Contributions of working muscle to whole body lipid metabolism are altered by exercise intensity and training

Anne L. Friedlander,1 Kevin A. Jacobs,2 Jill A. Fattor,2 Michael A. Horning,2 Todd A. Hagobian,1 Timothy A. Bauer,3 Eugene E. Wolfel,3 and George A. Brooks2

1Exercise Physiology Lab, Clinical Studies Unit, Veterans Affairs Palo Alto Health Care System, Palo Alto; 2Exercise Physiology Laboratory, Department of Integrative Biology, University of California Berkeley, Berkeley, California; and 3University of Colorado, Health Science Center, Division of Cardiology, Denver, Colorado

Submitted 29 March 2006; accepted in final form 21 July 2006

Whole body fat metabolism during exercise has been well characterized. Compared with rest, fat use increases during mild exertion but decreases at higher exercise intensities. Factors such as dietary energy balance, gender, exercise duration, and training status modulate the overall metabolic and substrate partitioning responses set by energy flux (6, 13, 16, 25, 35). Because of the high percentage of whole body energy expenditure accounted for by the working muscles during exercise, changes in fat use measured at the whole body level have generally been attributed to alterations in substrate kinetics occurring in the working muscle beds. However, because of the invasive nature of procedures, few data exist to confirm that whole body measurements of lipid metabolism can be used as a surrogate for working limb muscle measurements. Interestingly, the available data do not support a major role for working muscle in lipid metabolism. For instance, Roepstorff et al. (36) demonstrated that, during cycling exercise at 58% of peak oxygen consumption (VO2peak) in fasted, trained subjects, only ~35% of whole body free fatty acid (FFA) uptake was accounted for by the working limbs. Equivalent or smaller contributions of working muscle to whole body lipid kinetics have also been reported during cycling exercise in untrained subjects (8) or when smaller amounts of muscle mass are utilized (45). Because of the relatively small contribution of working muscle to whole body lipid metabolism, it is unknown whether the whole body changes observed in response to increasing intensity and/or training interventions would be fully accounted for by the exercising limbs. In this regard, using arteriovenous gas and metabolite concentration (a-v) differences, Bergman et al. (3) demonstrated that 9 wk of endurance training did not change working leg fat oxidation or net FFA balance despite significant increases in muscle respiratory capacity (11) and increases in whole body fat oxidation at the same absolute workload following training. A similar disconnect was observed when exercise intensity was increased from 45 to 65% of VO2peak, resulting in a significant decrease in whole body fat oxidation but no change in leg fat oxidation (3). In contrast, Kiens et al. (28) showed that 8 wk of one-leg training could increase net FFA uptake and lipid oxidation during 2 h of leg-kicking exercise when the same power output was used for the trained and untrained legs. As well, using stable isotopes, Turcotte et al. (44) demonstrated greater FFA uptake and oxidation in the active muscles of trained vs. untrained men during prolonged one-leg kicking exercise at 60% of maximal extension strength. However, it is understood that cross-sectional studies comparing trained athletes to sedentary subjects do not isolate training effects from genetic predisposition. To our knowledge, no investigations have used the combination of a longitudinal study design, [a-v] difference measurements, and isotope techniques to measure changes in lipid kinetics across working muscle beds in response to different exercise intensities before and after an endurance training program.

Therefore, to evaluate the contribution of working muscle to whole body lipid oxidation, we used stable isotopes and [a-v]...
differences to determine whole body and working muscle FFA disposal rates in untrained young men at two exercise intensities pretraining and then again at the same absolute and relative exercise intensities following 9 wk of endurance training. We hypothesized that lipid use would be inversely related to energy flux both before and after training in the working legs as well as at the whole body level. We also hypothesized that the active muscles would display greater carbohydrate dependence than the whole body regardless of intensity or training state.

METHODS

This paper is part of a larger study investigating different aspects of substrate metabolism during rest and exercise. Therefore, some of the data related to subject and workload characteristics have been, and will be, presented elsewhere. All data that are presented elsewhere is so noted in the tables.

Subjects. Eight healthy, nonsmoking, sedentary male subjects between the ages of 18 and 35 were recruited from the University of California campus through flyers and mailings. Subjects were considered untrained if they had participated in < 2 h of regular strenuous activity per week during the previous year and if they had a VO₂ peak between 35 and 50 ml · kg⁻¹ · min⁻¹ as determined by a continuous-progressive peak stress test on a leg cycle ergometer. To qualify for participation in the study, subjects were required to be diet and weight stable, to have a body fat percentage of < 25%, and to be disease and injury free as determined by medical questionnaire and physical examination. Subjects provided informed consent, and the protocol was approved by the Stanford University and University of California Committee for Protection of Human Subjects.

General experimental design. After an initial interview and screening tests, two stable isotope infusion trials were performed on a leg cycle ergometer for 1 h at 45 and 65% of VO₂ peak (hereafter referred to as 45UT and 65UT, respectively). The order of trials was randomized, and trials were performed exactly 1 wk apart. Subjects began training 2 days after their second isotope trial and continued for 9 wk. After training, anthropometric measurements and VO₂ peak tests were repeated and two more isotope trials performed, one at the same absolute workload (ABT) that elicited 65% of pretraining VO₂ peak, and the second at a workload that elicited 65% of the new posttraining VO₂ peak, the same relative exercise intensity (RLT). The two posttraining trials were 1 wk apart and randomized, and training was continued between the two trials. Isotope trials were performed following 36–48 h of inactivity and identical food consumption.

