Endurance training has little effect on active muscle free fatty acid, lipoprotein cholesterol, or triglyceride net balances

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PLASMA FREE FATTY ACIDS (FFAs) represent a fuel source whose turnover, availability, and rate of oxidation are highly adaptable. FFA concentration [FFA] can rise two- to fourfold during 60 min of moderate-intensity exercise (5, 10, 11, 21) and is accompanied by a two- to threefold rise in turnover (10, 11, 21). FFAs represent an important whole body fuel source contributing 10–60% of whole body lipid oxidation and 5–20% of total energy expenditure during rest and exercise (10, 11, 21). The importance of FFAs to active skeletal muscle metabolism has been investigated, with the inclusion of the arteriovenous (a-v) balance technique to distinguish between active limb skeletal muscle from whole body lipid metabolism. It appears that FFAs contribute to active skeletal muscle metabolism to some degree, because studies employing the a-v balance technique have demonstrated a shift from net leg FFA release at rest to uptake during one-legged knee extension (23, 45) and two-legged cycle ergometry (2, 5, 40, 41) performed at a variety of durations and intensities (45–240 min, 30–65% VO2 peak).

Although considerable attention has been directed toward describing changes in whole body lipid metabolism with endurance training, far less is known about training-induced changes in FFA, lipoprotein, or plasma triglyceride (TG) metabolism across the active limb. Cross-sectional studies indicate that endurance-trained subjects exhibit greater net leg FFA uptake than untrained subjects during prolonged one-legged knee extension exercise (45). As well, longitudinal studies have shown that endurance training results in an increase in net leg FFA uptake at the same absolute intensity during prolonged one-legged knee extension exercise (23) and at the same relative intensity during two-legged cycle ergometry (5). To date, a detailed examination of the influence of endurance training on net leg individual FFA balance has not been reported. Except for a slight preference for the uptake of the unsaturated fatty acids oleate and linoleate over that of the saturated fatty acid palmitate (16), the relative net uptake of individual FFAs across the exercising forearm has been shown to be highly related to FFA concentrations in the plasma. However, issues related to the effects of endurance training on individual and total FFA and lipoprotein exchange across large muscle groups responsible for the majority of energy flux during exercise have not been explored in detail.

Long-term (8 mo) endurance training has been shown to result in significant improvements in the plasma lipid and lipoprotein profiles of sedentary, overweight subjects with mild to moderate dyslipidemia (26). Additionally, 4 mo of endurance training has been shown to significantly increase HDL cholesterol (HDL-C) in healthy, sedentary men (24). However, little is known about the potential role of skeletal muscle in training-induced changes in plasma lipid and lipoprotein profiles. In the only study to date to examine net leg lipoprotein balance, Kiens and Lithell (25) demonstrated that 8 wk of one-legged knee extension training resulted in significantly
higher rates of net VLDL-TG uptake at rest and net HDL$_{2}$-C release at rest and after 110 min of exercise in the trained vs. untrained leg. Results of that study suggest that active skeletal muscle plays a significant role in training-induced improvements in plasma lipoprotein profiles. Moreover, because of constancy of the hormonal environment presented to trained and untrained legs in the same individuals, the results can be interpreted to implicate intramuscular as opposed to hormonally-mediated regulation of lipoprotein metabolism.

The purpose of this study was to employ a longitudinal study design to evaluate the effect of two-legged endurance training on net leg individual FFAs, lipoprotein, and TG balances at rest and during moderate-intensity exercise. We tested the hypothesis that net leg FFA, LDL-C, and TG uptake and HDL-C release during moderate-intensity cycling exercise would increase following endurance training.

**METHODS**

Detailed descriptions of many of the methods employed in this study have been published previously (5, 21), but relevant information is reiterated for the convenience of the reader. As well, results in the present report are ultimately to be considered with those contained in another report from the same study (Freiendlauer AL, Jacobs KA, Fattor JA, Horning MA, Hagobian TA, Bauer TA, Wolfel EE, and Brooks GA, unpublished observations).

**Subjects.** Eight healthy sedentary men age 18–32 yr were recruited from the University of California, Berkeley campus, by posted notices. Subjects were considered sedentary if they engaged in no more than 2 h of regular strenuous physical activity per week for 2 yr and had a peak oxygen consumption ($$\text{VO}_2$$ peak) of <50 ml·kg$^{-1}$·min$^{-1}$. Subjects were included in the study if they had <25% body fat, were nonsmokers, were diet and body weight stable, had a 1-s forced expiratory volume (FEV$$_1$$) that was >70% of forced vital capacity (FVC), and were injury and disease free as determined by health history questionnaires and physical examination. The procedures and risks were thoroughly explained to the subjects, and their written, informed consent was obtained. The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley (CPHS no. 2002-3-21).

**Experimental design.** After being screened, subjects completed a pair of experimental trials before and after 9 wk of endurance training. Each experimental trial consisted of 90 min of rest and 60 min of exercise. Prior to training, subjects were tested in random order at 45% (45% Pre) and 65% (65% Pre) $$\text{VO}_2$$ peak with ~1 wk between trials. After being trained, subjects were tested in random order at 65% pretraining $$\text{VO}_2$$ peak [same absolute intensity (ABT)] and 65% posttraining $$\text{VO}_2$$ peak [same relative intensity (RTL)] with ~1 wk between trials, during which subjects continued training.

