Leg free fatty acid kinetics during exercise in men and women

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Leg free fatty acid kinetics during exercise in men and women. Am. J. Physiol. Endocrinol. Metab. 278: E113–E117, 2000.—We previously reported that epinephrine stimulates leg free fatty acid (FFA) release in men but not in women. The present studies were conducted to determine whether the same is true during exercise. Six men and six women bicycled for 90 min at 45% of peak O2 consumption, during which time systemic and leg FFA kinetics ([9,10-3H]palmitate) were measured. The catecholamine and hormonal responses to exercise were not different in men and women. The baseline systemic and leg palmitate release was 94 ± 15 vs. 114 ± 5 µmol/min and 16 ± 2 and 20 ± 3 µmol/min, respectively, in men and women [P = nonsignificant (NS)]. Systemic and leg palmitate release increased (both P < 0.001) to 251 ± 18 vs. 212 ± 16 µmol/min and 73 ± 19 vs. 80 ± 12 µmol/min in men and women, respectively, during the last 30 min of exercise (P = NS, men vs. women). We conclude that the systemic and leg adipose tissue lipolytic response to exercise is not different in nonobese men and women.

THE REASON(S) FOR THE DRAMATIC DIFFERENCE in body fat distribution between nonobese men and women has been of interest to us because a male-type fat pattern, if maintained as obesity develops, is associated with adverse health consequences (16). It is possible that the processes that determine the gender-specific fat distribution patterns may help us to understand the mechanisms that participate in the creation of an upper-body or lower-body obese phenotype. The balance between the uptake and release of fatty acids should determine the net amount of fat stored in a given fat depot (de novo lipogenesis in adipose tissue is thought to be negligible in humans). Lower-body adipocytes from men and women have different lipolytic properties in vitro; women's fat cells have lesser lipolytic responses to physiological catecholamines (25). If these differences are present in vivo, they might favor greater net fat storage in this depot in women.

We have used isotope dilution and splanchnic/leg balance techniques to test the hypothesis that regional lipolysis [free fatty acid (FFA) release] differs between men and women in vivo (11, 12, 14). Basal (14) and postprandial (11) leg FFA release was similar in men and women; however, epinephrine-stimulated lower-body FFA release was substantially different in men and women; leg FFA release increased in men, but not in women (12). Physical activity is the most common circumstance that increases plasma catecholamine concentrations, and catecholamines are necessary for the increased FFA response to exercise (17). Previous studies have documented that leg adipose tissue FFA release increases during exercise in men (1, 24); however, no information is available regarding lower-body lipolysis during exercise in women. If lower-body adipose tissue FFA release fails to increase during exercise in women, this could contribute to the preferential lower-body fat accumulation in nonobese women. These studies were therefore designed to examine the possibility that exercise stimulates lower-body adipose tissue lipolysis in men, but not in women. If this were verified, it would be one of the few physiological circumstances in which differential regulation of lipolysis could help determine body fat distribution.

MATERIALS AND METHODS

Subjects. Written, informed consent was obtained from six nonobese, premenopausal women and six nonobese, age-matched men. Women were studied in the follicular phase of their menstrual cycle. The subjects' characteristics are provided in Table 1.

Materials. [9,10-3H]palmitate was obtained from Amer- sham (Arlington Heights, IL) and prepared for intravenous infusion as a 0.3% albumin in 0.9% NaCl solution. Indocya- nine green (CardioGreen; Becton-Dickinson, Cockeysville, MD) was used in these studies.

Assays and measurements. Plasma glucose was measured using a glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin (9) and growth hormone concentrations (20) were measured by radioimmunoassay. Epinephrine and norepinephrine were measured by high-performance liquid chromatography (HPLC) with electrochemical detection (4). Indocyanine green was measured by spectrophotometry on the day of the study. Plasma palmitate concentration and specific activity (SA), as well as the isotopic purity of the [3H]palmitate, were determined using HPLC (18). To measure leg oxygen uptake (V02), arterial and venous blood gases were measured using an Instrument Laboratories (Lexing- ton, MA) model 1301 blood gas analyzer and model 282 co-oximeter. Arterial and venous blood gas analyses were carried out in duplicate at each time point. Resting V02 and carbon dioxide excretion rates were measured with a Delta- Trac Metabolic Cart (Sensor Medics, Yorba Linda, CA). V02...


