Endurance exercise training attenuates leucine oxidation and BCOAD activation during exercise in humans

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McKenzie, Scott, Stuart M. Phillips, Sherry L. Carter, Stuart Lowther, Martin J. Gibala, and Mark A. Tarnopolsky. Endurance exercise training attenuates leucine oxidation and BCOAD activation during exercise in humans. Am J Physiol Endocrinol Metab 278: E580–E587, 2000.—We studied the effects of a 38-day endurance exercise training program on leucine turnover and substrate metabolism during a 90-min exercise bout at 60% peak O2 consumption (V̇O2peak) in 6 males and 6 females. Subjects were studied at both the same absolute (ABS) and relative (REL) exercise intensities posttraining. Training resulted in a significant increase in whole body V̇O2peak and skeletal muscle citrate synthase (CS; P < 0.001), complex I-III (P < 0.05), and total branched-chain 2-oxoacid dehydrogenase (BCOAD; P < 0.001) activities. Leucine oxidation increased during exercise for the pretraining trial (PRE, P < 0.001); however, there was no increase for either the ABS or REL posttraining trial. Leucine oxidation was significantly lower for females at all time points during rest and exercise (P < 0.01). The percentage of BCOAD in the activated state was significantly increased after exercise for both the ABS and REL exercise trials, with the increase in PRE being greater (P < 0.001) compared with REL (P < 0.05). Females oxidized proportionately more lipid and less carbohydrate during exercise compared with males. In conclusion, we found that 38 days of endurance exercise training significantly attenuated both leucine oxidation and BCOAD activation during 90 min of endurance exercise at 60% V̇O2peak, for both ABS and REL exercise intensities. Furthermore, females oxidize proportionately more lipid and less carbohydrate compared with males during endurance exercise.

gender differences; substrate oxidation; protein turnover; branched-chain 2-oxoacid dehydrogenase

ENDURANCE EXERCISE RESULTS in an acute increase in leucine oxidation (8, 22, 27). Amino acid oxidation may contribute 2–3% of the total energy cost during an acute bout of endurance exercise (8, 27). For well trained endurance athletes who are exercising at a high percentage of peak O2 consumption (V̇O2peak) on a daily basis, this may result in a small increase in the requirement for dietary protein (10, 25, 34). Conversely, for relatively untrained individuals exercising at a more modest exercise intensity, there is no increase in amino acid requirements on the basis of 24-h amino acid oxidation tracer studies (8). These observations are consistent with the fact that leucine oxidation is positively correlated with the intensity of exercise (25) but are at odds with the phylogenetic logic that would predict that repeated physiological stressors (i.e., exercise training) should attenuate the oxidative flux through pathways in an attempt to preserve proteins that are essential to structure and/or function.

It is currently unclear as to whether endurance exercise training increases, decreases, or does not change the oxidation of amino acids. Lamont et al. (24) showed that endurance exercise training did not affect resting (24) or the exercise-induced increase (23) in leucine oxidation. Two studies in rodents have demonstrated that the trained animals actually oxidized more leucine compared with untrained animals at the same absolute exercise intensity (7, 14). Conversely, Hood and Terjung (17) found an attenuation in leucine’s contribution to total energy consumption in trained rodents by use of in situ hindlimb model. This controversy is likely due to the use of different models (animal vs. human; in situ vs. in vivo), as well as the difficulty in matching the workload in trained vs. untrained muscle (i.e., absolute or relative intensity).

Leucine is oxidized in the mitochondria by the branched-chain 2-oxoacid dehydrogenase (BCOAD) enzyme (5). This enzyme is activated by dephosphorylation (1) and is rate limiting to branched-chain amino acid oxidation in primates (20). An acute bout of exercise increases skeletal muscle BCOAD activity by increasing the dephosphorylated form from 7 to 25% (19, 31, 37). This activation is correlated with a decrease in the phosphorylation potential, pH, and/or glycogen (19, 36). Given that endurance exercise training attenuates glycogen utilization at the same absolute intensity of exercise (28), one would predict that this may lessen the activation of BCOAD and, hence, attenuate the increase in leucine oxidation. An alternative hypothesis, however, is that the increase in mitochondrial volume that occurs with endurance exercise (15) would lead to an increase in total BCOAD volume and abolish any beneficial effect conferred by an attenuation of activation. To date, no study has determined the effect of endurance exercise training on human muscle BCOAD activity.

In addition to the paucity of information on the effect of endurance exercise training on BCOAD activity and...
amino acid oxidation, there is very little information on potential gender differences in amino acid metabolism during exercise (27, 32). We have shown that females oxidize proportionately less leucine during endurance exercise than males (27). To date, there have been no studies that have examined whether there are gender differences in amino acid oxidation after endurance training, and no studies have specifically examined the possibility of a gender difference in BCOAD activity.