**Screening tests.** $$\text{VO}_2$$ peak was determined before training, after 4–5 wk of training, and at the end of the 9-wk training program using a continual graded exercise test on an electronically braked cycle ergometer (Monark Ergometric 839E, Vansbro, Sweden). An initial power output of 50 W was increased by 25–50 W every 3 min until volitional exhaustion. Respiratory gases were analyzed by an open-circuit indirect calorimetry system (ParvoMedics TrueMax 2400; ParvoMedics, Sandy, UT). Body composition was determined by the skinfold method before and after training (20). Subjects were asked to maintain regular dietary regimens, and body weight was recorded on a daily basis to evaluate the need for adjustments in caloric intake to maintain baseline body weight. Three-day dietary records were collected to assess habitual dietary habits and monitor each subject’s caloric intake and macronutrient composition at baseline and every 2–3 wk during training using the Nutritionist III program (N-Squared Computing, Salem, OR). FEV$$_1$$ and FVC were determined with a 9-liter spirometer, and a 12-lead electrocardiogram (ECG) was recorded to screen for any cardiac arrhythmias.

**Testing protocol.** Subjects were admitted to the metabolic ward at the Clinical Studies Unit at the Palo Alto Veterans Affairs Health Care System the night before each experimental trial and remained there until testing was completed the following day. The night before each experimental trial, subjects were fed a standardized dinner [1,180 kcal: 69% carbohydrate (CHO), 21% fat, and 10% protein]. Two subjects were tested per day, and morning and afternoon testing was randomly assigned and then replicated in the remaining trials. Morning subjects were fed a standardized pretrial meal (434 kcal: 74% CHO, 10% fat, and 16% protein) 1 h before procedures started and 4–5 h before exercise. Afternoon subjects were fed a standardized breakfast (661 kcal: 55% CHO, 34% fat, and 11% protein) and the same standardized pretrial meal as the morning subjects 1 h before procedures started and 4–5 h before exercise. We chose to test our subjects in the 3- to 4-h postprandial state to control for the effects of meal size, composition, and timing and to mimic the eating practices of active individuals in a nonlaboratory environment. Although the ATP III lipoprotein analysis guidelines (1) recommend the use of blood samples from overnight-fasted subjects, recent reports (43) conclude that fasting conditions are not necessary for the direct assessment of total cholesterol, LDL-C, and HDL-C.

**Catheterization.** After local anesthesia with Lidocaine, the femoral artery and vein of the same leg were cannulated using standard percutaneous techniques, as previously described (50), with the following modifications. Localization and cannulation of the femoral artery and vein were performed using vascular ultrasound (Site-Rite 3, Bard Access Systems; Dymax, Pittsburgh, PA). A 5.0-French, 65-cm angiographic catheter (model 451-501V5; Cordis, Miami, FL) was inserted 25 cm and positioned in the distal abdominal aorta via the femoral artery. A 6-French thermodilution nonballoon venous catheter (model F06TNN001; American Edwards Laboratory, Irvine, CA) was placed with the tip in the distal iliac vein through a venous sheath. After insertion, the venous sheath and catheter were withdrawn such that the distal tip of the catheter was 15 cm and the proximal port (10 cm from the tip) was 5 cm from the skin insertion site. The proximal port, which was used for all femoral venous blood sampling and the cold saline injection for thermodilution blood flow measurements, was positioned ~2 cm from the entry site into the femoral vein. Both catheters were sutured to the skin and further secured by an Ace bandage wrap. The external portions of each catheter were directed toward the hip for easy access during exercise. Alternate legs were used for the two tests both pretraining and posttraining. One subject experienced pain in the groin related to the catheter placement when he was positioned on the cycle ergometer for exercise, and so that trial (45% Pre) was discontinued and the catheters were removed. A cardiologist experienced in vascular catheterization techniques performed all catheterizations.

**Blood sampling.** Arterial and venous blood samples were drawn simultaneously and anaerobically after 75 and 90 min of rest and 30, 45, and 60 min of exercise. Blood for FFA and lipoprotein analyses was placed in tubes containing EDTA and a preservative cocktail the night before each experimental trial and remained there until testing was completed the following day. The night before each experimental trial, subjectswere fed a standardized dinner [1,180 kcal: 69% carbohydrate (CHO), 21% fat, and 10% protein]. Two subjects were tested per day, and morning and afternoon testing was randomly assigned and then replicated in the remaining trials. Morning subjects were fed a standardized pretrial meal (434 kcal: 74% CHO, 10% fat, and 16% protein) 1 h before procedures started and 4–5 h before exercise. Afternoon subjects were fed a standardized breakfast (661 kcal: 55% CHO, 34% fat, and 11% protein) and the same standardized pretrial meal as the morning subjects 1 h before procedures started and 4–5 h before exercise. We chose to test our subjects in the 3- to 4-h postprandial state to control for the effects of meal size, composition, and timing and to mimic the eating practices of active individuals in a nonlaboratory environment. Although the ATP III lipoprotein analysis guidelines (1) recommend the use of blood samples from overnight-fasted subjects, recent reports (43) conclude that fasting conditions are not necessary for the direct assessment of total cholesterol, LDL-C, and HDL-C.

