Isotopic determination of amino acid-urea interactions in exercise in humans

ROBERT R. WOLFE, MARTA H. WOLFE, ETHAN R. NADEL, AND JAMES H. F. SHAW
Department of Surgery, Harvard Medical School and Shriners Burns Institute, Boston, Massachusetts 02114; Shriners Burns Institute, Galveston, Texas 77550; and the John B. Pierce Foundation Laboratory and Yale University, New Haven, Connecticut 06519

WOLFE, ROBERT R., MARTA H. WOLFE, ETHAN R. NADEL, AND JAMES H. F. SHAW. *Isotopic determination of amino acid-urea interactions in exercise in humans.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 56(1): 221–229, 1984.— We recently reported that in light exercise (30% $\dot{V}O_{2max}$) the oxidation of [1-^{13}C]leucine was significantly increased but the rate of urea production was unchanged (*J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 52: 458–466, 1982). We have therefore tested three possible explanations for this apparent incongruity. *1*) We infused $NaH^{13}CO_3$ throughout rest and exercise and found that, although altered bicarbonate kinetics in exercise resulted in greater recovery of $^{13}CO_2$, the difference between rest and recovery was small compared with the increase in the rate of $^{13}CO_2$ excretion during exercise when [1-^{13}C]leucine was infused. *2*) We infused [^{15}N]leucine and isolated plasma urea N to determine directly the rate of incorporation of the ^{15}N. During exercise there was no increase in the rate of ^{15}N incorporation. Simultaneously, we infused [2,3-^{13}C]alanine and quantified the rate of incorporation of ^{15}N in alanine. We found that [^{15}N]alanine production from [^{15}N]leucine more than doubled in exercise, and by deduction, alanine production from other amino acids also doubled. *3*) We tested our previous assumption that [1-^{13}C]leucine metabolism in exercise was representative of the metabolism of other essential amino acids by infusing [1-^{13}C] and [α-^{15}N]lysine throughout rest and exercise. We found that the rate of breakdown of lysine during exercise was not increased in a manner comparable to that of leucine. Thus, these data confirm our original findings that leucine decarboxylation is enhanced in light exercise but urea production is unchanged. It appears that, in exercise, leucine metabolism is not representative of the metabolism of all essential amino acids and a model of whole-body protein synthesis and catabolism based on that assumption is not valid.

stable isotopes; mass spectrometry; lysine, leucine; alanine; urea; bicarbonate recovery

THE CONCEPT that the increased energy demands during exercise are not met even in part by the oxidation of essential amino acids dates to 1900 (27). However, the results of recent studies involving the indirect estimation of urea production in exercise (17) as well as the determination of amino acid flux (2) suggest that protein catabolism and amino acid oxidation may be accelerated in exercise. For this reason we recently used stable isotope tracer techniques to determine the rate of leucine oxidation and urea production during light exercise [30% maximal O_2 consumption ($\dot{V}O_{2max}$)] to assess the effect of exercise on net protein catabolism (25). We found that, although urea production did not change during exercise, there was a 400% increase above the resting level in the rate of leucine oxidation. These results would seem to be incompatible in that the urea data lead us to conclude that there was no increase in net protein catabolism in exercise, whereas the leucine data suggested a marked increase in net protein catabolism (4). The present series of studies were performed in an attempt to resolve this apparent paradox.

One possible explanation for the discrepancy between the leucine and urea data is that we overestimated the rate of leucine oxidation due to unrecognized alterations in the bicarbonate pool dynamics during exercise. In this study we have therefore infused $NaH^{13}CO_3$ in rest and throughout exercise and determined the percent of infused ^{13}C recovered in expired $^{13}CO_2$. A second possibility is that we previously assumed that leucine metabolism was representative of the metabolism of all essential amino acids, and this assumption may not hold in exercise. Therefore, we have determined the rates of oxidation of [1-^{13}C]lysine and deamination of [α-^{15}N]lysine at rest and during exercise. Third, it is possible that the gas chromatograph mass spectrometry technique we used to measure urea enrichment during the infusion of [^{15}N]$_2$urea (23) was not sensitive enough to detect an increased rate of incorporation of N resulting from leucine deamination. We have consequently infused [^{15}N]leucine in a series of studies and determined the rate of incorporation of the ^{15}N into plasma urea by means of a sensitive analysis involving the use of an isotope-ratio mass spectrometer. In addition, we infused [2,3-^{13}C]alanine simultaneously with the [^{15}N]leucine and measured the ^{15}N enrichment as well as the ^{13}C enrichment of alanine. This enabled us to quantify the rate of transfer of leucine N to alanine in rest and exercise. Finally, we have combined the [^{15}N]leucine data with the [1-^{13}C]leucine data obtained previously in the same subjects (23) and calculated the rate of transamination-reamination reactions between leucine and α-ketoisocaproate (α-KIC) at rest and in exercise.