Screening tests. Body composition was determined by seven site skinfold measurements (22). VO₂ peak was determined to be the highest 30-s value obtained on subjects exercising on an electronically braked cycle ergometer (Monark Ergometric 839E; Monark, Vansbоро, Sweden) during a continual, progressive protocol that increased external power output 25 or 50 watts every 3 min until voluntary cessation. Respiratory gases were analyzed (TrueMax Metabolic cart; ParvoMedics, Sandy, UT) and recorded every 30 s by an online, real-time, PC-based system. Subjects underwent two VO₂ peak tests prior to commencement of the study, and the tests were evaluated on maximal heart rate, respiratory exchange ratio (RER) values (> 1.15), and VO₂ uniformity to assure a true maximum effort both before and after training. Three-day dietary records were kept at the beginning, 5 wk, and 10 wk into training to monitor the subjects’ dietary composition and caloric intake. Subjects were asked to keep their dietary composition the same throughout the study but to increase caloric intake to match new levels of energy expenditure. Analysis of food records was performed using the Nutritionist III program (N-Squared Computing, Salem, OR).

Tracer protocols. Subjects arrived at the Clinical Studies Unit at the Veterans Affairs (VA) Palo Alto Health Care System by 8 PM the night before isotope trials, having performed no exercise that day. Subjects were fed a standardized dinner [1,180 kcal, 10% protein, 69% carbohydrate (CHO), 21% fat] and were required to be in bed by 11 PM. Isotope trials were performed the following morning or afternoon (consistent between trials) in the postabsorptive state. Morning subjects were given a pretial meal (CHO set at 1.1 g/kg body wt; average meal = 434 kcal, 16% protein, 74% CHO, 10% fat) to consume at 5 AM, 3 h before the start of the rest study period. Afternoon subjects were given a standardized breakfast at 5 AM (661 kcal, 11% protein, 55% CHO, 34% fat) and then the same pretest meal as above at 11 AM, 3 h prior to the start of rest. Thus, we report data on resting subjects 4 h after a low-glycemic-index meal was taken, and we report data on the same exercising subjects 5 h after the meal was taken. We chose to test our subjects in a fed, postabsorptive state so that the results would be typical of exercise performed in a nonlaboratory environment. As well, we fed subjects, because our previous experience with untrained men exercising at 65% maximal oxygen consumption (VO₂ peak) when fasted for 12 h indicated falling blood glucose concentration and counterregulatory endocrine responses after 30 min of leg cycling exercise (41).

An antecubital venous catheter was placed in one arm for the infusion of tracers. Catheters were also placed in the femoral artery and vein to obtain samples across the working limb (see details below). After the collection of background blood and expired air samples, subjects rested semisupine for 90 min while 1,3-Cpalmitate tracers were infused continuously (Gemini PC-1 infusion pump; Immed, San Diego, CA). The resting infusion rate was set at 0.66 mg/min for palmitate, and upon initiation of exercise the palmitate infusion rate was doubled. Isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA) and prepared by the University of California at San Francisco School of Pharmacy. Tracer palmitate was combined with 100 ml of 25% human albumin and suspended in 0.9% saline. The palmitate tracer cocktail was tested for sterility and pyrogenicity, and all palmitate/albumin infuses were used within 16 days after completion of sterility testing. Glycerol tracer ([1,1,2,3,3-2H]glycerol) was also infused, and those results will be reported separately.

Anaerobic arterial and venous blood samples were drawn simultaneously and analyzed serially in duplicate at 0, 75, and 90 min of rest and at 30, 45, and 60 min of exercise. Blood temperature was obtained from a thermistor at the end of the venous thermodilution catheter immediately prior to blood sampling. BA Radiometer U-ABLS (Copenhagen, Denmark) was used for blood gas determinations, commencing with arterial samples (generally within 15 s of blood draw completion). Venous samples were debubbled, capped, iced, and analyzed following the arterial samples (typically within 2 min of blood withdrawal). Based upon results of Beaulieu et al. (1), the blood samples we obtained were analyzed well within the established period of sample stability. As well, steady-state exercise conditions minimized impact of transit time differences between limb O₂ uptake and CO₂ release. Direct measurements of hemoglobin, oxygen saturation, and oxygen content were also performed on the same blood samples (U-O3M Hemoximeter; Radiometer, Copenhagen, Denmark). At each of the blood sampling time points, respiratory gas exchange was determined using the caloriometry system described above, and a sample of expired air was collected in a 10-ml vacuum Exetainer tube to determine 13CO₂ isotopic enrichment. The expired air samples were stored at room temperature until they were analyzed using isotope ratio mass spectrometry by Metabolic Solutions (Nashua, NH).

Catheterizations. After local anesthesia with Lidocaine, the femoral artery and vein of the same leg were cannulated using standard percutaneous techniques, as previously described (48), 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 451–501 V5; Cordis, Miami, FL) was inserted 25 cm and positioned in the distal abdominal aorta via the femoral artery. A 6-French thermomediation, nonballoon venous catheter (model F06TN0011; 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 was used for all femoral venous blood sampling as well as for cold saline injection for thermomediation blood flow measurements, was positioned about 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 pre- and posttraining.