The primary purpose of this study was to examine the relationship between leucine oxidation and BCOAD activity during endurance exercise and to determine the adaptive response to a 38-day endurance exercise training program. A second major purpose was to determine the potential impact of gender on leucine oxidation.

**METHODS**

**Subjects and Recruitment**

We recruited a total of six males and eight females to participate in a longitudinal endurance exercise training study. All participants were young, healthy, and did not participate in any form of physical activity for ≥1 h/wk at the time of recruitment and had not participated in a formal endurance exercise training program for ≥6 mo before the study. All female subjects were eumenorrheic before entering the study, and none experienced menstrual dysfunction as a result of the training program. Three of the females were taking oral contraceptives for ≥6 mo before the study and continued to take them for the duration of the study. All individuals completed 4-day weighed diet records to ensure that no individuals were on a restrictive energy intake, and a medical history was taken to ensure that there were no medical contraindications to participation in an exercise training study. After this initial screen, the participants completed a maximal aerobic testing protocol on a cycle ergometer to determine VO2peak, and body composition was determined by dual-energy X-ray absorptiometry (DEXA; QDR 1000W, Hologic, Waltham, MA) (6a).

Of the original 14 participants, a total of 6 males and 6 females completed all aspects of training and analysis. One female was removed from final analysis because she became pregnant after 4 wk of training, and one female dropped out after 1 wk of training for personal reasons. As expected, males were taller, heavier (total and fat-free mass), and had lower percent body fat compared with females. The descriptive characteristics of the participants are found in Table 1.

**Design**

Training. The participants completed an initial pretesting (PRE) trial (see Testing trials), followed by 38 ± 2 days of cycle ergometry training. The training was performed for 60 min/day (5 days/wk) at an intensity of 60% VO2peak pretraining. Intervals were performed 1 day/wk for 30 min in the form of 1 min at 100% VO2peak followed by 4 min at 60% VO2peak. All training was performed on cycle ergometers (Monark, Varberg, Sweden). A heart rate monitor was used to ensure that individuals exercised at the target intensity. At the half-way point in the training study, all participants completed a second VO2peak, and the workload was reset according to the subject’s new VO2peak. All subjects performed a final VO2peak test after completion of the training program. Subjects completed two posttraining trials, one at the same relative (REL) intensity and another at absolute (ABS) intensity. The order of these trials was randomly allocated and separated by 4 days. Diet records were also completed for 4 days in the final week of training. Before all of the trials, the subjects received a 4-day individual dietary checklist that was flesh-free, isonitrogenous and isocaloric in its habitual diet. Furthermore, each subject received an individual prepackaged diet on the day before, and the day of, each exercise testing session. DEXA scans were completed on each subject at the midpoint between the two posttraining trials (same time of day and dietary status as for the pretraining DEXA scan). All female subjects were in the midfollicular phase of the menstrual cycle for PRE, ABS, and REL testing trials.

Testing trials. The PRE, ABS, and REL testing trials were identical, with the exception that only one posttraining preexercise muscle biopsy was performed, as we will describe. Each subject was tested after a 12-h fast at the same time of the day between trials (0800–1200). Twenty-four-hour urine collections were completed for the rest and exercise testing days and were stored at 4°C for 24 h. Urine samples were collected after the first urination of the rest and exercise days, up to and including the first urination of the following day. A 10-ml aliquot of each urine collection was stored at −50°C for subsequent analysis of urea nitrogen and creatinine.

On arrival at the laboratory, subjects had a 20-gauge plastic catheter inserted in an antecubital vein. A 5-ml blood sample (t = −120 min) was collected into a heparinized tube and spun immediately, and the plasma was stored at −50°C for subsequent analysis of baseline α-KIC isotopic enrichment. After this, a primed-continuous infusion of [L-1-13C]leucine (99% 13C enrichment; CDI Isotopes, Point Claire, QC, Canada) was started (prime = 1 mg/kg; continuous = 1 mg/kg·h−1) with a priming dose (0.295 mg/kg) of sodium bicarbonate (NaH13CO3, 99% enriched, CDI Isotopes). All isotopes were from the same batch; they were diluted in sterile 0.9% saline on the morning of the infusion and filtered twice through a 0.2-µm filter. Shortly after this, a second plastic catheter was placed into a contralateral distal forearm vein, and a heating pad was applied for the duration of the experiment to obtain "arterialized" blood samples.

After 60 min of isotope infusion, a muscle biopsy was taken from the vastus lateralis muscle with a modified Bergström needle (5 mm diameter) with suction modification. A piece (~30 mg) was immediately removed and placed on a moistened saline tissue for immediate processing to determine BCOAD activity, as described in BCOAD activity. Another
piece (~75 mg) was immediately quenched and stored in liquid nitrogen for subsequent analysis of NADH-cytochrome c oxidoreductase (complex I-II) and citrate synthase (CS) activities and for glycogen (all to follow). A second incision was also made at this time, 10 cm proximal to the first incision, for the pretraining and for the first of the two posttraining trials for a postexercise biopsy.