METHODS

Subjects

A total of eight subjects participated in one or more parts of this study. The number of subjects in each

0161-7567/84 $1.50

protocol is specified below. All subjects were moderately fit but not involved in intensive physical training. Informed consent was obtained after the experimental protocol had been explained in detail. The study was approved by the Human Studies Committees of both Yale University and the Massachusetts General Hospital.

Experimental Protocol

All experiments involved the infusion of stable isotopes and the collection of blood samples and/or expired-breath samples. The subjects reported to the laboratory in the postabsorptive state. A 17-gauge cathether for isotope infusion was inserted into a forearm vein and advanced 8 cm beyond the point of insertion. An 18-gauge Angiocath was inserted into a vein near the hand on the other arm. The hand was warmed before sampling to "arterialize" the venous sample (14). Before the start of the isotope infusion, samples of expired air were collected in 5-liter anesthesia bags for the determination of the background enrichment of CO_2, and a blood sample was drawn to determine the background enrichment of the plasma urea and amino acids of interest.

All studies consisted of two periods (*I* and *II*). At the start of *period I*, a primed constant infusion of isotope dissolved in saline (see below) was started and maintained for the duration of the experiment. In *period I* (120 min) the subjects were at rest, seated in the contour chair of the modified cycle ergometer (see below). During the first 90 min, O_2 consumption ($\dot{V}o_2$) and CO_2 production ($\dot{V}co_2$) were determined while an isotopic equilibrium was attained in the body pools of interest. Between 90 and 120 min, we drew four 12-ml blood samples at 10-min intervals for analysis of plasma enrichments and collected 10 expired air samples in 3-liter anesthesia bags for analysis of the enrichment of expired CO_2.

The exercise (*period II*) began at 120 min. Without interruption of the isotope infusion or change of position, the subjects began to pedal against a fixed resistance on the ergometer. The exercise intensity averaged 360 kpm·min^{-1} and was adjusted slightly so that a heart rate of 110 beats/min was maintained; this level of exercise was approximately 30% of $\dot{V}o_{2\,max}$. The exercise period lasted 105 min. Blood samples were drawn at 20, 40, 60, 75, 90, and 105 min of exercise. Expired air samples were collected in duplicate for $^{13}CO_2$-enrichment measurement at the same time each blood sample was drawn. $\dot{V}o_2$ and $\dot{V}co_2$ were measured during 6-min intervals four times throughout *period II*.

Isotope Infusions

Eight subjects were infused with a primed-constant infusion of $NaH^{13}CO_3$ (Prochem, Summit, NJ) at the rate of 9.9 μmol/min with a priming dose of 840 μmol given over 1 min. These subjects also received a primed-constant infusion of [^{15}N]leucine (KOR Isotopes, Cambridge, MA; infusion rate = 10 μmol/min, prime = 600 μmol). In this group of subjects we determined the rate of recovery of $^{13}CO_2$ in expired air, the plasma leucine enrichment, and the plasma urea enrichment.

