Comparison of leucine kinetics in endurance-trained and sedentary humans

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Lamont, Linda S., Arthur J. McCullough and Satish C. Kalhan. Comparison of leucine kinetics in endurance-trained and sedentary humans. *J. Appl. Physiol.* 86(1): 320–325, 1999.—Whole body leucine kinetics was compared in endurance-trained athletes and sedentary controls matched for age, gender, and body weight. Kinetic studies were performed during 3 h of rest, 1 h of exercise (50% maximal oxygen consumption), and 2 h of recovery. When leucine kinetics were expressed both per unit of body weight and per unit of fat-free mass, both groups demonstrated an increase in leucine oxidation during exercise ($P < 0.01$). Trained athletes had a greater leucine rate of appearance during exercise and recovery compared with their sedentary counterparts ($P < 0.05$) and an increased leucine oxidation at all times on the basis of body weight ($P < 0.05$). However, all of these between-group differences were eliminated when leucine kinetics were corrected for fat-free tissue mass. Therefore, correction of leucine kinetics for fat-free mass may be important when cross-sectional investigations on humans are performed. Furthermore, leucine oxidation, when expressed relative to whole-body oxygen consumption during exercise, was similar between groups. It is concluded that there was no difference between endurance-trained and sedentary humans in whole body leucine kinetics during rest, exercise, or recovery when expressed per unit of fat-free tissue mass.

amino acid metabolism; stable isotope tracers; exercise recovery; proteolysis

ALTHOUGH STUDIES have been performed on laboratory animals (7, 13, 15); experiments on the effects of endurance training on amino acid kinetics in humans are limited (11). The need for research on humans is supported by the fact that there are large interspecies differences in the turnover rate of the free amino acid pool (11). There is one previous study that reported that differences in endurance-trained and sedentary humans in whole body leucine kinetics during rest are related to the size of the anatomic muscle mass (20). There are no comparisons of whole body amino acid kinetics during exercise or recovery in trained and sedentary individuals. Therefore, we studied whole body leucine and lysine kinetics in endurance-trained athletes and sedentary controls that were pair matched for body weight. Leucine was studied because it is an essential ketogenic, branched-chain amino acid that can be oxidized by skeletal muscle (10, 30). Lysine kinetics were simultaneously studied because, in contrast to leucine, lysine is an essential amino acid that cannot be degraded or transaminated by skeletal muscle (32). Although leucine turnover appears to be increased in the resting endurance-trained athlete, we postulated that this increase may be due to a difference in the size and not the function of the fat-free tissue mass (20). Therefore, the hypothesis tested in this experiment was that there will be no difference in whole body leucine kinetics during exercise or recovery in the endurance-trained athlete and pair-matched sedentary control.

METHODS

Subjects. Fourteen healthy men ($n = 8$) and women ($n = 6$) were recruited for this experiment. Seven of these subjects (3 women and 4 men) were endurance-trained (Trn), and seven (3 women and 4 men) were nontrained, sedentary adults (Con). Five of the women were tested in the follicular phase of their menstrual cycle. Menstrual cycle phase was determined by counting days from the onset of menses and by using a monoclonal antibody self-test kit (Ovukit, Quidel San Diego, CA). The subjects were considered endurance trained if their cycle ergometry-obtained maximal oxygen consumption ($\dot{V}O_{2max}$) was >50 ml·kg^{-1}·min^{-1}. All athletes reported that they had a training frequency of five times a week or more and that their training sessions were at least 1 h/day. In addition, the trained subjects had been in training no less than 1 yr. These endurance-trained athletes were marathon runners, triathletes, or long-distance cyclists. All 14 participants had normal 12-lead electrocardiograms and were without a family or personal history of diabetes mellitus. This project was approved by the Investigational Review Board of the University Hospitals of Cleveland. A written informed consent was obtained from each subject before his or her participation in this experiment.