One subject experienced pain in the groin related to the catheter placement when he was positioned on the cycle ergometer for exercise, so that trial 45 UT was discontinued and the catheters were removed. Thus results presented for the exercise portion of the 45 UT trial include data from only seven subjects. All catheterizations were performed by a cardiologist experienced in the procedures employed.

**Hemodynamics.** Leg venous blood flow was determined by thermomediation technique, using a cardiac output computer (Model 9520; American Edwards Laboratories) with a 10-cc bolus injection of sterile saline cooled to 0°C via an ice slurry (Co-Set II, model 93600; American Edwards Laboratory). Measurements were made 3–4 times during rest and exercise immediately after blood sampling and averaged for estimation of limb blood flow. The validity and precautions associated with this measurement technique have been described previously (2). Heart rate and ECG were continuously recorded and displayed, using a 3-lead ECG connected to a MacLab A/D converter (AD Instruments, Castle Hill, Australia), and tracked on a Macintosh 7200/200 Power Mac computer (Apple Computer, Cupertino, CA). Arterial blood pressure was also continuously recorded and displayed using a Transpac pressure transducer (Baxter) positioned at the level of the heart connected to the MacLab system and calibrated prior to every trial.

**Training protocol.** Subjects were required to exercise with a personal trainer in the University of California Berkeley Exercise Physiology Laboratory 5 days/wk for 1 h each day on the cycle ergometer, with interval training sessions added (2 days/wk) after 6 wk of training. In addition to the supervised training, subjects were required to exercise for 1 additional hour on the weekend in any manner they desired. During the first 3 wk of training, exercise intensity was gradually increased from 50 to 75% of each participant’s VO2 peak. Subjects were asked to warm up for 5 min and stretch prior to their 1 h of exercise. Personal trainers used heart rate monitors and data from periodic evaluations of VO2 peak to adjust workloads as the subjects improved. Throughout training, subjects were weighed daily and to assure weight and body fat stability.

**Blood sample collection and analysis.** Blood samples drawn simultaneously from arterial and venous catheters were immediately placed on ice, centrifuged within 30 min of collection for 10 min at 2,500 g, decanted, and frozen. Plasma for FFA concentration determinations was collected in EDTA-treated tubes. Insulin, glucagon, and cortisol analyses were performed by radioimmunoassays (Instar, Stillwater, MN, and Diagnostic Laboratories, Los Angeles, CA) on arterial plasma samples that were collected with aprotinin and frozen at −80°C until analysis. Plasma for catecholamine analyses was collected with glutathione and EDTA and stored at −80°C until analyses by HPLC, as previously described (12).

Palmitate isotopic enrichments were measured by mixing 1 ml aliquot of plasma with a solution of heptane-isopropanol (30/70) containing H2SO4 (0.0033 M) that contained 100 nmol of pentadecanoic acid as an internal standard. The solution was stored frozen for subsequent thin-layer chromatography (TLC) separation of blood lipids. After isolation by TLC, FFAs were derivatized to fatty acid methyl esters to allow volatility by gas chromatography (GC). The gas chromatograph was equipped to detect simultaneously both the individual FFA concentrations by flame ionization detector (FID) and isotopic enrichment of palmitate by mass spectrometry (MS; GC model 6890 Series II and MS model 5989A; Hewlett-Packard).

**Whole body palmitate and FFA flux calculations.** Palmitate rates of appearance (Ra) and disappearance (Rd) were calculated using equations defined by Steele and modified for use with stable isotopes (47). Detailed calculations have been presented previously (15).

On the basis of a previous study in our laboratory (42) and modified equations of Sidossis et al. (40) accounting for energy expenditure adjusted for lean mass, the bicarbonate/acetate correction factor (k) was set at 0.5 for rest and 0.90, 0.96, 0.95, and 0.97 for 45 UT, 65 UT, ABT, and RLT intensities, respectively. The impact of the difference in k between 0.95 and 0.97 on plasma oxidation was negligible.

For both whole body and leg, FFA kinetics and oxidation were calculated by dividing the value for palmitate kinetics by the fraction of plasma palmitate concentration to total plasma FFA concentration, as determined by GC-FID. Rates of total FFA oxidation were calculated using the pulmonary respiratory exchange ratio and VO2 (assuming 4.7 kcal/l O2, 9 kcal/g triglyceride, 3 mol of FFA per mole of triglyceride, and an average of 860 g/mol for triglyceride) (14). Percentage of oxidative energy from lipid was calculated from the pulmonary RER. At rest, nitrogen balance was assumed, so VO2 was corrected to account for nitrogen excretion equivalent to average intake (12% of long-term dietary energy intake). During exercise, the contribution of protein oxidation was considered to be negligible relative to total energy expenditure, so a nonprotein respiratory quotient (RQ) was assumed to be equal to RER.