Four of the eight subjects in the above group returned to the laboratory on another day and were infused simultaneously with [1-^{13}C]lysine and [α-^{15}N]lysine (KOR Isotopes, Cambridge, MA). The [1-^{13}C]lysine was infused at the rate of 0.19 μmol·kg^{-1}·min^{-1} (prime = 16 μmol/kg), and the [α-^{15}N]lysine was infused at the rate of 0.08 μmol·kg^{-1}·min^{-1} (prime = 6.8 μmol/kg). In addition, the bicarbonate pool was primed with 3.4 μmol/kg of $NaH^{13}CO_3$ (3), and the urea N pool was primed with an infusion of 1.6 μmol/kg of [^{15}N]$_2$urea. We have previously discussed in detail the rationale that allows the successful priming of the urea N pool with doubly labeled urea when singly labeled [^{15}N]urea is produced as a result of [α-^{15}N]lysine catabolism (6). Briefly, the important point is that our analysis of the enrichment of plasma urea involves the hydrolysis of the molecule (see below). In the subsequent formation of N_2 gas before mass spectrometry analysis, the N atoms are scrambled at random such that the odds of two ^{15}N atoms combining are exceedingly low. Thus, we measure the enrichment of N atoms in urea, not the molecular enrichment of urea per se; our analysis does not distinguish between ^{15}N derived from doubly labeled urea or singly labeled urea. Consequently, it is irrelevant whether singly labeled or doubly labeled urea is used to prime the urea N pool, even though it is singly labeled urea that is being produced. Had we used singly labeled urea, twice as much urea would have been required for the priming dose (6).

The four subjects who participated in our original study of leucine and urea metabolism in exercise (25) were infused with [^{15}N]leucine at the rate described above and also with [2,3-^{13}C]alanine (Merck Isotopes, Montreal, Canada) (infusion rate = 7.64 μmol/min, prime = 760 μmol). In these experiments, we determined the rate of leucine N incorporation into alanine and the rate of alanine flux in addition to the rate of incorporation of ^{15}N into urea. Since two of these subjects were also in the bicarbonate study described above and thus were infused twice with ^{15}N, the overlapping data from the two studies were averaged to provide one value. Thus, the plasma urea enrichment during [^{15}N]leucine infusion was determined in 10 subjects, bicarbonate recovery was determined in eight subjects, lysine kinetics and oxidation were determined in four subjects, and the leucine-alanine interrelationship was investigated in four subjects.

All isotopes were dissolved in sterile normal saline and infused at 0.191 ml/min using a Harvard syrine pump. We measured the exact concentration of each isotope in the infusion solution so that the infusion rate was precisely known and corrected for the body weight of each subject.

Analysis of Samples

Expired air. $\dot{V}o_2$ and $\dot{V}co_2$ were calculated from continuous recordings of the fractions of O_2 and CO_2 in the expired air and continuous recording of the expired ventilatory volume fractions of O_2 and CO_2 were determined from mixed expired air using electronic analyzers calibrated against gases analyzed manometrically; values were corrected to STPD.

The expired air samples to be used for the determina-

tion of isotopic enrichment were bubbled through 0.1 N NaOH to trap CO_2 for later analysis. Immediately before analysis, the $NaHCO_3$ solution was acidified with H_3PO_4 under vacuum to release the CO_2 gas for quantification of enrichment on a dual-inlet, isotope-ratio mass spectrometer (IRMS; model 3-60 RMS, Nuclide, State College, PA) (25).

Blood. The samples were immediately centrifuged and the plasma separated. Proteins were precipitated with sulfosalicylic acid. The amino acids were separated from the protein-free supernatant by desorption with NaOH from Dowex 50W-X8 200/400 mesh hydrogen form cationic resin. The *N*-acetyl, *n*-propyl (NAP) esters were prepared by heating the dried eluate with 3.5 hypobromite in propanol for 20 min at 100°C (1). The esters were dried, dissolved in 2 ml of a 1:2:5 mixture of acetic anhydride:triethylamine:acetone, and heated for 3 min at 60°C. The acetyl esters were then dried and the residue was dissolved in 100 μl ethyl acetate for analysis.

Mass Spectrometry

The NAP amino acids were analyzed on a Hewlett-Packard 5985B gas chromatograph mass spectrometer (GCMS). The GC oven was isothermal at 250°C, the injection port was set at 250°C, and transfer and jet separator were set at 275°C. The methane carrier and reactant gas flow was set at 24 ml/min and the source pressure was 2×10^{-4} Torr. Packing material AA9713 (Alltech, Arlington Heights, IL) was used in a glass coil column.