Table 1. Subject characteristics

<table>
<thead>
<tr>
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<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>32 ± 3</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 3</td>
<td>169 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84.0 ± 6.6</td>
<td>65.4 ± 4.1</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>18 ± 3</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Leg fat, kg</td>
<td>5.5 ± 1.1</td>
<td>8.7 ± 1.1</td>
</tr>
<tr>
<td>Leg fat &amp; free mass, kg</td>
<td>22.6 ± 0.9</td>
<td>14.8 ± 0.8</td>
</tr>
<tr>
<td>Peak VO₂, ml·kg FFM⁻¹·min⁻¹</td>
<td>56 ± 3</td>
<td>51 ± 1</td>
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Values are means ± SE of 6 men and 6 women subjects. With the exception of age and peak oxygen consumption (VO₂peak) values are significantly (P < 0.05) different between men and women. Percent body fat, leg fat, and leg fat free mass (FFM) were measured using dual-energy X-ray absorptiometry; leg values refer to both legs.

and CO₂ production (VCO₂) during exercise were measured as previously described (21).

Protocol. Each volunteer’s peak O₂ consumption (VO₂peak) was measured with a continuous bicycle exercise test. Exercise was initiated at 25 and 50 W for women and men, respectively, and increased by 25 or 30 W, respectively, every 2 min until exhaustion. Breath-by-breath VO₂ and VCO₂ were measured throughout exercise. All volunteers were required to consume an isocaloric diet in the Mayo Clinic General Clinical Research Center (GCRC) for 7 days before this study. The diet provided 35% of calories as fat, 15% as protein, and 50% as carbohydrate. All subjects maintained their usual level of physical activity during the week before the study. Weight stability was achieved before the exercise/FFA turnover study. Total body and regional fat and fat free mass (FFM) were measured within 3 days of the study by use of dual-energy X-ray absorptiometry (model DPX-iQ, Lunar Radiation, Madison, WI). Each subject was scanned for 20 min, and whole body and regional analysis was performed using software version 4.1.

The volunteers were admitted to the GCRC on the evening before the study. An 18-gauge forearm intravenous infusion catheter was placed and kept patent with a controlled infusion of 0.45% NaCl. At 0700, under local anesthesia, 20-gauge catheters were placed using sterile technique into the femoral artery (for indocyanine green infusion) and a radial artery (for arterial blood sampling), and an 18-gauge catheter was placed in the ipsilateral femoral vein (for blood sampling). After the catheters were placed, a primed (1.2 mg) continuous (200 µg/min) femoral arterial infusion of indocyanine green was started for measurement of leg blood flow. An intravenous infusion of [9,10-³H]palmitate (0.3 µCi/min) was initiated simultaneously for measurement of systemic and leg FFA uptake and release. After 30 min for isotope and indocyanine green equilibration, a series of blood samples was obtained at 10-min intervals over 30 min for baseline (resting) values.

After the baseline samples were collected, the tracer and indocyanine green infusions were temporarily stopped while the volunteers were transferred to the Integrative Physiology Core of the GCRC for the exercise study. The continuous [9,10-³H]palmitate infusion was started as soon as the volunteers arrived in the exercise study room, whereas the indocyanine green infusion was restarted at ~3.0 mg/min 3 min before the collection of each exercise blood sample (15) and then discontinued after the sample was collected. The volunteers exercised at ~45% of their previously determined VO₂peak for 90 min. Arterial and femoral venous blood samples were obtained at 15-min intervals during exercise. Blood oxygen content, blood glucose, plasma FFA concentration and SA, and indocyanine green concentrations were measured in both arterial and femoral venous samples. Arterial plasma lactate, insulin, growth hormone, and catecholamine concentrations were measured on three of the baseline samples and the last three exercise samples. Systemic VO₂ and VCO₂ were measured throughout exercise by use of a breath-by-breath mass spectrometry system previously validated against the meteorological balloon collection technique (21). Heart rate was monitored throughout the study. The subjects were provided free access to drinking water and were cooled by an electric fan while bicycling. After completion of the study, the catheters were removed and the volunteers remained under observation in the GCRC until the following morning.