**Leg respiratory gas exchange.** Leg RQ was calculated from the ratio of venous-arterial CO2 difference ([v-a] CO2) and arterial-venous O2 difference ([a-v] O2). Blood PCO2, PO2, pH, and Hb were measured on both arterial and venous samples and used in the calculations by Douglas et al. (10) for determination of blood CO2 content. CO2 solubility and apparent dissociation constant were estimated from the equations of Kelman (27) as described previously (3). Blood O2 contents were directly measured using a hemoximeter for both arterial and venous samples. Where RQ was <0.71 or exceeded 1.0, a theoretical limit was assumed. Leg VO2 was calculated using the Fick equation as follows:

$$ \text{leg VO}_2 = 2 \cdot \text{one-leg blood flow} \cdot [\text{a-v}]\text{O}_2 $$

**Leg palmitate kinetics.** For palmitate calculations, flux values were based on plasma flow (PF), which was calculated as blood flow (1 − Hct/100):

$$ \text{net leg balance (\mu mol/min)} = [\text{C}_2\text{C} - \text{C}_2\text{C}(\text{Hct}/\text{Hctv})] \cdot 2 \cdot \text{one-leg PF} $$

$$ \text{fractional extraction (\%)} = \frac{[\text{IE}_a - \text{IE}_v - \text{IE}_c(\text{Hct}/\text{Hctv})]}{\text{IE}_a} \cdot 100 $$

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$$ \text{fractional extraction (\%)} = \frac{[\text{IE}_a - \text{IE}_v - \text{IE}_c(\text{Hct}/\text{Hctv})]}{\text{IE}_a} \cdot 100 $$

$$ \text{leg uptake (\mu mol/min)} = \text{fractional extraction} \cdot \text{C}_2 \cdot 2 \cdot \text{one-leg PF} $$

$$ \text{leg release (\mu mol/min)} = \text{leg uptake} - \text{net balance} $$

where C2 and Cc are the concentrations of palmitate in arterial and venous blood, respectively, and IEa and IEv are the arterial and venous enrichments of palmitate. Venous concentrations are corrected for change in hematocrit (Hct) across the working limb. FFA kinetics were calculated by dividing the value for palmitate kinetics by the fraction of plasma palmitate concentration to total plasma FFA concentration as determined by GC-FID (Table 2).

**Statistics.** Data are represented as means ± SE. Calculations of FFA kinetics were made using the last two (75, 90 min) and three (30,
45, 60 min) isotopic enrichment measurements obtained during rest and exercise, respectively. To assess significance of differences in metabolite concentration and flux rates among the four isotope trials, one-way and two-way (test × time) ANOVA with repeated measures were used, and, where appropriate, Fisher least significant difference tests were used for post hoc analyses. Student’s t-tests were used to determine differences in subject characteristics before and after training. Because one subject did not undergo the exercise portion of the 45% pretraining trial (see Catherizations), comparisons made with the 45UT trial use only seven subjects. Values presented for, and comparisons among, the three 65% trials (65UT, ABT, RLT) include the full complement of eight subjects. Statistical significance was set at α = 0.05.

**RESULTS**

**Subject and workload characteristics.** Subject characteristics obtained before and after 9 wk of endurance training are listed in Table 1. VO₂peak (ml·kg⁻¹·min⁻¹) increased over the course of training by 15%. Subjects remained body weight stable and did not experience a change in body fat. Workload and energy expenditure characteristics are listed in Table 2. Because of the training-induced changes in VO₂peak, work performed during the ABT trial (same absolute workload posttraining) represented 55% of posttraining VO₂peak.

**Whole body lipid kinetics.** Whole body FFA flux and oxidation values are presented in Table 3. Arterial FFA concentration did not change between rest and exercise and was not different during the last 30 min of exercise between any of the testing conditions. Palmitate was ~28% of total FFA concentration during rest and exercise both before and after training. FFA R₇ and R₈ were not significantly different from each other for all conditions (only R₇ is listed in Table 3) but increased between rest and exercise (P < 0.05). Pulmonary RER was higher during exercise than during rest both before and after training. Training also reduced RER during exercise in ABT, compared with 65UT (P < 0.05), but not during RLT. Total whole body fat oxidation increased between rest and exercise and tended to be elevated postraining for both ABT and RLT compared with the 65UT workload (~1,100 vs. 792 μmol/min, P = 0.065). Plasma FFA oxidation followed a pattern that was workload dependent, but the only significant difference between exercise conditions was between 45UT and RLT (Table 3). Percentage of R₇ oxidized was ~20% at rest but increased to 64% during the 45UT trial. For all three of the higher-intensity trials, percentage of R₇ oxidized was ≥90% (Table 3).

**Two-leg blood flow and energy expenditure.** Leg blood flow (LBF) increased more than 5 times between rest and exercise at 45UT (Table 4). Before training, LBF was also 40% greater (P < 0.05) at the higher intensity (65%) compared with 45% pretraining. After endurance training, LBF remained constant at ABT but tended to increase at RLT (11%, P = 0.06). As expected, leg VO₂ paralleled whole body VO₂ during exercise (Fig. 1), and represented a smaller portion of the total oxygen consumption at rest (~20%) than during exercise (~67%).