The physical characteristics of these subjects are shown in Table 1. The sedentary, nontrained subjects were chosen to match the gender, age, and body weight of the endurance-trained athletes [Table 1; P = not significant (NS)]. An $H_2{}^{18}O$ isotope dilution method was used to determine total body water and fat-free mass (22). Also, whole body anatomic muscle mass (kg) was calculated from urinary creatinine excretions. It was assumed that 1 g of creatinine excreted during a timed 24-h period equaled 18.5 kg of whole body muscle (14). Urine samples were obtained on the third day of a meat-free diet to eliminate exogenous sources of creatinine.

Experimental protocol. A 7-day weight-maintaining dietary-control period preceded the stable-isotope infusion experiment. The diets were formulated to meet daily caloric needs and consisted of ~58–60% carbohydrate, 30% fat, and 10–12% protein. Both groups received equal daily amounts of nitrogenous protein (Trn = 70.86 ± 5.30 g protein/day vs. Con = 70.50 ± 4.85 g protein/day). The equations of Harris and Benedict (12) were used to estimate each subject's resting

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Table 1. *Physical characteristics of the trained and of nontrained control subjects*

	Age, yr	$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	Body Weight, kg	Anatomic Muscle Mass, kg	Fat-Free Mass, kg
Trn	28.9 ± 2.2	54.4 ± 1.8	67.7 ± 4.9	24.6 ± 4.5	60.6 ± 4.7
Women	32.0 ± 3.6	51.4 ± 0.9	55.5 ± 3.5	17.8 ± 4.5	48.5 ± 0.5
Men	26.5 ± 2.6	56.6 ± 2.5	76.8 ± 3.7	29.7 ± 6.4	67.5 ± 4.5
Con	34.7 ± 3.6	39.7 ± 1.7	68.8 ± 4.5	19.8 ± 2.2	57.6 ± 5.2
Women	32.3 ± 6.7	35.9 ± 1.3	59.4 ± 3.7	14.6 ± 2.5	46.2 ± 4.2
Men	36.5 ± 4.6	42.5 ± 1.7	75.9 ± 3.7	26.4 ± 2.8	66.1 ± 5.3
P	NS	<0.0005	NS	NS	NS

Values are means ± SE for 7 trained (Trn) and 7 control (Con) subjects. $\dot{V}O_{2max}$, maximal oxygen consumption; NS, not significant. *P* values are for statistical comparisons between Trn and Con.

energy needs. Increases in energy expenditure due to daily exercise training sessions were determined for each athlete by using standard metabolic equations (2). The energy expenditures for exercise training were added to the resting energy requirements to compute daily caloric needs for each athlete. A registered dietitian designed the individual meal plans for all subjects and used a dietary-exchange procedure to individually counsel each subject on his or her nutritional plans (9). The protein sources allowed on *days 1–4* included meat proteins, and on *days 5–7* they were switched to meat-free proteins. This dietary substitution was made to eliminate exogenous nutritional creatinine so that an accurate measure of anatomic muscle mass could be obtained. Total 24-h urine volumes (*days 6* and *7*) were collected for the determination of creatinine excretion. The subjects were instructed to refrain from physical exercise on *days 5* and *6* to avoid an acute exercise recovery effect on amino acid kinetics during the experimental tracer infusion (*day 7*). Compliance with these dietary and experimental procedures were evaluated by reviewing the written dietary records and by interviewing each subject before the infusion experiment.

A progressive, incremental cycle ergometry protocol was used to determine $\dot{V}O_{2max}$. The metabolic cart (model 2900, Sensor Medics Yorba Linda, CA) was calibrated before each exercise test with standard gas mixtures that were previously verified by the Scholander technique. $\dot{V}O_{2max}$ was assumed if there was a plateau in oxygen uptake ($\dot{V}O_2$) and a respiratory exchange ratio (RER) of >1.0 at maximal workloads.

