Myocellular triacylglycerol breakdown in females but not in males during exercise

CHARLOTTE H. STEFFENSEN, CARSTEN ROEPSTORFF, MARIANNE MADSEN, AND BENTE KIENS
Department of Human Physiology, Copenhagen Muscle Research Centre, University of Copenhagen, DK-2100 Copenhagen, Denmark

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Steffensen, Charlotte H., Carsten Roepstorff, Marianne Madsen, and Bente Kiens. Myocellular triacylglycerol breakdown in females but not in males during exercise. Am J Physiol Endocrinol Metab 282: E634–E642, 2002.—The resting content and use of myocellular triacylglycerol (MCTG) during 90 min of submaximal exercise [60% of peak oxygen uptake (Vo2peak)] were studied in 21 eumenorrheic female and 21 male subjects at different training levels [untrained (UT), moderately trained (MT), and endurance trained (END)]. Males and females were matched according to their Vo2peak expressed relative to lean body mass, physical activity level, and training history. All subjects ingested the same controlled diet for 8 days, and all females were tested in the midfollicular phase of the menstrual cycle. Resting MCTG, measured with the muscle biopsy technique, averaged 48.4 ± 4.2, 48.5 ± 8.4, and 52.2 ± 5.8 mmol/kg dry wt in UT, MT, and END females, respectively, and 54.1 ± 4.9, 31.6 ± 3.3, and 38.4 ± 3.0 mmol/kg dry wt in UT, MT, and END males, respectively (P < 0.001, females vs. males in all groups). Exercise decreased MCTG content in the female subjects by an average of 25%, regardless of training status, whereas in the male groups MCTG content was unaffected by exercise. The arterial plasma insulin concentration was higher (P < 0.05) and the arterial plasma epinephrine concentration was lower (P < 0.05) in the females than in the males at rest and during exercise. MCTG use was correlated to the resting concentration of MCTG (P < 0.001). We conclude that resting content and use of MCTG during exercise are related to gender and furthermore are independent of training status.

MATERIALS AND METHODS

Twenty-one female and twenty-one male subjects were recruited to participate in the study. All subjects were young and healthy (although not screened for family history of type 2 diabetes) and were nonsmokers (Table 1). All subjects were fully informed about the nature of the study and the possible risks associated with it before they volunteered to participate, and written consent was given. The study was approved by the Copenhagen Ethics Committee and conformed to the code of ethics of the World Medical Association (Declaration of Helsinki).

Preexperimental protocol. All subjects initially performed an incremental exercise test on a Krogh bicycle ergometer to determine the peak oxygen uptake (Vo2peak). In addition, all subjects filled out a questionnaire and a training log regard-
Table 1. Subject and testing characteristics