**Two-leg FFA kinetics.** FFA net balance was negative (indicating release) at rest (~−36 μmol/l) but then increased significantly to net uptake for all exercise conditions (45UT 179 ± 75, ABT 236 ± 63, RLT 136 ± 110) except 65UT (52 ± 127). Percent extraction of FFA across the working legs was ~45% at rest and was lower (~20%) during exercise at all intensities (Fig. 2). Tracer-measured FFA uptake (TMU) increased between rest and exercise, but there was no pretraining intensity effect (Fig. 2A). TMU showed a significant training effect when measured at the same relative workload (716 ± 173 μmol/min vs. 461 ± 72 for RLT vs. 65UT, respectively, P < 0.05) but only tended to increase at the same absolute workload (ABT 643 ± 137). Tracer-measured FFA release demonstrated a workload-dependent pattern, but the only significant increase above resting values (and 45UT) was for RLT (Fig. 3B).

**Two-leg lipid oxidation.** Leg RQ was higher during exercise than during rest but did not differ significantly between exercise conditions (Table 4). RQ demonstrated a similar pattern as RER but tended to be elevated in the leg compared with whole body for each exercise intensity. Total leg lipid oxidation (R₉ox), calculated from leg RQ and leg oxygen consumption, demonstrated an intensity effect pretraining since 45UT, but not 65UT, was significantly elevated above resting values (Table 4). In addition, training resulted in a significant increase

Table 1. Subject characteristics before and after 9 wk of endurance training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26±1</td>
<td>27±1</td>
<td>4%</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.6±2.0</td>
<td>180±2.0</td>
<td>0.5%</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77.4±3.7</td>
<td>78.7±3.6</td>
<td>1.7%</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>11.9±1.6</td>
<td>11.7±1.7</td>
<td>1.7%</td>
</tr>
<tr>
<td>Fat free mass, kg</td>
<td>67.8±2.2</td>
<td>69.0±2.5</td>
<td>1.8%</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>9.4±1.8</td>
<td>9.4±1.5</td>
<td>0.0%</td>
</tr>
<tr>
<td>VO₂peak, ml·kg⁻¹·min⁻¹</td>
<td>45.2±1.2</td>
<td>52.0±1.8*</td>
<td>14.8%</td>
</tr>
<tr>
<td>VO₂peak, l/min</td>
<td>3.5±0.2</td>
<td>4.1±0.2*</td>
<td>17.1%</td>
</tr>
<tr>
<td>Maximal heart rate, bpm</td>
<td>194±9</td>
<td>193±9</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Values are means ± SE. VO₂ peak oxygen consumption. All data were previously reported (24). *Significantly different from pretraining values (P < 0.05).

Table 2. Whole body gas exchange and work characteristics during rest and exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Untrained</th>
<th>Trained</th>
<th>45UT</th>
<th>65UT</th>
<th>ABT</th>
<th>RLT</th>
</tr>
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<tbody>
<tr>
<td>VO₂, l/min</td>
<td>0.32±0.01</td>
<td>0.32±0.01</td>
<td>1.56±0.06</td>
<td>2.32±0.11</td>
<td>2.24±0.08</td>
<td>2.70±0.11</td>
</tr>
<tr>
<td>Workload, W</td>
<td>0±0</td>
<td>0±0</td>
<td>99±4</td>
<td>156±6†</td>
<td>156±6†</td>
<td>193±10†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>61±2</td>
<td>61±2</td>
<td>125±3</td>
<td>157±3†</td>
<td>143±3§</td>
<td>157±3§</td>
</tr>
<tr>
<td>Total EE, kcal/min</td>
<td>1.6±0.1</td>
<td>1.5±0.1</td>
<td>6.8±1.0</td>
<td>11.6±0.5</td>
<td>11.1±0.4</td>
<td>13.4±0.5§</td>
</tr>
</tbody>
</table>

Values are means ± SE. 45UT, 45% of VO₂ peak before training. EE, energy expenditure; 65UT, 65% of VO₂ peak before training; ABT, 65% of pretraining VO₂ peak (same absolute workload as pretraining); RLT, 65% of posttraining VO₂ peak (same relative workload). *All values significantly different between rest and exercise at P < 0.05; †significantly different from 45UT at P < 0.05; ‡significantly different from 65UT at P < 0.05; §significantly different from ABT at P < 0.05; ¶significantly different from untrained rest at P < 0.05. Note: VO₂, workload, and heart rate data have been previously reported (24).
in total leg fat oxidation at the same ABT relative to 65UT (P < 0.05). An intensity effect was also evident posttraining, with RLox in ABT higher than RLT (P < 0.05).

Leg vs. whole body lipid metabolism. Energy expenditure of the two working legs increased from ~20% at rest to between 62 and 69% of whole body expenditure during exercise (Table 5). Leg FFA TMU accounted for ~37% of whole body RLox at rest. Pretraining there was an intensity effect, since a larger percentage of whole body uptake was directed to the leg during exercise than was the body as a whole. At the lower work intensities (45UT and ABT) a substantial portion (~62%) of whole body lipid oxidation could be accounted for by leg fat oxidation, but at higher relative intensities (65UT and RLT) the leg contribution dropped to ~30% of whole body lipid oxidation. Therefore, caution is warranted when using whole body measures of lipid metabolism as a surrogate for changes in working muscle, especially at higher exercise intensities.