Tracer-infusion studies. All subjects reported to the Clinical Research Center after an overnight fast on the morning of *day 7*. Intravenous cannulas were placed into superficial hand veins of each arm. One cannula was used for the tracer infusions of L-[1-^{13}C]leucine (99 atom %excess ^{13}C), L-[α-^{15}N]-lysine (99 atom %excess ^{15}N), and sodium bicarbonate NaH[^{13}C] O_3 (99 atom %excess ^{13}C) (purchased from Merck, Dorval, Canada). The labeled tracers were weighed and dissolved in normal saline and sterilized by microfiltration (0.22-μm Millipore filter). Each labeled tracer was tested for sterility and pyrogenicity before the tracer infusions (16). The second intravenous cannula was used for blood sampling and was kept patent with an isotopic saline infusion (10 ml/h). A background sample of expired air and venous blood was obtained before the infusions were begun. Priming doses were administered to reach an early steady-state and were 1.2 μmol/kg of NaH$^{13}CO_3$, 4 μmol/kg of L-[1-^{13}C]leucine, and 6.8 μmol/kg of L-[α-^{15}N]lysine. This prime was followed by a 6-h constant-rate L-[1-^{13}C]leucine (5 μmol·kg^{-1}·h^{-1}) and L-[α-^{15}N]lysine (7 μmol·kg^{-1}·h^{-1}) infusion. An accurately weighed amount of labeled water ($H_2{}^{18}O$, 99 atom % excess ^{18}O; MSD Isotopes) was given orally to determine total body water (22, 26, 27). The first 3 h of the primed infusion resulted in the acquisition of an isotopic plateau (18). During this 3-h period, venous blood samples were withdrawn every 30 min. These blood samples were immediately centrifuged, and the plasma was stored at −70°C for later analyses. Breath samples were collected every 30 min by using a Hans-Rudolph, one-way nonrebreathing valve connected to a 5-liter anesthesia bag. An aliquot of each breath sample was trapped in an evacuated glass tube for the subsequent analysis of $^{13}CO_2$ (16). Carbon dioxide production ($\dot{V}CO_2$) and $\dot{V}O_2$ were determined throughout the 3 h of rest. The average isotopic enrichment for the last hour of the infusion during resting conditions was used to calculate leucine and lysine kinetics.

At the beginning of *hour 4*, each subject began to exercise at 50% of $\dot{V}O_{2max}$ by using a Monark cycle ergometer (Varberg, Sweden). Blood samples were withdrawn at 0, 15, 30, 45, 50, 55, and 60 min of exercise. Heart rates and ratings of perceived exertion (Borg scale) were periodically determined throughout the 1 h of submaximal exercise (20, 30, 40, 45, 50, and 60 min). $\dot{V}O_2$ and $\dot{V}CO_2$ were continuously measured with a Hans-Rudolph adult face mask that was interfaced with the metabolic cart. Aliquots of each breath sample were trapped in an evacuated glass tube at 0, 5, 13, 27, 43, 50, 55, and 57 min of exercise for the subsequent determination of $^{13}CO_2$ enrichments (16). The average isotopic enrichment value for the last 20 min of exercise was used to calculate leucine and lysine kinetics during submaximal exercise.

After the 1 h of submaximal exercise, the subjects again rested in a supine position for 2 h. Every 30 min during these 2 h, expired air was analyzed for CO_2 enrichments. Also, respiratory calorimetry was measured and blood samples were obtained during recovery. Leucine and lysine kinetics were calculated for the last 30 min of this 2-h recovery.

Analytic methods. Plasma glucose was determined on a glucose analyzer (model 2, Beckman Instruments, Fullerton, CA) by using the glucose oxidase method. Plasma urea nitrogen was measured with a urea nitrogen analyzer by using the urease reaction (model 2, Beckman Instruments). Plasma free fatty acid (FFA) levels were determined according to Laurell and Tebbling (21). Total plasma protein concentration was measured by using refractometry (model SPR-T2, Atago). The percent increases in plasma protein concentration above resting levels were used to correct the FFA, glucose, and urea nitrogen concentrations for fluid-volume shifts that occurred with exercise (25). Twenty-four-hour urinary creatinine excretions were determined with a colorimetric assay (kit no. 555A, Sigma Diagnostics, St. Louis, MO).

The method of Adams (1) was used to perform the plasma derivatization procedure and the *n*-propyl *N*-acetyl ester was used for these quantitative analyses. The analytic methods that were used to determine the ^{13}C enrichments of leucine and expired $^{13}CO_2$ have been previously described (16, 18–20). Plasma leucine, lysine, and α-ketoisocaproate (KIC) enrichments were measured on a Hewlett-Packard model 5985A gas chromatograph-mass spectrometer with a selective ion-monitoring software package. Isotopic plasma KIC enrichment was measured with electron-impact ionization and selected ion monitoring of the *N*-methylquinoxalone derivative at mass-to-charge ratio (*m*/*e*) of 174/175. Selected ion monitoring was performed at *m*/*e* 216/217 for leucine and 273/274 for lysine.