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
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<tr>
<td></td>
<td>UT</td>
<td>MT</td>
<td>END</td>
<td>UT</td>
<td>MT</td>
<td>END</td>
<td>UT</td>
<td>MT</td>
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<tr>
<td>Age, yr</td>
<td>27 ± 1</td>
<td>26 ± 1</td>
<td>25 ± 1</td>
<td>27 ± 2</td>
<td>23 ± 1</td>
<td>26 ± 1</td>
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<td>Height, m</td>
<td>1.69 ± 0.03a</td>
<td>1.66 ± 0.02a</td>
<td>1.75 ± 0.02a</td>
<td>1.85 ± 0.02</td>
<td>1.84 ± 0.02</td>
<td>1.84 ± 0.03</td>
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<tr>
<td>BM, kg</td>
<td>65.0 ± 2.8a</td>
<td>59.9 ± 2.5a</td>
<td>65.9 ± 3.3a</td>
<td>82.9 ± 5.7</td>
<td>76.2 ± 1.9</td>
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<tr>
<td>Body fat, %</td>
<td>26.2 ± 2.3a</td>
<td>18.6 ± 1.2a,b</td>
<td>17.5 ± 1.2a,b</td>
<td>16.7 ± 2.6</td>
<td>13.3 ± 2.1b</td>
<td>11.8 ± 1.3b</td>
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<tr>
<td>LBM, kg</td>
<td>46.7 ± 1.8a</td>
<td>47.2 ± 2.1a</td>
<td>54.1 ± 2.5a</td>
<td>69.3 ± 4.1</td>
<td>66.0 ± 2.0</td>
<td>66.3 ± 1.3</td>
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<tr>
<td>VO2peak l/min</td>
<td>2.7 ± 0.1a</td>
<td>3.0 ± 0.2a,b,c</td>
<td>3.8 ± 0.2a,b,c,d</td>
<td>3.7 ± 0.2</td>
<td>4.2 ± 0.1e</td>
<td>4.8 ± 0.1a,d</td>
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<tr>
<td>ml/kg BM 1·min⁻¹</td>
<td>41.3 ± 0.8a</td>
<td>50.7 ± 1.4a,b,c</td>
<td>58.1 ± 1.3a,b,c,d</td>
<td>44.8 ± 2.9</td>
<td>55.0 ± 1.0b</td>
<td>63.3 ± 0.8a,d</td>
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<tr>
<td>ml/kg LBM 1·min⁻¹</td>
<td>57.0 ± 2.0</td>
<td>63.3 ± 1.4a,b</td>
<td>71.0 ± 1.5a,b,c</td>
<td>53.2 ± 3.2</td>
<td>63.5 ± 1.2b</td>
<td>71.7 ± 0.6a,b,c</td>
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<tr>
<td>Training frequency, bouts/wk</td>
<td>NA</td>
<td>3.9 ± 0.6c</td>
<td>8.4 ± 0.9b,e</td>
<td>NA</td>
<td>2.0 ± 0.5c</td>
<td>6.7 ± 0.5a,b,c,e</td>
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<tr>
<td>Training duration, h/wk</td>
<td>NA</td>
<td>4.7 ± 1.2a,b</td>
<td>11.6 ± 0.9b,e</td>
<td>NA</td>
<td>2.1 ± 0.5c</td>
<td>9.6 ± 1.2a,b,c,e</td>
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<tr>
<td>Workload % VO2peak</td>
<td>60 ± 2</td>
<td>59 ± 2</td>
<td>58 ± 1</td>
<td>60 ± 2</td>
<td>59 ± 2</td>
<td>58 ± 1</td>
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<tr>
<td>ml O2·kg LBM 1·min⁻¹</td>
<td>33.9 ± 1.6</td>
<td>37.1 ± 1.2b</td>
<td>41.5 ± 1.5b,e</td>
<td>31.9 ± 1.6</td>
<td>35.4 ± 0.6b,c,e</td>
<td>41.7 ± 0.8b,c,e</td>
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</tbody>
</table>

Values are means ± SE; n = 7/group. UT, untrained; MT, moderately trained; END, endurance trained; BM, body mass; LBM, lean BM; VO2peak, peak O2 uptake; NA, not applicable. aP < 0.001, significantly different from males; bP < 0.01, significantly different from experimental diet; cP < 0.05, significantly different from UT; dP < 0.01, significantly different from MT.

Table 2. Composition of habitual and experimental diets

<table>
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<tr>
<th></th>
<th>Habitant Diet</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Energy intake, kJ</td>
<td>Carbohydrate, E%</td>
<td>Fat, E%</td>
<td>Protein, E%</td>
<td>Energy intake, kJ</td>
<td>Carbohydrate, E%</td>
<td>Fat, E%</td>
<td>Protein, E%</td>
</tr>
<tr>
<td></td>
<td>UT</td>
<td>9,146 ± 810a</td>
<td>58.7 ± 2.3b</td>
<td>25.4 ± 2.3b</td>
<td>14.4 ± 0.4b</td>
<td>9,692 ± 438a</td>
<td>66.3 ± 0.7</td>
<td>18.3 ± 0.3</td>
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<tr>
<td></td>
<td>MT</td>
<td>10,255 ± 576c</td>
<td>63.6 ± 2.5b</td>
<td>21.9 ± 2.3b</td>
<td>14.1 ± 0.7b</td>
<td>10,310 ± 500a</td>
<td>66.1 ± 0.2</td>
<td>19.3 ± 0.1</td>
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<tr>
<td></td>
<td>END</td>
<td>13,644 ± 1,229c,d</td>
<td>69.1 ± 3.8a,b</td>
<td>17.6 ± 3.8</td>
<td>13.1 ± 0.6b</td>
<td>13,792 ± 909c,d</td>
<td>65.7 ± 0.2</td>
<td>18.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>13,022 ± 1,284</td>
<td>60.6 ± 1.9a</td>
<td>23.7 ± 1.8b</td>
<td>15.1 ± 1.7b</td>
<td>13,145 ± 1,127</td>
<td>65.0 ± 0.2</td>
<td>19.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>UT</td>
<td>13,288 ± 408</td>
<td>57.4 ± 1.6b</td>
<td>26.2 ± 1.9b</td>
<td>14.3 ± 0.8b</td>
<td>13,181 ± 349</td>
<td>65.1 ± 0.3</td>
<td>18.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>END</td>
<td>16,240 ± 592d</td>
<td>63.4 ± 3.8</td>
<td>20.9 ± 3.5</td>
<td>14.1 ± 0.9b</td>
<td>16,414 ± 592d</td>
<td>65.5 ± 0.1</td>
<td>19.4 ± 0.0</td>
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</tbody>
</table>