After 105 min of isotope infusion (t = −15 min), another blood sample was taken for α-KIC enrichment determination. Subjects then rested on a cycle ergometer (Lode, The Netherlands) for 15 min before a final resting blood sample (t = 0). Both of these blood samples were collected into chilled heparinized tubes, and in addition to α-KIC enrichment, the t = 0 sample was also assayed for glucose and lactate concentration.

Resting (t = 0) measurements of oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER) were then obtained after a 5-min stabilization period, during which the subjects were seated and breathed into a 60-liter balloon (Econo-Tainer, Eindhoven, The Netherlands), and the amount of CO_2 produced during the 10-min incubation period was calculated. This biopsy was used for the determination of BCOAD activity and glycogen concentrations.

Skeletal Muscle Analyses

BCOAD activity. BCOAD activity was determined using a method developed by Wagenmakers et al. (37). Briefly, a 20- to 40-mg piece of wet muscle was immediately blotted and quickly weighed before being homogenized in an ice-cold buffer (0.25 M sucrose, 10 mM Tris base, pH 7.4). The homogenate was aliquoted into two halves and processed separately. The aliquot used for the determination of total enzymatic activity and active (i.e., dephosphorylated) enzyme fraction was preincubated with ADP (8.3 mM) and NaF (83.3 mM), inhibitors of the BCOAD kinase and phosphatase, respectively, whereas the aliquot used to determine total BCOAD activity was incubated with ADP only. After preincubation for 5 min at 37°C, 100 µl of 0.5 mM 2-oxo-[1-14C]isocaproate (54.0 mCi/mmol; Amersham Life Sciences, Buckinghamshire, UK) were added to the sealed reaction vials. The samples were incubated for 10 min at 37°C, and the reaction was terminated by injection of 3 M perchloric acid. The 14CO_2 produced during the incubation was collected with an ethanolamine-ethylene glycol (1:2, vol/vol) solution located in a plastic reservoir within the sealed reaction vials. After 90 min, the reservoir was removed and placed in a 20-ml scintillation vial with 10 ml of scintillation cocktail (Beckman, Fullerton, California). The specific activity of the 14CO_2 ethanolamine-ethylene glycol was then determined in a liquid scintillation β-counter (Philips, PW 4700 Liquid Scintillation, Eindhoven, The Netherlands), and the amount of CO_2 produced during the 10-min incubation period was calculated. Activities of BCOAD were calculated from the specific radioactivity of the substrate (10,000 dpm/nmol) and are expressed in nanomoles of 2-oxoisocaproate decarboxylated per minute per milligram wet weight of muscle. The percentage of active BCOAD was calculated by dividing the actual activity (activity of the dephosphorylated aliquot) by the total activity of the aliquot. Activities were determined in duplicate or triplicate, and a blank correction was subtracted (incubation of buffer without homogenate). The CV for triplicate samples averaged 16.2%.

Complex I-III and CS activity. A 10- to 30-mg piece of wet muscle was homogenized in 200 µl of buffer consisting of 5 mM potassium phosphate, 1 mM EDTA, and 0.1 mM dithiothreitol (DTT), in water, pH to 7.4.

One milliliter of reaction buffer (0.1 mM potassium phosphate and 1 mM azide, pH ~7.0) was placed into each of two cuvettes. Thirty microliters of cytochrome c (40 mg/ml) were then added to 5 µl of 1 mM rotenone in the rotenone-sensitive cuvette. The cuvettes were then mixed, and 20 µl of muscle homogenate were added to each. After an initial absorbance reading at 550 nm with an ultraviolet (UV) spectrophotometer (Shimadzu UV-1201, Tokyo, Japan), 10 µl of NADH (5 mg/ml) were added to each tube. The cuvettes were mixed, and absorbances were read at 30, 60, 90, and 120 s. Rotenone-sensitive complex I-III activity was calculated by subtracting the total rotenone values (µmol cytochrome c reduced·mg wet muscle·1·min⁻¹). The intra-assay CV was 7.6%.

One milliliter of reaction buffer (0.1 M pH 8.0), heated to 37°C, was added to a cuvette containing 10 µl of DTNB (4 mg/ml of Tris buffer) and 2 µl of acetyl-CoA (30 mM in H2O). To this, 10 µl of muscle homogenate were added and mixed. The spectrophotometer (Shimadzu UV-1201) was zeroed, and 10 µl of oxaloacetic acid (6.6 mg/ml Tris buffer) was added to start the reaction. Absorbances were recorded at 412 nm every 30 s for 2 min. CS activity was calculated and expressed as micromoles per minute per milligram wet weight. The intra-assay CV was 5.0%.