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**Blood sampling.** Arterial and venous blood samples were drawn simultaneously and anaerobically after 75 and 90 min of rest and 30, 45, and 60 min of exercise. Blood for FFA and lipoprotein analyses was placed in tubes containing EDTA and a preservative cocktail, respectively, and was centrifuged at 2,800 g for 18 min at 4°C. The lipoprotein preservative cocktail contained EDTA (0.15%), enzyme inhibitors (o-phenylalanyl-l-prolyl-l-arginyl-CHCl$_3$, 1 mM; aprotinin, 50 KU/ml), antibiotics (gentamicin sulfate, 50 μg/ml; chloramphenicol sodium succinate, 0.05 mg/ml), and a bacterioside (sodium azide, 0.01% wt/vol). Exactly 1 ml of plasma for FFA analysis was mixed with 4 ml of heptane-isopropanol (30:70) extraction solution, 2 ml of 3.3 mM H$_2$SO$_4$, and 100 nmol of pentadecanoic acid (PDA; 15:0) as an internal standard. Plasma for lipoprotein analysis was flushed with nitrogen prior to capping, and all plasma samples were stored at ~20°C until analysis. Hematocrit measurements were performed on both arterial and venous blood using a circular microcapillary tube reader (International Equipment, no. 2201).
steady-state training sessions per week were replaced with interval training during the last 2 wk of training. Two of the subjects were asked to perform an activity of their choosing on 1 day per week, and encouraged subjects to warm up, cool down, and stretch to enhance materials SP1 for apoA-I and SP3-07 for apoB. In-house controls and reference controls were assigned concentration values with the use of International Federation of Clinical Chemistry standard reference plasma controls, with and without elevated lipids, were included in the immunoturbidimetric assay reagent kit (Bacton Assay Systems, San Marcos, CA). Measurements were performed using the Express Plus 550 analyzer according to kit instructions. Calibrators and reference controls were assigned concentration values with the use of International Federation of Clinical Chemistry standard reference materials SP1 for apoA-I and SP3-07 for apoB. In-house controls measured in each group of 20 unknowns had a coefficient of variation <3%.

Calculations. Net leg individual FFA and lipoprotein balances were calculated as the product of leg plasma flow and a-v differences where arterial and venous hematocrit values were used to correct for changes in plasma volume:

\[
\text{net leg FFA balance (mol/min)} = 2(1-\text{leg } Q) \times ([\text{FFA}]_a - ([\text{Hct}_a/\text{Hct}_v][\text{FFA}]_v])
\]

\[
\text{net leg lipoprotein balance (mg/min)} = 2(1-\text{leg } Q) \times ([\text{lipo}]_a - ([\text{Hct}_a/\text{Hct}_v][\text{lipo}]_v))
\]

where \( Q \) is leg plasma flow, Hct is hematocrit, FFA is free fatty acid, lipoprotein and the subscripts a and v represent arterial and venous plasma, respectively.

### Table 2. Workload, heart rate, respiratory gas exchange, and blood flow at rest and during exercise before and after 9 wk of endurance training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest pretraining</th>
<th>Rest posttraining</th>
<th>Average During Last 30 min of Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>45% Pretraining</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>65% Pretraining</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>65% Old (ABT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>65% New (RLT)</td>
</tr>
<tr>
<td>Workload, W</td>
<td>92 ± 4</td>
<td>156 ± 6</td>
<td>156 ± 6</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>61 ± 2</td>
<td>56 ± 2*</td>
<td>156 ± 6</td>
</tr>
<tr>
<td></td>
<td>125 ± 3b</td>
<td>157 ± 3b</td>
<td>156 ± 6</td>
</tr>
<tr>
<td></td>
<td>125 ± 3b</td>
<td>157 ± 3b</td>
<td>156 ± 6</td>
</tr>
<tr>
<td></td>
<td>145 ± 3b</td>
<td>157 ± 3b</td>
<td>156 ± 6</td>
</tr>
<tr>
<td></td>
<td>156 ± 0.06b</td>
<td>2.32 ± 0.11b,c</td>
<td>2.24 ± 0.08b,c</td>
</tr>
<tr>
<td></td>
<td>156 ± 0.06b</td>
<td>2.32 ± 0.11b,c</td>
<td>2.24 ± 0.08b,c</td>
</tr>
<tr>
<td></td>
<td>145 ± 0.04b</td>
<td>2.19 ± 0.09b,c</td>
<td>2.07 ± 0.07b,c</td>
</tr>
<tr>
<td></td>
<td>145 ± 0.04b</td>
<td>2.19 ± 0.09b,c</td>
<td>2.07 ± 0.07b,c</td>
</tr>
<tr>
<td></td>
<td>1.93 ± 0.01b</td>
<td>0.95 ± 0.01b</td>
<td>0.92 ± 0.01b,d</td>
</tr>
<tr>
<td></td>
<td>1.93 ± 0.01b</td>
<td>0.95 ± 0.01b</td>
<td>0.92 ± 0.01b,d</td>
</tr>
<tr>
<td></td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>1-Leg blood flow, liters/min</td>
<td>3.6 ± 0.4e</td>
<td>5.0 ± 0.4e</td>
<td>5.3 ± 0.4e</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 8 \). ABT, absolute intensity; RLT, relative intensity; RER, respiratory exchange ratio. *Significantly different from rest pretraining; †significantly different from corresponding rest; ‡significantly different from 45% pretraining; §significantly different from 65% pretraining; ¶significantly different from all other workloads. \( P < 0.05 \).
residual/SD) were calculated to categorize the magnitude of the error.