Values are means ± SE; n = 7/group. E%, % of energy. aP < 0.001, significantly different from males; bP < 0.01, significantly different from experimental diet; cP < 0.001, dP < 0.05, significantly different from UT; eP < 0.001, significantly different from MT.
performed under conditions similar to those of the exercise experiment (after 12 h fasting and after having abstained from any physical activity the day before).

**Experimental protocol.** All subjects reported to the laboratory by either bus or car at 8 AM after an overnight fast. All subjects abstained from any physical activity for 2 days before the exercise experiment. After the subjects rested for 30 min in the supine position, resting pulmonary oxygen uptake (V\textsubscript{O\textsubscript{2}}) and CO\textsubscript{2} excretion (V\textsubscript{CO\textsubscript{2}}) were measured, and the respiratory exchange ratio (RER) was calculated. Thereafter, a catheter was inserted into the femoral artery under local anesthesia by use of aseptic techniques, and the tip was advanced proximally 2 cm above the inguinal ligamentum for blood sampling. After the catheter was inserted, the subjects rested for 60 min in the supine position before a second measurement of resting V\textsubscript{O\textsubscript{2}} and V\textsubscript{CO\textsubscript{2}} and subsequent calculation of RER. Resting blood samples were drawn, and a muscle biopsy was obtained from the vastus lateralis muscle with suction under local anesthesia before the subjects started to exercise on a Krogh bicycle ergometer for 90 min. All subjects exercised at the same relative workload (65% V\textsubscript{O\textsubscript{2} peak}). Expired air was collected in Douglas bags every 15 min during exercise for measurement of V\textsubscript{O\textsubscript{2}} and V\textsubscript{CO\textsubscript{2}} and calculation of RER. Blood samples were drawn at 15, 30, 60, 75, and 90 min of exercise. Heart rate was monitored throughout the experiment with a heart rate monitor (Vantage XL, Polar Electro). During exercise, subjects were allowed water ad libitum and were cooled by an electric fan. At the termination of exercise, another muscle biopsy was obtained through the same skin incision but with the needle pointing in a different direction.

All exercise experiments in females were carried out in the midfollicular phase of the menstrual cycle (between days 7 and 11; mean day was 9.0 ± 0.2, 9.1 ± 0.4, and 9.4 ± 0.6 in UT, MT, and END groups, respectively).

**Pulmonary oxygen uptake and RER.** Pulmonary oxygen uptake and RER were measured and calculated, respectively, during rest and exercise from the collection of expired air in the Douglas bags. The air volume in each Douglas bag was measured in a Collins bell spirometer (Tissot principle), and the O\textsubscript{2} and CO\textsubscript{2} content of the expired air was determined with a paramagnetic (Servomex) and an infrared (Beckman LB-2) system, respectively. Two gases of known composition were used to calibrate both systems regularly.

**Muscle analysis.** In the present study, the biopsies were obtained from the same depth of the vastus lateralis muscle to prevent differences in fiber type composition between the biopsies obtained before and at the termination of exercise (30). The biopsy samples were divided in two. One part was frozen in liquid nitrogen within 10–15 s and stored at –80°C for subsequent biochemical analysis. The other part was mounted in embedding medium and frozen in precooled isopentane and then stored at –80°C for subsequent histochecmical analysis. Serial transverse sections (10 μm) were cut and stained for myofibrillar ATPase to identify the different fiber types (2). Before biochemical analysis, ~30 mg wet wt of muscle tissue were freeze dried and dissected free of all visible adipose tissue, connective tissue, and blood with the use of a stereo microscope, leaving the muscle fibers for further analysis. The muscle fibers were mixed, and ~1 mg dry wt of the pooled fibers was used for measurement of the MCTG concentration according to the method of Kiens and Richter (24). Glycerol from the degraded triacylglycerol was assayed fluorometrically as described previously by Kiens and Richter (24). A coefficient of variation (CV) of 4% for the MCTG concentration was obtained between five samples from the mixed freeze-dried pool of fibers as described above.

