Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects

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Roepstorff, Carsten, Charlotte H. Steffensen, Marianne Madsen, Bente Stallknecht, Inge-Lis Kanstrup, Erik A. Richter, and Bente Kiens. Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects. Am J Physiol Endocrinol Metab 282: E435–E447, 2002; 10.1152/ajpendo.00266.2001.—Substrate utilization across the leg during 90 min of bicycle exercise at 58% of peak oxygen uptake (Vo2peak) was studied in seven endurance-trained males and seven endurance-trained, eumenorrheic females by applying arteriovenous catheterization, stable isotopes, and muscle biopsies. The female and male groups were matched according to Vo2peak per kilogram of lean body mass, physical activity level, and training history of the subjects. All subjects consumed the same diet, well controlled in terms of nutrient composition as well as energy content, for 8 days preceding the experiment, and all females were tested in the midfollicular phase of the menstrual cycle. During exercise, respiratory exchange ratio (RER) and leg respiratory quotient (RQ) were similar in females and males. Myocellular triacylglycerol (TG) degradation was negligible in males but amounted to 12.4 ± 3.2 mmol/kg dry wt in females and corresponded to 25.0 ± 6.0 and 5.0 ± 7.3% of total oxygen uptake in females and males, respectively (P < 0.05). Utilization of plasma fatty acids (12.0 ± 2.5 and 9.6 ± 1.5%), blood glucose (13.6 ± 1.5 and 14.3 ± 1.5%), and glycogen (48.5 ± 4.9 and 42.8 ± 2.1%) were similar in females and males. Thus, in females, measured substrate oxidation accounted for 99% of the leg oxygen uptake, whereas in males 28% of leg oxygen uptake was unaccounted for in terms of measured oxidized lipid substrates. These findings may indicate that males utilized additional lipid sources, presumably very low density lipoprotein-TG or TG located between muscle fibers. On the basis of RER and leg RQ, it is concluded that no gender difference existed in the relative contribution from carbohydrate and lipids to the oxidative metabolism across the leg during submaximal exercise at the same relative workload. However, an effect of gender appears to occur in the utilization of the different lipid sources.

[13C]palmitate; plasma fatty acids; myocellular triacylglycerol; glucose; glycogen

Recently, it has been investigated in several studies whether a gender difference exists in the relative utilization of carbohydrates and lipids as fuel sources during submaximal exercise. Some studies have shown that females derive a relatively larger contribution from lipids to oxidative metabolism during exercise than males (6, 11, 19, 36). However, others have observed a similar relative utilization of carbohydrates and lipids in females and males exercising at the same relative workload (3, 17, 26).

The relative contribution from carbohydrates and lipids as fuel during submaximal exercise is a result of the sum of utilization of the different carbohydrates (blood glucose and muscle glycogen) and lipids (albumin-bound long-chain fatty acids (FA) from the blood plasma, FA from circulating very low density lipoprotein-triacylglycerols (VLDL-TG), and FA from myocellular triacylglycerols (MCTG)). It has previously been observed in a few studies that females and males have a similar systemic turnover of plasma FA during exercise expressed relative to body mass (BM) (3, 7) or lean body mass (LBM) (26). Furthermore, Friedlander et al. (7) found that systemic plasma FA oxidation during exercise did not differ between untrained females and males. It has also previously been observed that systemic turnover of glucose was similar in females and males exercising at the same relative workload (6, 17, 26).

The study of substrate utilization locally in the active muscles provides a much more detailed picture than whole body measurements, which do not reflect metabolism only in skeletal muscle. Recently, Burguera et al. (3) observed that plasma FA total uptake and release across the leg were similar in untrained females and males during bicycle exercise at 45% of peak oxygen uptake (Vo2peak). However, whether this gender similarity exists at a higher relative workload and/or in trained individuals is not known. Furthermore, the oxidation of plasma FA in the exercising leg has not yet been compared in females and males.

So far, the three methods most often applied to quantify the respective utilization of blood glucose, glycogen, plasma FA, VLDL-TG, and MCTG during exercise are the muscle biopsy technique, the stable
isotope tracer technique, and net balances across the active muscle tissue bed, respectively. However, up to now, the relative utilization of these energy sources during exercise has not been determined by applying the three mentioned methods simultaneously in a single gender comparative study. Particularly, gender comparisons of net balances across the active muscles are lacking. Such studies would be expected to yield valuable information on possible gender differences in substrate utilization during exercise.

Therefore, the purpose of the present study was specifically to determine the utilization of blood glucose, glycogen, plasma FA, and MCTG, when applying simultaneously the muscle biopsy technique, the stable isotope tracer technique, and net balances across the active muscles.

**MATERIALS AND METHODS**

From screening of 48 young endurance-trained females and males, seven females and seven males were recruited to participate in the study (Table 1) on the basis of the training and oxidative capacity criteria given in Prescreening that all females were eumenorrheic with a cycle length between 28 and 35 days and that none of them were taking oral contraceptives. Preliminary tests and the main exercise experiment in females were carried out in the midfolllicular phase of their menstrual cycle (determined as days 7–11 from onset of menstruation, mean day 9 ± 1 (mean ± SE) for the exercise experiment).

All subjects were fully informed of the risks associated with the study, and all of them gave written, informed consent. The study was approved by the Copenhagen Ethics Committee and was carried out in accordance with the Declaration of Helsinki II.

**Experimental Design**

**Prescreening.** $\dot{V}O_{2\text{peak}}$ was initially determined in an incremental bicycle exercise test, where respiratory measurements were carried out with the Douglas bag technique. Training history and present weekly training were determined from self-reports (questionnaire and activity record).

Furthermore, body composition was calculated from body density (31) determined by hydrostatic weighing with a correction for residual lung volume measured by the oxygen dilution method (16). Single-leg composition was determined by means of dual-energy X-ray absorptiometry (Lunar, Madison DPX-IQ version 4.6.6) from a whole body scan by a pelvis cut angled through the femoral necks according to the manufacturer's directions (Table 1).

