Relationship between leucine oxidation and oxygen consumption during steady-state exercise

LINDA S. LAMONT, ARTHUR J. McCULLOUGH, and SATISH C. KALHAN

Exercise Science Program, the University of Rhode Island, Kingston, RI 02881; and The Schwartz Center for Metabolism and Nutrition, Case-Western Reserve University School of Medicine, Cleveland, OH 44109

ABSTRACT

LAMONT, L. S., A. J. McCULLOUGH, and S. C. KALHAN. Relationship between leucine oxidation and oxygen consumption during steady-state exercise. *Med. Sci. Sports Exerc.*, Vol. 33, No. 2, pp. 237–241, 2001. **Purpose:** The purpose of this study was to assess the relationship between whole-body leucine oxidation and oxygen consumption during steady-state exercise. Our hypothesis was that leucine oxidation will be responsive to increased whole-body energy needs. **Methods:** Sixteen healthy individuals (7 women and 9 men) were infused with a stable isotope of leucine and, for comparison purposes, lysine during 60 min of moderate-intensity exercise. **Results:** Leucine oxidation was increased ($P < 0.05$) and nonoxidative leucine disposal was decreased ($P < 0.05$), whereas leucine and lysine rate of appearance remained unchanged (P = NS) during exercise. Linear regression analysis indicated a modest relationship between leucine oxidation and steady-state oxygen consumption ($R = 0.69$; $P < 0.003$) during steady-state exercise. The coefficient of determination ($R^2 = 0.49$) indicates that approximately half of the variance in whole-body leucine oxidation during exercise can be explained by whole-body oxygen consumption. **Conclusion:** In a statistically appropriate sample size of humans whose dietary intake was controlled, the whole-body rate of leucine oxidation during exercise was only partially influenced by energy demands. **Key Words:** L-[1-^{13} C] LEUCINE, L-[α-^{15} N]LYSINE, MODERATE INTENSITY EXERCISE, METABOLIC RATE

Although there are many human studies of leucine oxidation during steady-state exercise (4,7,12,17,19,26,27), only one used a sample size larger than four to six subjects (7). These small sample sizes do not allow for an examination of the relationship between metabolic rate and oxidation of leucine. Furthermore, the relationship between leucine oxidation and oxygen consumption during exercise has been determined in laboratory animals (9,10), but in humans only one study is available (17). These human data should be considered preliminary because of the small sample size ($N = 4$). Statistical power analysis reveals that a minimal sample size of 14 would be needed to find a relationship between leucine oxidation and oxygen consumption (R of 0.70; power of 0.80; alpha of 0.05). The purpose of the present study was to examine the relationship between whole-body leucine kinetics and steady-state oxygen consumption with a statistically appropriate sample size. Lysine kinetics were also studied for comparison purposes because lysine cannot be transaminated by skeletal muscle. Our hypothesis was that there will be a linear relationship between leucine oxidation and oxygen consumption during steady-state exercise.

0195-9131/01/3302-0237/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Submitted for publication January 2000.
Accepted for publication May 2000.

METHODS

Subjects. We recruited 16 individuals for this experiment (7 women and 9 men). The physical characteristics of this group are located in Table 1. These subjects had varying exercise habits, ranged from young to middle-aged, and had varying body compositions. Each participant was medically screened, and all had normal electrocardiograms and were without a family or personal history of diabetes mellitus. Our Investigational Review Board approved this project and requested a written informed consent from each subject before his or her participation in this experiment.

Experimental protocol. A registered dietitian used a dietary exchange procedure to design a weekly meal plan for each of our subjects (6). These meal plans were designed to be weight maintaining and employed the Harris and Benedict equation to determine daily caloric needs (8). Each diet had a standard composition of 58–60% carbohydrate, 30% fat, and 10–12% protein. Any increase in daily energy expenditure due to exercise training was computed for those subjects who regularly exercised. The increase in energy expenditures due to exercise were determined by standard metabolic equations (1) and were added to the resting energy requirements to compute daily caloric needs. Subjects who regularly exercised were instructed to refrain from physical activity on days 5 and 6 of the experiment to avoid an acute exercise recovery effect on leucine and lysine kinetics. Tracer infusions of the stable-isotopes of leucine and lysine were performed after 7 d of dietary equilibrium.