DISCUSSION

The main finding of this investigation was that [a-v] and tracer-derived measures of leg muscle lipid metabolism paralleled whole body patterns based on energy flux, but working muscle tended to be more carbohydrate dependent during exercise than was the body as a whole. At the lower work intensities (45UT and ABT) a substantial portion (~62%) of whole body lipid oxidation could be accounted for by leg fat oxidation, but at higher relative intensities (65UT and RLT) the leg contribution dropped to ~30% of whole body lipid oxidation. Therefore, caution is warranted when using whole body measures of lipid metabolism as a surrogate for changes in working muscle, especially at higher exercise intensities. Second, although TMU and RLox increased with training at RLT and ABT, respectively, fat oxidation in working limb muscles still made up a small percentage (5–17%) of the total energy used by young, postabsorptive individuals to sustain exercises and as little as 4% (65UT), to total energy expenditure. Furthermore, 9 wk of endurance training increased the contribution to 17% for ABT but did not change RLT (5%).

Hormonal responses. The hormone analyses will be discussed elsewhere in detail (Fattor JA, Friedlander AL, Jacobs KA, Wollfer EE, and Brooks GA, unpublished data). However, arterial hormone concentration data are included in Table 6 to facilitate the reader’s interpretation of the lipid data presented here.

Table 3. Whole body fat metabolism during rest and exercise*

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>RER</td>
<td>0.85±0.01</td>
<td>0.85±0.02</td>
</tr>
<tr>
<td>Arterial [FFA], μmol/l</td>
<td>554±72</td>
<td>489±91</td>
</tr>
<tr>
<td>Palmitate as %total FFA</td>
<td>26.6±0.5</td>
<td>27.7±0.6</td>
</tr>
<tr>
<td>FFA RLox, μmol/min</td>
<td>507±53</td>
<td>468±75</td>
</tr>
<tr>
<td>Total FFA oxidation, μmol/min</td>
<td>262±25</td>
<td>270±39</td>
</tr>
<tr>
<td>Plasma FFA oxidation, μmol/min</td>
<td>100±14</td>
<td>110±10</td>
</tr>
<tr>
<td>Other fat oxidation, μmol/min</td>
<td>162±15</td>
<td>160±24</td>
</tr>
<tr>
<td>%RLox oxidized</td>
<td>19±1</td>
<td>25±2</td>
</tr>
</tbody>
</table>

Values are means ± SE. [FFA], free fatty acid concentration; RER, respiratory exchange ratio; RLox, rate of disappearance. *All values significantly different between rest and exercise except for [FFA] and %palmitate. †Significantly different from 45UT at P < 0.05; §significantly different from 65UT at P < 0.05. See Table 2 legend for details.

Table 4. Gas exchange, blood flow, and fat oxidation values for 2 legs at rest and exercise

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Leg VO2, l/min</td>
<td>0.10±0.04</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>2-Leg blood flow, l/min</td>
<td>1.4±0.1</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Leg RQ</td>
<td>0.89±0.03</td>
<td>0.84±0.02</td>
</tr>
<tr>
<td>2-Leg EE, kcal/min</td>
<td>0.3±0.04</td>
<td>0.3±0.02</td>
</tr>
<tr>
<td>Total leg fat oxidation, μmol/min</td>
<td>36±12</td>
<td>59±8</td>
</tr>
</tbody>
</table>

Values are means ± SE. RQ, respiratory quotient. *Significantly different between rest and exercise at P < 0.05; †Significantly different from 45UT at P < 0.05; §significantly different from 65UT at P < 0.05; ¶significantly different from ABT at P < 0.05. See Table 2 legend for details. Blood flow data previously reported (24).
similar to previous studies in men conducted by our group (3, 15, 16) and others (19, 37) using similar designs and relative exercise intensities. However, there were some notable differences between studies coming from our laboratory. For example, in the present investigation, despite obtaining a similar pattern as earlier studies, we did not find significant differences in FFA $R_a$ or $R_d$ between exercise intensities. Previously, we had shown both an intensity effect pretraining and an endurance training effect in young men (16) following the same protocol (but without leg a-v measurements or biopsies). We surmise that the invasive nature of the present investigation added a variable of stress that overrode some of the previously observed differences among trials.