Glycogen. Approximately 40 mg of frozen wet muscle was lyophilized, powdered to disintegrate nonmuscle elements, and stored at −50°C. Muscle glycogen determination was made using a method modified from that described by Bergmeyer et al. (3). One hundred sixty microliters of 0.1 M NaOH were added to 2–4 mg of powdered muscle. Samples were then incubated for 10 min at 80°C. After cooling, a combined solution of 0.1 M HCl, 0.2 M citric acid, and 0.2 M disodium hydrogen phosphate was added to the sample solution in a ratio of 1:4 (pH 7.4 ± 0.4). Forty microliters of amyloglucosidase were then added and allowed to sit at room temperature for 80 min. Liberated glucose was then assayed spectrophotometrically at 340 nm by adding 25 µl of sample and 250 µl of reagent that consisted of 100 mM triethanolamine, 40 mM KOH, 30 mM Mg(OAc)2·2 H2O, 1 mM Na2 EDTA·2 H2O, 0.75 mM ATP, 1 mM DTT, and 1 mM NAD, brought to a pH of 8.2 with KOH. Absorbances were then measured at 340 nm. Four microliters of a hexokinase (HK)/glucose-6-phosphate dehydrogenase (G6PDH) solution (prepared by diluting 228 units of G6PDH and 200 units of HK, with 200 µl of each, and combining them into one solution) were then added. The cuvette was then mixed, and the absorbance was read after 15 min. Glycogen concentrations were calculated using a standard curve from bovine glycogen and expressed as millimoles per kilogram dry wt. The intra-assay CV was equal to 3.8%.

Urine analysis. Urea nitrogen was determined colorimetrically with the urease/urea-nitroprusside reaction (procedure no. 640, Sigma). Urine creatinine was determined using a picric acid method (procedure no. 555, Sigma). The intra-assay CV values were 4.5 and 1.8%, respectively.
Plasma analysis. Plasma glucose was determined colorimetrically by the glucose oxidase method (kit no. 315, Sigma). Plasma insulin was determined with a standard single-antibody RIA (Coat-A-Count no. TK1NS, Intermedico). Plasma lactate was determined with a lactate analyzer (model 23L, YSI Scientific). The intra-assay CV values for the three assays (glucose, insulin, and lactate) were 5.3, 2.5, and 3.2%, respectively.

Protein turnover. Total leucine flux (Q) and oxidation (O) were calculated from the enrichment of plasma [13C]KIC (reciprocal pool model) with steady-state equations, as has been previously described (27). Briefly, Q = synthesis (S) + O, and S is estimated as the nonoxidative leucine disposal (NOLD) by use of the equation, NOLD = Q - O. Plasma KIC enrichment was determined by the O-trimethylsilyl quinoxalinol derivative and gas chromatography-mass spectrometry (CG-MS) analysis with selected ion monitoring (232.1/233.1) (GC = 6890; MS = 5973, Hewlett-Packard). Breath \( ^{13} \text{CO}_2/^{12} \text{CO}_2 \) enrichment was determined by isotope ratio mass spectrometry (VG Isogas, SIRA 10, Cheshire, UK) after cryogenic extraction, as described previously (27). To account for the proportion of \( ^{13} \text{CO}_2 \) that remained within slowly turning over bicarbonate pools (bicarbonate retention factor c), we performed resting and exercise bicarbonate infusions (NaH\(^{13}\)CO\(_3\), CDN Isotopes) in three males and three females at the midpoint of the exercise training. We also measured the changes in breath \( ^{13} \text{CO}_2/^{12} \text{CO}_2 \) enrichment in three males and three females at the same time point to further correct our breath enrichments for changes in the proportion of endogenous substrate use. Individual data were used for the individuals in whom the measurements were made, and a mean value was used for the others (resting c = 0.81 and exercise c = 1.00). Calculations for the resting leucine turnover data were made at isotopic plateau by using an average of the t = 15 and t = 0 min time points; exercise leucine turnover data were taken as the average of the 60-, 75-, and 90-min exercise values (breath and plasma isotopic plateau). Isotopic plateau was confirmed in each individual both at rest and during exercise, with a slope not significantly different from 0 and the CV of the measurements <5%.

Statistics

Statistical significance was determined by ANOVA with repeated-measure design. When a significant main effect and/or interaction occurred, the location of pairwise differences was determined using Tukey post hoc analysis. The level taken to indicate significance was P < 0.05. All data in tables are presented as means ± SD, except where indicated otherwise. Statistica 5.1 (Statsoft, Tulsa, OK) was used for all statistical analyses.

