Regulation of plasma long-chain fatty acid oxidation in relation to uptake in human skeletal muscle during exercise

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CIRCULATING LONG-CHAIN FATTY ACIDS (LCFA) are an important energy source for human skeletal muscle during moderate-intensity exercise. On the whole body level, previous studies have suggested that when LCFA availability or exercise intensity is manipulated, systemic plasma LCFA oxidation during exercise is regulated at the entrance of LCFA into mitochondria (6, 35). However, whole body measurements of plasma LCFA kinetics do not accurately reflect plasma LCFA kinetics in skeletal muscle (12, 31); therefore, studies in human skeletal muscle are warranted.

In human skeletal muscle, the cellular LCFA content increased 54% when exercise intensity increased from 65 to 90% peak oxygen uptake (VO\textsubscript{2 peak}) (16), indicating that the uptake of LCFA exceeds LCFA utilization in skeletal muscle during high-intensity exercise when carbohydrate utilization is very high. Thus regulation of LCFA oxidation in contracting human skeletal muscle seems to occur at a step downstream from the entry of LCFA into the muscle cell. However, this hypothesis has not yet been investigated through direct measurements of plasma LCFA kinetics in skeletal muscle of exercising human volunteers.

On the contrary, it has recently been suggested that cellular lipid-binding proteins play a very important role in regulation of LCFA utilization in several tissues, including heart and skeletal muscle (9). The most important lipid-binding proteins in skeletal muscle are the fatty acid translocase (FAT/CD36) (1) and plasma membrane fatty acid-binding protein (FABP\textsubscript{pm}) (36, 39), both located at the plasma membrane, and the cytosolic fatty acid-binding protein (FABP\textsubscript{c}) (9, 40). Muscle-specific overexpression of FAT/CD36 in mice led to enhanced plasma LCFA utilization in contracting muscle (13), and CD36 knockout mice had diminished uptake of plasma LCFA in skeletal muscle (5), suggesting a link between FAT/CD36 content and plasma LCFA utilization. Furthermore, in rats, muscle contractions induced a relocation of FAT/CD36 from an intracellular compartment to the sarcolemma, which was proposed to enhance the capacity to transport LCFA across the sarcolemma (2). In human volunteers, exercise training led to an increase in skeletal muscle FABP\textsubscript{pm} (14). Also in humans, long-term ingestion of a high-fat diet induced an increase in skeletal muscle FAT/CD36, FABP\textsubscript{pm}, and FABP\textsubscript{c} content (30). Thus it seems that these three lipid-binding proteins are regulatable in human skeletal muscle in a long-term perspective. However, their possible role in the acute regulation of plasma LCFA utilization in skeletal muscle during exercise in humans has never been addressed. Another putative lipid-binding protein located to the plasma membrane is caveolin (37), which is a necessary protein for the formation of caveolae (41). Several recent studies have suggested a role of caveolae and/or one of the three isoforms of caveolin, caveolin-1, in cellular LCFA uptake in adipocytes and endothelial cells (28, 29, 37). It is presently unknown whether caveolae and/or the muscle-specific caveolin isoform caveolin-3 plays a role in LCFA uptake in skeletal muscle cells.
The purpose of the present study was to investigate possible sites responsible for regulation of LCFA oxidation in contracting human skeletal muscle. The relations between leg plasma LCFA uptake and oxidation and the skeletal muscle content of FAT/CD36, FABPpm, FABPc, and caveolin-1 and -3 were determined in eight moderately trained men during bicycle exercise at 65% \( \dot{V}O_2 \text{peak} \). Two trials were performed, with markedly different leg total fat oxidation during exercise: high fat oxidation (H-FOX) and low fat oxidation (L-FOX). These two trials were achieved by manipulating preexercise muscle glycogen stores via exercise and dietary manipulation on the preceding day. Furthermore, via ingestion of a light meal on the morning of the experimental day and a controlled variable intravenous glucose infusion during the exercise test, we kept several potentially confounding blood metabolites and hormones nearly similar between the two trials at rest as well as during exercise. We hypothesized that sarcolemmal lipid-binding proteins would not limit leg plasma LCFA oxidation during exercise, consistent with a major role of intracellular sites in regulation of plasma LCFA oxidation in contracting human muscle.

**MATERIALS AND METHODS**

**Subjects.** Eight young, healthy, moderately trained men (Table 1) were recruited to participate in the study. All subjects were nonsmokers and were engaged in regular exercise training 4–6 h per wk (running, bicycling, and resistance training). Before inclusion into the study, subjects were fully informed about the nature and possible risks of the study and gave their written consent. The study was approved of the study and given their written consent. The study was approved by the Copenhagen Ethics Committee (KF-01-078/01) and carried out according to the code of ethics of the World Medical Association (Declaration of Helsinki II).

**Preexperimental testing.** All subjects initially performed an incremental exercise test on a bicycle ergometer (Monark 839 Electronic Ergometer, Monark Exercise, Vansbro, Sweden) to determine their \( \dot{V}O_2 \text{peak} \). Respiratory measurements were carried out with the Douglas bag technique. Percent body fat was measured by dual-energy X-ray absorptiometry (Lunar, Madison DPX-IQ version 4.6.6).

**Experimental design.** Subjects underwent two experimental trials separated by 2–3 wk. In both trials, subjects completed glycogen-depleting bicycle exercise followed by a controlled diet for the rest of the day (11–12 h). The controlled diet consisted primarily of fat (H-FOX) in one trial and primarily of carbohydrate (L-FOX) in the other (see Dietary control). The next day, subjects performed a bicycle exercise test for 60 min at \( \sim 65\% \dot{V}O_2 \text{peak} \). The order of the H-FOX and L-FOX trials was randomized and stratified.

**Dietary control.** During 3 days before the first trial, subjects’ habitual energy and nutrient intakes were determined by a self-reported dietary record (Table 1). During those 3 days, subjects were instructed to avoid food items with a high ratio of \( ^13 \text{C} \) to \( ^12 \text{C} \) to keep the background enrichment of \( ^13 \text{CO}_2 \) in breath and blood as low as possible during the exercise experiment. All food and beverage intakes were weighed to the accuracy of 1 g and recorded. Subsequently, the energy intake and composition of the habitual diet were recalculated by means of a computer database (Dankost 2000, Danish Catering Center, Copenhagen, Denmark). For 3 days before the second trial, subjects followed a diet identical to the one ingested before the first trial (Table 1).