Table 4. Net leg individual FFA fractional extraction and balance at rest and during exercise across all trials

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Rest</th>
<th>Exercise Time, min</th>
<th>%Total [FFA] Across All Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>211.0 ± 25.4</td>
<td>175.6 ± 20.8*</td>
<td>199.8 ± 26.3†</td>
</tr>
<tr>
<td>Myristate 14:0</td>
<td>10.9 ± 1.7</td>
<td>9.5 ± 1.1</td>
<td>11.0 ± 1.4†</td>
</tr>
<tr>
<td>Palmitate 16:0</td>
<td>139.6 ± 17.8</td>
<td>126.9 ± 16.0</td>
<td>146.3 ± 20.0†</td>
</tr>
<tr>
<td>Stearate 18:0</td>
<td>60.3 ± 6.6</td>
<td>39.2 ± 4.1*</td>
<td>42.4 ± 5.4*</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>233.1 ± 35.0</td>
<td>193.8 ± 27.8*</td>
<td>228.1 ± 33.5†</td>
</tr>
<tr>
<td>Palmitoleate 16:1 (n−7)</td>
<td>11.7 ± 2.5</td>
<td>12.4 ± 2.2</td>
<td>14.8 ± 2.5†</td>
</tr>
<tr>
<td>Oleate 18:1 (n−9)</td>
<td>221.4 ± 32.5</td>
<td>181.3 ± 25.8*</td>
<td>213.3 ± 31.2†</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>80.7 ± 14.4</td>
<td>72.4 ± 11.5</td>
<td>84.4 ± 13.3†</td>
</tr>
<tr>
<td>Linoleate 18:2 (n−6)</td>
<td>74.2 ± 13.3</td>
<td>66.5 ± 10.8</td>
<td>77.7 ± 12.4†</td>
</tr>
<tr>
<td>Linolenate 18:3 (n−3)</td>
<td>3.8 ± 0.8</td>
<td>3.5 ± 0.6</td>
<td>4.1 ± 0.8†</td>
</tr>
<tr>
<td>Arachidonate 20:4 (n−6)</td>
<td>2.8 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Unsaturated/saturated</td>
<td>1.42 ± 0.08</td>
<td>1.47 ± 0.07</td>
<td>1.52 ± 0.06†</td>
</tr>
<tr>
<td>Total FFA</td>
<td>524.8 ± 74.3</td>
<td>441.7 ± 59.2*</td>
<td>512.3 ± 71.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE, averaged over the 4 trials; n = 8 subjects. %Total FFA concentration ([FFA])/ is average across both intensities and all time points. *Significantly different from rest; †significantly different from preceding exercise time points; ‡significantly different from 30 min of exercise. P < 0.05.

Net leg FFA fractional extraction was calculated from the ratio of a-v [FFA] and arterial [FFA] with correction of venous [FFA] for blood volume shifts, as determined from hematocrit measurement:

\[
\text{net leg FFA fractional extraction (\%)} = \left[\frac{[\text{FFA}]_a - \left(\frac{[\text{Hct}]_v}{[\text{Hct}]_a}\right)[\text{FFA}]_v}{[\text{FFA}]_a}\right] \times 100
\]

Statistics. Data are represented as means ± SE. Results of Shapiro-Wilk tests indicated that the data were normally distributed. The wilks tests indicated that the data were normally distributed. The significance of within- and between-condition mean differences was assessed by ANOVA, with repeated measures followed by post hoc analyses using the Least Significant Difference Test. The 95% confidence intervals of net leg FFA fractional extraction and balance data were examined to determine whether or not they contained zero. Those data points whose 95% confidence intervals did not contain zero were ascertained to have reached significant levels of net fractional extraction, release, or uptake. The adequacy of the estimation of net leg total FFA balance from net leg palmitate balance was evaluated with multiple approaches. Regression analyses were performed to examine the strength of the relationship between measured and estimated net leg total FFA balance. The error associated with the estimation of the net leg total FFA balance was quantified with the calculation of residuals (estimated minus measured values) that were then standardized by dividing by the appropriate SD of the residuals to account for the much smaller net leg total FFA balance at rest compared with exercise. Finally, Cohen’s d scores (average residual/SD) were calculated to categorize the magnitude of the error of estimation as large (>0.8), moderate (0.5–0.8), or small (<0.5) (6)

Table 3. Individual arterial [FFA] and ratio of unsaturated to saturated fatty acids at rest and during exercise across all trials

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Rest</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>%Total [FFA] Across All Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitate 16:0</td>
<td>−10.4 ± 5.2</td>
<td>9.0 ± 2.6*‡‡</td>
<td>7.3 ± 2.2*‡‡</td>
<td>3.8 ± 1.8*</td>
<td>−9.9 ± 3.9</td>
</tr>
<tr>
<td>Stearate 18:0</td>
<td>−13.2 ± 8.2</td>
<td>8.8 ± 3.7*‡‡</td>
<td>9.7 ± 3.2*‡‡</td>
<td>1.5 ± 3.5</td>
<td>−4.2 ± 3.0</td>
</tr>
<tr>
<td>Oleate 18:1 (n−9)</td>
<td>−14.5 ± 6.9</td>
<td>11.5 ± 3.2*‡‡</td>
<td>11.6 ± 3.0*‡‡</td>
<td>3.1 ± 3.0*</td>
<td>−22.7 ± 8.1</td>
</tr>
<tr>
<td>Linoleate 18:2 (n−6)</td>
<td>−3.4 ± 3.3</td>
<td>6.9 ± 6.1</td>
<td>−0.5 ± 4.8*</td>
<td>7.2 ± 3.1</td>
<td>−3.0 ± 2.1</td>
</tr>
<tr>
<td>Total FFA</td>
<td>−11.9 ± 5.5</td>
<td>10.2 ± 2.3*‡‡</td>
<td>8.3 ± 2.1*‡‡</td>
<td>4.3 ± 2.1*</td>
<td>−44.0 ± 15.5</td>
</tr>
</tbody>
</table>

Values are means ± SE in minutes, averaged over the 4 trials; n = 8 subjects. *Significantly different from rest; †significantly different from preceding exercise time points; ‡significantly different from 30 min of exercise; §significantly different from zero. P < 0.05.