Only subjects engaged in endurance-type physical training for a minimum of 5–7 h/wk for ≥2 yr were enrolled in the study. Furthermore, a $\dot{V}O_{2\text{peak}}>55\, \text{ml·kg}^{-1} \cdot \text{min}^{-1}$ and $>60\, \text{ml·kg}^{-1} \cdot \text{min}^{-1}$ was required for females and males, respectively. The male group was matched to the female group according to their $\dot{V}O_{2\text{peak}}$/LB and training history (Table 1).

**Dietary control.** When enrolled in the study, all subjects recorded their food intake by weighing on 5 nonconsecutive days (consisting of 1 day without training, 1 day with heavy training, 1 weekend day, and 2 weekdays with normal training) to determine the amount of energy and the nutrient composition of their habitual diet. Food records were analyzed using a computer program (Dankost 2000, Danish Catering Center, Copenhagen, Denmark). On the basis of these individual food records, subjects consumed a controlled, isocaloric diet during the 8 days preceding the exercise experiment. The diet consisted of ~15 energy percent (E%) protein, ~20 E% fat, and ~65 E% carbohydrate. This nutrient composition was similar to the habitual diet of the subjects. Food items with a high ratio of $^{13}$C to $^{12}$C were avoided in the controlled diet to keep the background enrichment of $^{13}$CO$_2$ in breath and blood as low as possible during the exercise experiment. During the 8 days, all food intake was strictly controlled, and all food was weighed to an accuracy of 1 g and registered in a dietary record. Throughout the 8 days, adherence to the diet was evidenced by the close resemblance between the prescribed dietary content and the actual intake.

**Exercise experimental protocol.** The exercise test consisted of 30 min bicycling on a Krogh bicycle ergometer for 90 min at 58% $\dot{V}O_{2\text{peak}}$.

On the morning of the exercise experiment, subjects arrived at the laboratory at 8:00 AM by either bus, train, or car after an overnight fast from 11:30 PM the night before. Furthermore, the subjects had abstained from exercise training during the 2 days preceding the exercise experiment. After 30 min of rest in the supine position, Teflon catheters were inserted under local anesthesia into the femoral artery and the contralateral femoral vein by use of aseptic techniques. The tips of the catheters were advanced proximally to the midfolllicular position. Then, arterial and venous resting blood samples were obtained simultaneously for determination of background enrichments of the isotopes. Expired air was collected in Douglas bags. Then, a muscle biopsy was obtained from vastus lateralis muscle under local anesthesia with the needle introduced in a distal angle.

With the subjects still in the supine position, infusion of stable isotopes was initiated and continued during the following 90 min at rest. After a primer infusion of $[6,6-^{2}\text{H}_2]$glucose (3.203 mg/kg BM) and NaH$^{13}$CO$_3$ (0.085 mg/kg BM), glucose and glycogen metabolism were studied by means of a stable isotope tracer technique, and net balances across the active muscles.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Females $(n = 7)$</th>
<th>Males $(n = 7)$</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>25 ± 1</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.75 ± 0.02</td>
<td>1.84 ± 0.03*</td>
</tr>
<tr>
<td>BM, kg</td>
<td>65.9 ± 3.3</td>
<td>75.2 ± 1.8*</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>17.5 ± 1.2</td>
<td>11.8 ± 1.3*</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>54.1 ± 2.5</td>
<td>66.3 ± 1.3*</td>
</tr>
<tr>
<td>One-leg LLM, kg</td>
<td>8.3 ± 0.5</td>
<td>10.4 ± 0.2*</td>
</tr>
<tr>
<td>$\dot{V}O_{2\text{peak}}$</td>
<td>3.84 ± 0.18</td>
<td>4.76 ± 0.11‡</td>
</tr>
<tr>
<td>l/min</td>
<td>58.1 ± 1.3</td>
<td>63.3 ± 0.8*</td>
</tr>
<tr>
<td>ml·kg·BM$^{-1}$</td>
<td>71.0 ± 1.5</td>
<td>71.7 ± 0.6</td>
</tr>
<tr>
<td>ml·LBM$^{-1}$</td>
<td>317 ± 15</td>
<td>390 ± 12‡</td>
</tr>
<tr>
<td>Maximal work load, W</td>
<td>5.2 ± 0.9</td>
<td>7.0 ± 1.5</td>
</tr>
<tr>
<td>Training history</td>
<td>8.4 ± 0.9</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>Duration, h/wk</td>
<td>11.6 ± 0.5</td>
<td>9.6 ± 1.2</td>
</tr>
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Data are means ± SE. BM, body mass; LBM, lean body mass; LLM, lean leg mass; $\dot{V}O_{2\text{peak}}$, peak oxygen uptake. Gender difference, *$P < 0.05$; ‡$P < 0.01$; †$P < 0.001$.  

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mg/kg BM) into the antecubital vein of one arm, within 1 min, constant infusions of [U-13C]palmitate (0.011 μmol·kg BM−1·min−1) and [6,6-2H2]glucose (0.055 mg/kg BM−1·min−1) into the antecubital veins of contralateral arms were initiated using calibrated syringe pumps (Harvard Apparatus, Plymouth Meeting, PA, and VIAL Medical SE 200 B, St. Etienne, France). [6,6-2H2]glucose and NaH[13C]O3 infusates were passed through a 0.22-μm sterile filter (Millex-Or, Millipore, Molsheim, France) before infusion, whereas [U-13C]palmitate was not, because of the preparation described below.

During the last 10 min of the infusion period at rest, femoral venous blood flow was measured by the thermodilution method with bolus injections of 5 ml of ice-cold sterile saline (1). Furthermore, expired air was collected in Douglas bags, and blood samples were obtained twice at 5-min intervals for determination of basal isotope enrichment in blood and expiratory air as well as for determination of basal resting blood concentrations. To avoid dehydration during the rather long preexercise resting and exercising periods, a slow intravenous saline drip was started at the same time that the infusion of stable isotopes was initiated. During the experiment, a total of ~800 ml of isotonic saline was administered.