Lysine. The methane chemical ionization (CI) spectrum was used for all measurements. Selected ion monitoring was used to scan ion ratios at *m/e* 185.1 and 186.1 for ^{15}N enrichments, and at *m/e* 313.2 and 314.2 for total ^{15}N and ^{13}C enrichments. Figure 1 shows the unlabeled, the 99% [α–^{15}N]labeled, and the 99% [1–^{13}C]labeled NAP lysine CI spectra, respectively. The molecular ion ($M + H^+$) at *m/e* 273.2 was enriched either with ^{13}C or ^{15}N, as was the ($M + C_3H_5$) molecular adduct at *m/e* 313.2. The fragment at *m/e* 185.1 contained the α–nitrogen but not the first carbon. The ($M + C_3H_5$) ion was in the same dynamic range as the ion at *m/e* 185.1 in these spectra, so both of these ions were monitored simultaneously. In this way the ^{15}N enrichment and the total ^{13}C plus ^{15}N enrichments were measured directly, and the ^{13}C enrichment was then calculated by subtraction.

Leucine. All the GCMS conditions were the same as above, except the oven was temperature-programmed from 150 to 250° at 20°/min. The methane CI pseudomolecular ion at *m/e* 216.1 and isotope at 217.1 were monitored for determination of ^{15}N enrichment.

Alanine. All GCMS conditions were the same as for leucine. The pseudomolecular ion at *m/e* 174.1 and isotopes at 175.1 and 176.1 were selected for monitoring the ^{15}N and 2,3–^{13}C enrichments, respectively.

Plasma Urea Enrichment

The general approach to the procedure involved the isolation and hydrolysis of urea and trapping of the resulting ammonia, followed by reaction with hypobromite to produce N_2 gas and then analysis of the ^{15}N enrichment of the N_2 on a nuclide IRMS. The first step of the procedure was to measure the urea concentration of the plasma (4). We then added a measured volume of an unlabeled urea solution of known concentration and natural enrichment, as determined relative to the standard gas, to serve as a "carrier" so that there would be enough N_2 gas for IRMS analysis. The plasma proteins were precipitated with sulfosalicylic acid and the protein-free supernatant passed through a cation exchange column (Dowex 50-X8 (H+), 100/200 mesh) that had been conditioned by the addition of HCl and then water. The urea was eluted with 2 N NH_4OH and the eluant was