RESULTS

One subject did not complete a 45% Pre trial due to discomfort experienced at rest following catheterization. As a result, the data from the remaining seven subjects were used in comparisons made between trials, whereas the data from all subjects were used in comparisons made before and after training (subject characteristics) and when data were averaged across all four trials (individual FFA concentrations, net leg fractional extraction, and net leg balance).

Subject characteristics, hemodynamics, and pulmonary gas exchange. Dietary macronutrient composition (46 ± 2% CHO, 35 ± 2% fat, and 16 ± 1% protein) did not change due to training. Subjects remained body weight and composition stable during the study despite an increase in energy expenditure (Table 1). Heart rate at rest was lower after training than before and increased from rest to exercise in a RLT-dependent manner (45% Pre < ABT < 65% Pre and RLT, P < 0.05; Table 2). VO2 and VCO2 increased significantly from rest to exercise in an ABT-dependent manner (45% Pre < 65% Pre and ABT < RLT, P < 0.05). Respiratory exchange ratio increased from rest to exercise in all conditions and was lower at the same absolute workload after training (ABT) than at 65% Pre (P <
As a result of the significant 15% increase in relative \( \dot{V}_\text{O}_2 \text{peak} \) with endurance training \((P < 0.05)\), the workload that elicited 66% of \( \dot{V}_\text{O}_2 \text{peak} \) before training \((156 \pm 6 \text{ W})\) elicited only 55% of the new \( \dot{V}_\text{O}_2 \text{peak} \) after training in the ABT trial (Tables 1 and 2). Leg blood flow increased from rest to exercise in all trials and was significantly higher during exercise at 65% Pre, ABT, and RLT than at 45% Pre \((P < 0.05)\).

[FFA]. The plasma concentrations of total and individual FFA tended to be higher during exercise at 45% Pre than all other trials, but these differences never reached statistical significance, and therefore, the data were pooled (Table 3). Consequently, as assessed over the course of a 60-min exercise bout, neither exercise intensity nor endurance training significantly affected total [FFA]. However, plasma stearate, oleate, and total [FFA] decreased significantly from rest to 30 min of exercise \((P < 0.05)\), whereas plasma myristate, palmitate, palmitoleate, oleate, linoleate, linolenate, and total [FFA] increased ~26% between 30 and 60 min of exercise \((P < 0.05)\). Oleate and palmitate were the most abundant FFAs \((41 \text{ and } 28\% \text{ of total FFAs, respectively})\). The percentage of oleate did not change from rest to exercise \((41.4 \pm 0.5 \text{ vs. } 40.7 \pm 0.5\% \text{ of total FFA})\), whereas the percentage of palmitate increased significantly \((27.2 \pm 0.3 \text{ vs. } 28.7 \pm 0.2\% \text{ of total FFA}, P < 0.05)\). Unsaturated FFA (palmitoleate, oleate, linoleate, linolenate, and arachidonate; 62% of total FFA pool) concentration increased ~30% from 30 to 60 min of exercise, whereas the concentration of saturated FFA (myristate, palmitate, and stearate; 38% of total FFA pool) increased only ~22%. Consequently, the ratio of unsaturated to saturated fatty acids increased significantly from rest to the last 30 min of exercise (Table 3).

Net leg FFA fractional extraction and balance. The plasma concentrations of myristate, palmitoleate, linolenate, and arachidonate were too small (together representing 7% of total FFA concentration) to accurately determine [a-v] differences, net leg fractional extraction, or balance. Therefore, only the net leg fractional extraction and balance data of palmitate, oleate, oleate, linoleate, and total FFA are presented (Table 3 and Fig. 1). Net leg plasma palmitate, stearate, oleate, and total FFA fractional extraction increased significantly from rest throughout exercise \((P < 0.05)\) (Table 4). Net leg plasma palmitate, stearate, oleate, and total FFA fractional extraction during exercise at 30 and 45 min were significantly greater than zero and the corresponding values at 60 min of exercise \((P < 0.05)\). Net leg plasma linoleate fractional extraction did not change significantly from rest but decreased significantly at 45 min of exercise to the point that it was significantly different from 60 min of exercise \((P < 0.05)\). Net leg plasma palmitate, oleate, and total FFA balance increased significantly from rest through 45–60 min of exercise \((P < 0.05); \text{Table 4})\). Net leg plasma palmitate, oleate, and total FFA balance during exercise at 45 min were significantly greater than 60 min of exercise, and values from 30 to 60 min of exercise were significantly greater than zero net balance \((P < 0.05)\). Although the net leg balance of plasma stearate and linoleate tended to increase from rest to exercise, they never reached statistical significance.

Plasma lipid and lipoprotein concentrations and net leg balance. All subjects were normolipidemic with TG, total cholesterol, and LDL-C concentrations meeting the ATP III normal/optimal classifications set by the National Cholesterol Education Program (Table 5) (1). Nine weeks of endurance

Fig. 1. Effect of exercise intensity and endurance training on arteriovenous (a-v) differences in total free fatty acid [FFA]: (A), net leg total FFA fractional extraction (B), and net leg total FFA balance (C). Values are means ± SE; \(n = 7\) subjects. *Significantly different from zero at 45% pretraining (Pre); †significantly different from zero at 65% Pre; ‡significantly different from zero at absolute intensity (ABT); §significantly different from zero at relative intensity (RLT). \(P < 0.05\). Main effects: min 30, 45, and 60 > rest; min 45 > min 60; \(P < 0.05\).
training significantly increased plasma HDL-C concentration by ~6%, decreased plasma LDL-C concentration by ~7%, and decreased LDL peak particle size by 0.7% (P < 0.05; Table 5). Only minor changes were noted between rest and exercise in some of the lipoprotein variables, and neither exercise intensity nor endurance training significantly influenced plasma lipid or lipoprotein concentrations during exercise. The net leg balances of plasma TG, HDL-C, and LDL-C did not change from rest to exercise, were not affected by endurance training, and were not different from zero at rest or during exercise.