**Glycogen depletion protocol.** On the day of the glycogen depletion exercise, subjects arrived at the laboratory at 7:30 AM. All subjects abstained from any heavy physical activity on the day before the glycogen-depletion exercise. A breakfast meal was served consisting of 30 percent of energy (% E) from fat, 55 E% from carbohydrate (CHO), and 15 E% from protein, and containing 25% of each subject’s daily energy intake (DEI). Subjects initiated the glycogen-depletion bicycle exercise at 10 AM by performing 45 min of bicycling at 75% \( \dot{V}O_2 \text{peak} \) and arm cranking for 10 min at 50 W. Then subjects completed a period of intermittent exercise consisting of 1.5 min of bicycle exercise bouts at high workload followed by 2 min at 40% \( \dot{V}O_2 \text{peak} \). Initially, the high workload was set to elicit 100% \( \dot{V}O_2 \text{peak} \), and each time subjects were unable to maintain the high workload for 1.5 min the following bouts were performed at an intensity five percentage points lower until the high workload reached 75% \( \dot{V}O_2 \text{peak} \). When subjects were unable to complete 1.5 min at 75% \( \dot{V}O_2 \text{peak} \), they exercised for 5 min at 40% \( \dot{V}O_2 \text{peak} \) and then completed four bouts of 1.5 min at 90, 85, 80, and 75% \( \dot{V}O_2 \text{peak} \), with 2 min at 40% \( \dot{V}O_2 \text{peak} \) between bouts. Then subjects performed 20 min of bicycle exercise at 50% \( \dot{V}O_2 \text{peak} \) with simultaneous arm cranking at 25 W for two 6-min periods. Finally, subjects completed five 1-min bicycle exercise bouts at 80% \( \dot{V}O_2 \text{peak} \), with 3 min at 40% \( \dot{V}O_2 \text{peak} \) between bouts. The glycogen-depletion exercise lasted for 3–4.5 h and was well tolerated by all subjects. To determine the muscle glycogen concentration after the glycogen-depletion protocol, with subjects under local anesthesia a muscle biopsy was obtained from the vastus lateralis muscle by the needle biopsy technique. For the following 6 h, subjects stayed at the laboratory while allowed to move freely around. Meals were served immediately after the biopsy procedure and 1, 2, 3, 5 h after subjects completed the glycogen-depletion exercise. Then subjects were given a light snack (53 E% from fat and 47 E% from protein in H-FOX, and 100 E% from CHO in L-FOX) to ingest later at 11 PM, and they left the laboratory at ~7 PM. The controlled diet during the recovery period consisted of 85 E% from fat, 2 E% from CHO, and 13 E% from protein in H-FOX and 8 E% from fat, 80 E% from CHO, and 12 E% from protein in L-FOX. To secure a low background enrichment of \( ^13 \text{CO}_2 \) in breath and blood during the exercise experiment, the controlled diet contained no food items with a high ratio of \( ^13 \text{C} \) to \( ^12 \text{C} \). The total amount of energy to ingest during the recovery period was calculated as the individual DEI as reported from the dietary record minus the breakfast (25% DEI) plus the estimated extra energy consumption during the bicycle exercise to ensure that subjects were in energy balance. During the glycogen-depletion exercise and for the rest of the day, subjects were allowed to drink water ad libitum.

**Exercise experimental protocol.** The next morning, subjects arrived at the laboratory at 7:30 AM, having abstained from any physical activity during the morning. To avoid large differences in preexercise blood glucose and plasma fatty acid concentrations between H-FOX and L-FOX trials, a light breakfast was served containing 10% of the individual DEI and consisting of 4 E% from fat, 78 E% from CHO, and 18 E% from protein. The 60 ± 6 g of CHO in the light breakfast meal had a high glycemic index, and the meal was ingested 4.5 h later.

### Table 1. Subject characteristics and habitual dietary intake

<table>
<thead>
<tr>
<th></th>
<th>Means ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.80 ± 0.02</td>
</tr>
<tr>
<td>BM, kg</td>
<td>76 ± 3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.5 ± 0.7</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>14.6 ± 1.9</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{peak} ) ( \text{l/min} )</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>( \text{mL/kg BM}^{-1} \text{min}^{-1} )</td>
<td>53.7 ± 1.6</td>
</tr>
<tr>
<td>Habitual dietary intake (3-day period)</td>
<td></td>
</tr>
<tr>
<td>Energy intake, MJ/day</td>
<td>13.1 ± 1.3</td>
</tr>
<tr>
<td>E% protein</td>
<td>16.5 ± 0.7</td>
</tr>
<tr>
<td>E% fat</td>
<td>23.5 ± 2.7</td>
</tr>
<tr>
<td>E% carbohydrate</td>
<td>60.0 ± 2.8</td>
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</tbody>
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Values are means ± SE of 8 subjects. BM, body mass; MJ, megajoule; E%, energy percentage.
before initiation of the subsequent exercise bout. After subjects had rested for 30 min in the supine position, Teflon catheters were inserted under local anesthesia into the femoral artery and the contralateral femoral vein with a thermistor probe inserted into the vein, as described previously (31). During the second trial, femoral catheters were inserted in opposite legs. Two venous catheters were inserted into antecubital veins for infusion of glucose and [U-13C]palmitate.

Then subjects rested for ~30 min in the supine position.Expired air was collected in a Douglas bag, and arterial and venous resting blood samples were obtained for determination of background enrichments of 13CO2 and [U-13C]palmitate. Then, a primer infusion of NaH[13CO3][1 μmol/kg body mass (BM)] was administered into an antecubital vein; subsequently, a constant infusion of [U-13C]palmitate (0.008 μmol·kg BM–1·min–1) into the same antecubital vein was initiated and continued during the following 90 min at rest.

After 75 min of the infusion period at rest, femoral venous blood flow was measured by the thermodilution technique by use of bolus injections of 5-ml ice-cold sterile saline (31). Then, expired air was collected in a Douglas bag, and blood samples were obtained in duplicate for determination of resting isotope enrichments in blood collected in a Douglas bag, and blood samples were obtained in duplicate for determination of background enrichments of 13CO2 and [U-13C]palmitate. Then, a primer infusion of NaH[13CO3][1 μmol/kg body mass (BM)] was administered into an antecubital vein; subsequently, a constant infusion of [U-13C]palmitate (0.008 μmol·kg BM–1·min–1) into the same antecubital vein was initiated and continued during the following 90 min at rest.