The subjects commenced a 90-min bicycle exercise test on a Kroghe bicycle ergometer at a workload corresponding to 58% V02peak. Males and females exercised at the same relative workload. Infusion of stable isotopes was continued during the exercise period, with the infusion rate of [6,6-2H2]glucose doubled, whereas the infusion rate of [U-13C]palmitate remained unchanged compared with that at rest. Blood was sampled simultaneously from the femoral artery and vein at 15, 30, 60, 75, and 90 min of exercise. Femoral venous blood flow was determined immediately before each blood sampling by means of a constant infusion of ice-cold saline in the gaseous form. Each injection introduced 20 μl of samples into the column in split mode (split ratio 1:2 for breath and 1:5 for blood samples). A fused silica 25 m × 0.32 mm CP-Poraplot Q column (Chrompack, VARIAN, Analytical Instruments, Værløse, Denmark) was used for chromatography. A deactivated, fused silica 5 m × 0.32 mm column coated with cyanoethylmethyl (Chrompack, VARIAN) was used as a postcolumn. Helium was the carrier gas at 1.8 ml/min. Injector and oven temperatures were set at 35 and 30°C, respectively.

Derivatization of palmitate to its methyl ester was modified from the method described by Patterson et al. (21). Briefly, heptadecanoic acid was added to plasma samples or blanks and mixed for 10 min. Precipitation of proteins was carried out by adding ice-cold acetone, mixing for 1 min, placing at −20°C for 15 min, and centrifuging. After centrifugation, the supernatant was transferred to a new tube. After H2O and hexane were added, mixed for 15 min, and centrifuged, the supernatant was transferred to a new tube, and samples were evaporated under nitrogen. To form FA methyl esters, buffer and isododecane were added, samples were mixed for 10 min, hexane was added, and samples were mixed for 15 min and centrifuged. The supernatant was transferred to a new tube and evaporated under nitrogen. To separate FA methyl esters by solid phase extraction (SPE) chromatography, hexane was added to the dried samples, mixed for 2 min, and transferred to SPE tubes (Supelclean LC/SC, 3-ml tubes, Supelco, Bellefonte, PA) previously washed twice with hexane. SPE tubes were rinsed with hexane, and FA methyl esters were eluted with two rinses of 3% ethyl acetate in hexane and collected into a new tube.
Finally, samples were evaporated under nitrogen, and hexane was added before transfer to GC vials.

The enrichment of [U-13C]palmitate was measured by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS; Hewlett-Packard 5890, Palo Alto, CA, and GC Combustion III/Delta plus+, Finnigan MAT) on the methyl ester derivatives. A 30 m × 0.32 mm ID column coated with 0.2 μm of 10% cyanopropyl-90% biscyanopropyl polysiloxane (Rtx 2330, Restek, Bellefonte, PA) was used for chromatography. A deactivated fused silica 5 m × 0.32 mm column coated with cyanophenylmethyl (Chrompack, VARIAN) was used as a precolumn. Injection of samples (2 μl) into the column occurred by large-volume injection (programmed temperature vaporization). Helium was the carrier gas at 1.8 ml/min. Temperatures were set at the following: injector, initially 50°C, stayed for 1 min, increased to 285°C at 20°C/min, and stayed for 5 min; oven, initially 50°C, stayed for 4 min, increased to 150°C at 10°C/min, increased to 160°C at 2°C/min, increased to 250°C at 20°C/min, and stayed for 10 min; oxidation reactor, 960°C; reduction reactor, 650°C.

For determination of 13CO2 enrichment, a CO2 gas with known 13CO2 enrichment was used as a reference gas. Based on this reference gas, the enrichment of 13CO2 was obtained as a 5-value with reference to the enrichment of 13CO2 in Pee Dee Belemnite limestone. From the 5-value, the ratio between 13CO2 and 12CO2 was calculated for every sample.

Plasma palmitate concentrations were determined by GC (AutoSystem XL, Perkin-Elmer, Wellesley, MA) on the methyl ester derivatives by using heptadecanoic acid as an internal standard. Each injection introduced 2 μl of samples into the column in split mode (split ratio 1:5). A 30 m × 0.32 mm ID column coated with 0.2 μm 10% cyanopropyl-90% biscyanopropyl polysiloxane (Rtx 2330) was used for chromatography. A fused silica 5 m × 0.32 mm column coated with deactivated cyanophenylmethyl (Chrompack, VARIAN) was used as a precolumn. Helium was the carrier gas at 1.8 ml/min. Temperatures were set at the following: injector, 300°C; oven, initially 50°C, stayed for 4 min, increased to 130°C at 10°C/min, increased to 160°C at 4°C/min, and then increased to 250°C at 20°C/min and stayed for 10 min.

Derivatization of glucose to its butyrylboronic acid derivative was modified from Pickert et al. (24). Briefly, precipitation of proteins was carried out by adding a methanol-chloroform solution (2.3:1 vol/vol) to the plasma samples, centrifuging, and transferring the supernatant to a new tube. Lipids were then removed by adding chloroform and H2O at pH 2.0, centrifuging, and transferring the supernatant to a new tube. After being frozen at −70°C in liquid nitrogen and vacuum centrifuged in a SpeedVac centrifugal concentrator (MAXI dry lyo P.D. 1.0, Heto-Holten, Allerød, Denmark), the extracted glucose was derivatized by adding butyrylboronic acid in pyridine and nitrogen gas, heating, and finally adding acetic anhydride and nitrogen gas. After 90 min of reaction at 25°C, the liquid was transferred to a new tube, evaporated to dryness with nitrogen, redissolved in ethyl acetate, and transferred to a GC vial.