**Estimation of net leg total FFA balance from net leg palmitate balance.** Studies employing a combination of fatty acid tracer infusion and a-v balance techniques commonly estimate net leg total FFA balance by dividing the measured net leg palmitate balance by the proportion of total [FFA] accounted for by palmitate (46, 47). This estimation assumes that the net leg balances of the remaining individual FFA (72% of total FFA pool) will be in the same direction as palmitate (i.e., negative or positive) with a magnitude that is in direct proportion to their respective concentrations. Data from the present study indicated that relative individual arterial [FFA] accounted for by palmitate (0.28) both at rest (r = 0.9622; P < 0.01) and during exercise (r = 0.8899, P < 0.01; Fig. 3A). The absolute residuals between measured and estimated net leg total FFA balance were smaller at rest than during exercise (Fig. 3B and Table 6). The average residual (estimated minus measured net leg total FFA balance) was +6.3 ± 7.3 μmol/min (+13.9%) at rest and ranged from −14.2 ± 47.8 to −29.4 ± 50.0 μmol/min (−6.8 to −21.3%) during exercise (Table 6). Examination of Cohen’s d values indicated a small effect on peak particle size at rest and during exercise (Cohen’s d = ±0.31 and −0.11 to −0.25, respectively). The residuals were standardized by dividing rest and exercise values by their respective average SD of the residuals to account for the much smaller net leg total FFA balance values at rest compared with exercise (~44 vs. 193 μmol/min; Fig. 3C). These standardized residuals were similar at rest and during exercise.

**DISCUSSION**

As part of our effort to describe the magnitude and adaptability of human lipid metabolism, we employed a longitudinal study design to examine the effects of endurance training on net leg lipid and lipoprotein balances at rest and during moderate-intensity exercise. Despite significant improvements in aerobic capacity and arterial HDL-C and LDL-C concentrations, the net leg balances of individual FFAs, lipoproteins, and TG at rest and during moderate-intensity exercise were unaffected by endurance training. The results led to the following conclusions that will be subsequently discussed. 1) Relative availability is the predominant determinant of individual FFA use by active skeletal muscle, 2) endurance training—induced improvements in the lipoprotein profiles of normolipidemic men are not attributable to changes in HDL-C or LDL-C exchange across active skeletal muscle, and 3) the measurement of net leg palmitate balance provides a reasonably accurate means for estimating net leg total FFA balance at rest and during exercise.

**Table 5. Plasma lipid and lipoprotein parameters at rest and during exercise before and after 9 wk of endurance training**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest Pretraining</th>
<th>Rest Posttraining</th>
<th>45% Pretraining</th>
<th>65% Pretraining</th>
<th>65% Old (ABT)</th>
<th>65% New (RLT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG, mg/dl</td>
<td>82±9</td>
<td>87±9</td>
<td>69±9</td>
<td>83±9</td>
<td>88±11</td>
<td>85±8</td>
</tr>
<tr>
<td>Net leg TG balance, mg/min</td>
<td>-0.9±0.8</td>
<td>-0.8±0.6</td>
<td>-5.2±7.2</td>
<td>-5.8±6.7</td>
<td>-2.3±6.7</td>
<td>-6.9±6.7</td>
</tr>
<tr>
<td>Total C, mg/dl</td>
<td>147±14</td>
<td>146±13</td>
<td>143±13</td>
<td>150±15</td>
<td>151±13</td>
<td>146±15</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>49±5</td>
<td>52±5</td>
<td>47±5</td>
<td>49±5</td>
<td>54±5</td>
<td>52±5</td>
</tr>
<tr>
<td>Net leg HDL-C balance, mg/min</td>
<td>-0.4±0.3</td>
<td>0.9±0.5</td>
<td>-3.6±3.1</td>
<td>-5.2±2.9</td>
<td>2.4±2.9</td>
<td>-1.5±2.9</td>
</tr>
<tr>
<td>apoA-I, mg/dl</td>
<td>110±8</td>
<td>113±7</td>
<td>105±8</td>
<td>110±7</td>
<td>118±6</td>
<td>116±8*</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>82±9</td>
<td>76±9</td>
<td>82±9</td>
<td>85±10</td>
<td>80±9*</td>
<td>76±13</td>
</tr>
<tr>
<td>Net leg LDL-C balance, mg/min</td>
<td>-0.2±0.8</td>
<td>-1.1±1.2</td>
<td>5.1±6.8</td>
<td>-8.7±6.4</td>
<td>-2.8±6.4</td>
<td>-10.6±6.4</td>
</tr>
<tr>
<td>apoB, mg/dl</td>
<td>63±5</td>
<td>62±5</td>
<td>61±5*</td>
<td>64±5</td>
<td>62±5</td>
<td>60±6</td>
</tr>
<tr>
<td>LDL peak particle size, nm</td>
<td>27.5±0.2</td>
<td>27.3±0.1</td>
<td>27.4±0.1</td>
<td>27.5±0.2</td>
<td>27.3±0.1</td>
<td>27.4±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. TG, triglyceride; C, cholesterol; HDL-C, HDL cholesterol; apoA-I, apolipoprotein A-I; LDL-C, LDL cholesterol; apoB, apolipoprotein B. *Significantly different from corresponding rest; †significantly different from rest pretraining. P < 0.05.
Dietary controls. Individuals with odd dietary habits were excluded from the subject pool, and participants were coun-
sed on appropriate dietary habits. Evening and morning
meals were standardized and provided in the metabolic unit
before trials. Three-day food records were kept before and after
training, and subjects were weight stable across the training
period. As already noted, analysis of dietary macronutrient
composition indicated no change during training. Surprisingly,
dietary energy intake did not rise with training, so subjects
either compensated by altering discretionary physical activity
or they underreported food consumption.