In contrast, when five small samples of wet muscle were dissected and analyzed separately, a CV of 31% resulted between the samples. As suggested by Wendling et al. (47), the relatively high variability in MCTG concentration between different locations in the muscle is a concern when detecting expected differences of 20–30%. To circumvent this potential problem, two or more biopsies might be obtained. However, the large number of subjects and the consistency in our findings regardless of training status indicate that our findings are not masked by methodological errors. To further avoid the impact of methodological errors as well as day-to-day variation in the analysis, we analyzed muscle tissue from both female and male subjects in one assay run.

**Blood analysis.** Blood was sampled in heparinized syringes, immediately transferred to plastic test tubes containing EGTA, and centrifuged. Plasma was immediately frozen at –80°C until further analysis. Blood for the analysis of progesterone and estradiol was sampled in dry syringes and transferred to dry test tubes in which the blood coagulated for a few hours before it was centrifuged. Serum was frozen at –80°C until further analysis. The plasma concentration of insulin was determined using an RIA kit (insulin RIA 100, Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). The plasma concentrations of epinephrine and norepinephrine were also determined using an RIA kit (KatCombi RIA, Immuno-Biological Laboratories, Hamburg, Germany) as were the serum concentrations of progesterone (progesterone 125I RIA, DGR Instruments) and estradiol (estradiol ultrasensitive RIA, DGR Instruments).

**Statistical evaluation.** Results are given as means ± SE. A three-way ANOVA with repeated measures for the time factor was used to determine whether variables were influenced by gender, training status, or time as well as to test for a possible interaction between these three factors. For the variables independent of time, a two-way ANOVA was used to determine any influences of gender and training status and a possible interaction between these two factors. Because females and males were not pairwise matched, gender was not considered to be a repeated factor. In the case of a significant main effect of one or more factors, Tukey’s post hoc test was used to detect pairwise differences between the means. Correlations were evaluated by means of linear regression analysis (Pearson product moment correlations). In all cases, an α of 0.05 was used as the level of significance.

**RESULTS**

**Diet.** As we intended, the actual experimental diet averaged 65.5 E% carbohydrates, 19 E% fat, and 15.5 E% protein in all groups (Table 2). There were no differences in energy intake in any groups for habitual vs. experimental diet. The energy intake was significantly higher in males than in females and in END vs. UT and MT subjects. In UT and MT females and males, the nutrient composition of the habitual diet was similar and slightly but significantly different from the experimental diet. In END females and males, the energy percentages of carbohydrates and fat in the habitual diet were similar to those of the experimental diet. Furthermore, the energy percentage from dietary carbohydrates was higher for END than for UT subjects.

**Workload.** The average workloads during the bicycle exercise test were expressed relative to V\textsubscript{O\textsubscript{2} peak} and as V\textsubscript{O\textsubscript{2} per kilogram of LBM (Table 1). All subjects com-
completed the 90-min bicycle exercise test at a workload corresponding to 59% of \( \dot{V}O_2 \)peak. Furthermore, at each training level no gender differences were observed in \( \dot{V}O_2 \) expressed relative to LBM. However, in females as well as males, the UT, MT, and END groups differed significantly in \( \dot{V}O_2 \) expressed relative to LBM (\( P < 0.01 \)).

**RER.** At rest, RER was similar in all groups (0.80 ± 0.02, 0.81 ± 0.02, and 0.79 ± 0.02 in UT, MT, and END females, respectively, and 0.85 ± 0.05, 0.80 ± 0.03, and 0.79 ± 0.01 in UT, MT, and END males, respectively; Fig. 1). RER remained constant throughout the exercise period in UT and MT subjects, averaging 0.87 ± 0.02 and 0.89 ± 0.02 in UT females and males, respectively, and 0.87 ± 0.02 and 0.89 ± 0.02 in MT females and males, respectively. However, RER remained constant during the first 60 min of exercise in END subjects (0.90 ± 0.02 and 0.91 ± 0.02 at 60 min in females and males, respectively) and subsequently decreased (\( P < 0.05 \)) to 0.87 ± 0.02 and 0.88 ± 0.01 at 90 min in females and males, respectively. No gender differences or effects of training status were observed at any time point.