TABLE 1. Physical characteristics of the sample group.

	Age (yr)	Body Weight (kg)	$\dot{V}O_{2max}$ mL·kg/min	Fat-Free Mass (kg)	% Body Fat (%)
Mean ± SEM	30.5 ± 2.1	68.9 ± 2.8	46.3 ± 2.4	59.5 ± 3.0	14.56 ± 1.69
Range	19–46	51.6–89.2	29.8–62.4	41.4–80.1	5–28

A progressive, incremental cycle ergometer protocol was used to determine maximal oxygen consumption ($\dot{V}O_{2max}$) and was performed on days 3 or 4 of the experiment. The metabolic cart (Sensor Medics Model 2900,Yorba Linda, CA) was calibrated before each exercise test with standard gas mixtures that were previously verified by the Scholander technique (22). $\dot{V}O_{2max}$ was assumed if there was a plateau in oxygen uptake and/or a respiratory exchange ratio of greater than one at maximal workloads.

Tracer infusion studies. Subjects reported to the Clinical Research Center in a postabsorptive state (~15-h) on the morning of day 7. A nurse placed two intravenous cannulae into superficial hand veins, one in each hand. One cannula was used for the tracer infusions of: L- [1-^{13}C] leucine (99 atom % excess of ^{13}C), and L- [α-^{15}N] lysine (99 atom % excess of ^{15}N), and sodium bicarbonate NaH [^{13}C] O_3 (99 atom % excess of ^{13}C). These isotopes were purchased from Merck, Inc. of Dorval, Canada. We weighed and dissolved the tracers in normal saline and then sterilized this solution by micro-filtration (0.22 μm Millipore filter). The tracers were tested for sterility and pyrogenicity before their infusion. The second intravenous cannula was used for collecting blood samples and was kept patent with an isotopic saline infusion (10 mL·h^{-1}). Priming doses were administered to reach an early steady-state and were: 1.2 μmol/kg of NaH$^{13}CO_3$, 4.0 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 infusion of L- [1-^{13}C] leucine at a rate of 5.0 μmol·kg^{-1}·h^{-1} and of L- [α -^{15}N] lysine at a rate of 7.0 μmol·kg^{-1}·h^{-1}. We obtained a background sample of expired air and venous blood from each subject before beginning the infusion. A weighed amount of labeled water ($H_2{}^{18}O$; 99 atom % ^{18}O excess; MSD Isotopes) was orally given to determine total body water (18).

Tracer infusion during rest. The first 3 h of the primed, constant-rate infusion were used to obtain an isotopic plateau so that leucine and lysine kinetics during rest could be determined. During these 3 h, 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 using a Hans-Rudolph (Kansas City, MO), one-way nonrebreathing valve connected to a 5-L anesthesia bag. An aliquot of each breath sample was trapped in an evacuated glass tube for the subsequent analysis of $^{13}CO_2$. Carbon dioxide production (VCO_2) and oxygen consumption ($\dot{V}O_2$) were determined throughout the 3 h of rest. The average isotopic enrichment during the 3rd h was used to calculate leucine and lysine kinetics during rest.

Tracer infusion during exercise. At the beginning of the 4th h, the subjects began to exercise at 50% of their $\dot{V}O_{2max}$ using a constant-load pan weight Monark cycle ergometer (Varberg, Sweden). Blood samples were withdrawn at 0, 15, 30, 45, 50, 55, and 60-min of exercise. We continuously determined both $\dot{V}O_2$ and $\dot{V}CO_2$ using a Hans-Rudolph adult face mask interfaced with the metabolic cart. In addition, 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 $^{13}CO_2$ enrichment. The average isotopic enrichment for the last 20 min of exercise was used to calculate leucine and lysine kinetics during exercise.

Analytical methods. We determined plasma urea nitrogen concentration with a urea nitrogen analyzer (Beckman Instruments, Model 2, Fullerton, CA) using the urease reaction (16). Plasma glucose was determined on a glucose analyzer (Beckman Instruments, Model 2). Plasma free fatty acid (FFA) levels were determined according to Laurell and Tebbling (15). Total plasma protein concentration was measured with refractometry (model SPR-T2, Atago). The percent increase in plasma protein concentration above rest was used to correct the FFA and urea nitrogen concentrations for fluid volume shifts that occurred with exercise (20).