Calculations and statistics. In a separate 7-h control experiment, we determined that our stable-isotope infusion procedures resulted in steady-state enrichments (data not shown). Enrichments for $^{13}CO_2$, [^{13}C]KIC, and [^{15}N]lysine are shown in Figs. 1, 2, and 3, respectively. Isotopic plateaus were

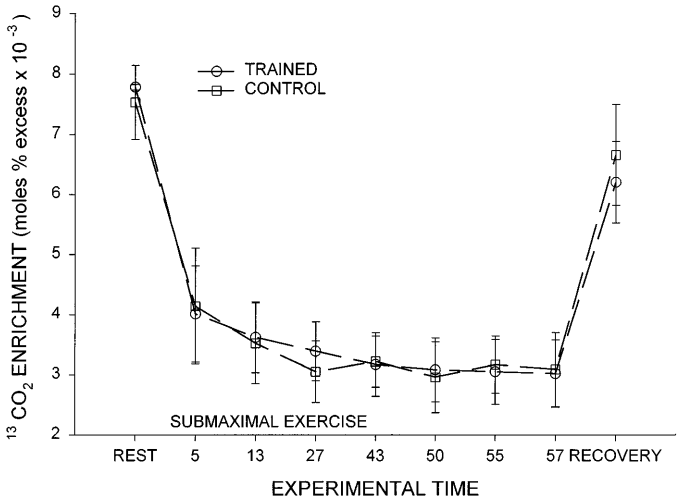

Fig. 1. Breath $^{13}CO_2$ enrichments during rest, exercise, and recovery in endurance-trained athletes and sedentary, nontrained controls. Values are means ± SE for rest and recovery. Time is in min.

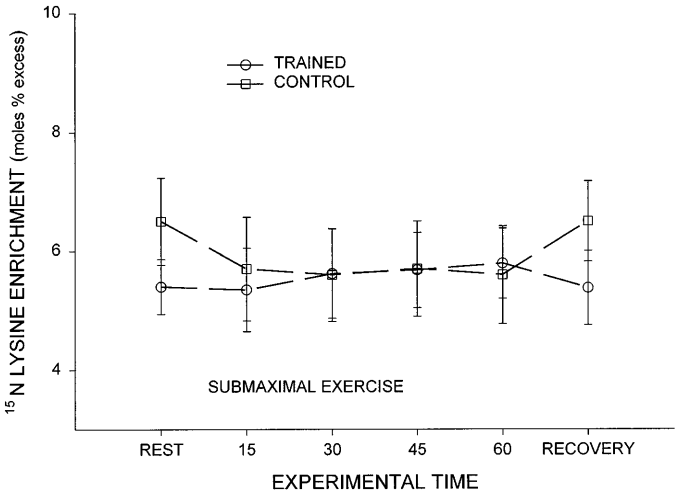

Fig. 3. Plasma [^{15}N]lysine enrichments during rest, exercise, and recovery in endurance-trained athletes and sedentary, nontrained controls. Values are means ± SE for rest and recovery. Time is in min.

observed for breath $^{13}CO_2$, plasma [^{13}C]leucine, [^{13}C]KIC, and plasma [^{15}N]lysine after 2.5 and 3 h of rest. In addition, isotopic plateaus were observed for $^{13}CO_2$, plasma [^{13}C]leucine, [^{13}C]KIC, and plasma [^{15}N]lysine in both the trained and control groups between *minutes 40* and *60* of exercise. Therefore, steady-state tracer kinetic equations were used (20). Plasma KIC enrichments and the reciprocal pool model was used for the calculations of leucine kinetics. Leucine kinetics were corrected for bicarbonate retention. The bicarbonate retention factors employed were 83.1% for rest and 98.9% for exercise in the trained athletes and 83.1% for rest and 96.6% for exercise in the control subjects (4).