RESULTS

There was a significant decrease in percent body fat for both males and females after the 38-day exercise training protocol (Table 1). There was a significant increase in VO\(_{2\text{peak}}\) (P < 0.05, whether expressed as l/min, ml·kg\(^{-1}\)·min\(^{-1}\), or ml·kg FFM·min\(^{-1}\)) after exercise training for both males and females (Table 1). Dietary protein intake was higher for males than females (P < 0.001), and there was no effect of training on protein intake (Table 1).

By design, the exercise intensity (%VO\(_{2\text{peak}}\)) was the same for the exercise trial before training (PRE) and the postexercise trial at the same relative (REL) intensity, and it was similar between genders (Table 2).

### Table 2. Testing characteristics before and after training

<table>
<thead>
<tr>
<th></th>
<th>Males (n = 6)</th>
<th>Females (n = 6)</th>
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<tbody>
<tr>
<td>Exercise intensity, % VO(_{2\text{peak}})</td>
<td>PRE 61.8 ± 3.7</td>
<td>59.9 ± 9.4</td>
</tr>
<tr>
<td></td>
<td>ABS 56.7 ± 1.9</td>
<td>59.9 ± 9.8</td>
</tr>
<tr>
<td></td>
<td>REL 59.5 ± 2.9</td>
<td>58.7 ± 5.0</td>
</tr>
<tr>
<td>Exercise intensity, W</td>
<td>PRE 132.5 ± 22.1</td>
<td>80.8 ± 17.2*</td>
</tr>
<tr>
<td></td>
<td>ABS 132.5 ± 22.1</td>
<td>80.8 ± 17.2*</td>
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<tr>
<td></td>
<td>REL 160.5 ± 23.8</td>
<td>110.5 ± 15.1*</td>
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</table>

Values are means ± SD. PRE, pretesting trial; ABS and REL, absolute and relative exercise intensities posttraining, respectively. *Main effect for gender for average exercise intensity (females < males, P < 0.001). †Main effect for exercise intensity, REL > ABS and PRE (P < 0.0001).

Similarly, the power output was identical for the PRE and the postexercise training trial at the same absolute (ABS) intensity, and the absolute power output was higher for males and females at all time points (Table 2).

The increase in VO\(_{2\text{peak}}\) was paralleled by an increase in the maximal enzyme activities for marker enzymes of the electron transport chain [NADH-cytochrome c oxidoreductase (complex I–III; P < 0.05)] and the tricarboxylic acid cycle (CS; P < 0.001) after endurance exercise training (Table 3).

Leucine oxidation (Table 4) increased significantly during exercise over resting values during the last 30 min of the 90-min exercise bout before the endurance exercise training program (PRE), P < 0.001. After the training program, there was no significant increase in leucine oxidation during the final 30 min of exercise for either the REL or ABS exercise intensities. Males showed higher leucine oxidation compared with females both at rest and during exercise for all trials (P < 0.01). Both leucine flux and NOLD were significantly lower during exercise compared with rest for all three trials. Females showed a lower leucine flux compared with males at all time points (P < 0.05; Table 4).

There was a significant increase in total BCOAD activity (Fig. 1) when the PRE was compared with both ABS and REL trials (P < 0.001 and P < 0.0001, respectively). The percentage of BCOAD activation was significantly increased from 0 to 90 min in both the PRE and REL exercise trials, with the increase in PRE

### Table 3. Maximal mitochondrial enzyme activities

<table>
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<tr>
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<th>Pre</th>
<th>Pst</th>
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<tbody>
<tr>
<td>NADH-Cytochrome c oxidoreductase (complex I–III), µmol·min(^{-1})·mg wet wt(^{-1})</td>
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<tr>
<td>Male</td>
<td>1.6 ± 0.9</td>
<td>2.6 ± 0.9*</td>
</tr>
<tr>
<td>Female</td>
<td>1.4 ± 0.4</td>
<td>2.1 ± 0.9*</td>
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<tr>
<td>Citrate synthase, µmol·min(^{-1})·mg wet wt(^{-1})</td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>12.0 ± 3.9</td>
<td>16.1 ± 3.2†</td>
</tr>
<tr>
<td>Female</td>
<td>11.9 ± 0.6</td>
<td>15.1 ± 1.4†</td>
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</table>

Values are means ± SD (n = 6 males, n = 6 females, for VO\(_{2\text{peak}}\) and n = 5 males, n = 5 females for citrate synthase and Complex I–III). *†Main effect for training (Prevs. Pst); †for Complex I–III (P < 0.05); †for CS (P < 0.001).
being greater [P < 0.001 (PRE), and P < 0.05 (REL); Fig. 2]. There was no significant increase from 0 to 90 min in the ABS exercise. There was a significant correlation between the percentage of BCOAD activation and leucine oxidation (P = 0.42; P < 0.05; data not shown).