The enrichment of [6,6-2H2]glucose was determined by GC-MS (Finnigan Trace GC 2000 - Automass III) on the butyrylboronic acid derivative. A fused silica 30 m × 0.25 mm ID CP-Sil 8 CB low bleed/MS column (Chrompack, VARIAN) was used for chromatography. A deactivated fused silica 5 m × 0.32 mm column coated with cyanophenylmethyl (Chrompack, VARIAN) was used as a precolumn. Injection of samples (2 μl) into the column occurred by large volume injection (programmed temperature vaporization). Helium was the carrier gas at 1.8 ml/min. Temperatures were set at the following: injector, initially 60°C, stayed for 1 min, increased to 285°C at 60°C/min, and stayed; oven, initially 105°C, stayed for 6.5 min, increased to 206°C at 25°C/min, increased to 207°C at 0.5°C/min, increased to 300°C at 15°C/min, and stayed for 5 min. Ions of mass-to-charge ratio (m/z) 296–304 were monitored on the mass spectrometer in profile mode.

Before the analyses of palmitate concentration and [6,6-2H2]glucose enrichment were carried out, systems were calibrated with a series of standards containing known amounts of palmitate or known enrichments of [6,6-2H2]glucose. Regression analysis was performed on the observed concentrations and enrichments vs. the actual concentrations and enrichments of the standards. The slope was used to correct the raw concentration and enrichment data of plasma samples.

Calculations

Plasma FA and glucose kinetics. Enrichments are reported in units of tracer-to-trace ratio (TTR), which is defined as TTR = ratioenrichment − ratiobackground, where sa is sample and bk is background sample. At rest, systemic rates of appearance (Ra) and disappearance (Rd) were calculated using steady-state equations (39). Non-steady-state equations modified for use with stable isotopes were applied during exercise (25). The effective volume of distribution was assumed to be 40 ml/kg BM for palmitate and 165 ml/kg BM for glucose (25). For glucose, however, it did not affect the results whether a distribution volume of 40, 100, or 165 ml/kg BM was applied. Across the exercising leg, palmitate uptake and release were calculated using the following equations provided by Wolfe (39)

\[
\text{leg palmitate uptake} = \frac{E_{A}[A] - E_{V}[V]}{E_{A}[A]} \cdot PF
\]

\[
\text{leg palmitate release} = \text{leg palmitate uptake} - ([A] - [V]) \cdot PF
\]

where EA and EV are the enrichments of [U-13C]palmitate in the plasma of the femoral artery and vein, respectively, [A] and [V] are the plasma concentrations of palmitate in the femoral artery and vein, respectively, and PF is femoral venous plasma flow. Systemic and leg palmitate oxidation during exercise were calculated using the following equations provided by Wolfe (39)

\[
\text{systemic palmitate oxidation} = \frac{E_{CO_{2}} \cdot V_{CO_{2}}}{E_{FO_{2}} \cdot c \cdot 16}
\]

\[
\text{leg palmitate oxidation} = \frac{[U^{13}CO_{2}]_{V} - [^{13}CO_{2}]_{A}}{E_{FO_{2}} \cdot c \cdot 16} \cdot BF
\]

where ECO2 is the enrichment of 13CO2 in the expiratory air and VCO2 is the excretion of CO2 in breath. EV is the enrichment of [U-13C]palmitate in the plasma of the femoral vein, and the factor 16 accounts for the fact that oxidation of 1 mol of palmitate results in 16 mol of CO2. [13CO2]A and [13CO2]V are the blood concentrations of 13CO2 in the femoral artery and vein, respectively, and PF is femoral venous plasma flow, and c is the acetate correction factor. FA kinetics were calculated as the palmitate kinetics divided by the ratio between the plasma palmitate concentration and the plasma FA concentration.

Background correction. Enrichments of 13CO2 in breath and blood during exercise were corrected for the increase in background enrichment after initiation of exercise observed.
during background experiments in three subjects. No increase in background enrichment at initiation of exercise was observed for [U-13C]palmitate or [6,6-2H2]glucose.

**Acetate correction factor.** The acetate correction factor c was applied when calculating plasma FA oxidation rates systemically as well as across the exercising leg to account for the amount of 13CO2 label from [U-13C]palmitate lost by fixation at any step between the entrance of labeled acetyl-CoA into the tricarboxylic acid cycle and recovery in the breath or venous blood, respectively, despite complete oxidation of [U-13C]palmitate (37). A correction factor of 0.23 at rest and 0.91 during exercise was applied in the calculation of palmitate oxidation rates. The values of this factor were based on studies by Schrauwen and colleagues (27, 28) and a study by Van Loon et al. (38), where the acetate correction factor was determined in endurance-trained males bicycling at the same relative and absolute workload as the males in the present study. In one of the studies by Schrauwen et al. (28), it was shown that 13CO2 label recovery was practically based on studies by Schrauwen and colleagues (27, 28) and a factor was applied in the calculation of plasma FA oxidation rates.

**RESULTS**

**Workload**

Females as well as males completed the 90-min bicycle exercise test at a workload averaging 58 ± 1% VO2 peak. The workload expressed relative to LBM averaged 41.5 ± 1.5 and 41.7 ± 0.8 ml O2·kg LBM−1·min−1 in females and males, respectively [not significant (NS)]. The average absolute workload was 170 ± 6 W in females and 196 ± 6 W in males (P < 0.01).

**Respiratory and Cardiovascular Parameters**

Resting femoral venous blood flow was 0.22 ± 0.02 and 0.32 ± 0.05 l/min in females and males, respectively (NS). During exercise, a constant blood flow was observed in the female subjects (averaging 5.6 ± 0.2 l/min). On the other hand, in the male subjects, femoral venous blood flow increased (P < 0.01) continuously from 15 min (5.9 ± 0.2 l/min) to 60 min (6.5 ± 0.3), after which it leveled off (averaging 6.3 ± 0.3 l/min during the last 30 min of exercise). During exercise, a main effect of gender was observed (P < 0.05).

No measurable differences in the Hct of femoral arterial and venous blood were observed at rest or during exercise (P > 0.05). At rest, the Hct was 39 ± 1 and 43 ± 1% in females and males, respectively. After 15 min of exercise, an increase (P < 0.001) in Hct was observed to 42 ± 1% in females and 47 ± 1% in males, whereas Hct decreased slightly but significantly (P < 0.001) to 40 ± 1 and 44 ± 1% at 90 min of exercise in females and males, respectively. A main effect of gender was observed in Hct (P < 0.05).