The method of Adams was used to perform the plasma derivatization procedure, and the n-propyl N-acetyl ester was used for these quantitative analyses (2). The analytical methods that were used to determine (13) C α-ketoisocaproate and expired $^{13}CO_2$ enrichments have been described elsewhere (13,14,18). We measured plasma α-ketoisocaproate (α-KIC) and lysine enrichments on a Hewlett-Packard model 5985A gas chromatograph-mass spectrometer with selective ion-monitoring software. Selected ion monitoring was performed at a mass-to-charge ratio (m/z) of 273/274 for lysine and m/z of 174/175 for α-KIC. Expired CO_2 was separated from the breath samples by cryogenic distillation, and the $^{13}C/^{12}C$ ratio was measured on an isotope ratio mass spectrometer.

We obtained background enrichments of expired $^{13}CO_2$ before each isotopic infusion procedure. This background enrichment was subtracted from the isotopic plateau value in the calculations of leucine kinetics. We used bicarbonate retention factors of 83.1% for rest and 98.9% for exercise in those subjects who regularly exercised and 83.1% for rest and 96.6% for exercise in those individuals who were identified as sedentary (5).

The calculation of total body water and FFM were determined using labeled water with an $H_2[^{18}O]$ tracer dilution method described previously described (18). An isotopic plateau for expired C [$^{18}O_2$] was achieved within 3 h when using this tracer dilution technique (18,21). Body composition was calculated under the assumption that water constitutes a fixed fraction (73.25%) of the fat-free body mass (21,23).

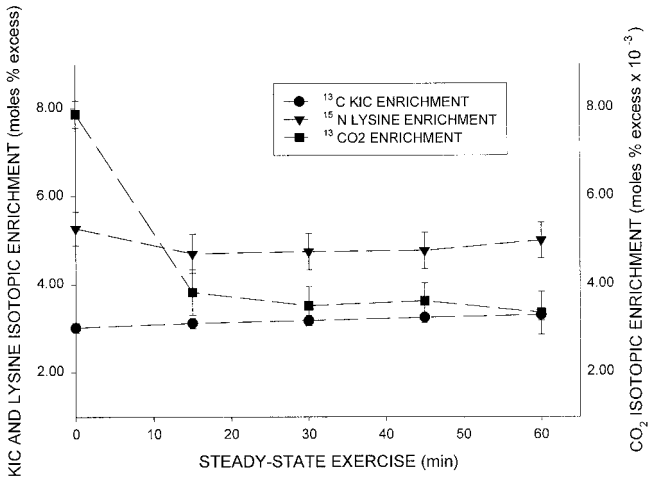

Figure 1—Breath CO_2 and plasma leucine and lysine enrichments during rest and steady-state exercise. KIC and lysine enrichments are graphed as moles % excess, whereas CO_2 enrichment is graphed as moles % excess × 10^{-3}.

Calculations and statistics. Steady-state tracer kinetic equations were used to calculate leucine and lysine kinetics. The reciprocal pool model was used in the calculation of leucine kinetics.

Repeated measures analyses of variance with Newman-Keuls *post hoc* tests and Student's *t*-tests were employed for these data analyses. Pearson-product moment correlations and Kolmogorov-Smirnov normality tests were also performed. The coefficient of determination (R^2) was also calculated. Throughout this text, the data are expressed as mean ± standard error of the mean (SE). A probability value of $P < 0.05$ was considered statistically significant.

RESULTS

Isotopic steady-state. Labeled carbon dioxide, α-KIC, and lysine exhibited isotopic plateaus between 2.5 and 3 h of supine rest. We observed isotopic plateaus for labeled carbon dioxide, α-KIC, and lysine between 40 and 60 min of steady-state exercise (see Fig. 1 for graphs of isotopic enrichments during exercise).

Steady-state oxygen consumption. Table 2 lists the expired gas values during rest and exercise. Oxygen consumption remained unchanged throughout exercise (P = NS). The respiratory exchange ratio increased significantly during this exercise bout (Table 2; $P < 0.05$).

Plasma substrate concentrations. Figure 2 indicates that there were significant decreases in plasma urea nitrogen and glucose during this exercise ($P < 0.05$). FFA concentrations showed an initial decline with exercise ($P < 0.05$) and a gradual increase toward the end of the hour of exercise (45 and 60 min).