Analysis of urinary excretion indexes showed a main effect for gender for both absolute urea N excretion (g/day) and creatinine excretion (g/day). For both outcome measures, males were significantly higher than females (P < 0.05, and P < 0.01, respectively). Regression analysis demonstrated a positive correlation between urea N excretion (g/day) and leucine oxidation (r = 0.46; P < 0.0001; data not shown).

Plasma glucose was unchanged within any condition during exercise. Plasma insulin concentration showed a main effect for time, with a decrease throughout exercise, and with t = 0 and t = 30 being greater than all other time points (P < 0.001 and P < 0.05, respectively). The time points t = 60 and t = 90 did not differ from one another. No main effects for trial or gender were found (data not shown).

Plasma lactate (Fig. 3) responses demonstrated a significant trial-by-time interaction. The ABS trial lactate concentration was significantly lower than either the PRE or REL trials at all time points (P < 0.01). The lactate concentration at 30 min was significantly greater than at 0 min during all trials (P < 0.001) and was greater at this time for PRE vs. REL and ABS (P < 0.001).

Muscle glycogen concentration (Fig. 4) was significantly lower at t = 90 than at t = 0 during all trials (P < 0.001). In addition, the ABS and REL trial glycogen concentrations (collapsed across time) were significantly higher than the PRE trial values (P < 0.001).

Exercise RER (Table 5) showed main effects for all variables. Males had a significantly higher RER than females (P < 0.05). ABS trial RER was significantly greater than either the PRE or REL RER values (P < 0.05), and t = 30 RER was significantly higher than RER at all other time points (P < 0.05; Table 5).

The percentage of energy derived from carbohydrate (CHO) and fat during exercise (as calculated from RER; Table 6) showed a main effect for gender, with males using proportionately more CHO and females using

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**Table 4. Leucine turnover**

<table>
<thead>
<tr>
<th></th>
<th>PRE Rest</th>
<th>PRE Ex</th>
<th>ABS Rest</th>
<th>ABS Ex</th>
<th>REL Rest</th>
<th>REL Ex</th>
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<tr>
<td><strong>Oxidation, µmol·kg⁻¹·h⁻¹</strong></td>
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<tr>
<td>Male</td>
<td>43.9 ± 12.1</td>
<td>85.2 ± 21.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.6 ± 8.8</td>
<td>39.6 ± 27.3</td>
<td>48.5 ± 19.1</td>
<td>59.8 ± 24.4</td>
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<tr>
<td>Female&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.4 ± 8.1</td>
<td>38.8 ± 24.7</td>
<td>25.8 ± 7.9</td>
<td>21.5 ± 14.7</td>
<td>29.0 ± 10.6</td>
<td>28.5 ± 14.4</td>
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<tr>
<td><strong>Flux, µmol·kg⁻¹·h⁻¹</strong></td>
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<tr>
<td>Male</td>
<td>185.3 ± 66.2</td>
<td>196.5 ± 32.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>186.4 ± 36.9</td>
<td>163.6 ± 26.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>192.9 ± 26.4</td>
<td>178.5 ± 28.6&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Female&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154.9 ± 21.6</td>
<td>140.4 ± 19.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>160.6 ± 29.3</td>
<td>136.3 ± 20.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>162.7 ± 41.8</td>
<td>136.0 ± 25.0&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td><strong>NOLD, µmol·kg⁻¹·h⁻¹</strong></td>
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<tr>
<td>Male</td>
<td>141.1 ± 58.6</td>
<td>111.5 ± 21.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>138.6 ± 32.2</td>
<td>124.1 ± 23.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>145.5 ± 17.7</td>
<td>118.2 ± 39.5&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>Female&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.0 ± 23.8</td>
<td>101.6 ± 37.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>134.8 ± 31.9</td>
<td>114.8 ± 24.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>133.6 ± 40.1</td>
<td>107.5 ± 19.4&lt;sup&gt;e&lt;/sup&gt;</td>
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</tbody>
</table>

Values are means ± SD (n = 6 males; n = 6 females). Ex, exercise; NOLD, nonoxidative leucine disposal. Main effect for gender (male-female: *P < 0.01, **P < 0.05);<sup>a</sup><sup>b</sup>, Interaction (trial × time), Ex > Rest only in PRE trial (P < 0.001), whereas PRE Ex is significantly greater than all trials and times (P < 0.05).<sup>a</sup><sup>e</sup>, Main effect for time (Rest > Ex collapsed across gender).

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Fig. 1. Total branched-chain 2-oxoacid dehydrogenase (BCOAD) activity. PRE, pretesting trial; ABS and REL, absolute and relative exercise intensities, respectively. Values are means ± SD. *Main effect for trial, PRE < ABS and REL (P < 0.001).