Resting oxygen uptake across the leg was 13 ± 2 and 20 ± 4 ml/min in females and males, respectively (NS). During exercise, leg oxygen uptake was constant in both groups, averaging 838 ± 48 ml/min and 999 ± 39 ml/min in females and males, respectively (P < 0.05).

Leg RQ increased at initiation of exercise from 0.79 ± 0.01 and 0.80 ± 0.01 at rest to 0.91 ± 0.02 and 0.89 ± 0.02 at 15 min of exercise in females and males, respectively. During the 1st h of exercise, leg RQ was constant, averaging 0.90 ± 0.02 and 0.88 ± 0.02 in females and males, respectively, but then decreased (P < 0.01) to 0.87 ± 0.02 and 0.82 ± 0.01 at 90 min in females and males, respectively. No gender differences were observed in leg RQ (NS).

At rest, RER was similar in females (0.79 ± 0.02) and males (0.79 ± 0.02) (NS) (Fig. 1). At initiation of exercise, RER increased (P < 0.001) in females as well as in males and did not change during the 1st h of exercise (averaging 0.89 ± 0.02 and 0.91 ± 0.01 in females and males, respectively). However, from 60 to 90 min, RER decreased significantly to 0.87 ± 0.02 in fe-
males and 0.88 ± 0.01 in males. No gender difference was observed in RER during exercise at any time point (NS).

Metabolite Concentrations

The arterial concentrations of blood glucose, plasma FA, and glycerol are shown in Fig. 2. After an increase \( (P < 0.001) \) in arterial blood glucose concentration (Fig. 2A) at initiation of exercise, it did not change significantly from 15 to 30 min. During the next 30 min, arterial glucose concentration decreased \( (P < 0.05) \) to a lower level, which was maintained throughout exercise.

During the first 15 min of exercise, arterial plasma FA concentration decreased in males \( (P < 0.05) \) but not in females (NS) (Fig. 2B). Then, it increased \( (P < 0.01) \) continuously until the end of exercise in males, whereas a borderline significant increase \( (P = 0.06) \) was observed in females.

At initiation of exercise, an increase \( (P < 0.001) \) was observed in arterial plasma glycerol concentration (Fig. 2C), followed by a further significant increase throughout exercise.

The arterial blood lactate concentration averaged \( 0.5 \pm 0.1 \) mM at rest in both groups. During exercise, small increases to a maximum value of \( 1.1 \pm 0.2 \) mM after 15 min were observed in both groups.

There were no significant gender differences in arterial glucose, plasma FA, glycerol, or lactate concentrations during exercise.

Glucose Kinetics

The enrichment of \([6,6-\text{H}_2]\)glucose in arterial plasma is shown in Fig. 3A. The \([6,6-\text{H}_2]\)glucose enrichment did not change significantly during the first 75 min of exercise, whereafter it decreased from 75 to 90 min \( (P < 0.01) \). Furthermore, a main effect of gender was observed \( (P < 0.05) \).

The systemic glucose \( R_a \) and \( R_d \) are provided in Table 2 and were similar in females and males at rest (NS), but lower in females than in males during exercise \( (P < 0.05) \).

At initiation of exercise, the glucose net uptake across the leg (Fig. 4A) increased \( (P < 0.001) \). However, throughout exercise, it did not change significantly, averaging \( 114 \pm 24 \) and \( 110 \pm 21 \mu\text{mol} \cdot \text{kg lean leg mass (LLM)}^{-1} \cdot \text{min}^{-1} \) during the last hour of exercise in females and males, respectively. No significant gender differences were observed at rest or during exercise.

Plasma FA Kinetics

The enrichments of \([U,^{13}\text{C}]\)palmitate in plasma and \(^{13}\text{CO}_2\) in the expiratory air and blood at rest and during exercise are shown in Fig. 2B. After an increase \( (P < 0.001) \) at initiation of exercise, arterial plasma FA concentration decreased \( (P < 0.05) \) to a lower level, which was maintained throughout exercise.

At initiation of exercise, an increase \( (P < 0.001) \) was observed in arterial plasma glycerol concentration (Fig. 2C), followed by a further significant increase throughout exercise.

The enrichments of \([U,^{13}\text{C}]\)palmitate in plasma and \(^{13}\text{CO}_2\) in the expiratory air and blood at rest and during exercise are shown in Fig. 2B. After an increase \( (P < 0.001) \) at initiation of exercise, arterial plasma FA concentration decreased \( (P < 0.05) \) to a lower level, which was maintained throughout exercise.

At initiation of exercise, an increase \( (P < 0.001) \) was observed in arterial plasma glycerol concentration (Fig. 2C), followed by a further significant increase throughout exercise.
exercise are shown in Fig. 3. The only significant changes in the enrichments during the last 60 min of the exercise period occurred in the femoral venous enrichment of [U-13C]palmitate, which decreased slightly from 30 min

Table 2. Systemic blood glucose and plasma FA kinetics at rest and during exercise

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Ra, µmol·kg LBM⁻¹·min⁻¹</td>
<td>21.5 ± 1.0</td>
<td>22.6 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>38.2 ± 2.2</td>
<td>46.3 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose Rd, µmol·kg LBM⁻¹·min⁻¹</td>
<td>21.5 ± 1.0</td>
<td>22.6 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>38.2 ± 2.2</td>
<td>46.3 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma FA Ra, µmol·kg LBM⁻¹·min⁻¹</td>
<td>8.7 ± 1.7</td>
<td>11.0 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rest</td>
<td>19.7 ± 3.2</td>
<td>18.7 ± 4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma FA Rd, µmol·kg LBM⁻¹·min⁻¹</td>
<td>8.7 ± 1.7</td>
<td>11.0 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rest</td>
<td>19.5 ± 3.2</td>
<td>18.5 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma FA oxidation, µmol·kg LBM⁻¹·min⁻¹</td>
<td>3.8 ± 0.3</td>
<td>4.2 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rest</td>
<td>18.8 ± 2.1</td>
<td>15.9 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
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</tbody>
</table>

Data are means ± SE. FA, fatty acid; Ra, rate of appearance; Rd, rate of disappearance. Exercise data are averaged during the last 60 min of the exercise period. *Gender difference, P < 0.05.
cantly different gender difference was observed in leg plasma FA net uptake (averaging 9.1 ± 2.6 and 14.2 ± 3.8 μmol·kg LLM⁻¹·min⁻¹ during the last hour of exercise in females and males, respectively, Fig. 4B).