Background enrichment values of expired $^{13}CO_2$ were obtained before each isotopic infusion procedure. These background values were subtracted from the isotopic plateau to calculate leucine oxidation. In a separate experiment we determined that the change in background enrichment of $^{13}CO_2$ due to submaximal exercise was extremely small (0.059%). This small change in background due to the submaximal exercise has been previously reported (4, 18, 31), and no calculation adjustments were made.

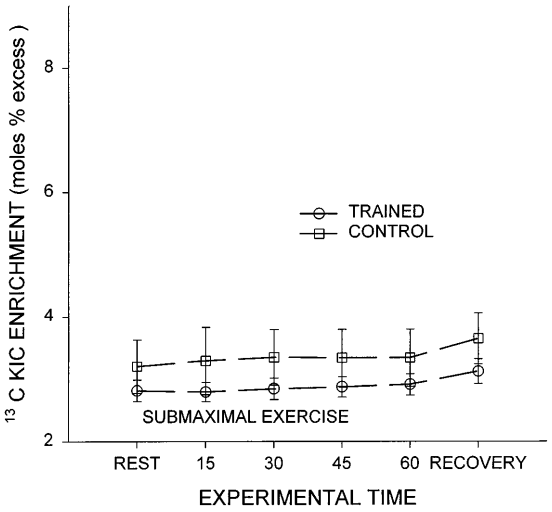

Fig. 2. Plasma [^{13}C]α-ketoisocaproate (KIC) enrichments during rest, exercise, and recovery in endurance-trained athletes and sedentary, nontrained controls. Values are means ± SE for rest and recovery. Time is in min.

Total body water and fat-free mass were determined with the $H_2{}^{18}O$ tracer-dilution data as previously described (22). An isotopic plateau for expired $C^{18}O_2$ was achieved within 3 h by using this technique (22). Body composition was computed using the assumption that water constitutes a fixed fraction (73.25%) of the fat-free body mass (22, 26, 27). Body compositions were also measured with bioelectrical impedance analysis (bioelectrical impedance analysis-obtained fat-free mass was Trn = 62.1 ± 5.8 kg and Con = 52.0 ± 4.0 kg).

When measures were taken repeatedly, such as plasma substrate determinations and RERs, repeated measures analyses of variance and Newman-Keuls post hoc tests were used to make between-group comparisons. The Student's *t*-test was used to compare differences in amino acid kinetics within and between pair-matched subjects. The statistical power for the between-group comparisons [ratio of leucine-to-lysine rate of appearance (R_a)] at an α level of 0.05 was 0.992. Correlations were determined by using the Pearson product-moment correlation. Throughout the text, the data are expressed as means ± SE. A probability value of < 0.05 was considered statistically significant.

RESULTS

Each endurance-trained subject was matched by age, gender, and body weight to a nontrained control subject (Table 1). As expected, the trained athletes had lower resting heart rates (Trn = 55.14 ± 6.2 vs. Con = 69.43 ± 6.2 beats/min; $P < 0.05$) and exercise heart rates ($P < 0.05$). Ratings of perceived exertion (Borg scale) were also lower throughout exercise in the trained compared with the nontrained subjects ($P < 0.05$). Although both groups exercised at the same percentage of $\dot{V}O_{2max}$, the endurance-trained athletes had a higher mean $\dot{V}O_2$ during exercise (Trn = 1.63 ± 0.18 l/min vs. Con = 1.30 ± 1.4 l/min; $P < 0.05$). Plasma substrate and RER data are displayed in Table 2. The RER was significantly lower during rest and exercise in the trained group. Plasma FFA concentrations were lower and plasma urea nitrogen concentrations were greater during rest, exercise, and recovery in the trained group.