Fig. 2. Percent BCOAD activation (%BCOAD activity). Values are means ± SE of 12 subjects, 6 male and 6 female (pooled). *Significant increase between 0 and 90 min for PRE and REL. Increase for PRE > that for REL (P < 0.001 and P < 0.05, respectively).
proportionately more fat during exercise \((P < 0.05)\). A main effect for trial showed that the proportionate oxidation of CHO was lower, and fat was higher, for the ABS trial compared with PRE and REL \((P < 0.01); Table 6\).

**DISCUSSION**

The major finding of this study was that a 38-day endurance exercise training program resulted in a significant attenuation of the exercise-induced increase in leucine oxidation, with a concomitant attenuation of BCOAD percent activation in both males and females. Finally, females oxidized proportionately more lipid, less CHO, and less protein during exercise at 65\% \(\text{VO}_{2}\text{peak}\) compared with males, both before and after endurance exercise training.

Several previous studies have shown acute increases in leucine oxidation during endurance exercise similar to that in the current study \((13, 29, 30)\). To date, however, no study has examined the effects of a longitudinal endurance training program on protein oxidation in humans. Previous authors have investigated the effects of endurance training on rodents and have found equivocal results. Henderson et al. \((14)\) observed that training substantially increased whole body leucine turnover and oxidation at rest as well as during exercise at a given intensity. These authors suggested that a training-induced increase in BCOAD enzyme and subsequent activation may have accounted for the increase in whole body leucine oxidation. In contrast to these findings, Hood and Terjung \((17)\) were able to show that training reduced the relative contribution of leucine oxidation to \(\text{VO}_{2}\) in electrically stimulated, perfused rat hindquarters. These authors speculated that BCOAD may have been activated less in trained muscle during exercise because of smaller perturbations of energy homeostasis \(\text{I.e.}, \) lower ADP and Pi\). Hood and Terjung therefore suggested that the training-induced increases in whole body leucine oxidation observed previously \((14)\) must have occurred in the liver, which is a plausible explanation when we consider that rodents have a much higher distribution of BCOAD enzyme within liver than do humans \(70\%\) compared with 30\%, respectively \((20)\).

In humans, Lamont et al. \((23)\) recently examined the cross-sectional differences in leucine kinetics in trained and untrained individuals. Results showed an increase in leucine oxidation for both groups during exercise when expressed per kilogram body weight. However, all between-group differences were eliminated when leu-

### Table 5. Exercise RER

<table>
<thead>
<tr>
<th>Time During Exercise, min</th>
<th>Male*</th>
<th>Female*</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.95 ± 0.04</td>
<td>0.91 ± 0.04</td>
</tr>
<tr>
<td>60</td>
<td>0.92 ± 0.02</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>75</td>
<td>0.91 ± 0.02</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>90</td>
<td>0.91 ± 0.02</td>
<td>0.91 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SD \(n = 6\) males; \(n = 6\) females. *Main effect for gender, males > females \((P < 0.05)\). †Main effect for trial, ABS < PRE and REL \((P < 0.05)\).

### Table 6. Calculated substrate oxidation (from RER)

<table>
<thead>
<tr>
<th>% Expenditure</th>
<th>PRE</th>
<th>ABS</th>
<th>REL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male*</td>
<td>86.5 ± 4.6</td>
<td>78.8 ± 3.6</td>
<td>88.5 ± 7.5</td>
</tr>
<tr>
<td>Female</td>
<td>80.6 ± 8.5</td>
<td>73.6 ± 9.0</td>
<td>81.0 ± 4.9</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male*</td>
<td>13.5 ± 4.6</td>
<td>21.2 ± 3.6</td>
<td>11.5 ± 7.5</td>
</tr>
<tr>
<td>Female</td>
<td>19.4 ± 8.5</td>
<td>26.4 ± 9.0</td>
<td>19.0 ± 4.9</td>
</tr>
</tbody>
</table>

Values are means ± SD \(n = 6\) males; \(n = 6\) females. RER, respiratory exchange ratio. CHO, carbohydrate. *Main effect for gender, males > females \((P < 0.05)\) and males < females \((P < 0.05)\). †Main effect for trial, ABS < PRE and REL \((P < 0.01)\).
Kinetics were corrected for fat-free tissue mass (23). Our results were not altered by the expression of leucine kinetics relative to fat-free tissue mass. Although Lamont et al. did not show a decrease in leucine oxidation in trained individuals, they did not show the increase seen by Henderson et al. (14) in rodents.

