plasma FFA concentration. The rate of oxidation of nonplasma fatty acids was calculated as the difference between the rates of whole body and plasma FFA oxidation. We assumed that intramuscular triglycerides were the primary source of nonplasma fatty acids and that plasma triglycerides were not an important source of fuel during exercise (31, 34, 51).

Statistical Analyses

A power analysis, based on our previous data (25), suggested that five subjects would be needed to detect a 30% difference in substrate kinetics between men and women with an α value of 0.05 and a power of 0.80. A two-way ANOVA (gender × time) with repeated measures was performed to test the significance of differences in substrate kinetics and hormone concentrations between men and women. Student’s t-test for independent samples was used to test the significance of differences in whole body substrate oxidation and total lipolytic response between men and women during the last 30 min of exercise. A P value of ≤0.05 was considered to be statistically significant. All data are expressed as means ± SE.

RESULTS

Exercise Characteristics

During the last 30 min of exercise, Vo2 and Vco2 were constant. Cycle ergometer exercise in men and women was performed at the same absolute (24.5 ± 1.9 and 24.8 ± 1.4 ml Vo2·kg FFM−1·min−1, respectively) and relative (52 ± 3 and 53 ± 2% Vo2peak, respectively) intensities.

Plasma Hormone and FFA Concentrations

No significant differences were observed in plasma hormone concentrations between men and women at rest (Table 2). During exercise, plasma epinephrine and norepinephrine concentrations increased (P < 0.05), whereas plasma insulin concentrations decreased (P < 0.05) in both men and women (Table 2). After a transient decline in plasma FFA concentration during the first 10 min of exercise, plasma FFA concentration increased progressively throughout exercise in men (rest and end of exercise: 347 ± 33 and 637 ± 104 µM) and women (rest and end of exercise: 450 ± 48 and 778 ± 110 µM). On average, palmitate comprised 28 ± 1% of total plasma FFA concentration in men and 27 ± 2% of total plasma FFA concentration in women. The relative contribution of palmitate to total plasma FFA concentration did not change during exercise in either men or women.

Glycerol and Palmitate Kinetics

Glycerol Ra increased progressively during exercise in both men (from 2.0 ± 0.3 µmol·kg body wt−1·min−1 at rest to 5.7 ± 0.8 µmol·kg body wt−1·min−1 at the end of exercise) and women (from 2.3 ± 0.3 µmol·kg body wt−1·min−1 at rest to 7.6 ± 0.8 µmol·kg body wt−1·min−1 at the end of exercise; Fig. 1). However, glycerol Ra (expressed per kg body wt, FFM, and FM) during exercise was ~30% higher in women than in men (significant effect of gender and time; P < 0.05; Fig. 1). The lipolytic response to exercise, defined as the total increase in glycerol Ra during exercise above baseline, was ~60% greater in women (317 ± 40 µmol/kg body wt) than in men (195 ± 33 µmol/kg body wt; P < 0.05; Fig. 2).

Palmitate Ra also increased progressively from rest to exercise in both groups but was ~30% higher in women than in men at rest (1.26 ± 0.14 and 0.86 ± 0.10 µmol·kg body wt−1·min−1, respectively) and dur-

Table 2. Plasma hormone concentrations during rest and exercise

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<th>Rest</th>
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<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td></td>
<td>Exercise</td>
<td>Women</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>54 ± 11</td>
<td>48 ± 12</td>
</tr>
<tr>
<td>Norepinephrine, ng/ml</td>
<td>238 ± 51</td>
<td>209 ± 31</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>9.0 ± 1.1</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>5.9 ± 1.8</td>
<td>3.9 ± 0.4</td>
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Values are means ± SE. *Value significantly different from corresponding value at rest, P < 0.05.
ing exercise ($3.3 \pm 0.4$ and $2.6 \pm 0.4$ μmol·kg body wt$^{-1}$·min$^{-1}$ at the end of exercise, respectively, significant effect of gender and time; $P < 0.05$; Fig. 3). Similarly, Palmitate $R_d$ increased progressively from rest to exercise in both groups but was $\sim 40\%$ higher in women than in men ($4.1 \pm 0.2$ and $2.7 \pm 0.5$ μmol·kg FFM$^{-1}$·min$^{-1}$ during the last 30 min of exercise, respectively; significant effect of gender and time; $P < 0.05$). The increase in total FFA availability during exercise, defined as the total increase in FFA $R_a$ above baseline, was $\sim 70\%$ greater in women ($652 \pm 46$ μmol/kg body wt) than in men ($453 \pm 70$ μmol/kg body wt; $P < 0.05$; Fig. 2).

Substrate Oxidation

During the last 30 min of exercise, the RER was the same in men ($0.87 \pm 0.02$) and women ($0.87 \pm 0.02$). The whole body total fatty acid oxidation rate was also similar in men ($20.5 \pm 3.7$ μmol·kg FFM$^{-1}$·min$^{-1}$) and women ($18.5 \pm 1.1$ μmol·kg FFM$^{-1}$·min$^{-1}$). However, the source of fatty acids that were oxidized differed between genders. The relative contribution of the oxidation of plasma FFAs to total fatty acid oxidation was higher in women than in men ($76 \pm 5$ and $46 \pm 5\%$, respectively; $P < 0.05$), whereas the contribution of nonplasma fatty acids to total fatty acid oxidation was greater in men than in women ($54 \pm 5$ and $24 \pm 5\%$, respectively; $P < 0.05$; Fig. 4).