**Pulmonary \( \dot{V}O_2 \).** \( \dot{V}O_2 \) remained constant throughout the exercise period, averaging 1.56 ± 0.07, 1.70 ± 0.05, and 2.19 ± 0.11 in UT, MT, and END females, respectively, and 2.17 ± 0.13, 2.31 ± 0.10, and 2.7 ± 0.08 in UT, MT, and END males, respectively. The average \( \dot{V}O_2 \) during exercise was higher in END subjects compared with UT and MT subjects (\( P < 0.001 \)). Furthermore, the average \( \dot{V}O_2 \) during exercise was lower in females than in males within each training group (\( P < 0.001 \)).

**MCTG.** At rest, the MCTG content in the vastus lateralis muscle was significantly higher in the female subjects vs. the male subjects, regardless of training status (48.4 ± 4.2, 48.5 ± 8.4, and 52.2 ± 5.8 mmol/kg dry wt in UT, MT, and END females, respectively, and 34.1 ± 4.9, 31.6 ± 3.3, and 38.4 ± 3.0 mmol/kg dry wt in UT, MT, and END males, respectively; \( P < 0.001 \)); however, in the male subjects the MCTG content remained unchanged after vs. before exercise, regardless of training level; \( P > 0.05 \). Differences were not observed in the percentage of type IIA fibers. In END males the percentage of type I fibers was higher (\( P < 0.001 \)) and the percentage of type IIB fibers was lower (\( P < 0.001 \)) compared with UT and MT females. Differences were not observed in the percentage of type IIA fibers.

**Fiber type distribution.** The fiber type distribution in the vastus lateralis muscle was not different between UT and MT females (Table 3). However, in END females the percentage of type I fibers was higher (\( P < 0.001 \)) and the percentage of type IIB fibers was lower (\( P < 0.001 \)) compared with UT and MT females. Differences were not observed in the percentage of type IIA fibers.
GENDER DIFFERENCES IN MCTG USE

Table 3. Muscle fiber distribution in vastus lateralis muscle in females and males

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
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<th>Males</th>
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<tbody>
<tr>
<td></td>
<td>UT</td>
<td>MT</td>
<td>END</td>
<td>UT</td>
</tr>
<tr>
<td>%/no.</td>
<td>58.8±2.3 alc</td>
<td>53.5±3.0</td>
<td>70.8±3.1 abc</td>
<td>48.3±3.5</td>
</tr>
<tr>
<td>Area, μm²</td>
<td>4.274±0.720 f</td>
<td>3.760±0.242 f</td>
<td>4.215±0.248 a</td>
<td>5.298±0.674</td>
</tr>
<tr>
<td>%/area</td>
<td>63.9±3.3 a</td>
<td>58.9±2.7</td>
<td>74.5±1.7 abc</td>
<td>44.2±3.6</td>
</tr>
<tr>
<td>Type I</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>%/no.</td>
<td>27.9±3.1 f</td>
<td>28.4±2.4</td>
<td>24.8±2.8</td>
<td>31.2±3.5</td>
</tr>
<tr>
<td>Area, μm²</td>
<td>3.631±0.540 f</td>
<td>3.216±0.314 f</td>
<td>3.844±0.258 f</td>
<td>6.558±0.709</td>
</tr>
<tr>
<td>%/area</td>
<td>26.1±3.6 f</td>
<td>26.4±2.2 a</td>
<td>22.2±1.7 a</td>
<td>35.6±3.7</td>
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<tr>
<td>Type IIB</td>
<td></td>
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</tr>
<tr>
<td>%/no.</td>
<td>10.6±1.4 f</td>
<td>15.7±1.8</td>
<td>3.9±0.9 abc</td>
<td>18.6±2.7</td>
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<tr>
<td>Area, μm²</td>
<td>3.380±0.738 f</td>
<td>2.815±0.334 f</td>
<td>2.960±0.271 f</td>
<td>5.755±0.906</td>
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<tr>
<td>%/area</td>
<td>8.3±0.9 a</td>
<td>12.6±1.3 a</td>
<td>2.9±0.7 abc</td>
<td>18.5±3.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7/group except n = 6 for END females. abcP < 0.01, defP < 0.001, significantly different from males; hP < 0.001, iP < 0.01, significantly different from UT; cP < 0.001, cP < 0.05, significantly different from MT.

was lower (P < 0.001) compared with UT and MT males. The percentages of type I and IIB fibers did not differ between UT and MT males. No effect of training status was observed in the percentage of type IIA fibers in males.

Gender differences in fiber type distribution were not observed in MT or END groups, whereas UT females had a higher percentage of type I fibers than UT males (P < 0.01). The area of all fiber types tended to be smaller in END males compared with both UT and MT males (Table 3). Furthermore, the area of the different fiber types was larger in all male groups than in the corresponding female groups (P < 0.01).