TABLE 2. Expired gas values during rest and exercise.

	Oxygen Consumption ($L \cdot h^{-1}$)	Respiratory Exchange Ratio
Rest	0.28 ± 0.36	0.79 ± 0.019
15 min	1.29 ± 0.98	0.84 ± 0.009*
30 min	1.30 ± 0.11	0.84 ± 0.010*
45 min	1.34 ± 0.10	0.83 ± 0.011*
60 min	1.43 ± 0.48	0.84 ± 0.001*

* Value significantly different from rest ($P < 0.05$).

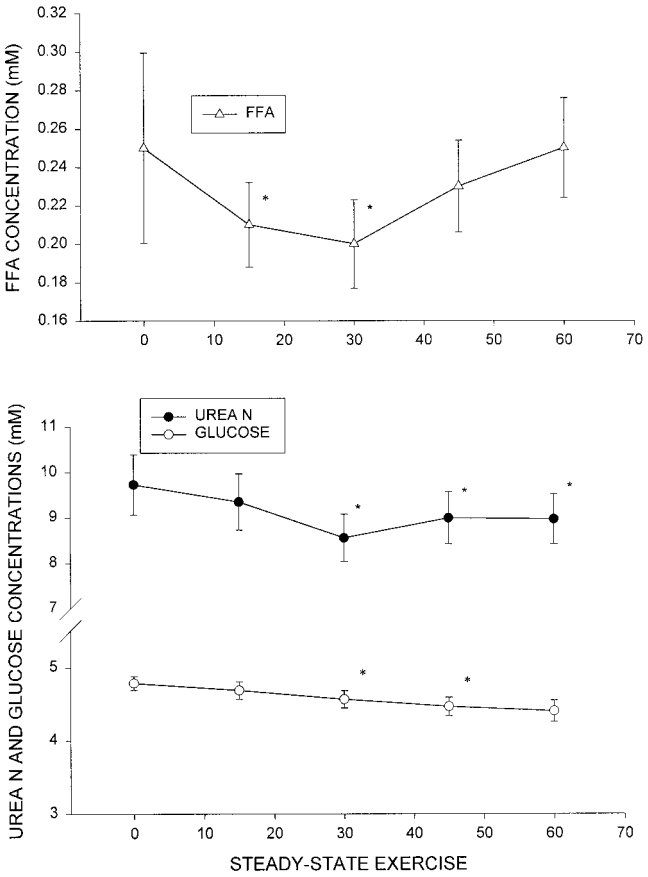

Figure 2—Urea nitrogen, glucose, and FFA concentrations during rest and steady-state exercise. Values are means ± SE * Exercise values are significantly different from rest ($P < 0.05$).

Leucine and lysine kinetics. The leucine kinetic data expressed per kilogram of fat-free mass are presented in Figure 3. There was a significant increase in leucine oxidation ($P < 0.05$) and a significant decrease in nonoxidative leucine disposal during exercise ($P < 0.05$). Leucine rate of appearance did not change from rest to exercise. Furthermore, lysine rate of appearance remained unchanged from rest to exercise (lysine Ra rest = 108.07 ± 5.33 vs lysine Ra exercise = 106.37 ± 4.26 $\mu mol \cdot kg^{-1} \cdot h^{-1}$; P = NS).

Relationship between leucine kinetics and metabolic rate. Figure 4 shows the relationship between whole-body leucine oxidation and oxygen consumption during steady-state exercise ($R = 0.69$; $N = 16$, $P < 0.003$). The power of this relationship at an alpha of 0.05 was computed to be 0.87. Both leucine oxidation and oxygen consumption during exercise passed the Kolomogorov-Smirnov normality test, indicating that these data were drawn from a normally distributed population. The coefficient of determination between these two variables (R^2) was 0.48. Linear regression analysis indicated that the rate of whole-body leucine oxidation could be predicted with the following formula: leucine $oxidation_{exercise}$ ($\mu mol \cdot h^{-1}$) = $10.75 + (0.39 \times \dot{V}O_2)$; where $\dot{V}O_2$ is in $L \cdot h^{-1}$. Correlations