The mechanism for the attenuated whole body leucine oxidation after training may relate to the attenuation of BCOAD activation. These variables showed some correlation, in that the training-induced attenuation of leucine oxidation was positively correlated with percent BCOAD activation. The correlation between percent BCOAD activation and leucine oxidation was not high \((r = 0.42)\), which supports the conclusion by Bowtell et al. (4) that factors such as leucine availability may also determine leucine oxidation. The mechanism for this attenuation after training may relate to the higher muscle glycogen concentration observed in the current study after training, compared with the lower pretraining basal muscle glycogen concentration. An increase in muscle glycogen concentration in the basal state after endurance exercise training has been observed by others (11, 12). Although the absolute and percentage decrease in glycogen, across exercise, was similar before and after training in the current study, the absolute glycogen concentration postexercise and after training was within the normal "basal" range in an untrained individual and would not likely be limiting to energy homeostasis. Kasprzak (19) suggested that exercise training resulted in a smaller exercise-induced reduction in energy charge \((ATP/ADP + P_i)\) and that this may attenuate BCOAD activation. It would be expected that mitochondrial energy charge would bear a higher correlation with BCOAD activation given its intramitochondrial location; thus it would be of interest to determine whether endurance training results in lesser mitochondrial energy charge alteration and may explain the observed attenuation of BCOAD activation.

In addition to changes in protein oxidation and BCOAD activation, we also found mitochondrial enzyme changes after training. CS, cytochrome c oxidoreductase, and total BCOAD enzyme activities were found to increase after training, indicating an increase in the total mitochondrial oxidative potential and aerobic capacity (2, 15, 16). It is interesting that, although total BCOAD capacity increased after training, the percentage in the active form was attenuated. This may represent an adaptive strategy for the organism to "spare" critical proteins in response to a metabolic stress, i.e., after exercise training. This finding may also partially explain the apparent paradox between studies finding that endurance training increases (26, 34) and those finding that it does not alter (6, 8, 35) dietary protein requirements. For example, an elite athlete who has upregulated mitochondrial potential to a large degree would also have an increased total BCOAD capacity, and, if exposed to a rigorous training program, may increase leucine oxidation/protein requirements (34). Conversely, the current study supports the concept that more modest endurance exercise results in homeostatic adaptations that ultimately do not tax the enhanced BCOAD capacity. Overall, this study supports the results of studies finding that moderate exercise does not result in an increase in dietary protein requirements (6, 8), yet the increase in total BCOAD activity provides a mechanism for a higher "capacity" for leucine oxidation in the trained state. This may explain the finding of a higher dietary protein requirement for the very elite endurance athlete (34) and may also explain the finding of a higher leucine oxidation after training in rodents (7, 14).

In the current study, females oxidized proportionally more lipid and less CHO and protein than males, both pre- and posttraining. Similar results were found in recent studies by Horton et al. (18) and Freidlander et al. (9), who were able to show that females utilized a greater proportion of lipid during exercise postraining (9, 18). Considering that these differences were observed before and after training, one cannot imply that the genders were not properly matched; therefore, the results of the current study further support the evidence that gender differences do exist in substrate utilization during exercise. Studies with properly matched males and females have shown similar increases in lipid and decreases in protein oxidation during endurance exercise (27, 32).

Females oxidized less protein than males at rest as well as during exercise. This result is identical to that shown by Phillips et al. (27) in a study that examined leucine kinetics of male and female endurance athletes during exercise. Even though protein oxidation was less for females at rest and during exercise, this lower level of oxidation was not due to differences in the percent BCOAD activation in skeletal muscle. However, the difference in protein oxidation could be a result of a differential activation of the hepatic BCOAD enzyme, such that females may have a lesser activation than seen in males. In addition, females had a lower RER at all time points compared with males, which implied greater lipid and less CHO utilization. The fact that females did not significantly differ from males in their muscle glycogen utilization \((\%\text{decrease})\) during exercise implied that any CHO sparing was hepatic (differences in glycogenolysis/glucogenesis).

Finally, a recent study by Bowtell et al. (4) pooled male and female protein oxidation data on the basis that gender differences in protein oxidation were due to dietary intake of protein. Citing a previous study by Phillips et al. (27), they rationalized that gender differences in protein oxidation at rest and during exercise were the result of a higher protein intake for males vs. females \((0.94 \text{ and } 0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}, \text{respectively})\). When data from their study were used directly (4), it can be shown that the increase in rate of protein oxidation accounted for by each increment of \(0.1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}\) of protein intake is \(0.71 \text{ µmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\) (at rest) and \(2.31 \text{ µmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\) (during exercise). When these calculations, and the dietary protein intake difference of \(0.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}\) \((1.6 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \text{ for males and } 1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \text{ for females})\) found between males and females are used in the current study, dietary protein
intake accounted for ~12% (at rest) and 19% (during exercise) of total protein oxidation. Clearly, this does not account entirely for the observed gender differences in protein oxidation in the current study.

In summary, both genders exhibited an acute increase in protein oxidation and BCOAD activation during exercise that was attenuated after a 38-day endurance exercise training program (at both ABS and REL intensity). The present study has confirmed that females oxidized proportionately more lipid and less CHO and protein during exercise compared with their male counterparts. These gender differences were present before and after the training program and thus implied that the gender differences in metabolism were not due to a difference in training status.

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