Dynamics of individual FFA availability, net leg fractional
extraction, and balance. The percent contribution of each
individual FFA to the total concentration at rest agrees well
with results of other studies on both men (13, 17, 19, 30, 32)
and women (14, 15, 21, 33). Additionally, the percent contri-
bution of each individual FFA to the total concentration during
exercise agrees well with a previous study in our laboratory
(21) of women exercising at the same relative intensities for the
same duration. The absolute concentrations of total and indi-
vidual FFA at rest are two- to threefold higher than other
reports of 3- to 4-h postprandial men (32) and women (21) and
agree more closely with those of overnight-fasted subjects (13,
15, 19, 30, 33) and 3- to 4-h postprandial men studied in our
laboratory following nearly identical femoral catheterization
procedures (5). These results are likely explained by an eleva-
tion in the rate of lipolysis due to the psychological stress
experienced by the subjects during the femoral catheterization
performed 75–90 min prior to the blood draws. In support of
this contention, standardized 20–30 min mental stress proto-
cols have been shown to result in pronounced increases in FFA
and glycerol concentrations (18, 31, 49) that are similar in
magnitude to those seen during moderate-intensity exercise
(31).

Net leg FFA fractional extraction was not affected by exer-
cise intensity or endurance training and averaged 7.6% across
all trials and time points (Table 4 and Fig. 1B) and is similar to
previously reported values calculated without isotope tracers
during one-legged knee extension (6–7%) (23) and two-legged
cycle ergometry (6.0–6.4%) (5). Hagenfeldt and Wahren (16)
performed the first study on individual FFA net limb fractional
extraction and balance and determined that the exercising
forearm has a slight preference for the uptake of the unsatur-
ated fatty acids oleate and linoleate over that of the saturated
fatty acid palmitate. However, in a subsequent investigation in
the same laboratory, no differences in individual FFA net
forearm fractional extraction were found during exercise (17).

The present study is the first to evaluate the influence of
relative individual FFA availability on uptake across large
muscle groups responsible for the majority of energy flux
during exercise. It appears that the unsaturated fatty acids
oleate and linoleate are above the line of best fit, whereas the
saturated fatty acid palmitate is below the line of best fit (Fig.
2) supporting the initial results of Hagenfeldt and Wahren (16).
However, regardless of the effect of saturation status, it is clear
that relative availability is the predominant determinant of
individual FFA use by active skeletal muscle. These observa-
ations are consistent with the interpretation that FFA disposal in
active muscle is limited by mitochondrial translocation (42),
but that for a given rate of muscle FFA uptake, sarcolemmal
fatty acid translocators do not discriminate among FFA moi-

Fig. 3. A: relationship between measured and estimated net leg total FFA
balance at rest and during exercise. B: absolute difference (estimated minus
measured) at rest and during exercise. C: standardized difference (absolute
difference divided by either average rest or exercise SD of the absolute
difference) at rest and during exercise.
et al. (10, 35) have reported that endurance training increases net leg total FFA uptake during 2–3 h of one-legged knee extension exercise in healthy young men. In the present study, we did not determine net leg total FFA balance during the first 30 min of exercise in the previous study. In the present study, the first 30 min of exercise, but values for net leg total FFA uptake were similar to those in our previous report at 45 and 60 min of exercise.

In comparing our results with those of others, the absence of a training effect in active net leg total FFA balance during exercise was also observed in a cross-sectional report comparing endurance-trained and sedentary subjects (22). Others have reported that endurance training increases net leg total FFA uptake during 2–3 h of one-legged knee extension exercise in the 9- to 12-h-fasted state using both cross-sectional (45) and longitudinal research designs (23). The disparity in the present findings with those of previous studies is likely explained by differences in nutritional status (3–4 h postprandial vs. 9–12 h fasted), exercise modality (two-legged cycle ergometry vs. one-legged knee extension), exercise duration (60 vs. 120–180 min), absolute power output (156–193 vs. 23–34 W), relative exercise intensity (65 vs. ~15% whole body \( V_{O2peak} \)), and magnitude of autonomic stimulation. In combination, results of our two studies as well as the results of others lead to the conclusion that the training effect on active skeletal muscle net total FFA uptake is small and easily overridden by recent CHO nutrition, high muscle power output, and other factors favoring CHO utilization. However, the results obtained as a consequence of the present investigation do not preclude a role of skeletal muscle for lipid oxidation during recovery from exercise (27).

Role of skeletal muscle in endurance training-induced improvements in the lipoprotein profile. We chose to test our subjects in the 3- to 4-h postprandial state to mimic nonlaboratory conditions despite the ATP III recommendation to use blood samples from overnight-fasted subjects for lipoprotein analyses (1). It has recently been reported (43) that concentrations of total cholesterol, LDL-C, and HDL-C 4 h after a large, high-fat meal (880 kcal, 57% fat) were only 2–5% lower compared with a 12-h fast. Therefore, we feel confident that the standardized pretrial low-fat meals (434 kcal, 10% fat) fed to our subjects had little influence on our lipoprotein data or our conclusions that endurance training significantly decreased LDL-C and increased HDL-C concentrations (Table 5).