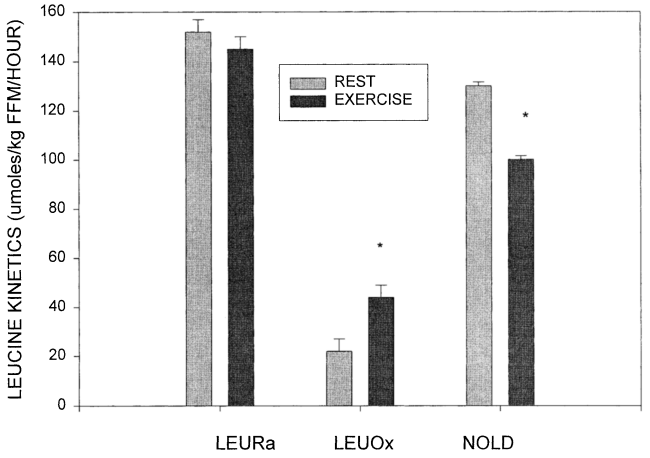

Figure 3—Leucine kinetics during rest and steady-state exercise. LEURa, leucine rate of appearance; LEUOx, leucine oxidation; NOLD, nonoxidative leucine disposal. Values are means ± SE * Exercise values are significantly different from rest ($P < 0.05$).

between leucine rate of appearance and nonoxidative leucine disposal with oxygen consumption were not statistically significant [(leucine rate of appearance vs oxygen consumption; R = 0.10, $P = 0.24$) and (nonoxidative leucine disposal vs oxygen consumption; R = 0.42, $P = 0.11$)]. The correlation between lysine rate of appearance and oxygen consumption was also not significant (lysine rate of appearance versus oxygen consumption; R = 0.34, $P = 0.20$).

DISCUSSION

The purpose of this investigation was to determine the relationship between whole-body leucine oxidation and steady-state oxygen consumption during prolonged exercise in a statistically appropriate sample size of humans. Our sample had generalized physical characteristics and exercise habits and the statistical analyses indicated that the source population for this sample was normally distributed. In addition, we controlled for the pre-experimental diet of our subjects and had adequate statistical power to examine the relationship between metabolic rate and leucine oxidation.

We found that the rate of leucine oxidation (decarboxylation) almost doubled from rest to moderate intensity, steady-state exercise in this sample. The magnitude of this leucine oxidation increase was slightly less but consistent with all of the previous studies of leucine metabolism during endurance exercise (7,12–14,17,26,27). Wolfe and colleagues (26) reported a 3.7-fold increase in leucine oxidation, Hagg et al. (7) reported a 4.8-fold increase, whereas Millward et al.(17) found a 3.1-fold increase in leucine oxidation with prolonged exercise. These three studies used exercise intensities of 30–50% of $\dot{V}O_{2max}$. However, a more recent study reported findings that directly concur with ours: a two-fold increase in leucine oxidation that was accompanied by a two-fold increase in fractional activation of skeletal muscle branched-chain keto acid dehydrogenase (3). The exercise intensity used in this study by Bowtell et al. (3) was 60% of maximum. The above-reported differences in the rate of leucine oxidation during exercise may relate to the variation in exercise intensities used in these experiments [between 30 and 60%] (3,7,12,17,26,27). Although these studies found that exercise caused a two- to four-fold increase in leucine oxidation, leucine's contribution to the exercise induced increase in energy expenditure has been found to be small (17). In contrast to the increased leucine oxidation, leucine rate of appearance remained unaltered from rest to steady-state exercise. Our lysine tracer results were consistent with those provided by the leucine tracer, in that lysine rate of appearance remained unchanged from rest to exercise. Wolfe et al. (26) found that if [^{13}C] leucine was used as an isotopic tracer, the rate of appearance did not change from rest to exercise. But when [^{15}N] leucine was infused as the tracer, an increased rate of appearance of leucine was found during exercise (26,27). Our sample had a decrement in nonoxidative leucine disposal, a finding that is consistent with other studies (3,12,14) and the concept that leucine incorporation into proteins is reduced during exercise (a reduction in whole-body protein synthesis).