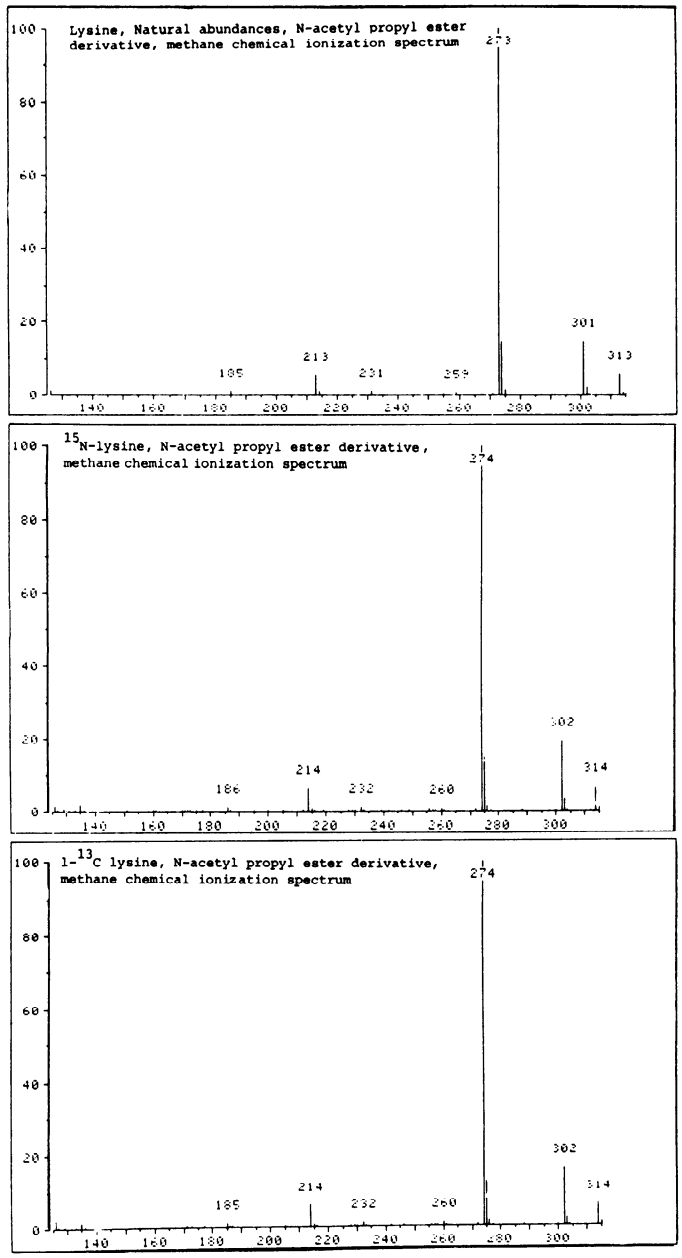

FIG. 1. Chemical ionization spectra for unlabeled, 99% α-^{15}N, and 99% 1-^{13}C-*N* acetyl, *n*-propyl lysine. Selected ion monitoring was used to scan ion ratio at *m/e* 185.1 and 186.1 for ^{15}N enrichments, and *m/e* 313.2 and 314.2 for total ^{15}N and ^{13}C enrichments.

placed in a 50-ml Erlenmeyer flask to which 2 g of Permutit were added to remove nonurea N compounds (9). The Conway procedure was used for the liberation of ammonia from the urea by means of incubation with urease and subsequent trapping of the ammonia in H_2SO_4 (10). The ammonia was then reacted with hypobromite and the resulting N_2 gas was analyzed for enrichment and the enrichment was expressed as atom percent excess (APE) compared with the enrichment of a standard of tank N_2 gas. Enrichment was then determined and corrected for the amount and enrichment of the urea added as carrier.

Kinetic Calculations

The rate of recovery of $^{13}CO_2$ was determined by multiplying the enrichment of the expired CO_2 by the total $\dot{V}CO_2$. The μmol $^{13}CO_2$ excreted/min was then expressed as a percent of the $NaH^{13}CO_3$ infusion rate in μmol/min.

The plasma flux of all substrates was calculated by the modification of the Steele equation (20) as applied to stable isotope tracers. The modification is to account for the contribution of the isotope to the endogenous amino acid kinetics.

$$\text{amino acid flux } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) = \frac{\text{isotope infusion rate } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1})}{\text{plasma amino acid enrichment (APE)} \times 0.01} - \text{tracer infusion rate } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1})$$

This equation only applies in an isotopic steady state (i.e., when the enrichment of the plasma amino acid is not changing with time). Rather than use the nonsteady-state modification of this equation to quantify the amino acid kinetics in the early phase of the transition from rest to exercise, we have instead used the enrichment values obtained later in exercise when an isotopic plateau existed.

Lysine oxidation was calculated as follows

$$\text{lysine oxidation } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) = \frac{CO_2 \text{ enrichment (APE)} \times \dot{V}CO_2}{\text{plasma } [^{13}\text{C}]\text{lysine enrichment (APE)} \times \text{bicarbonate correction factor}}$$

The bicarbonate correction factor is discussed in RESULTS.

The rate of lysine catabolism determined from the incorporation of α-^{15}N into urea was calculated as follows

$$\text{lysine catabolism } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) = \frac{\text{urea production } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) \times 2\ \mu\text{mol N}/\mu\text{mol urea} \times \text{urea N enrichment (APE)}}{\text{plasma lysine enrichment (APE)}}$$

The value for the rate of urea production was taken from our previous study (25) in which the same was determined by means of the infusion of $[^{15}N_2]$urea.

Both the [^{13}C] and the [^{15}N]lysine data can be used to estimate rates of whole-body synthesis (S) and catabolism (C). When the ^{13}C data was used (22)

$$S(\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}) = \frac{[^{13}\text{C}]\text{lysine flux} - \text{lysine oxidation } (\text{mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}) \times 6.25 \text{ g protein/g N}}{3.4 \text{ mmol lysine/g N}}$$

and

$$C(\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}) = \frac{[^{13}\text{C}]\text{lysine flux}}{3.4 \text{ mmol lysine g/N}} \times 6.25 \text{ g protein/g N}$$