Recent meta-analyses have revealed that endurance exercise training is most commonly associated with moderate (4.6%), but inconsistent (range of −5.8 to +25%), increases in HDL-C and little or no change in either total or LDL-C (8, 28). The variability of the HDL-C response may be due to methodological differences, such as exercise intensity and duration (26) as well as various biological, behavioral, and lifestyle characteristics (28). Regardless, the 6% increase reported in the present study agrees well with other training interventions on healthy, sedentary males (25). Modest increases in HDL-C with endurance training are also likely due to a shift in HDL subspecies characterized by an increase in HDL2 and a decrease in HDL3 in both normal-weight (37) and obese subjects (7). The importance of this exercise-induced shift in HDL subspecies is highlighted by the fact that HDL2 is the final cholesterol carrier in reverse cholesterol transport and, therefore, plays a pivotal role in reducing cardiovascular disease risk.

Although the mechanisms for endurance training-induced increases in concentrations of total HDL-C and HDL2-C are unclear, cross-sectional studies of active and sedentary males and females (36) have found that HDL-C concentration is highly correlated to the activity of both adipose tissue and skeletal muscle lipoprotein lipase (LPL). Patsch et al. (38) first described the potential mechanism for the shift in HDL subspecies with in vitro evidence that hydrolysis of VLDL-TG by LPL resulted in the transfer of protein, phospholipids, and cholesterol from VLDL to HDL3, leading to its transformation into HDL2. Taken together, these results highlight the potential important role of peripheral tissue, such as skeletal muscle in endurance training-induced improvements in the plasma lipoprotein profile. In the only study to date to examine net leg lipoprotein balance, Kiens and Lithell (25) demonstrated that 8 wk of one-legged knee extension training resulted in significa-

### Table 6. Error in estimation of net leg total FFA balance at rest and during exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest</th>
<th>Exercise</th>
<th>Exercise Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated net leg total FFA balance</td>
<td>−39.2 ± 26.0</td>
<td>164.6 ± 97.1</td>
<td>30</td>
</tr>
<tr>
<td>Measured net leg total FFA balance</td>
<td>−45.5 ± 26.8</td>
<td>187.5 ± 91.3</td>
<td>45</td>
</tr>
<tr>
<td>Residual</td>
<td>6.3 ± 7.3</td>
<td>−22.9 ± 44.5</td>
<td>60</td>
</tr>
<tr>
<td>%Difference</td>
<td>13.9</td>
<td>−12.2</td>
<td></td>
</tr>
<tr>
<td>Cohen’s d scores</td>
<td>0.31</td>
<td>−0.18</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE in μmol/min for estimated and measured net leg total FFA balances as well as residuals. For the difference between estimated and measured net leg total FFA balance, \( n = 8 \). Residual, estimated − measured; %difference, [average residual/average measured net leg total FFA balance] × 100; Cohen’s d scores, average residual/SD.
ificantly higher rates of net VLDL-TG uptake at rest and net HDL₂-C release at rest and after 110 min of exercise in the trained vs. untrained leg. The higher VLDL-TG uptake in the trained leg was associated with a 70% higher muscle LPL activity and greater capillary density than the untrained leg, thereby increasing the capacity and surface area for TG hydrolysis, respectively. However, the applicability of the results is limited due to the use of a one-legged training model in which the hormonal environment is not greatly altered and power output is low compared with two-legged exercise. Additionally, the importance of skeletal muscle as a site for VLDL-TG output is low compared with two-legged exercise. Taken together, the results of the present study and those of others (25) can be interpreted to indicate that endurance training-induced adaptations in skeletal muscle are responsible in part for the migration in the HDL subfractions from HDL₃ to HDL₂ without any great net changes in total HDL-C production across the limb. The impact of endurance training on lipoprotein balance across other extrahepatic tissues, such as adipose and cardiac tissue during exercise and postexercise recovery (27), warrants investigation.

Adequacy of the estimation of net leg total FFA balance from net leg palmitate balance. The study of limb fatty acid kinetics is complicated by the fact that fatty acids undergo simultaneous uptake and release. This leads to an underestimation of leg total FFA uptake in studies relying solely on limb a-v balance techniques. More recently, this methodological shortcoming has been overcome with the combination of fatty acid tracer infusion and a-v balance techniques allowing for the measurement of tracer-measured leg FFA uptake and release. A potential shortcoming of this approach, however, is that it relies on the estimation of net leg total FFA balance, uptake, and release by dividing the measured net leg balance, uptake, and release of an individual FFA (most commonly palmitate) by the proportion of total FFA concentration accounted for by the measured individual FFA (46, 47). This estimation assumes that the flux of the remaining individual FFAs (72% of total FFA pool), the error in the estimation was not improved, resulting in Cohen’s d scores of 0.29 and 0.16 at rest and during exercise, respectively. The error involved in the estimation of tracer-measured leg total FFA uptake and release from palmitate data could be examined with the infusion of multiple isotopically labeled fatty acids. However, the results of the present study can likely be extended to assume that the error involved in the estimation of tracer-measured leg total FFA uptake and release from leg palmitate uptake and release is also small.

In conclusion, endurance training favorably affects blood lipoprotein profiles even in young, healthy normolipidemic men, but muscle contractions per se have little effect on net leg LDL-C, or TG uptake or HDL-C release during moderate-intensity cycling exercise. Therefore, the favorable effects of physical activity on the lipid profiles of young, healthy normolipidemic men in the postprandial state are not attributable to changes in HDL-C or LDL-C exchange across active skeletal muscle.

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