DISCUSSION

In this study, we examined the effect of gender on lipid metabolism during moderate-intensity endurance exercise. Men and women were matched on adiposity and aerobic fitness to eliminate the confounding influence of gender-related differences in these factors on substrate metabolism. We found that whole body lipolytic rate and plasma FFA availability and uptake during exercise were greater in women than in men. The rate of whole body total fatty acid oxidation was similar in men and women, but the source of fatty acids used as fuel during exercise differed between genders. Compared with men, women oxidized more plasma FFA, presumably derived from adipose tissue triglycerides, and less nonplasma fatty acids, derived primarily from intramuscular and possibly plasma triglycerides.

The mechanism(s) responsible for the gender differences in lipolysis of adipose tissue triglycerides observed in our subjects during exercise is not known. Most of the increase in lipolytic activity that occurs during exercise is mediated through stimulation of adipose tissue β-adrenergic receptors by circulating catecholamines (1, 23, 38). In addition, activation of α-adrenergic receptors, which inhibits lipolysis, may also be involved in determining the net lipolytic response to exercise (23, 48). Plasma catecholamine concentrations during exercise were similar in our male and female subjects. Therefore, the higher rate of lipolysis in women than in men was likely caused by increased adipose tissue sensitivity to β-adrenergic
stimulation, decreased adipose tissue sensitivity to α-adrenergic stimulation, or a combination of the two.

It is unlikely that differences in lipolytic sensitivity to β-adrenergic stimulation were responsible for the gender differences in lipid kinetics we observed during exercise. Several studies that evaluated the direct effects of catecholamines on lipolytic activity have found that adipose tissue lipolytic sensitivity to catecholamines is similar in men and women. Studies performed in vitro in isolated human adipocytes exposed to physiological concentrations of catecholamines (10, 33, 35, 54) and in vivo in human subjects during catecholamine infusion in conjunction with microdialysis (37) or isotope tracer methods (28) found that adipose tissue lipolytic sensitivity was similar in men and women. However, gender differences in α-adrenergic receptor activity may have influenced lipolytic rates during exercise. By using the microdialysis technique, it has been shown that local adipose tissue α-adrenergic receptor blockade during endurance exercise increased regional glycerol release from abdominal subcutaneous adipose tissue in men but not in women (23). These results suggest that α-adrenergic receptor activity inhibits lipolysis during exercise in men but is not involved in the regulation of lipolysis during exercise in women.

The higher rate of fatty acid release into the systemic circulation was probably responsible for the higher rate of plasma fatty acid uptake in our female than in our male subjects. Several studies have shown that whole body fatty acid uptake during short-term (<2 h) moderate-intensity exercise depends on the availability of fatty acids from plasma (18, 38, 39, 51). Although muscle FFA uptake is carrier mediated and saturable (4, 52), the close relationship between FFA Ra and Rd in our subjects suggests that fatty acid uptake was not limited by muscle fatty acid transport in either men or women.

Despite greater tissue uptake of FFAs from plasma in our women than men, total fat oxidation was similar in both groups. This observation suggests that total fat oxidation during moderate-intensity exercise is not entirely regulated by plasma FFA availability. Data from other studies provide evidence that the rate of fat oxidation during moderate-intensity exercise is primarily influenced by energy requirements (53), exercise intensity (43), aerobic fitness (14, 27, 53), muscle oxidative capacity (17), glucose availability (9), and body composition (25).
Therefore, exercise training, which increases muscle oxidative capacity (17, 32, 50), increases fat oxidation during exercise without increasing plasma FFA availability (14, 27, 53). In addition, increasing plasma FFA availability by administration of lipids and the infusion of heparin during moderate-intensity exercise is not accompanied by a corresponding increase in fat oxidation (21, 39, 42). Thus total fat oxidation during exercise was similar in our men and women because they were matched on percent body fat and aerobic fitness, so their exercise bout was performed at the same absolute and relative intensities. Given the similar requirement for fatty acids as a fuel, it is likely that the increased availability and uptake of plasma fatty acids in our women resulted in a decrease in their use of intramuscular triglycerides. Carefully controlled studies conducted in isolated skeletal muscle demonstrate that there is a reciprocal relationship between the oxidation of plasma and intramuscular fatty acids during exercise (11, 12).

Our finding that total fat oxidation during exercise is similar in men and women contradicts the results of several studies that found the rate of fat oxidation was higher in untrained women than in untrained men (3, 6, 15, 30). However, previous studies conducted in untrained men and women, who had similar aerobic fitness levels and percent body fat, also found similar rates of fat oxidation during exercise in both genders (30). Therefore, it is possible that differences in body composition may be responsible for some of the gender differences in fat oxidation rates reported previously. In fact, we have recently found that, within the same gender, increased adiposity is associated with increased fat oxidation during exercise (25).

Our study has several potential limitations. We purposely matched our men and women on adiposity and aerobic fitness to eliminate the possible influence of these factors on substrate metabolism. Although this matching allowed us to evaluate gender independently of gender-related differences in body composition and fitness, it resulted in selecting men who were slightly fatter and less fit than the average lean man (3, 15, 26, 30, 49). Therefore, our results should not be generalized to different cohorts of men or women. In addition, our female subjects were slightly fatter and less fit than the average lean man (3, 15, 26, 30, 49). Therefore, our results may not be representative of the general population of women.

In summary, the results of the present study demonstrate the presence of sexual dimorphism in lipid kinetics during exercise. We found that lipolysis of adipose tissue triglycerides and fatty acid release into plasma during moderate-intensity exercise were higher in women than in men who were matched on adiposity and fitness. The increased availability of plasma fatty acids led to a greater rate of plasma FFA tissue uptake and oxidation in women than in men. However, total fat oxidation was the same in men and women because of a decreased oxidation rate of fatty acids derived from nonplasma sources, presumably intramuscular and possibly plasma triglycerides, in women.

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