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Breath samples. Expired air in the Douglas bags was handled as described previously (31). The respiratory exchange ratio (RER) was calculated as

\[ \text{RER} = \frac{V_{\text{CO}_2}}{V_{\text{O}_2}}, \]

where \(V_{\text{CO}_2}\) and \(V_{\text{O}_2}\) are the systemic CO2 excretion rate and the systemic O2 uptake rate, respectively. Total systemic fat oxidation rate was calculated by the following equation, with the nonprotein respiratory quotient (26):

\[ \text{total fat oxidation (g/min)} = 1.695 \times V_{\text{O}_2} - 1.701 \times V_{\text{CO}_2}. \]

Total systemic fat oxidation rate was expressed in micromolar fatty acid equivalents per minute, with the assumption for molar weights of fatty acids and triacylglycerols of 269 and 861 g/mol, respectively, and that fat oxidation not covered by plasma LCFA was covered by oxidation of circulating VLDL-triaclylglycerol (VLDL-TG) or intramuscular TG (IMTG).

Muscle biopsies. The biopsies were quick-frozen (~10 s) in liquid nitrogen while still in the biopsy needle and were stored at ~–80°C for subsequent analysis. Thirty milligrams wet weight were frozen-embedded and dissected free of all visible adipose tissue, connective tissue, and blood under a microscope. The dissected muscle fibers were pooled and then divided into subpools for the respective analyses.

Muscle glycogen. The glycogen concentration was determined by a fluorometric method (19) on 1–2 mg of dry muscle tissue.

Tissue fractionation and Western blotting. Protein content of FAT/CD36, FABPpm, and caveolin-1 and -3 was measured by Western blotting on total crude membrane (TCM) fractions, and FABPc content was also measured by Western blotting on a cytosolic fraction. Briefly, the freeze-dried muscle tissue (3–4 mg) was homogenized in 450 μl of ice-cold Tris-HCl buffer (pH 7.4) containing 25 mM Tris-HCl, 150 mM NaCl, and 2 mM EDTA. The homogenates were centrifuged at 10,000 g (4°C) for 2 h. Then, all of the supernatant, which contained cytosolic proteins, was harvested, and 5 μl of protease inhibitor cocktail (PIC, Sigma-Aldrich, Steinheim, Germany) and 45 μl of Tris-HCl buffer containing 25% SDS were added before storage at ~–80°C for subsequent Western blotting. The pellets were resuspended in 5 μl of PIC (Sigma-Aldrich) and 495 μl of Tris·HCl buffer containing 2.5% SDS and centrifuged at 1,000 g (4°C) for 10 min. The resulting supernatant, which contained membrane proteins (TCM), was stored at ~–80°C for subsequent Western blotting, and the pellet, which contained fragments of nuclei and cytoskeleton, was discarded.

To determine the linearity range of the primary antibodies, Western blotting with protein gradients of a few samples was performed, and the optimal sample protein concentrations were assessed. The cytosolic and TCM fractions, respectively, were diluted to the correct protein concentration and boiled in Laemmli buffer with Triton X-100 before being subjected to SDS-PAGE and immunoblotting. The polyvinylidene difluoride membrane (Millipore, Bedford, MA) was blocked in either skimmed milk or bovine serum albumin in Tris-buffered saline with Triton X-100 buffer before incubation with antibodies. The primary antibodies against FAT/CD36, FABPpm, and FABPc, have been described elsewhere (3, 21, 33), and the primary antibodies against caveolin-1 and -3 were purchased from BD Biosciences (Palo Alto, CA, cat. nos. 610060 and 610421, respectively). The secondary antibodies were horseradish peroxidase-conjugated anti-mouse, anti-rabbit (both from DAKO, Copenhagen, Denmark), or anti-goat (Zymed, San Francisco, CA). Antigen-antibody complexes were visualized using enhanced chemiluminescence (ECL+, Amersham Biosciences, Little Chalfont, UK) and quantified by a Kodak Image Station E440CF (Kodak, Glostrup, Denmark).

Blood samples. Hematocrit and blood O2 and CO2 concentrations were determined as described previously (31). The leg respiratory quotient (RQ) was calculated from the following equation:

\[ \text{leg RQ} = \frac{[\text{CO}_2]_V - [\text{CO}_2]_A}{[\text{O}_2]_V - [\text{O}_2]_A} \]

where \([\text{CO}_2]_V\) and \([\text{O}_2]_V\) are the blood concentrations of CO2 and O2 in venous (V) and arterial (A) blood, respectively. Total leg fat oxidation rate was calculated using the nonprotein RQ (26) and was expressed in micromolar FA equivalents, as described above for total systemic fat oxidation rate. Blood glucose concentration was measured automatically (ABL510; Radiometer Medical, Copenhagen, Denmark). Plasma LCFA concentration was measured by a colorimetric commercial assay kit (Wako Chemicals, Richmond, VA), which was performed by a COBAS FARA autoanalyzer (COBAS FARA 2; Roche Diagnostic, Basel, Switzerland). Glycerol concentration in plasma was measured spectrophotometrically on a COBAS FARA autoanalyzer (Roche Diagnostic) by use of the glycerol kinase, pyruvate kinase, and lactate dehydrogenase reactions. Concentrations of insulin (Pharmacia Insulin Radioimmunoassay 100; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) as well as epinephrine and norepinephrine (KatCombi Radioimmunoassay, Immuno-Biological Laboratories, Hamburg, Germany) in plasma were determined by radioimmunoassay.

Isotope analyses. Potassium [U-13C]palmitate (98% enriched) and NaH13CO3 (99% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA). On the day of the exercise experiment,
the palmitate tracer was dissolved in sterile water at 60°C, passed through a 0.22-µm sterile filter (Millipore-Or; Millipore, Molsheim, France), and added, and thereby complexed, to sterile 20% (wt/vol) human albumin (State Serum Institute, Copenhagen, Denmark).