The relationship between leucine oxidation and oxygen consumption during this steady-state exercise appears to be modest and consistent with two previous animal studies (9,10). However, the only available human study (17) reported a stronger relationship (R = 0.99) between leucine oxidation and oxygen consumption. It should be underscored that the exercise duration used in this previous human study (30 min) may not have allowed for an isotopic steady state and the sample size was smaller than needed for adequate statistical power. In addition, no mention was made in this study of a pre-experimental control for the subjects dietary intake (17). On the other hand, the previous animal data are consistent with ours ($R_{untrained\ rats} = \sim 0.70$ and $R_{trained\ rats} = \sim 0.81$ between leucine oxidation and $\dot{V}O_2$; see refs. 9,10). We found that approximately half of the variance in leucine oxidation can be explained by a change in whole-body oxygen consumption. Therefore, our hypothesis that leucine oxidation will be responsive to an increased whole-body energy demand in the human was accepted. However, the remaining half of the variance in

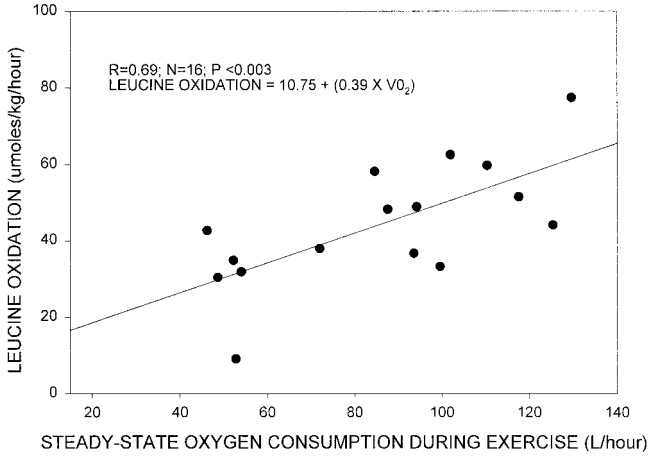

Figure 4—Statistical relationship between leucine oxidation and oxygen consumption during steady-state exercise.

leucine oxidation must be explained by other regulatory factors. Some of the other known regulators of leucine oxidation are carbohydrate availability (24,25), the NADH/NAD ratio, the acyl-CoA/CoA ratio, ketoacid availability (11), and the extent of β-adrenergic stimulation (14).

In conclusion, there was no effect of moderate intensity, steady-state exercise on leucine or lysine rate of appearance. But leucine oxidation was increased by two-fold, and non-oxidative leucine disposal was reduced during steady-state exercise. Approximately half of the variance in leucine oxidation during exercise can be explained by whole-body oxygen consumption. Therefore, the rate of leucine oxidation in the exercising human was only partially influence by whole-body energy demands.

This study was supported by the following grants: AHA91007450 from the American Heart Association, Dallas; GCRC RR00080 from the National Institutes of Health; and HD11089 from the National Institutes of Health.

Address for correspondence: Linda S. Lamont, Ph.D., 25 West Independence Way, Kingston, RI 02881; E-mail: lla4983u@postoffice.uri.edu.