Table 2. *Plasma substrate and respiratory exchange ratios for the Trn and Con groups*

	Trn	Con
	Respiratory exchange ratio	
Rest	0.74 ± 0.01*	0.83 ± 0.01
15 min	0.79 ± 0.01*	0.85 ± 0.01
30 min	0.82 ± 0.01*	0.84 ± 0.01
45 min	0.81 ± 0.01*	0.85 ± 0.01
60 min	0.81 ± 0.01*	0.84 ± 0.01
Recovery	0.73 ± 0.01	0.74 ± 0.01
	Plasma glucose, mM	
Rest	4.58 ± 0.16	4.84 ± 0.53
15 min	4.47 ± 0.14	4.74 ± 0.34
30 min	4.29 ± 0.11	4.65 ± 0.07
45 min	4.19 ± 0.09	4.52 ± 0.07
60 min	4.13 ± 0.09	4.39 ± 0.07
Recovery	4.33 ± 0.09	4.66 ± 0.03
	Plasma FFA, mM	
Rest	0.23 ± 0.12	0.24 ± 0.39
15 min	0.15 ± 0.20*	0.24 ± 0.02
30 min	0.16 ± 0.14*	0.23 ± 0.17
45 min	0.18 ± 0.15*	0.26 ± 0.16
60 min	0.21 ± 0.13*	0.29 ± 0.19
Recovery	0.22 ± 0.15*	0.33 ± 0.02
	Plasma urea nitrogen, mM	
Rest	11.37 ± 0.94*	8.58 ± 0.70
15 min	10.57 ± 0.68*	8.44 ± 0.43
30 min	9.98 ± 0.55*	7.94 ± 0.25
45 min	9.86 ± 0.45*	8.05 ± 0.28
60 min	10.29 ± 0.35*	7.90 ± 0.30
Recovery	11.29 ± 0.32*	8.58 ± 0.31

Values are means ± SE. *Significantly different from Con for comparable time periods ($P < 0.05$). FFA, free fatty acid.

Plasma glucose decreased with exercise, but the decrement was not significant and there were no differences between groups at any time points.

Leucine kinetics expressed relative to body weight. Leucine kinetics were calculated by using the reciprocal pool model (plasma KIC enrichments) and are shown in Table 3. The nontrained control subjects showed a significant decrease in leucine R_a during exercise and recovery in contrast with their resting R_a ($P < 0.01$). However, in the athletes there was no change in leucine R_a due to the stimulus of exercise (exercise vs. rest), but leucine R_a did decrease during recovery compared with rest ($P < 0.01$). A between-group comparison indicated that leucine R_a was greater during both exercise and recovery in the trained compared with the nontrained group ($P < 0.05$).

Leucine oxidation was increased during exercise above resting levels in both the trained and control subjects (Table 3, $P < 0.01$). Only the trained subjects had a decrease in leucine oxidation during recovery compared with rest ($P < 0.03$). The between-group comparisons showed that the trained subjects had a higher rate of leucine oxidation at all times (rest, exercise, and recovery) in contrast with their pair-matched sedentary controls ($P = 0.05$). Nonoxidative leucine disposal (nonoxidative leucine disposal = leucine R_a − leucine oxidation) was reduced during exercise in both groups (Table 3; $P < 0.01$). Only the nontrained control subjects had a nonoxidative leucine disposal that remained significantly reduced during the first few hours of recovery from exercise ($P < 0.01$). There were no between-group differences in nonoxidative leucine disposal.

Leucine kinetics corrected for fat-free body mass and $\dot{V}O_2$. Leucine kinetics corrected for fat-free body mass are shown in Table 4. When leucine R_a, oxidation, and nonoxidative leucine disposal rates were corrected for fat-free tissue mass, the exercise and recovery effects were similar to those found when leucine kinetics rates were expressed relative to body weight. However, when leucine R_a and oxidation, and nonoxidative leucine disposal were corrected for fat-free mass, there were no differences between groups (P = NS; Table 4). Also, the correlation between exercise leucine oxidation and whole body fat-free mass was significant ($R = 0.80$; $P < 0.001$; $n = 14$). Therefore, there was a physiological relationship between whole body leucine kinetics and the size of the fat-free tissue mass.

When the leucine oxidation rate during exercise was expressed relative to whole body $\dot{V}O_2$, leucine oxidation was not different between groups (Trn = 28.00 ± 9.21 vs. Con = 34.18 ± 13.02 μmol·kg body $wt^{-1}\cdot l\ O_2^{-1}$; P = NS). The correlation between mean $\dot{V}O_2$ and leucine oxidation during exercise was not significant ($R = 0.46$; $P = 0.09$; $n = 14$).