Enrichments of [13]CO2 in expiratory air samples and blood samples were determined by gas chromatography-isotope ratio mass spectrometry (GC-IRMS; DeltaPlus, Finnigan MAT, Bremen, Germany) as described previously (31). Before injection into the gas chromatograph, phosphoric acid was added to the blood samples to release CO2 in gaseous form, and all samples were brought to pressure with pure helium.

Derivatization of palmitate to its methyl ester was modified from Patterson et al. (25), as previously described (31). The enrichment of [U-13]C-palmitate in plasma was measured by GC-combustion-IRMS (GC-C-IRMS; Hewlett-Packard 5890, Palo Alto, CA, and GC Combustion III/Deltaplus, Finnigan MAT) on the methyl ester derivatives, as described previously (31). Plasma palmitate concentrations were determined by GC (AutoSystem XL; Perkin-Elmer, Foster City, CA) on the methyl ester derivatives by use of heptadecanoic acid as an internal standard (31).

**Acetate recovery.** An acetate correction factor was applied when calculating plasma LCFA oxidation rates systemically as well as across the exercising leg. This factor accounts for the amount of 13C label originating from [U-13]C-palmitate that is 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 the [U-13]C-palmitate. The acetate correction factors applied (see Results) were based on experiments in two of the eight subjects and in a new third subject. Those subjects completed the same protocols (H-FOX and L-FOX) without biopsy sampling and [U-13]C-palmitate infusion but with 13C-label infusion in the form of sodium [1,2-13]C-acetate in amounts corresponding to the 13C enrichment of plasma LCFA during [1,2-13]C-acetate infusion was calculated using the following equations:

\[
\text{systemic acetate recovery} = \frac{E_{\text{CO}_2} \cdot V_{\text{CO}_2}}{F \cdot 2}
\]

\[
\text{leg acetate recovery} = \frac{\left(100 - \frac{100}{Hct}\right) \cdot \left(100 - \frac{100}{Hct}\right)}{v_{\text{in}}}
\]

where \(E_{\text{CO}_2}\) is the enrichment of 13CO2 in the expiratory air, \(V_{\text{CO}_2}\) is the excretion of CO2 in breath, and \(F\) is the infusion rate of [1,2-13]C acetate. \([13]CO_2\) and \([13]CO_2\) are the blood concentrations of 13CO2 in the femoral artery and vein, calculated as the enrichment of 13CO2 times the blood concentration of CO2 and Hct is the hematocrit in femoral venous blood. \([13]C\)-acetate\) and \([13]C\)-acetate\) are the plasma concentrations of [1,2-13]C acetate in the femoral artery and vein, calculated as the enrichment of [1,2-13]C acetate times the plasma concentration of acetate, and the factor 2 in both equations accounts for the fact that oxidation of one mole of acetate results in two moles of CO2.

**Background correction.** Two of the eight subjects and a new third subject underwent two background trials (H-FOX and L-FOX) in which the same protocol was followed but without infusion of isotopes and sampling of biopsies. Background enrichments of 13CO2 in breath and blood increased significantly (4-14% depending on breath, arterial blood, or venous blood) at initiation of the exercise test, whereas background enrichment of [U-13]C-palmitate in plasma did not change significantly throughout the trials (data not shown). Therefore, enrichments of 13CO2 in breath and blood during exercise in the main trials and in the acetate recovery experiments were corrected on the basis of results from the background experiments. Those corrections influenced plasma LCFA oxidation rates during exercise by ~10% irrespective of trial.

**Plasma LCFA kinetics.** At rest, systemic plasma LCFA rates of appearance (Rv) and disappearance (Rd) were calculated using steady-state equations (48). Non-steady-state equations modified for use with stable isotopes were applied during exercise (32). The effective volume of distribution for palmitate was assumed to be 40 ml/kg BM (48). Palmitate uptake across the exercising leg and systemic and leg palmitate oxidation were calculated using equations by Wolfe (48) provided previously (31). Plasma LCFA kinetics were calculated as the plasma palmitate kinetics divided by the ratio between the plasma palmitate concentration and the plasma LCFA concentration. Plasma LCFA clearance across the leg was calculated by dividing plasma LCFA uptake across the leg by the arterial plasma LCFA concentration. Plasma LCFA fractional extraction across the leg was calculated by dividing plasma LCFA uptake across the leg by the leg plasma LCFA delivery calculated as arterial plasma LCFA concentration multiplied by femoral venous plasma flow.

**Statistics.** Data are presented as means ± SE. For variables independent of time, a paired t-test was performed to test for differences between H-FOX and L-FOX trials. For variables measured at several time points, a two-way analysis of variance (ANOVA), with repeated measures for the time and trial factors, was performed to test for differences between trials or changes due to time. When a significant main effect of time was found, significant pairwise differences were assessed using a Tukey post hoc test. In all cases, a probability of 0.05 was used as the level of significance.

**RESULTS**

**Workload.** The exercise intensity during the bicycle test averaged 68 ± 1 and 62 ± 1% VO2peak in H-FOX and L-FOX, respectively (P < 0.001), despite the identical workload (180 ± 7 W in both trials).

**Intravenous glucose infusion.** The glucose infusion rate averaged 39 ± 5 and 15 ± 6 µmol·kg BM⁻¹·min⁻¹ during the bicycle exercise in H-FOX and L-FOX, respectively (P < 0.01), resulting in similar and almost constant arterial plasma glucose concentrations clamped at the resting level in the two trials [4.9 ± 0.2 and 5.1 ± 0.1 mM in H-FOX and L-FOX, respectively (nonsignificant, NS); Fig. 1A].

**Respiratory and cardiovascular parameters.** At rest, femoral venous blood flow was 0.5 ± 0.1 and 0.4 ± 0.1 l/min in H-FOX and L-FOX, respectively (NS). At onset of exercise, femoral venous blood flow increased (P < 0.001) to 6.5 ± 0.4 and 6.1 ± 0.4 l/min in H-FOX and L-FOX, respectively (NS), and remained at these levels throughout exercise. Leg VO2 was 23 ± 3 and 18 ± 2 ml O2/min at rest in H-FOX and L-FOX, respectively (NS). It increased (P < 0.001) at initiation of exercise and then remained constant during the exercise period [averaging 1.007 ± 67 and 917 ± 64 ml O2/min during exercise in H-FOX and L-FOX, respectively (NS)].