Calculations and statistics. All values are presented as means ± SE unless otherwise stated. Systemic palmitate rate of appearance (R₀) was calculated using non-steady-state formulas (13), although because palmitate flux was increasing in response to exercise, steady-state formulas provided near identical results (13). Leg plasma flow was measured using the indocyanine green dye infusion and the calculations described by Jorfeldt and Wahren (15). Steady-state plasma palmitate concentrations and SA were used together with leg plasma flow to measure baseline leg palmitate uptake and release (1). Regional substrate balance measurements are ideally performed under steady-state conditions, which were not uniformly achieved during the last 30 min of exercise (slight changes in plasma palmitate concentrations were observed throughout exercise). This would result in significant problems for amino acid or glucose kinetics because of their longer tissue equilibration times. Fortunately, the half-life of FFA is brief (~3.5 min) (7) and decreases further with exercise (24). In addition to the high fractional FFA turnover rates during exercise, leg blood flow increases to such an extent that the circulation time of the leg is <10 s (19). This combination of factors should allow accurate leg balance measurements during exercise when steady-state assumptions are used, despite the gradual changes in FFA concentrations we observed.

Between-group differences in subject characteristics were tested for statistical significance using a non-paired Student’s t-test. A P < 0.05 was considered significant. Comparisons of baseline and exercise values (mean of values between 60 and 90 min) between men and women were performed using a 2 × 2 repeated-measures analysis of variance.

RESULTS

Subject characteristics. Men and women volunteers for this study were well matched for age and were of comparable fitness levels when peak VO₂ was assessed relative to FFM (Table 1). As expected, men were significantly taller and heavier, with a smaller percentage of body fat than women. Leg FFM was significantly greater in men than in women, whereas leg fat was significantly less in men. The baseline respiratory quotient (RQ) was 0.83 ± 0.02 in both men and women.

Exercise responses. Although men exercised at a higher absolute VO₂, they exercised at the same percentage of VO₂peak as women. The systemic RQ values were not significantly different during exercise in men and women. Consistent with their greater size and exercise capacity, the average leg plasma flow was greater in men than in women during exercise (Table 2). Baseline plasma catecholamine, insulin, and growth hormone concentrations were not different between men and women (Table 3). Plasma catecholamine and lactate concentrations increased significantly in both groups in
response to exercise, whereas plasma insulin concentrations decreased significantly. There were no statistically significant between-group (gender) differences in these values during exercise. Baseline arterial plasma glucose concentrations were 5.2 ± 0.2 and 4.8 ± 0.1 mmol/l in men and women and averaged 5.1 ± 0.3 and 4.9 ± 0.2 mmol/l over the last 30 min of exercise [P = nonsignificant (NS), men vs. women and baseline vs. exercise]. The baseline leg arteriovenous oxygen differences were 57 ± 11 and 30 ± 2 ml/l in men and women, respectively, increasing to 138 ± 10 and 123 ± 9 ml/l during exercise.

Palmitate kinetics. The changes in arterial and femoral venous plasma palmitate concentrations are depicted in Fig. 1. After an initial period of stable or declining concentrations, a gradual rise was noted throughout the remainder of exercise. Over the last 30 min of exercise, arterial plasma palmitate concentrations averaged 158 ± 14 and 195 ± 17 µmol/l in men and women, respectively (P = NS). Baseline systemic palmitate $R_a$ was 94 ± 15 and 114 ± 5 µmol/min in men and women (P = NS), and, in contrast to palmitate concentrations, $R_a$ increased by 15 min of exercise in both groups (Fig. 2C). Over the last 30 min of exercise, systemic palmitate $R_a$ increased to an average of 251 ± 18 µmol/min in men and 212 ± 16 µmol/min in women, respectively. The increase from baseline was significant (P < 0.001) in both groups, as indicated by a significant time effect; however, no gender effect was observed in the response, either in the absolute flux or in the percent increase from baseline values.