Table 3. *Leucine kinetics for both groups expressed relative to body weight*

	Leucine R_a	Leucine Oxidation	Nonoxidized Leucine Disposal
Trn			
Rest	157.0 ± 8.1	25.3 ± 2.2	131.6 ± 7.1
Exercise	154.4 ± 8.9	52.1 ± 6.5†	102.4 ± 10.9†
Recovery	144.0 ± 8.7†	19.2 ± 2.2*	124.8 ± 9.4
Con			
Rest	149.2 ± 6.8	18.3 ± 1.7‡	130.9 ± 6.2
Exercise	137.7 ± 5.9†‡	36.7 ± 5.2†‡	100.9 ± 8.5†
Recovery	125.6 ± 4.8†‡	13.9 ± 1.7‡	111.6 ± 5.0†

Values are means ± SE in μmol·kg body $wt^{-1}\cdot h^{-1}$. R_a, rate of appearance. *Significantly different from rest, $P < 0.05$. †Significantly different from rest, $P < 0.01$. ‡Significantly different between groups for comparable time periods, $P < 0.05$.

Table 4. *Leucine kinetics for both groups expressed relative to fat-free tissue mass*

	Leucine R_a	Leucine Oxidation	Nonoxidized Leucine Disposal
Trn			
Rest	175.0 ± 7.2	28.5 ± 2.8	146.4 ± 5.5
Exercise	172.5 ± 9.4	59.0 ± 8.2†	113.5 ± 10.8†
Recovery	160.5 ± 8.3†	22.0 ± 2.8*	138.8 ± 8.9
Con			
Rest	180.2 ± 7.8	22.3 ± 2.1	158.0 ± 6.7
Exercise	166.2 ± 6.2†	44.2 ± 6.0*	122.0 ± 10.4†
Recovery	151.7 ± 5.6†	16.7 ± 1.8	135.0 ± 6.1†

Values are means ± SE given in μmol·kg fat-free $mass^{-1}\cdot h^{-1}$. *Significantly different from rest, $P < 0.05$. †Significantly different from rest, $P < 0.01$. Between-group differences for leucine R_a, leucine oxidation, and nonoxidative leucine disposal were not statistically significant (P = NS).

Lysine kinetics. There was no difference between groups in lysine R_a at any experimental time point. Similarly, there was no change in lysine R_a with exercise regardless of aerobic training. However, both groups showed a decrease in lysine R_a during the initial few hours of recovery from exercise (Trn rest = 104.92 ± 5.11 and Trn recovery = 88.36 ± 5.30 $\mu mol \cdot kg^{-1} \cdot h^{-1}$; $P < 0.01$ vs. Con rest = 111.16 ± 7.47 and Con recovery = 94.49 ± 3.45 $\mu mol \cdot kg^{-1} \cdot h^{-1}$; $P < 0.007$).

DISCUSSION

The purpose of this investigation was to compare whole body leucine and lysine kinetics in endurance-trained and sedentary adults. Both groups were matched by gender, age, and body weight. Both groups also had similar body compositions, reflecting an equality in whole body protein mass. The similarity between groups in anatomic muscle and fat-free body mass is underscored because of a previous report that the protein pool size may alter the expression of whole body leucine turnover and oxidation in the resting human (20). The present data indicate that making comparisons between whole body amino acid kinetics in groups who differ in fat-free body mass may be problematic. With a few exceptions, most investigators express leucine kinetics relative to body weight and do not consider specific body compartments such as the skeletal muscle or fat-free tissue compartment. The present investigation supports the concept that correcting whole body leucine kinetics for fat-free tissue mass may be important when cross-sectional studies on humans are performed (20). It should be underscored that correcting leucine oxidation for fat-free mass equalized any between-group difference due to state of training. The elimination of an experimental effect when leucine oxidation was corrected for lean tissue mass (protein pool) may indicate that training effects on leucine metabolism are localized within skeletal muscle and are minimal when viewed at the whole body level.