RER and leg RQ did not differ significantly from each other at rest or during exercise, irrespective of trial. At rest, RER was 0.76 ± 0.02 and 0.83 ± 0.03, and leg RQ was 0.75 ± 0.01 and 0.85 ± 0.03 in H-FOX and L-FOX, respectively. Within the first 10 min of exercise, an increase (P < 0.01) was observed in RER (to 0.82 ± 0.02 and 0.91 ± 0.01 in H-FOX and L-FOX, respectively) as well as in leg RQ (to 0.85 ± 0.02 and 0.93 ± 0.02 in H-FOX and L-FOX, respectively). No further significant changes were seen in RER and leg RQ during exercise. There was a main effect (P < 0.001) of trial on RER and on leg RQ, both being lower in H-FOX than in L-FOX.
main effect ($P < 0.01$) of trial was observed on arterial plasma LCFA concentration, being slightly higher in H-FOX than in L-FOX.

At rest, arterial plasma glycerol concentration was $89 \pm 21$ and $69 \pm 7$ μM in H-FOX and L-FOX, respectively (NS; Fig. 1C). During exercise, a continuous increase ($P < 0.05$) was observed to $566 \pm 43$ and $244 \pm 47$ μM at 60 min in H-FOX and L-FOX, respectively. Arterial plasma glycerol concentration was significantly higher in H-FOX than in L-FOX at all time points during exercise ($P < 0.001$).

**Plasma hormone concentrations.** At rest, arterial plasma insulin concentration was $6.6 \pm 1.1$ and $6.5 \pm 0.6$ μU/ml in H-FOX and L-FOX, respectively (NS). In both trials the arterial plasma insulin concentration decreased ($P < 0.001$) continuously during the 60-min bicycle exercise to $3.4 \pm 0.2$ and $4.2 \pm 0.3$ μU/ml at 60 min of exercise in H-FOX and L-FOX, respectively. A minimal difference between trials was seen in arterial plasma insulin concentration at 20, 30, 50, and 60 min of exercise ($P < 0.05$).

At rest, arterial plasma epinephrine concentration was $0.60 \pm 0.10$ and $0.54 \pm 0.08$ nM in H-FOX and L-FOX, respectively (NS). At initiation of exercise it increased ($P < 0.001$) to $1.79 \pm 0.19$ and $1.54 \pm 0.19$ nM at 10 min in H-FOX and L-FOX, respectively, whereafter a further continuous increase ($P < 0.001$) was observed to $2.92 \pm 0.49$ and $2.05 \pm 0.31$ nM at 60 min of exercise in H-FOX and L-FOX, respectively. During exercise, arterial plasma epinephrine concentration did not differ significantly between H-FOX and L-FOX.

Resting arterial plasma norepinephrine concentration was $1.22 \pm 0.29$ and $1.49 \pm 0.29$ nM in H-FOX and L-FOX, respectively (NS). At onset of exercise, it increased ($P < 0.001$) and averaged $14.88 \pm 1.39$ and $11.04 \pm 0.87$ in H-FOX and L-FOX, respectively, during the 60-min exercise bout. Arterial plasma norepinephrine concentration was higher in H-FOX than in L-FOX at 10 min ($P < 0.05$) and 20, 40, 50, and 60 min ($P < 0.01$) of exercise.

**Acetate recovery.** Recovery of $^{13}$C label in $^{13}$CO$_2$ in expired air during infusion of [1,2-$^{13}$C]acetate in three subjects averaged $17 \pm 2$ and $16 \pm 1\%$ at rest and $85 \pm 4$ and $83 \pm 2\%$ during the last 30 min of exercise in H-FOX and L-FOX, respectively. Across the leg, the amount of $^{13}$CO$_2$ recovered in femoral venous blood averaged $95 \pm 2$ and $100 \pm 6\%$ of [1,2-$^{13}$C]acetate uptake across the leg during the last 30 min of exercise in H-FOX and L-FOX, respectively. There were no significant differences in acetate recovery between H-FOX and L-FOX trials.

**Systemic plasma LCFA kinetics.** Palmitate-to-total LCFA ratio in plasma was $28 \pm 2$ and $31 \pm 2\%$ at rest and $33 \pm 3$ and $35 \pm 3\%$ during the last 30 min of exercise in H-FOX and L-FOX, respectively (NS). Systemic plasma LCFA kinetics at rest and during exercise are shown in Table 2. Systemic plasma LCFA $R_a$, $R_d$, oxidation, and percentage of $R_d$ oxidized were all lower ($24, 42, 24\%$, respectively) in L-FOX than in H-FOX during exercise ($P < 0.05$) but not at rest (NS).

**Leg plasma LCFA kinetics.** Plasma LCFA uptake, clearance, fractional extraction, oxidation, and percentage of uptake oxidized across the leg during exercise are shown in Table 3. Plasma LCFA uptake, clearance, and fractional extraction did not differ significantly between trials. Plasma LCFA oxidation and percent uptake oxidized were both lower (50 and 38\%, respectively) in L-FOX than in H-FOX ($P < 0.05$).

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**Blood metabolite concentrations.** At rest, the arterial concentration of plasma LCFA was $674 \pm 110$ and $596 \pm 112$ μM in H-FOX and L-FOX, respectively, decreasing ($P < 0.05$) at initiation of exercise (to $468 \pm 61$ and $334 \pm 34$ μM at 10 min in H-FOX and L-FOX, respectively), after which it increased ($P < 0.05$) continuously during exercise to $703 \pm 58$ μM in H-FOX and $618 \pm 58$ μM in L-FOX at 90 min (Fig. 1B).

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[Figure 1: Arterial blood or plasma concentrations of glucose (A), long-chain fatty acids (LCFA, B), and glycerol (C) during 60 min of bicycling at 65% peak oxygen uptake (V̇O$_2$peak) with high (H-FOX) or low (L-FOX) fat oxidation. A: significantly different from 40 min, $\uparrow P < 0.05$; $\uparrow\uparrow P < 0.001$. B: main effect of trial, $* P < 0.01$; significantly different from 10 min, $\uparrow P < 0.05$; significantly different from 20 min, $\uparrow\uparrow P < 0.05$; significantly different from 40 min, $\# P < 0.05$. C: effect of trial, $* P < 0.01$; significantly different from previous time point, $\uparrow P < 0.05$; $\uparrow\uparrow P < 0.001$; different from rest and 50 and 60 min, $\uparrow\uparrow P < 0.01$; different from rest and 10 and 20 min, $\# P < 0.01$.]
before the 60-min bicycle exercise test. H-FOX and L-FOX, respectively (P < 0.001) to 197 ± 21 and 504 ± 25 mmol/kg dry wt in H-FOX and L-FOX, respectively (P < 0.001), immediately before the 60-min bicycle exercise test.