When the ^{15}N data were used (6)

$$S(\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}) = \frac{[^{15}\text{N}]\text{lysine flux} - \text{lysine deamination } (\text{mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}) \times 6.25 \text{ g protein/g N}}{3.4 \text{ mmol lysine/g N}}$$

and

$$C(\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}) = \frac{[^{15}\text{N}]\text{lysine flux} \times 6.25 \text{ g protein/g N}}{3.4 \text{ mmol lysine/g N}}$$

This calculation assumes an equilibrium in plasma urea N (see RESULTS) and also assumes the absence of transamination reactions before incorporation into urea. The rate of incorporation of N from [^{15}N]leucine into urea was not calculated because an equilibrium in the urea N pool was not achieved due to the absence of a priming dose of [^{15}N]urea. We did not prime the urea pool when [^{15}N]leucine was infused because the rationale described for priming the urea pool when [α-^{15}N]lysine is infused (6) only applies to the situation in which the labeled N is not involved in transamination reactions; this is not the case with [^{15}N]leucine.

The rate of transfer of the leucine N to alanine was calculated as described by Haymond and Miles (11).

$$\text{rate of appearance of } [^{15}\text{N}]\text{alanine } (R_{a\,[^{15}\text{N}]\text{ala}}\ \mu\text{mol/kg}^{-1}\cdot\text{min}^{-1}) = \text{alanine flux } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) \times {}^{15}\text{N enrichment of alanine}$$

where the alanine flux was derived from [2,3-^{13}C]alanine data.

$$\text{percent of alanine N derived from leucine N} = \frac{^{15}\text{N enrichment of alanine}}{^{15}\text{N enrichment of leucine}} \times 100$$

$$\text{rate of leucine N conversion to alanine N } (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) = \frac{R_{a\,[^{15}\text{N}]\text{ala}}}{^{15}\text{N enrichment of leucine}}$$

$$\text{percent of leucine N converted to alanine N} = \frac{\text{leu N} \rightarrow \text{ala N}}{\text{leucine flux}} \times 100$$

where the leucine flux was determined from the [^{15}N]-leucine data.

In the four subjects in whom we had previously determined the rates of leucine oxidation and flux by means

of [1-^{13}C]leucine, we applied that data as well as the [^{15}N]leucine data obtained in this study to the model described by Matthews et al. (13) to describe leucine α–KIC interactions. The difference in our calculations was that whereas Matthews et al. assumed the enrichment of α–KIC to be in equilibrium with plasma leucine, we directly measured α–KIC enrichment. Given the values for leucine carbon flux ($R_{a\,[^{13}C]leu}$), leucine N flux ($R_{a\,[^{15}N]leu}$), and α–KIC oxidation, the rate of leucine transamination to α–KIC and the rate of α–KIC reamination can be calculated

α–KIC reamination to leucine (μmol·kg^{-1}·min^{-1})

$$= R_{a\,[^{15}N]leu} - R_{a\,[^{13}N]leu}$$

leucine transamination to α–KIC

$$= \alpha\text{–KIC oxidation} + \alpha\text{–KIC reamination.}$$

Statistical Analysis

For all factors a single value was obtained in the steady-state situation that represented the mean of at least three different enrichment values over the last 30–40 min of exercise; that value was compared against the resting value by means of a paired t test.

RESULTS

Bicarbonate Infusion

At rest, 78.0 ± 6.4% of the infused ^{13}C was recovered as expired $^{13}CO_2$. At the onset of exercise, the enrichment of expired CO_2 dropped rapidly, apparently approaching a new plateau at about 90–100 min of exercise (Fig. 2).