The plasma FA total uptake and oxidation across the active muscles were similar in females and males (NS), whereas females had a higher release of FA to plasma (P < 0.01) (Table 3).

Muscle Samples

Muscle tissue samples were obtained from only six of the female subjects. Glycogen concentrations in vastus lateralis muscle before exercise were not significantly different between females and males (Fig. 5A). At termination of exercise, a significant decrease (P < 0.001) in glycogen concentration was observed in both groups. No significant gender difference was observed after exercise, and the glycogen utilization was similar in the two groups (NS).

Concentrations of MCTG in vastus lateralis before exercise were higher in females than in males (P < 0.05) (Fig. 5B). At termination of exercise, a decrease (P < 0.01) in MCTG concentration was observed in females; however, no significant change occurred in the males. Thus the change in MCTG concentration during exercise was significantly different between females and males.

Substrate Utilization Across the Leg

On the basis of the substrate utilizations across the leg provided above, relative contributions to the oxidative metabolism across the leg during 90 min of exercise derived from blood glucose, plasma FA, muscle glycogen, and MCTG were estimated (Fig. 6).

The active muscle mass in one of the exercising legs was estimated from the decrease in glycogen concentration in vastus lateralis during exercise and the glycogen oxidation during exercise. The glycogen oxi-

**Table 3. Plasma FA kinetics across the leg during exercise**

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma FA total uptake,</td>
<td>25.0 ± 3.2</td>
<td>20.4 ± 4.9</td>
</tr>
<tr>
<td>μmol·kg LLM⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma FA total release,</td>
<td>15.1 ± 2.1</td>
<td>4.6 ± 2.4*a</td>
</tr>
<tr>
<td>μmol·kg LLM⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma FA oxidation,</td>
<td>25.2 ± 4.6</td>
<td>17.1 ± 3.2</td>
</tr>
<tr>
<td>μmol·kg LLM⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE. Data are averaged during the last 60 min of exercise. *Gender difference, P < 0.01.
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Fig. 6. Estimated relative contributions to the oxidative metabolism across the leg during 90 min of exercise derived from blood glucose, muscle glycogen, plasma FA, and MCTG. Different from females, *P < 0.05; **P < 0.01.

Fig. 7. Arterial plasma concentrations of insulin, epinephrine, and norepinephrine. A: arterial insulin concentration. Different from rest and 30 min, †P < 0.05; †††P < 0.001. B: arterial epinephrine concentration. Different from rest, ††P < 0.01; †††P < 0.001; ‡different from 30 min, P < 0.01. C: arterial norepinephrine concentration. †Different from rest, P < 0.001.

In males, 9.6 ± 1.5 and 5.0 ± 7.3% in males. Thus measurable lipid utilization contributed 37.0 ± 6.4 and 14.6 ± 6.7% to the oxidative metabolism in females and males, respectively. The relative contribution from MCTG to the oxidative metabolism as well as the total measurable lipid utilization differed between females and males (P < 0.05 for both). None of the other energy sources differed significantly between females and males in their relative contribution to the oxidative metabolism during the bicycle exercise test.

In females, 0.9 ± 6.1% of the total oxygen consumption across the leg could not be accounted for as measurable substrate utilization. In males, the amount of oxygen that could not be accounted for amounted to 28.3 ± 6.5% of the total oxygen consumption across the leg. Thus the amount of oxygen not accountable for differed between females and males (P < 0.01).

Hormones

At initiation of exercise, the arterial insulin concentration did not change significantly, but it decreased (P < 0.001) continuously from 30 to 90 min of exercise in females as well as in males (Fig. 7A). The arterial epinephrine concentration did not change significantly from rest to exercise, but it increased (P < 0.01) continuously from 30 to 90 min of exercise in both females and males (Fig. 7B). The norepinephrine concentration increased (P < 0.001) from rest to exercise and did not change significantly during the exercise period (Fig. 7C). No gender differences were observed at any of the
time points in plasma concentrations of insulin, epinephrine, and norepinephrine (NS).

**DISCUSSION**

The present study revealed an equal relative contribution from carbohydrates and lipids as fuel across the leg (as estimated from leg RQ) during submaximal prolonged bicycle exercise at the same relative workload in endurance-trained females and males. However, a marked gender difference in the utilization of the different lipid sources was observed. Thus females degraded MCTG during exercise, whereas males did not. Furthermore, in females, the oxygen uptake across the leg for lipid oxidation was accounted for solely by MCTG and plasma FA oxidation. On the other hand, in males, MCTG and plasma FA oxidation did not cover the total amount of oxygen uptake for lipid oxidation, indicating that males oxidized additional lipid sources. Finally, we observed that females released a significantly higher amount of FA to plasma across the leg during exercise than did the male subjects.

The finding that RER was also similar in females and males is in line with some previous studies (3, 17, 26) but in contrast to others (7, 11, 19, 34, 36), where females utilized more lipids than males during exercise. In the present study, females were all tested in the midfollicular phase of their menstrual cycle, and all subjects ingested a well controlled experimental diet for 8 days before the main exercise experiment. Besides the menstrual status of the females and the dietary status of the subjects, a possible gender difference in the relative oxidation of carbohydrates and lipids might be influenced by the type and intensity of exercise, which differ markedly among some of the previous studies (3, 7, 17, 36).

It might be argued that the lack of a gender difference in the relative contribution from carbohydrates and lipids to the oxidative metabolism in the present study might be ascribed to the relatively small number of subjects. However, we have recently found similar RER during exercise in a larger number of females \((n = 20)\) and males \((n = 21)\), including both trained and untrained subjects (33). Moreover, we have also recently observed that breakdown of MCTG in females is independent of training status (33). Thus the conclusions reached in the present study are not to any major extent dependent on the training status of the subjects.