An even more important finding may be that the rate of whole body leucine oxidation, when corrected for mean $\dot{V}O_2$ during exercise, was not different between these two groups. Isolated muscle studies of single hindlimbs of rats have demonstrated small reductions in leucine oxidation relative to $\dot{V}O_2$ in trained compared with nontrained animals (15). This animal study, however, employed a prospective experimental design and a 9-wk exercise training protocol. The present data are the first to indicate that there was no difference between whole body leucine oxidation when endurance-trained and sedentary humans are exercising at the same relative intensity and are studied in a cross-sectional manner.

In the present study, the endurance-trained athletes had an accelerated leucine R_a (whole body proteolysis) compared with their nontrained counterparts. Submaximal exercise in these athletes provided less perturbation of whole body proteolysis (expressed relative to body weight) when leucine was the amino acid marker used and must be reconciled with the fact that there were no between-group differences in lysine R_a. As others have indicated, the response of leucine and lysine kinetics during exercise may be disparate (18, 32). However, it seems unlikely that leucine was selectively hydrolyzed from protein pools due to the stimulus of exercise. The discrepant R_a for these two essential amino acids may be due to a greater proteolytic rate occurring in those proteins that contain relatively more leucine than lysine. Many different protein pools contribute to the increased proteolysis with exercise (3, 6, 17), and the proteolytic mechanisms involved are still unknown (17). One recent study indicates that nonmyofibrillar protein proteolysis may be caused by an enhanced flux rate through lysosomal pathways (17). However, studies of endurance-training effects on proteolytic enzyme activity are conflicting (23, 28).

Others have reported that the leucine R_a decreases or remains unchanged and that leucine oxidation increases with prolonged exercise (20, 24, 29, 30, 32). Previous studies also indicate that lysine R_a remains unchanged or decreases with submaximal exercise (20, 32). The present study extends those findings and suggests that the underlying state of aerobic conditioning may influence the response of leucine kinetics expressed relative to body weight during aerobic exercise. In fact, the disparity between experimental outcomes in past research may actually reflect the variability in aerobic capacity of the study participants.

The present data collected during exercise recovery are consistent with past research in this area. Whole body leucine oxidation, leucine R_a, and lysine R_a expressed relative to body weight have been reported to decrease during the initial few hours of exercise recovery (5, 18). However, these previous studies did not examine the effects of endurance-training on leucine kinetics during recovery. The present study indicates that the state of aerobic conditioning can effect the fate of leucine during the immediate hours after endurance exercise. Specifically, leucine R_a and oxidation (relative to body weight) are greater in the recovering endurance-trained athlete.

In conclusion, when leucine kinetics were expressed relative to body weight, prolonged submaximal exercise increased the rate of leucine oxidation while simultaneously decreasing nonoxidative leucine disposal in both endurance-trained athletes and sedentary humans. Also, when the data were expressed relative to total body weight, endurance training was associated with an increased leucine oxidation at all times and a heightened leucine R_a and hence proteolysis during exercise and recovery. However, when leucine kinetics were expressed relative to fat-free mass, these intergroup differences were eliminated. Therefore, whole body leucine kinetics are similar in trained and sedentary individuals when expressed relative to fat-free tissue mass. These data indicate that correcting whole body leucine kinetics for fat-free mass may be important when cross-sectional studies on humans are performed. Finally, whole body leucine oxidation relative to metabolic rate was found to be the same in exercising, endurance-trained athletes and sedentary controls. Therefore, it is concluded that there was no

difference in whole body leucine kinetics between endurance-trained and sedentary humans when exercising at the same relative intensity.

The authors thank Karen Rossi, Rainbow Babies and Childrens Hospital, Anne Conrad, MetroHealth Medical Center, and the nurses of the General Clinical Research Center, University Hospitals (Cleveland, OH) for expert assistance. Dr. Claudia Villabona performed the gas chromatography-mass spectometry and other laboratory analyses. Rochelle A. Romito performed all the dietary planning and nutritional counseling for these experiments.

This study was supported by American Heart Association, Dallas Affiliate, Grant-in-Aid AHA 91007450 (to L. S. Lamont) and by National Institutes of Health Grants (GCRC RR-00080 and HD-11089).

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Received 6 April 1998; accepted in final form 3 September 1998.

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