In addition, the percentage of FFA $R_d$ oxidized was significantly higher during 65UT than during 45UT due to a non-significant increase in plasma FFA $R_a$, and a nonsignificant decrease in FFA $R_d$. The differing effects of intensity on FFA oxidation and $R_d$ are consistent with the hypothesis that FFA oxidation is regulated at a site distal to sarcolemmal uptake, likely during the mitochondrial translocation step (37, 38). However, in the present investigation we observed a larger percentage of $R_d$ oxidized at the higher work intensities, which appears to contradict our understanding that FFA oxidation is downregulated by high rates of carbohydrate flux (7, 38, 46). It could be that a work intensity of 65% is still within the peak range of fat use in this group of subjects, but our finding that as much as 96% of FFA $R_d$ was oxidized at the higher intensities (Table 3) does not appear to be consistent with what we observed in the active muscles (see Leg FFA kinetics). Still, high values for %FFA oxidized are consistent with results of other invasive studies that have also reported high rates of plasma oxidation relative to uptake (sometimes exceeding 100%) at moderate-to-high cycling intensities of relatively short duration (e.g., <90 min) (36, 37). In our calculations we tried to account for changes in label retention between workloads by employing bicarbonate/acetate correction factors adjusted for rate of oxygen consumption and lean mass (39, 40) such that values at the higher intensities were close to 1.0 (0.95–0.97). However, it may be that, during these highly invasive short-term studies, when subjects are “stressed,” labeled bicarbonate losses could drive the correction above 1.0. Alternatively, prelabeling of other nonplasma FFA pools during the equilibrium period could also result in an overestimation of plasma oxidation rates. Cycling of FFA through human skeletal muscle intramuscular triglyceride pools has been observed at rest and during exercise (17, 18). Increased stimulus for lipolysis mediated through the stress hormones in the
absence of increased use at rest could result in greater cycling through nonplasma lipid pools. Therefore, one or all of the reasons above might explain why the percentage of $R_d$ oxidized in the present investigation was substantially higher (64–96%) than during our previous studies (50–83%) (13, 16, 23). The preceding discussion could also explain why we found relatively small amounts of calculated “other” fat use (2–30% of total fat oxidation) in this investigation at the whole body level (Table 3) relative to our previous investigations in men and women reporting values of 40–75% (13, 23). Based upon results of the present investigation and others, difficulties in calculating other lipid oxidation from the divergent techniques of plasma isotope dilution and indirect calorimetry are evident, and problems in attributing physiological relevance to the estimate are likely (3, 13, 37).

Leg FFA kinetics. Despite maintenance of arterial FFA concentration during exercise (Table 3), leg FFA extraction fell from $110^-45$ to $110^5$% between rest and all four exercise intensities studied (Fig. 2). Similar decrements in extraction rate during exercise have been reported previously (37, 44, 45). Despite the decrease in fractional extraction, significant elevations in flow (Table 4) permitted increased FFA TMU during exercise relative to rest and posttraining at RLT (Fig. 3A). Thus plasma flow appears to be a critical determinant of FFA uptake by working skeletal muscle in healthy young men both before and after endurance training.

Compared to the few previous studies that have measured FFA TMU during cycling exercise (8, 19, 36, 37), our uptake values were moderately higher. These higher rates of uptake were surprising given that our subjects were less trained than some of the subjects used previously in cross-sectional studies (19, 36). Others have found training to increase sarcolemmal FFA binding and transport protein expression as well as increase FFA uptake (28, 29, 31, 43). Furthermore, our subjects were postabsorptive rather than fasted. It has been shown that both FFA uptake and release are suppressed by glucose infusion (9) or a meal (26) in the resting forearm and leg, respectively. Although higher than those previously reported by others in the range of 33–50% (19, 36, 37), the present values seem reasonable given that leg energy expenditure comprised $110^-67$% of whole body energy expenditure (Table 5).

In general, the limb FFA release rates we observed in exercising men also appeared to be higher than previously reported. For example, leg release rates of $110^-185$ and $110^-260$ μmol/min have been reported for young men cycling at 68 and 45% of $V_{O2Peak}$, respectively (8, 19). Several factors, such as muscle mass, exercise power output, energy status, and extra-cellular leg muscle adiposity, may be involved. In our reading of the literature, it is also often difficult to tell when investigators report data on flux across one or two working legs. As well, there is concern over the effect of catheter placement. For example, van Hall et al. (45) compared ante- and retrograde catheter placement during one-leg kicking exercise and found that leg release, but not uptake, of FFA was significantly higher using antegrade placement. On that basis the authors asserted that antegrade placement results in contamination of blood samples by drainage from leg subcutaneous adipose tissue. However, during cycling exercise, we were concerned that...
tween 65 and 90% of V˙O\textsubscript{2} max in subjects exercising on a Krogh vascular long-chain nonesterified fatty acid availability rose be-
 tween FFA. Previously, Kiens et al. (30) demonstrated that intracellular intensities is likely not a result of decreased availability of tissue. The fall in FFA oxidation in working muscles at higher percentage of total fat oxidation is accounted for by nonactive working muscle, lipid metabolism is suppressed and a greater relative intensities where carbohydrate flux is accelerated in RLT), leg fat oxidation was closer to 30%. Thus, at higher percentage of whole body energy expenditure accounted for by the higher relative intensities before and after training (65UT, 62%, a value seemingly appropriate that is based on the 
 whole body V\textsubscript{O}2 in response to changes in exercise power output (Fig. 1). However, we are mindful that, regardless of catheter placement, the extent of drainage from various adipose depots into femoral venous blood is unknown.