**Lipid Sources Utilized**

In the present study, males had a significantly higher net uptake of plasma FA across the leg during exercise than females despite an equal arterial concentration and delivery of plasma FA in females and males. However, when expressed relative to LLM, no gender difference was observed in net plasma FA uptake, indicating that the amount of muscle mass involved during exercise is responsible for plasma FA net uptake. Moreover, we observed that the systemic turnover of plasma FA, determined by tracer technique, was similar in females and males when expressed relative to LBM. The arterial glycerol and FA concentrations, where no gender difference was observed, are also in line with the finding of similar systemic plasma FA turnover in females and males. Expressing systemic plasma FA turnover relative to BM in the present study (data not shown) did not result in a gender difference either, supporting previous studies (3, 7). However, it is probably more correct to express plasma FA turnover relative to LBM to correct for the possible influence of the apparent gender difference in body composition on the turnover of plasma FA. Across the exercising leg, the total uptake and oxidation of plasma FA were similar in females and males, whereas the release of FA to plasma was higher in females than in males. The observation of a partial gender difference in plasma FA kinetics across the exercising leg is in immediate contrast to findings in a recent study by Burguera et al. (3), where the plasma FA total uptake and release across the leg were similar in females and males during exercise. However, there might be no contradiction between the two findings, because in the study by Burguera et al., subjects were untrained and bicycle exercise was performed at 45% VO\(_{2}\) peak, indicating that training status of the subjects and relative workload may influence plasma FA kinetics.

Even though the amount of plasma FA taken up systemically as well as across the leg was in the same range as in previous studies (3, 26), only 32–34% of the systemic plasma FA total uptake occurred across the two legs. This is in accord with previous observations in our laboratory, where 40–50% of the systemic plasma FA total uptake occurred across the legs during the last 30 min of 1-h bicycle exercise at 70% VO\(_{2}\) peak in moderately trained males (10). That such a relatively small fraction of the systemic plasma FA total uptake occurs in the active muscles implies that more than one-half of the systemic plasma FA total uptake would presumably occur in adipose tissue, liver, heart, and accessory as well as in inactive muscles during exercise, which may seem unlikely. At present, we are not able to explain these observations. A possibility to consider is contamination of the sampled femoral venous blood with blood draining tissues other than skeletal muscle, e.g., subcutaneous adipose tissue, which would have a high concentration of FA. Therefore, even if the contamination in terms of blood volume were small compared with the large flow perfusing the active muscles, the femoral venous concentration of FA might be artificially increased. Still, blood from adipose tissue would be expected to have a rather low level of enrichment with \([^{13}\text{C}]\)palmitate, since the FA would be derived from intracellular, mainly unlabeled triacylglycerols. Thus the arteriovenous difference of \([^{13}\text{C}]\)palmitate would be only slightly underestimated if contamination with blood draining adipose tissue was significant. The effect of such possible contamination on the calculation of plasma FA total uptake is therefore expected to be minor. Furthermore, even if contamination were an issue, it would not to any major extent influence the calculation of leg plasma FA oxidation and consequently the estimation of relative uti-
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...lization of the different energy sources, because plasma FA oxidation rates across the leg depend primarily on femoral arterial and venous $^{13}$CO$_2$ concentrations. The femoral venous $^{13}$CO$_2$ concentration would not be expected to be changed much by contamination of small volumes of blood from adipose tissue.

At rest only ~40% of systemic plasma FA total uptake was oxidized in the present study irrespective of gender. This supports previous studies in resting isolated rat muscle that used the pulse-chase technique, where a significant incorporation of exogenous palmitate into MCTG occurred (4, 22). During exercise, however, 86–96% of systemic plasma FA total uptake was oxidized in both females and males according to plasma FA total uptake and oxidation calculated from the tracer technique. This was slightly higher than in previous studies calculating this percentage in moderately trained females (5) and males (18). Across the leg in the present study, 100 and 84% of the plasma FA total uptake was oxidized in females and males, respectively. In the study by Guo et al. (8), the pulse-chase technique was applied to evaluate the kinetics of plasma palmitate after its entrance into the muscle cell. The authors showed that a significant incorporation of plasma palmitate into MCTG in vastus lateralis muscle occurred during exercise at 45% $\dot{V}O_2$ peak in untrained females and males. The finding of a 100 and 84% oxidation of plasma FA total uptake in the present study, in contrast to the study by Guo et al., might, however, be expected, due to the higher workload in the present study. Furthermore, the higher training level and, consequently, the higher skeletal muscle oxidative capacity of the subjects in the present study compared with that in the study by Guo et al., presumably increase their ability to oxidize a large fraction of the plasma FA taken up into skeletal muscle rather than incorporate plasma FA into MCTG. Therefore, it is not at all unexpected that, during exercise at 58% $\dot{V}O_2$ peak, a very high fraction of the plasma FA taken up across the leg was oxidized irrespective of gender. Finally, during submaximal exercise, parts of the motor neurons in the leg are inactive; therefore, part of the incorporation of plasma FA into MCTG may occur in nonactive muscle fibers. Obviously, the lower the relative exercise intensity, the higher the proportion of inactive fibers.