REFERENCES

1. American College of Sports Medicine. *Guidelines for Exercise Testing and Prescription*, 5th Ed., Baltimore: Williams & Wilkins, 1995, pp. 269–287.
2. Adams, R. F. Determination of amino acid profiles in biological samples by gas chromatography. *J. Chromatogr*. 95:189–212, 1974.
3. Bowtell, J. L., G. P. Leese, K. Smith, et al. Modulation of whole body protein metabolism, during and after exercise, by variation of dietary protein. *J. Appl. Physiol.* 85:1744–1752, 1998.
4. Carraro, F., W. G. Hartl, C. A. Stuart, D. K. Layman, F. Jahoor, and R. R. Wolfe. Whole body and plasma protein synthesis in exercise and recovery in human subjects. *Am. J. Physiol.* 258:E821–E831, 1990.
5. Coggan, A. R., D. L. Habash, L. A. Mendenhall, S. C. Swanson, and C. L. Kien. Isotopic estimation of CO_2 production during exercise before and after endurance training. *J. Appl. Physiol.* 75:70–75, 1993.
6. Franz, M. J., P. Bar, H. Holler, M. A. Powers, M. L. Wheeler, and J. Wylie-Rosett. Exchange lists: revised 1986. *J. Am. Diet. Assoc.* 87:28–40, 1987.
7. Hagg, S. A., E. L. Morse, and S. A. Adibi. Effect of exercise on rats of oxidation, turnover, and plasma clearance of leucine in human subjects. *Am. J. Physiol.* 242:E407–E410, 1982.
8. Harris, J. A., and F. G. Benedict. A biometric study of basal metabolism in man. Washington, DC: Carnegie Institute, 1919 (Pub. No. 279).
9. Henderson, S. A., A. L. Black, and G. A. Brooks. Leucine turnover and oxidation in trained rats during exercise. *Am. J. Physiol.* 249(*Endocrinol. Metab*.12): E137–E144, 1985.
10. Hood, D. A., and R. L. Terjung. Effect of endurance training on leucine metabolism in perfused rat skeletal muscle. *Am. J. Physiol.* 253(*Endocrinol. Metab*.16): E648–E656, 1987.
11. Hood, D. A., and R. L. Terjung. Effect of α ketoacid dehydrogenase phosphorylation on branched-chain amino acid metabolism in muscle. *Am. J. Physiol.* 261(*Endocrinol. Metab.* 24): E628–E634, 1991.
12. Knapik, J, C. Meredith, B. Jones, R. Fielding, V. Young, and W. Evans. Leucine metabolism during fasting and exercise. *J. Appl. Physiol.* 70:43–47, 1991.
13. Lamont, L. S., D. G. Patel, and S. C. Kalhan. Leucine kinetics in endurance-trained humans. *J. Appl. Physiol.* 69:1–6, 1990.
14. Lamont, L. S., A. J. McCullough, and S. C. Kalhan. β-Adrenergic blockade heightens the exercise-induced increase in leucine oxidation. *Am. J. Physiol.* 268(*Endocrinol. Metab.* 31): E910–E916, 1995.
15. Laurell, S., and G. Tebbling. Colorimetric microdetermination of free fatty acids in plasma. *Clin. Chim. Acta* 16:57–62, 1967.
16. Marsh, W. H., B. Fingerhut, and H. Miller. Automated and manual direct methods for the determination of blood urea. *Clin. Chem.* 11:624–627, 1965.
17. Millward, D. J., C. T. M. Davies, D. Halliday, S. L. Wolman, D. W. Matthews, and M. J. Rennie. Effect of exercise on protein metabolism in humans as explored with stable isotopes. *Fed. Proc.* 21:2686–2691, 1982.
18. Mullen, K. D., S. C. Denne, A. J. McCullough, et al. Leucine metabolism in stable cirrhosis. *Hepatology* 6:622–630, 1986.
19. Rennie, M. J., R. H. T. Edwards, S. Krywawuch, et al. Effect of exercise on protein turnover in man. *Clin. Sci. Lond.* 65:217–225, 1983.
20. Schlein, E. M., D. Jensen, and J. P. Knochel. Effect of plasma water loss on assessment of muscle metabolism during exercise. *J. Appl. Physiol.* 34:568–572, 1973.
21. Schoeller, D. A., E. van Santene, D. W. Peterson, W. Deitz, J. Jaspan, and P. D. Klein. Total body water measurement in humans with ^{18}O- and ^{2}H- labeled water. *Am. J. Clin. Nutr.* 33:2686–2693, 1980.
22. Scholander, P. F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. Biol. Chem.* 167: 235–250, 1947.
23. Sheng, H. P., and R. A. Huggins. A review of body composition studies with emphasis on total body water and fat. *Am. J. Clin. Nutr.* 32:630–647, 1979.
24. Wagenmakers, A. J. M, J. H. Brookes, J. H. Coakley, T. Reilly, and R. H. T. Edwards. Exercise-induced activation of the branched-chain 2-oxy acid dehydrogenase in human muscle. *Eur. J. Appl. Physiol.* 59:159–167, 1989.
25. Wagenmakers, A. J. M., E. J. Beckers, F. Brouns, et al. Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise. *Am. J. Physiol.* 260(*Endocrinol. Metab.* 23):E883–E890, 1991.
26. Wolfe, R. R., R. D. Goodenough, M. H. Wolfe, G. T. Royle, and E. R. Nadel. Isotopic analysis of leucine and urea metabolism in exercising humans. *J. Appl. Physiol.* 52:458–466, 1982.
27. Wolfe, R. R., M. H. Wolfe, E. R. Nadel, and J. H. F. Shaw. Isotopic determination of amino acid-urea interactions in exercise in humans. *J. Appl. Physiol.* 56:221–229, 1984.