The protein content of FAT/CD36, FABPpm, FABPc, and caveolin-1 and -3 in the vastus lateralis muscle immediately after the glycogen-depletion trial was 35 ± 10 and 84 ± 42 mmol/kg dry wt in H-FOX and L-FOX, respectively (NS). During the following experimental diet, the glycogen concentration in the vastus lateralis muscle increased respectively (NS). During the following experimental diet, the glycogen concentration in the vastus lateralis muscle increased respectively (NS). During the following experimental diet, the glycogen concentration in the vastus lateralis muscle increased respectively (NS). During the following experimental diet, the glycogen concentration in the vastus lateralis muscle increased respectively (NS).

**Muscle samples.** Glycogen concentration in the vastus lateralis muscle immediately after the glycogen-depletion trial was 35 ± 10 and 84 ± 42 mmol/kg dry wt in H-FOX and L-FOX, respectively (NS). During the following experimental diet, the glycogen concentration in the vastus lateralis muscle increased respectively (P < 0.001) to 197 ± 21 and 504 ± 25 mmol/kg dry wt in H-FOX and L-FOX, respectively (P < 0.001), immediately before the 60-min bicycle exercise test.

The protein content of FAT/CD36, FABPpm, FABPc, and caveolin-1 and -3 in the vastus lateralis muscle immediately after completion of the glycogen-depletion trial did not differ significantly between H-FOX and L-FOX (Figs. 2 and 3). During the subsequent ~21 h of dietary manipulation, the protein content of FABPpm, FABPc, and caveolin-3 did not change significantly, whereas FAT/CD36 and caveolin-1 expression increased irrespective of diet (FAT/CD36: 29 and 15% in H-FOX and L-FOX, P < 0.01; caveolin-1: 21 and 5% in H-FOX and L-FOX, P < 0.05).

**DISCUSSION**

In exercising humans, we investigated leg plasma LCFA kinetics during submaximal exercise in two different conditions. By manipulating preexercise muscle glycogen concentration to very low levels and comparing results to those of a trial with replenished muscle glycogen, we achieved two exercise trials (H-FOX and L-FOX, respectively) with marked difference in total fat oxidation, as also demonstrated in previous studies (46, 47). For the most part we were able to eliminate confounding differences between trials in circulating substrate and hormone levels, otherwise inherent in the present study design (46, 47), by combining a small preexercise meal, ingested 4.5 h before exercise, with a variable glucose infusion during the exercise trials. The major important findings were that, although muscle capacity for transsarcolemmal transport of plasma LCFA during exercise was identical in the two trials, as judged by the similar plasma LCFA clearance and fractional extraction across the leg, oxidation of plasma LCFA was reduced by 50% in the L-FOX trial compared with the H-FOX trial (Table 3). The higher plasma LCFA oxidation in the H-FOX trial was likely influenced by higher plasma LCFA concentration, rendering leg uptake of LCFA slightly higher (NS) in H-FOX than in L-FOX (Table 3). Nevertheless, the fact that leg plasma LCFA oxidation expressed as a percentage of leg plasma LCFA uptake was significantly higher in H-FOX than in L-FOX indicates that control of LCFA oxidation to some extent is regulated at a step downstream from the entry of LCFA into the muscle cell. Accordingly, the preexercise muscle content of the membrane fatty acid transporters FAT/CD36 and FABPpm was similar in the two trials.

On the whole body level, some previous studies based on quantifying systemic plasma LCFA oxidation during exercise by stable isotope tracer methodology have suggested that plasma LCFA oxidation is regulated mainly at the entry of LCFA into mitochondria (6, 35). Thus limitations in whole body plasma oleate oxidation during high-intensity vs. low-intensity exercise were suggested to be at the entry of oleate into the mitochondria (35). Furthermore, it has been shown that preexercise ingestion of glucose induced a larger (~50%) reduction in whole body plasma LCFA oxidation compared

**Table 2. Systemic total fat oxidation and plasma LCFA kinetics at rest and during exercise**

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
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<tbody>
<tr>
<td></td>
<td>H-FOX</td>
<td>L-FOX</td>
</tr>
<tr>
<td>Total fat oxidation, μmol FA/kg BM⁻¹min⁻¹†</td>
<td>6.0±0.5</td>
<td>5.5±0.6</td>
</tr>
<tr>
<td>Plasma LCFA Rₘ, μmol/kg BM⁻¹min⁻¹†</td>
<td>8.5±1.2</td>
<td>7.3±1.3</td>
</tr>
<tr>
<td>Plasma LCFA oxidation, μmol/kg BM⁻¹min⁻¹†</td>
<td>5.7±0.6</td>
<td>5.3±1.2</td>
</tr>
<tr>
<td>Percent plasma LCFA Rₘ oxidized, %</td>
<td>71±5</td>
<td>76±10</td>
</tr>
<tr>
<td>Value are means ± SE. H-FOX and L-FOX, high and low fat oxidation, respectively. FA, fatty acids; LCFA, long-chain fatty acids. Rₘ and Rₚₘ rates of appearance and disappearance, respectively. Exercise data are averaged during the last 30 min of the exercise period. Difference between rest and exercise in both trials, †P &lt; 0.001; different from rest within H-FOX, ‡P &lt; 0.001; different from H-FOX, *P &lt; 0.05; ††P &lt; 0.001.</td>
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</table>