In the present study, females had a significantly higher resting concentration of MCTG in vastus lateralis muscle than males, and females utilized significant amounts of MCTG during exercise, whereas males did not. This observation has recently been shown in our laboratory on a larger number of females and males, including both trained and untrained subjects (33). Other studies where the muscle biopsy technique was applied have also shown that males do not utilize MCTG during prolonged submaximal exercise (2, 14, 32). Furthermore, Guo et al. (8) observed no change in MCTG palmitate concentration in vastus lateralis during 90-min bicycle exercise at 45% $\dot{V}O_2$ peak in untrained females and males. A gender comparison was not made in their study. On the other hand, in a recent study by Romijn et al. (26), the difference between systemic lipid oxidation (determined by indirect calorimetry) and systemic plasma FA total uptake (determined by tracer technique) was similar in females and males during exercise, leading the authors in that study to conclude that, during exercise, no gender difference in whole body MCTG oxidation exists. However, in the present study, the difference between systemic lipid oxidation (averaging 28.7 ± 4.8 and 23.8 ± 2.5 μmol FA equivalents·kg LBM$^{-1}$·min$^{-1}$ during exercise in females and males, respectively) and systemic plasma FA oxidation (averaging 15.1 ± 1.8 and 12.4 ± 3.0 μmol FA equivalents·kg LBM$^{-1}$·min$^{-1}$ during exercise in females and males, respectively) was also similar in females and males [i.e., a difference of 13.6 ± 4.2 μmol FA equivalents·kg LBM$^{-1}$·min$^{-1}$ in females and 11.4 ± 4.7 μmol FA equivalents·kg LBM$^{-1}$·min$^{-1}$ in males (NS)], despite the obvious gender difference in MCTG utilization in vastus lateralis muscle. Therefore, a methodological problem must exist when indirectly estimating whole body MCTG oxidation as the difference between systemic lipid oxidation and systemic plasma FA total uptake or oxidation. As mentioned before, the fact that only one-third of the systemic plasma FA uptake occurred in the legs during exercise further indicates that systemic plasma FA uptake is a poor measure of leg plasma FA metabolism during exercise. Therefore, subtraction of systemic plasma FA turnover from total lipid oxidation cannot be expected to produce a reasonable estimate of MCTG breakdown, in accord with our findings that whole body nonplasma FA oxidation is not equivalent to MCTG utilization. This point of view has been voiced before (2, 10). Thus increasing evidence suggests that the indirect estimation of MCTG utilization by subtraction of systemic plasma FA turnover from total lipid oxidation is not valid.

Carbohydrate Sources

In contrast to previous studies (17, 26), we observed a higher systemic glucose turnover during exercise in endurance-trained males than in endurance-trained females. Furthermore, we observed a generally higher systemic glucose turnover than Romijn et al. (26), despite comparable workloads and training status of the subjects. These discrepancies are difficult to explain. However, from the data presented it is possible to calculate that 93 and 75% of the systemic glucose total uptake occurred across the two legs during exercise in females and males, respectively, indicating that our determination of systemic glucose turnover was not too high compared with the glucose uptake across the legs. Despite the observed gender difference in systemic glucose turnover expressed relative to LBM, we found a similar glucose net uptake across the leg in females and males expressed in absolute terms (data not shown) as well as relative to LLM. This suggests that males might have a higher total uptake of glucose in adipose tissue, liver, heart, brain, or inactive muscles during exercise compared with females.

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In the present study, the content of glycogen in vastus lateralis was similar in females and males before exercise. During exercise, the utilization of glycogen in vastus lateralis was also similar in females and males. This is in contrast to the study by Tarnopolsky et al. (36), where the utilization of muscle glycogen in vastus lateralis was higher in males than in females. It should, however, be noted that the study by Tarnopolsky et al., where subjects completed 95 min of treadmill running at 63% \(V_{\text{O}_2\text{peak}}\), revealed a significantly higher systemic oxidation of total carbohydrate in males than in females, thus differing from the conditions in the present study.

**Substrate Utilization Across the Leg**

The estimated active muscle mass plays an important role when the total utilization of muscle glycogen and MCTG is calculated. The estimation of active muscle mass is based on the assumptions that the difference in glycogen concentration between the two muscle tissue samples obtained before and after exercise, respectively, is representative of all active leg muscle tissue and that all of the glucose taken up across the leg during exercise is oxidized (12). Therefore, the estimation of active muscle mass is subject to potentially large variation, which might influence the variability especially of the total MCTG utilization and therefore also the amount of oxygen not accountable for as utilized substrate. For instance, an active muscle mass 0.5 kg (12%) higher than originally estimated in the male subjects would change the contributions from blood glucose, glycogen, plasma FA, and MCTG to the oxidative metabolism across the leg to 14, 53, 10, and 6%, respectively, decreasing the percentage of oxygen uptake across the leg not accountable for as substrate utilized from 28 to 17%. Despite these considerations, the MCTG utilization (25.0 \(\pm\) 6.0 and 5.0 \(\pm\) 7.3% of total oxygen uptake in females and males, respectively) and the sum of plasma FA and MCTG utilization (37.0 \(\pm\) 6.4 and 14.6 \(\pm\) 6.7%) differed significantly between females and males. On the other hand, the utilization of blood glucose (13.6 \(\pm\) 1.5 and 14.3 \(\pm\) 1.5%) and glycogen (48.5 \(\pm\) 4.9 and 42.8 \(\pm\) 2.1%) was similar in females and males. Finally, a significant gender difference was also observed in the percentage of oxygen uptake across the leg not accountable for as substrate utilized (0.9 \(\pm\) 6.1 and 28.3 \(\pm\) 6.5%). It might be speculated that the unaccountable oxygen consumption in males originated from oxidation of VLDL-TG or TG located between the muscle fibers, as has been suggested earlier (9, 12). Furthermore, a contribution from protein to the oxidative metabolism during exercise should not be disregarded (12) and seems to be larger in males than in females (29).

In conclusion, in the present study, the oxidative metabolism during prolonged submaximal exercise was evaluated in endurance-trained males compared with endurance-trained females in their midfollicular phase of the menstrual cycle. Despite similar relative contributions from carbohydrates and lipids to the oxidative metabolism during exercise at the same relative workload in endurance-trained females and males, we observed a gender difference in the relative utilization of the different lipid sources. Thus, although the plasma FA oxidation across the exercising leg was similar in females and males, MCTG was significantly degraded in females but not in males. In females, measured substrate oxidation accounted for 99% of the leg oxygen uptake, whereas in males, 28% of leg oxygen uptake was unaccounted for in terms of measured oxidized lipid substrates. These findings thus may indicate that males utilized a third lipid source, presumably VLDL-TG or TG located between muscle fibers.

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