The calculated percentage of fiber types relative to fiber area was significantly larger for type I fibers in females compared with males in the UT and END groups but not in the MT group. The calculated percentages of type IIA and IIB fibers relative to area were significantly smaller in all female groups compared with the respective male groups (Table 3). The average percentages of the different fiber types did not differ significantly between the biopsies obtained before and at the termination of exercise (data not shown).

Circulating hormones. At rest, the arterial plasma concentration of insulin averaged 8.3 ± 0.9, 8.3 ± 1.2, and 5.9 ± 0.9 μU/ml in UT, MT, and END females, respectively, and 7.1 ± 1.2, 6.2 ± 0.7, and 5.9 ± 0.8 μU/ml in UT, MT, and END males, respectively (Fig. 4A). The arterial plasma insulin concentration decreased continuously throughout exercise to 5.4 ± 0.6, 6.2 ± 2.1, and 4.3 ± 1.1 μU/ml at 90 min in UT, MT, and END females, respectively, and 3.2 ± 0.4, 3.1 ± 0.4, and 3.1 ± 0.5 μU/ml at 90 min in UT, MT, and END males, respectively (P < 0.001). The arterial plasma insulin concentration was significantly higher in females than in males at rest and during exercise regardless of training status (P < 0.05).

The resting arterial plasma concentration of epinephrine averaged 0.3 ± 0.1, 0.3 ± 0.1, and 0.4 ± 0.1 nmol/l in UT, MT, and END females, respectively, and 0.7 ± 0.2, 0.6 ± 0.2, and 0.6 ± 0.2 nmol/l in UT, MT, and END males, respectively (Fig. 4B). In UT and MT subjects, the arterial plasma epinephrine concentration did not change during the first 60 min of exercise but then increased throughout the last 30 min of exercise. In END subjects, the arterial plasma epinephrine concentration remained unchanged until 30 min of exercise but then increased throughout the rest of the exercise period. At 90 min, the arterial plasma epinephrine concentration averaged 1.9 ± 0.4, 2.9 ± 0.8, and 1.9 ± 0.6 nmol/l in UT, MT, and END females, respectively, and 3.6 ± 0.9, 3.9 ± 1.0, and 1.9 ± 0.4 nmol/l in UT, MT, and END males, respectively. The arterial plasma epinephrine concentration was significantly lower in females than in males at rest and during exercise regardless of training status (P < 0.05).

At rest, the arterial plasma concentration of norepinephrine was similar in females and males despite training status (Fig. 4C). The arterial plasma norepinephrine concentration increased significantly in all groups (P < 0.001) after the start of exercise and remained at a constant level until 60 min when it increased further during the last 30 min of exercise (P < 0.05). No gender differences or effects of training status were observed.

The serum concentration of progesterone averaged 0.19 ± 0.07, 0.12 ± 0.05, and 0.20 ± 0.14 ng/ml in UT, MT, and END females, respectively, and 0.23 ± 0.08, 0.26 ± 0.06, and 0.16 ± 0.06 in UT, MT, and END males, respectively. The serum concentration of progesterone was similar in females and males regardless of training status.

The serum concentration of estradiol averaged 49.1 ± 11.8, 57.6 ± 14.7, and 58.0 ± 10.6 pg/ml in UT, MT, and END females, respectively, and 34.8 ± 4.0, 35.7 ± 3.5, and 30.9 ± 2.8 in UT, MT, and END males, respectively. The serum estradiol concentration was significantly higher in females compared with males regardless of training status (P < 0.01).
The results of the present study demonstrate significant gender-based differences in resting content and utilization of MCTG during prolonged submaximal exercise at the same relative workload. At rest, the content of MCTG was significantly higher in females compared with males. During exercise, females utilized MCTG, whereas males did not. The observations of a higher resting content and MCTG use during exercise in females vs. males were made under conditions in which several parameters that could potentially affect substrate oxidation, including the pretest diet and the menstrual status and cycle phase of the female subjects, were standardized and carefully controlled.

**MCTG content at rest.** It has previously been demonstrated (23) that diet influences the MCTG content in human skeletal muscle, i.e., the consumption of a fat-rich diet increases MCTG content at rest. The higher MCTG content at rest in the female subjects compared with the male subjects in the present study is presumably not ascribed to the diet as all subjects ingested the same carbohydrate-rich diet for 8 days preceding the exercise experiment. Actually, due to their larger energy intake, males consumed a larger absolute amount of fat compared with females. However, when expressed relative to LBM, the fat ingestion was similar in females and males.