**Table 3. Leg total fat oxidation and plasma LCFA kinetics during exercise**

<table>
<thead>
<tr>
<th></th>
<th>H-FOX</th>
<th>L-FOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg total fat oxidation, μmol FA/min</td>
<td>1,098±140</td>
<td>494±84†</td>
</tr>
<tr>
<td>Leg plasma LCFA uptake, μmol/min</td>
<td>379±39</td>
<td>303±38</td>
</tr>
<tr>
<td>Leg plasma LCFA clearance, ml/min</td>
<td>620±61</td>
<td>604±83</td>
</tr>
<tr>
<td>Leg plasma LCFA fractional extraction, %</td>
<td>17.8±1.6</td>
<td>18.2±1.8</td>
</tr>
<tr>
<td>Leg plasma LCFA oxidation, μmol/min</td>
<td>421±47</td>
<td>212±37†</td>
</tr>
<tr>
<td>Percent leg plasma LCFA uptake oxidized, %</td>
<td>116±16</td>
<td>72±10*</td>
</tr>
</tbody>
</table>

**Values are means ± SE, averaged during the last 30 min of exercise period. Different from H-FOX, *P < 0.05; †P < 0.001.**
with the reduction in plasma LCFA $R_d$ (~33%) during bicycle exercise at 50% $V_{O_2, peak}$ (6). However, whole body measurements of plasma LCFA kinetics do not accurately reflect plasma LCFA kinetics locally in skeletal muscle during exercise, because, as we have previously demonstrated (12, 31), only 35–45% of systemic plasma LCFA $R_d$ was taken up by exercising muscle. This is also evident from the present study, in which the relation between systemic plasma LCFA $R_d$ and exercising muscle. This is also evident from the present study, in which leg plasma LCFA oxidation was not closely linked to leg plasma LCFA uptake, further supports that a major limiting step in plasma LCFA oxidation in contracting human skeletal muscle occurs further downstream from the entry of LCFA into the muscle cell. This is an essential realization, because it has recently been suggested that translocation of FAT/CD36 to the sarcolemma plays a very important role in regulation of LCFA utilization during contractile activity in rat skeletal muscle and heart (2, 18, 20). While the present findings do not exclude that an increase in uptake of circulating LCFA is related to translocation of FAT/CD36 to the sarcolemma, they suggest that translocation of FAT/CD36 is not the limiting step in plasma LCFA oxidation in exercising human skeletal muscle with different muscle glycogen stores and, consequently, different LCFA oxidation rates. Also, other models to manipulate carbohydrate and lipid oxidation indicate that, during contraction, LCFA oxidation is limited at intracellular sites rather than at the transsarcolemmal transport of LCFA into the muscle cell. Thus, in isolated contracting rat soleus muscle, addition of insulin to the incubation medium induced a 24% reduction in oxidation but a 21% increase in uptake of exogenous palmitate (8). These effects were probably not a direct effect of insulin but rather a result of an increased reliance on carbohydrate due to a higher glucose uptake (49). On the other hand, Turcotte et al. (38) showed that palmitate uptake and oxidation during contraction in isolated perfused rat hindquarters were not influenced by lowered precontraction muscle glycogen when glucose concentration in the perfusate was kept similar. However, comparison between that study (38) and the present study is difficult, since in the study by Turcotte et al. perfused muscles were dominated by type IIb/X fibers (38).

The contribution from nonplasma LCFA sources, i.e., VLDL-TG and IMTG, across the exercising leg could be estimated in the present study by subtracting leg plasma LCFA oxidation from leg total fat oxidation. This estimation also revealed that nonplasma LCFA sources contributed more ($P < 0.01$) to total energy expenditure during exercise in H-FOX (676 ± 145 μmol FA equivalents/min) than in L-FOX (282 ± 82 μmol FA equivalents/min).

In the present study, we found that, during exercise in the H-FOX trial, 115 ± 3 and 116 ± 16% of plasma LCFA uptake was oxidized systemically and across the leg, respectively, which indicates that plasma LCFA oxidation during exercise might have been slightly overestimated both systemically and across the leg. Underestimation of the acetate recovery factor would lead to overestimation of plasma LCFA oxidation. Although this might explain why systemic plasma LCFA oxidation exceeded $R_d$ during exercise in H-FOX, it could not fully explain that leg plasma LCFA oxidation slightly exceeded uptake, since the acetate recovery factor across the leg during exercise in H-FOX was ~95%. Alternatively, and more plausibly, $^{13}$C labeling of the nonplasma LCFA stores during the preexercise $[U^{13}C]$palmitate infusion and subsequent nonplasma LCFA oxidation during exercise would lead to overestimation of $[U^{13}C]$palmitate oxidation and, thus, plasma LCFA oxidation. Incorporation of $[U^{13}C]$palmitate into the IMTG pool of resting skeletal muscle during $[U^{13}C]$palmitate infusion has been observed in both rats (11) and humans (34). In the present study, assuming that 50% of the $[U^{13}C]$palmitate taken up across the leg during the preexercise $[U^{13}C]$palmitate infusion period was esterified (34, 43) and
that all this [U-13C]palmitate was oxidized during exercise, it could be calculated that leg plasma LCFA oxidation during exercise would have been overestimated by 44 ± 7 μmol/min in the H-FOX trial. The same calculation in the L-FOX trial revealed that leg plasma LCFA oxidation during exercise would have been overestimated by 34 ± 4 μmol/min. Consequently, if leg plasma LCFA oxidation were corrected accordingly in both trials, this would clearly not influence the conclusions of the present study but, importantly, in the H-FOX trial it would imply that the percentage of leg plasma LCFA uptake being oxidized during exercise approached ~100% (104 ± 15%). Alternatively, in the scenario that significant preexercise esterification of [U-13C]palmitate into IMTG did only occur in the H-FOX trial, then it would be appropriate to compare the corrected leg plasma LCFA oxidation during exercise in the H-FOX trial (377 ± 45 mol/min) with the noncorrected leg plasma LCFA oxidation during exercise in the L-FOX trial (212 ± 37 μmol/min), still revealing significantly higher leg plasma LCFA oxidation in the H-FOX than in the L-FOX trial (P < 0.01). Moreover, the percentage of leg plasma LCFA uptake being oxidized during exercise would also remain higher in H-FOX than in L-FOX (104 ± 15 and 72 ± 10%, respectively, P < 0.05). Thus, even when corrected for possible methodological limitations, the difference in intracellular partitioning of leg plasma LCFA uptake between H-FOX and L-FOX trials persists.

The present study showed that, in the L-FOX trial, at least 28% of the plasma LCFA that were taken up across the leg during exercise were not oxidized. A possible fate of these plasma LCFA in L-FOX was esterification into IMTG. Accordingly, in the study by Dyck et al. (8), addition of insulin to the incubation medium, which probably induced a higher reliance on carbohydrate as substrate, increased the esterification of palmitate in isolated contracting rat soleus muscle.