Baseline leg palmitate release was 16 ± 2 and 20 ± 3 µmol/min in men and women (P = NS). During exercise

Table 2. Exercise indirect calorimetry data and leg blood flow

<table>
<thead>
<tr>
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<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>$\dot{V}O_2$, ml/min</td>
<td>1,728 ± 71*</td>
<td>984 ± 49</td>
</tr>
<tr>
<td>$\dot{V}O_2$, % of max</td>
<td>46 ± 1</td>
<td>46 ± 1</td>
</tr>
<tr>
<td>Systemic RQ</td>
<td>0.89 ± 0.01</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>Leg plasma flow, ml/min</td>
<td>2,258 ± 186*</td>
<td>1,766 ± 79</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 men and 6 women subjects. Measured $\dot{V}O_2$, $\dot{V}O_2$ relative to peak $\dot{V}O_2$, average respiratory quotient (RQ), and leg blood flow during 90 min of exercise in study participants. *P < 0.05 men vs. women.

Table 3. Plasma catecholamine, hormone, and lactate concentrations

<table>
<thead>
<tr>
<th></th>
<th>Men Baseline</th>
<th>Men Exercise</th>
<th>Women Baseline</th>
<th>Women Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine, pmol/l</td>
<td>373 ± 95</td>
<td>1954 ± 1240*</td>
<td>351 ± 103</td>
<td>2155 ± 613*</td>
</tr>
<tr>
<td>Norepinephrine, nmol/l</td>
<td>0.91 ± 0.09</td>
<td>5.12 ± 0.09*</td>
<td>0.87 ± 0.06</td>
<td>3.67 ± 0.44*</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>23 ± 6</td>
<td>14 ± 6*</td>
<td>20 ± 2</td>
<td>13 ± 2*</td>
</tr>
<tr>
<td>hGH, µg/l</td>
<td>1.32 ± 0.61</td>
<td>3.68 ± 0.57*</td>
<td>0.65 ± 0.16</td>
<td>3.48 ± 0.48*</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.72 ± 0.08</td>
<td>0.94 ± 0.08*</td>
<td>0.52 ± 0.04</td>
<td>1.35 ± 0.19*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 men and 6 women subjects. hGH, human growth hormone. Average concentrations during baseline interval and final 30 min of exercise. *P < 0.05 vs. baseline.

Fig. 1. Arterial and femoral venous plasma palmitate concentrations (means ± SE) in women (n = 6, A) and men (n = 6, B) before and during 90 min of exercise at ~45% of peak $O_2$ consumption. The zero time point value is the mean of 4 resting, steady-state samples obtained over 30 min.

Fig. 2. Leg palmitate uptake (A), leg palmitate release (B), and systemic palmitate flux (C) (means ± SE) in women (n = 6) and men (n = 6) before and during 90 min of exercise at ~45% of peak $O_2$ consumption. The zero time point value is the mean of 4 resting, steady-state samples obtained over 30 min.
Leg palmitate release increased in both groups (Fig. 2B), averaging 73 ± 19 and 80 ± 12 µmol/min in men and women, respectively, during the last 30 min of exercise. A significant time effect (P < 0.001) was observed, but no significant gender effect (P = 0.62) was apparent. Leg palmitate release relative to leg fat mass was somewhat less in women than in men during the baseline (5.1 ± 1.1 vs. 7.0 ± 1.8 µmol·kg fat⁻¹·min⁻¹, respectively) and exercise (19.5 ± 2.8 vs. 29.9 ± 7.2 µmol·kg fat⁻¹·min⁻¹, respectively) conditions; however, these differences were not statistically significant. There were no statistically significant correlations between leg tissue composition (percent fat) and leg palmitate release under basal or exercise conditions for either group separately or the combined groups.

Leg palmitate uptake in men and women under baseline conditions was 12 ± 2 and 9 ± 2 µmol/min (P = NS), increasing (P < 0.001, Fig. 2A) to an average of 75 ± 19 and 69 ± 10 µmol/min during the last 30 min of exercise (P = NS, men vs. women).