To explain the finding of a higher resting MCTG content in females compared with males, we examined the muscle fiber composition, since it has previously been shown (7) in male subjects that type I fibers contain more MCTG than type II fibers. Furthermore, in a group of females and males, it has recently been shown (20) that MCTG content in soleus, tibialis anterior, and tibialis posterior muscles varied consistently with the expected fraction of type I fibers in these muscles. One might also consider the possibility that females have a higher content of MCTG in type I fibers and/or other fiber types than males. To our knowledge, this has not yet been investigated. END females and males had more type I fibers compared with both MT and UT females and males. There was no effect of gender in the percentage of type I fibers in MT and END subjects. However, UT females had a higher percentage of type I fibers than UT males. Previously, a similar fiber type composition in females and males has been found in UT subjects (5, 35, 39) as well as in physical education students (40). Other studies have found that in UT subjects (42), middle-distance runners (5), trained cyclists (11), and subjects representing a wide range of physical activity levels (33), females had a higher percentage of type I fibers than males. At all training levels in the present study, the area of all the fiber types was smaller in females than in males, especially for type II fibers, which is in accordance with previous observations (5, 35, 39, 40, 42). As a consequence, the calculated fiber composition expressed relative to fiber area revealed that type I fibers accounted for a relatively larger area in females than in males. Thus the higher percentage of type I fibers expressed relative to area might partly explain the higher resting content of MCTG in females. This is further supported by the findings in the present study of a modest but significant correlation between the percentage of type I fibers...
fibers relative to area and the resting concentration of MCTG ($P < 0.05, r = 0.39$).

In the present study, no effect of training status on MCTG concentrations at rest was observed in females or males. Previous studies in males have reported inconsistent results, with some studies (22, 34) showing an increase in resting MCTG with training and others (19) showing no such increase. The strict dietary control may explain why an effect of training status on the resting MCTG concentrations was not observed in the present study. The experimental diet was a low-fat diet and differed from the diet in our (22) previous study. The possibility that the low-fat diet has concealed a training effect on MCTG concentration in the present study can therefore not be excluded. Another possible explanation of the increase in MCTG concentration with training in our (22) previous study might be that the subjects had exercised the day before the measurements were done. Because MCTG has been shown (25) to be utilized in the postexercise period, the difference in the reported resting concentration between trained and UT muscle (22) might be influenced by different degrees of postexercise MCTG use in trained and UT muscle.

**MCTG utilization during exercise.** A major novel finding in the present study was that female subjects, regardless of training status, utilized a significant amount of MCTG during prolonged exercise, whereas male subjects did not. The fact that male subjects did not utilize MCTG during exercise to any measurable extent is in accord with our (22, 25) previous findings as well as other studies (1, 12, 21, 44) but does contrast with some studies (4, 19, 34) in which MCTG utilization was determined by applying the muscle biopsy technique. In recent studies (17, 38) in females in which a combination of isotope tracer technique and indirect calorimetry was used, it was suggested that the additional source of fatty acids oxidized during exercise at the same absolute workload after training (vs. before training) was provided by MCTG. Applying the same indirect methodology, several studies (27, 37) in males have indicated a similar significant utilization of MCTG during exercise. However, recent investigations in our laboratory revealed that combining isotope tracer technique and indirect calorimetry does not provide an accurate measure of MCTG use in males (13, 36) but does in females (36). This suggests that fat utilized during exercise is recruited from different sources in females and males (36).

To our knowledge, this study is the first to compare MCTG utilization in matched females and males at different training levels during prolonged exercise by applying the muscle biopsy technique. Recently, Guo et al. (12) evaluated the kinetics of intramuscular triacylglycerol fatty acids during exercise. Even though both female and male subjects participated in the study (12), a gender comparison was not made. Furthermore, net breakdown of MCTG in the vastus lateralis muscle was not observed during exercise, supporting our finding in male subjects. However, that study (12) revealed simultaneous esterification of plasma fatty acids to MCTG and MCTG hydrolysis when subjects exercised at 45% of $V_{\text{O2 peak}}$. Whether these events take place concurrently at higher exercise intensities, as in the present study, is not known. If this is the case, however, interpretation of our data might be that the balance between the two processes is displaced toward a higher net breakdown of MCTG in females than in males.