The present findings suggest that transsarcolemmal transport is not limiting plasma LCFA oxidation during exercise, which gives support to the notion that oxidation of LCFA during exercise is limited at sites beyond the sarcomella. After entering the cell, LCFA, converted to long-chain fatty acyl-CoA, may be either β-oxidized or esterified into complex lipids including TG. Accordingly, regulation of long-chain fatty acyl-CoA oxidation may take place directly at the mitochondria and/or indirectly by regulation of TG synthesis, thereby determining the channeling of long-chain fatty acyl-CoA toward oxidation. Recent studies in isolated soleus muscle from mice incubated with AICA riboside (23) revealed an inhibition of TG synthesis that channeled long-chain fatty acyl-CoA toward β-oxidation. It was suggested that AMP-activated protein kinase (AMPK) was the mediator by phosphorylating and inactivating glycerol-3-phosphate acyltransferase (GPAT), the enzyme catalyzing the initial step in TG synthesis (23). In accord, α2-AMPK activity was shown to vary reciprocally with muscle glycogen content during exercise in human skeletal muscle (47). Consequently, AMPK may have inhibited GPAT activity and glycerolipid synthesis more during exercise in the H-FOX than in the L-FOX trial in the present study. Thus it cannot be ruled out that GPAT activity may take part in the regulation of the percentage of LCFA uptake that is channeled toward oxidation rather than storage during exercise.

Another possible intracellular site in skeletal muscle where plasma LCFA oxidation could be regulated is at the entry of LCFA into mitochondria. Because the inner mitochondrial membrane is not readily penetrable by long-chain fatty acyl-CoA, entry of long-chain fatty acyl-CoA into mitochondria requires transfer of the acyl moiety to carnitine, a process catalyzed by carnitine palmitoyltransferase I (CPT I) located at the outer mitochondrial membrane (27). CPT I activity is regulated by the allosteric inhibitor malonyl-CoA and by the cosubstrate free carnitine among other factors (22). Because muscle malonyl-CoA concentration has been shown either not to change (24) or to decrease slightly (7) at high- compared with moderate-intensity exercise, when fat oxidation decreases markedly, it seems unlikely that malonyl-CoA should play any major role in regulation of LCFA oxidation in exercising human skeletal muscle. On the other hand, muscle free carnitine concentration has been shown to correlate well with the fat oxidation rate during graded intensity exercise in humans (44).

In the present study, FAT/CD36 and caveolin-1 expression increased during recovery from the bout of prolonged exercise that subjects performed on the day before the main experiment to deplete their glycogen stores. This increase in FAT/CD36 and caveolin-1 protein occurred irrespective of the type of diet consumed during the recovery period. In contrast, FABPₚₚ, FABPₗ, and caveolin-3 levels did not change during recovery from the glycogen-depletion exercise bout. Because the protein content of the five lipid-binding proteins did not respond to the two different postexercise diets, we conclude that a 21-h dietary manipulation is not enough to significantly change the expression of these proteins. This is in accord with the findings by Cameron-Smith et al. (4) that ingestion of a high-fat diet for 5 days did not significantly change the muscle content of FAT/CD36 and FABPₚₚ compared with the ingestion of a high-carbohydrate diet for 5 days. Apparently, up to 4 wk of dietary manipulation is necessary to detect significant changes in levels of lipid-binding proteins in human skeletal muscle (30).

The increase in FAT/CD36 and caveolin-1 protein during recovery from the glycogen-depletion exercise bout in the present study is probably caused by the very strenuous preceding exercise bout per se. During recovery from such an exercise bout, a high reliance on fat sources to energy production has been observed (15), presumably as a consequence of the priority given to replenishment of the depleted muscle glycogen stores. An increase in lipid-binding proteins during the recovery period is a possible means to enhance the capacity for LCFA transport and thereby the potential for high fat utilization. Accordingly, we have recently shown an increase during 90 min of submaximal bicycle exercise in FAT/CD36 mRNA (17), which presumably translated into a higher amount of protein a few hours into recovery. The increase in FAT/CD36 and caveolin-1 protein during recovery from the glycogen-depletion exercise bout implies the existence of signaling from a factor, which is changed by heavy exercise, toward enhanced synthesis of FAT/CD36 and caveolin-1. Several candidates may serve this function, but further research is needed on this issue.

The remarkably similar responses of FAT/CD36 and caveolin-1 protein during recovery from the glycogen depletion exercise bout prompted us to speculate whether FAT/CD36 and caveolin-1 responded in a coordinated manner. We detected caveolin-1 in TCM fractions of human muscle biopsies dissected free of blood, connective tissue, and adipose tissue.
Thus the caveolin-1 detected by us in human skeletal muscle was expected to be of endothelial origin. In rodent (10, 50) as well as in human skeletal muscle (45), FAT/CD36 was very abundantly expressed in endothelial cells compared with their parenchymal muscle cells. It is therefore possible that coordination of FAT/CD36 and caveolin-1 expression would occur in endothelial cells. Accordingly, a role of FAT/CD36 in transport of LCFA across the endothelial membranes has been suggested (40). Furthermore, a role of caveolin in cellular LCFA uptake has been suggested from studies in human microvascular endothelial cells (29). Therefore, we speculate that the increase in FAT/CD36 and caveolin-1 expression during recovery from the glycogen-depletion exercise bout in the present study is of endothelial origin and serves to increase the potential for LCFA transport into muscle for energy when glycogen repletion is given high priority.

In conclusion, the present study has shown that, with different preexercise muscle glycogen content, the oxidation of circulating LCFA in exercising human skeletal muscle can vary by a factor of two, without similar marked changes in plasma LCFA uptake and with no influence on the plasma LCFA fractional extraction. These results imply that, during exercise, an important limiting step in regulation of plasma LCFA oxidation in human skeletal muscle occurs downstream from, rather than at the entry of, plasma LCFA into the muscle cell. In addition, we have demonstrated increased skeletal muscle protein levels of FAT/CD36 and caveolin-1, but not of FABPpm, FABPc, and caveolin-3, during recovery from prolonged glycogen-depleting exercise regardless of the amount of carbohydrate in the diet consumed during the recovery period.

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