**DISCUSSION**

These studies were designed to examine whether mobilization of FFA from lower-body adipose tissue is impaired in women compared with men. We measured systemic and leg FFA release in groups of men and women of comparable age and fitness, at exercise levels known to result in moderate stimulation of lipolysis. Both groups had comparable exercise responses in terms of catecholamines, insulin, and growth hormone, each of which changed in a manner that should increase lipolysis. The increase in systemic and leg FFA (palmitate) release in response to this stimulus was virtually identical in both groups, suggesting that there is no gender difference in leg FFA mobilization in response to exercise. Our previous observation that the leg lipolytic response to epinephrine is impaired in women (12) cannot be extrapolated to physiological circumstances such as exercise.

A number of factors are thought to participate in the lipolytic response to exercise. The increase in epinephrine and norepinephrine during exercise appears to be important in the FFA response, because an adrenergic blockade inhibits the increase in FFA concentrations normally observed during moderate exercise (17). The results of our previous epinephrine infusion study (12) led us to believe that, if epinephrine were a critical component of the lower-body adipose tissue lipolytic response, women would fail to increase leg palmitate release during exercise. Arterial epinephrine concentrations in women in that study (1,605 ± 62 pmol/l) were not much different from those observed in the present study (Table 3). It should be noted, however, that arterial norepinephrine concentrations were ~2.5-fold greater during exercise than during the epinephrine infusion (12). Perhaps a threshold level of catecholamines was achieved during exercise in the women participants in this study that permitted the brisk lower-body adipose tissue lipolysis response. The decrease in plasma insulin concentrations with exercise also appears to be important for the normal increase in FFA to occur (10). Perhaps this hormonal response to exercise is critical in allowing leg adipose tissue, rather than catecholamines, to increase FFA release. Finally, growth hormone also has important lipolytic properties (3) and may have been an essential factor that allowed near-identical leg FFA release rates in men and women. Most likely, the integrated hormonal and catecholamine response to exercise is sufficiently robust to allow a generalized mobilization of FFA from all adipose tissue depots (1).

A number of investigators have reported differences between men and women in the metabolic response to exercise (2, 8, 23). We noted a statistically nonsignificant trend for the systemic RQ to be less in women than in men during exercise, but no such trend for differences in systemic lipid fuel mobilization. It is difficult to make direct comparisons with previous studies, however. This appears to be one of the few studies to directly measure FFA mobilization in men and women; most previous studies have used indirect indexes, such as plasma glycerol and/or FFA concentrations (23), or adipose tissue dialysate glycerol concentrations (2, 8). Other between-study differences include variations in the intensity and duration of exercise (2, 8, 23), the fitness level of the participants (23), and variations in the control of the prestudy diet (2, 8), a factor known to influence the metabolic response to exercise (5, 6).

In summary, we tested the hypothesis that leg adipose tissue mobilization of FFA is impaired in women compared with men by measuring systemic and leg FFA kinetics before and during 90 min of moderate-intensity exercise in both groups. Men and women had comparable hormonal and catecholamine responses to exercise, as well as similar systemic and leg FFA release. We conclude that the gender differences in catecholamine-stimulated leg lipolysis that we previously reported (12) do not extend to exercise. This finding, combined with the lack of substantive gender differences in basal (14) and postprandial (11) regional lipolysis, suggests that body fat distribution variations might relate more to regional fatty acid storage than to fatty acid release. Studies to determine whether the greater lipoprotein lipase activity in leg adipose tissue of women (22) is associated with relatively greater triglyceride uptake will be helpful in this regard.

We acknowledge the technical assistance of Joan Aikens, Carol Siverling, Tammi Eickhoff, and the staff of the Mayo Clinic General Clinical Research Center, and the editorial assistance of Susan Leachman.

This study was supported by National Institutes of Health Grants DK-45353 and RR-00985, the Minnesota Obesity Center (DK-50456), and the Mayo Foundation.

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Received 18 May 1999; accepted in final form 2 September 1999.

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