The enzymatic regulation of triacylglycerol breakdown in skeletal muscle is poorly understood. It has been suggested (41) that a hormone-sensitive triacylglycerol lipase (HSL) similar to the adipose tissue HSL might regulate MCTG hydrolysis. Support for this hypothesis was provided when HSL protein and mRNA were detected in rat skeletal muscle by Western and Northern blotting, respectively (15, 16, 28, 29). Recently, Kjaer et al. (26) also demonstrated the existence of HSL in human skeletal muscle, and evidence was provided that $\beta$-adrenergic stimulation increased the activity of HSL in skeletal muscle cells (26, 28, 29). However, in the present study, the arterial plasma concentration of epinephrine was significantly lower in females than in males during exercise, whereas the arterial plasma concentration of norepinephrine was similar in females and males (45). If HSL is responsible for the hydrolysis of MCTG, it might be speculated that females have a higher HSL activity compared with males due to a higher content of HSL and/or a higher sensitivity of HSL to potential stimulators. A gender difference in the content of HSL might exist, based on the indication of a higher content of HSL in type I fibers (28) and the fact that a greater percentage of type I fibers relative to area was found in females compared with males in the present study. Regarding lipolytic sensitivity to catecholamines, Hellström et al. (14) found, by means of the microdialysis technique, that during exercise, females have a higher release of glycerol and fatty acids from the abdominal adipose tissue than males despite the fact that both genders had similar concentrations of norepinephrine and that females had a lower concentration of epinephrine than males. The sensitivity of lipolytic activity toward these two catecholamines might be higher in females than in males in skeletal muscle tissue as well. It has previously been shown that the initial content of MCTG influences the use of MCTG during both exercise (8) and infusion of norepinephrine at rest (10). In the present study, the gender difference in MCTG utilization during exercise is at least partly explained by the gender difference in resting content of MCTG, because we observed a fair ($r = 0.61$) and significant ($P < 0.001$) correlation between these two parameters. Other factors such as sex hormones might, however, also influence MCTG hydrolysis. At rest, the circulating level of progesterone was identical in females and males, whereas the estradiol level was higher and displayed a greater variation in females than in males. Thus it might be speculated that estradiol exerts an influence on the resting MCTG content as well as on the degree of MCTG utilization during exercise in females. However, in the present study, the concentration of estra-
diol did not correlate with the MCTG content at rest or its utilization during exercise, rendering it unlikely that estradiol is a major regulatory factor acting directly on the storage and breakdown of MCTG in females.

It is controversial whether MCTG hydrolysis increases with training. In studies measuring the concentration of MCTG by the muscle biopsy technique, MCTG hydrolysis has been found to be similar in the trained and UT leg in males (22) and higher in END males (19, 34) than in UT males during exercise at the same absolute workload. However, in the present study, in which exercise was performed at the same relative workload, training status did not have any effect on the degree of MCTG breakdown. A similar finding was reported previously in males by Bergman et al. (1).

In the present study, we observed a gender difference in MCTG utilization at all training levels despite RER being similar during exercise in females and males. Even though we observed that RER decreased during the last 30 min of exercise in END subjects but not in UT and MT subjects, we were not able to observe any increased MCTG use in END vs. UT and MT subjects. The higher lipid oxidation during the last 30 min of exercise in END subjects seemed, however, to be covered primarily by plasma fatty acids, since oxidation of plasma fatty acids increased to a greater extent in END subjects than in MT and UT subjects during the last third of the exercise test (unpublished data).

A gender difference in MCTG utilization despite similar RER during exercise indicates that females and males obtain lipids from different sources during exercise. It has previously been observed (13, 22) that fatty acids derived from plasma very low density lipoprotein-triacylglycerol might contribute to the oxidative metabolism. Furthermore, it has been suggested (22, 36) that intermyocellular lipids are possibly mobilized during exercise, and plasma fatty acids are also known to contribute to the oxidative metabolism. Thus a gender difference might also exist in the use of one or more of these three additional lipid sources.

In summary, the present study revealed a higher resting content of MCTG in females than in males despite training level. Furthermore, regardless of training status, females utilized significant amounts of MCTG during prolonged exercise whereas males did not. Training had no effect on the resting MCTG content or the use of MCTG during exercise in either females or males. Females had a lower concentration of epinephrine in plasma than males, so if HSL is responsible for MCTG hydrolysis it might be speculated that a gender difference exists in the concentration of HSL and/or the sensitivity of HSL toward epinephrine. Other factors such as the resting content of MCTG might influence the degree of MCTG hydrolysis as well.

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