**Leg FFA oxidation.** The working muscle RQ and whole body RER values we obtained in the present investigation are consistent with those obtained by others obtained on human subjects exercising at similar exercise intensities (34). On the basis of RER and RQ values, the pattern of fat and carbohydrate oxidation of the leg was similar to that of the whole body, although the balance of substrate was shifted more toward carbohydrate in the leg (leg RQ > whole body RER) during exercise. At the lower relative intensities (45UT, ABT), leg oxidation as a percentage of whole body fat oxidation was ~62%, a value seemingly appropriate that is based on the percentage of whole body energy expenditure accounted for by the leg but higher than values reported previously (8, 36). At the higher relative intensities before and after training (65UT, RLT), leg fat oxidation was closer to 30%. Thus, at higher relative intensities where carbohydrate flux is accelerated in working muscle, lipid metabolism is suppressed and a greater percentage of total fat oxidation is accounted for by nonactive tissue. The fall in FFA oxidation in working muscles at higher intensities is likely not a result of decreased availability of FFA. Previously, Kiens et al. (30) demonstrated that intracellular long-chain nonesterified fatty acid availability rose between 65 and 90% of V\textsubscript{O}2\textsubscript{max} in subjects exercising on a Krogh knee extension ergometer. As well, in the present investigation, FFA delivery to working muscle was similar or increased at the higher intensities due to similar FFA concentrations and elevated limb blood flow (Table 4). Such evidence supports energy flux (ATP turnover) rather than FFA availability as the governor of utilization (33). Unfortunately, we were unable to determine plasma FFA oxidation rates across the limb. Although 1\textsuperscript{13}CO\textsubscript{2} was detected in both femoral arterial and venous blood, use of [1-\textsuperscript{13}C]palmitate yielded data difficult to interpret. As previously described (3), our results were that leg RQ was high, causing large dilution of 1\textsuperscript{13}CO\textsubscript{2} from palmitate by carbohydrate fuel sources. And, because of large intersubject variability, the [a-v]1\textsuperscript{13}CO\textsubscript{2} was not significant. In this regard, we note that there are few studies of FFA oxidation rates across the working limb, and, in those reports, [U-\textsuperscript{13}C]- or [\textsuperscript{14}C]palmitate was used (36, 37, 44, 45). One study by Helge et al. (19) utilized [1-\textsuperscript{13}C]palmitate to investigate the effects of a fat-rich diet on lipid kinetics in the working leg, but directly-measured leg plasma FFA oxidation values were not reported.

**Table 6. Arterial plasma hormone concentrations during rest and exercise**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Untrained</th>
<th>Trained</th>
<th>45UT</th>
<th>65UT</th>
<th>ABT</th>
<th>RLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, pmol/ml</td>
<td>48±8</td>
<td>30±7†</td>
<td>31±5*</td>
<td>23±3*</td>
<td>23±3</td>
<td>18±2†</td>
</tr>
<tr>
<td>Glucagon, pmol/ml</td>
<td>16±2</td>
<td>20±3</td>
<td>16±2</td>
<td>21±3*†</td>
<td>21±3†</td>
<td>23±3†</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>430±48</td>
<td>452±72</td>
<td>627±70*</td>
<td>636±82*</td>
<td>572±90</td>
<td>520±44</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>94±25</td>
<td>85±42</td>
<td>160±94*</td>
<td>216±96*†</td>
<td>212±80*</td>
<td>209±79*</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>216±25</td>
<td>230±42</td>
<td>504±92</td>
<td>1,120±136*†</td>
<td>889±84†</td>
<td>1,274±193*†§</td>
</tr>
</tbody>
</table>

*Significantly different between rest and exercise at \(P < 0.05\); †significantly different from 45UT at \(P < 0.05\); §significantly different from ABT at \(P < 0.05\); ‡significantly different from untrained rest. See Table 2 legend for details.

Chronic physical activity (endurance training) as employed in the present investigation almost doubles muscle mitochondrial respiratory capacity (11, 20). Surprisingly, although endurance training results in a major increase in the capacity of working muscle to oxidize all substrates, including fatty acids, results of the present investigation as well as previous studies (3, 4) show preferential carbohydrate use in working muscle. Still, it would be a mistake to overlook the clearly established role of physical exercise as a means to mitigate adiposity and the associated risk of chronic disease (21). Furthermore, although working muscle is not a major sink for lipid disposal during exercise, the balance of energy substrate use shifts toward lipids after exercise (5, 32). Hence, the major effects of exercise on body lipid mobilization and catabolism occur both during and after exercise, since the recovery period may persist for 24 h (21).

In conclusion, patterns of lipid use in the working muscle are similar to those observed at the whole body in response to intensity and training, although the leg tends to use a higher percentage of carbohydrate regardless of exercise intensity or training state. Overall, our results suggest that, similar to what has been established with the whole body, energy flux is the major determinant of energy substrate balance in the leg, and exercise training has small effects on energy substrate oxidation when relative level of effort is considered. In terms of total energy expenditure, leg lipid represents a minor energy source relative to carbohydrate when trained and untrained nonathletes exercise at moderate intensities (45–65% \(\text{V}\textsubscript{O}2\text{peak}\)) for relatively short (≤1 h) durations in the postabsorptive state. And finally, because exercise and training as generally prescribed (30–60 min duration and moderate intensity) have significant, but quantitatively small, effects on whole body or muscle lipid use, both prudent dietary and physical activity habits are necessary to control total and regional lipid storage.

**ACKNOWLEDGMENTS**

We thank the study participants for their dedication and compliance to this difficult protocol. We are also grateful to the nurses and Sharon Moynihan in the Clinical Studies Unit at the VA Palo Alto Health Care System for their assistance with the subject testing and dietary controls, respectively. We thank G. C. Henderson and T. Mau for reading and commenting on our paper.
REFERENCES