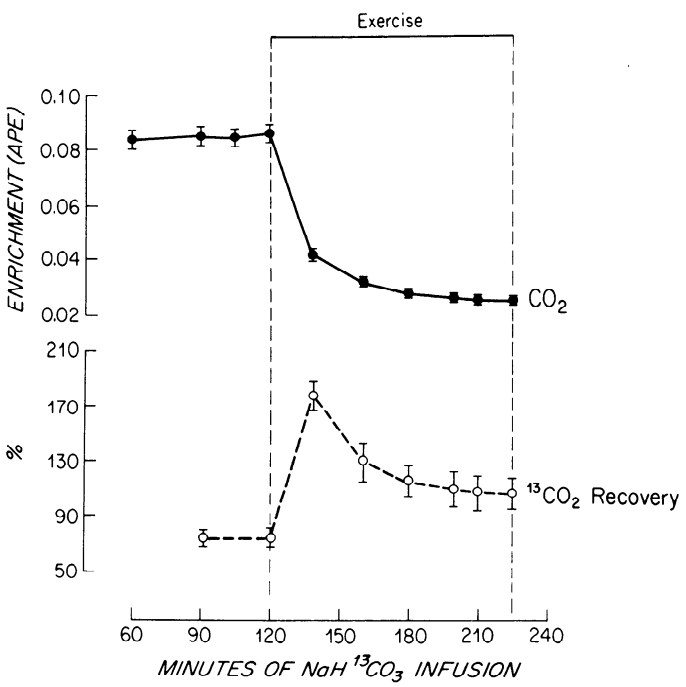

FIG. 2. $^{13}CO_2$ enrichment and percent of infused $NaH^{13}CO_3$ recovered as expired $^{13}CO_2$ at rest and during exercise. $NaH^{13}CO_3$ infusion rate was 9.9 μmol/min, prime = 890 μmol.

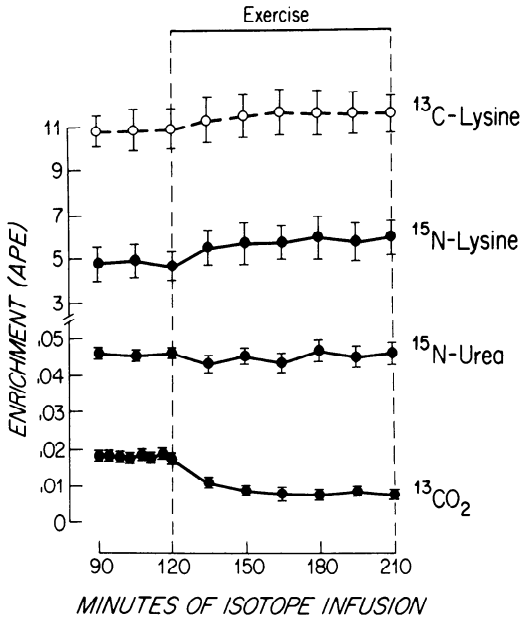

FIG. 3. Plasma enrichment of [^{13}C] and [α-^{15}N]lysine during simultaneous infusion of 2 isotopes as well as resulting enrichments of [^{15}N]urea and expired CO_2. Isotopic steady state was assumed to exist both at rest and during last 45 min of exercise.

The percent ^{13}C recovery rose dramatically in the early phases and then reached a plateau at approximately 106% of the infusion rate. The $^{13}CO_2$ data from the oxidation studies were thus appropriately corrected.

Lysine Infusions

During the lysine infusion, an isotopic equilibrium was achieved during *period I* in the plasma [^{13}C]- and [^{15}N]-lysine pools and also in the expired CO_2 and the plasma urea N (Fig. 3). During exercise, there were no significant changes in the enrichment of either [^{13}C]- or [^{15}N]lysine or of urea N, but there was a drop in enrichment of expired CO_2 (Fig. 3). The rates of appearance of lysine as determined from the ^{13}C data and ^{15}N data were 1.68 ± 0.19 and 1.64 ± 0.06 (SE) μmol·kg^{-1}·min^{-1}, respectively, at rest, and both declined slightly but not significantly in exercise (Fig. 4). At rest, lysine oxidation was 0.21 ± 0.05 μmol·kg^{-1}·min^{-1}, and that value increased to 0.37 ± 0.06 μmol·kg^{-1}·min^{-1} ($P < 0.05$). Lysine deamination at rest was 0.17 ± 0.04 μmol·kg^{-1}·min^{-1} and was not significantly altered in exercise. When rates of whole-body protein synthesis and catabolism were calculated from the two sets of data, the rates of both S and C were virtually identical at rest (Table 1). In exercise, the rate of synthesis declined significantly with both approaches. The rate of catabolism also declined significantly when the ^{15}N data were used, but the reduction calculated from the ^{13}C data was not statistically significant (Table 1).

[^{15}N]Leucine and [2,3-^{13}C]Alanine Infusions

The ^{15}N enrichment of leucine was at plateau when the first blood sample was drawn at 30 min (Fig. 5). From 30 min to 120 min of *period I* (rest), the enrichment

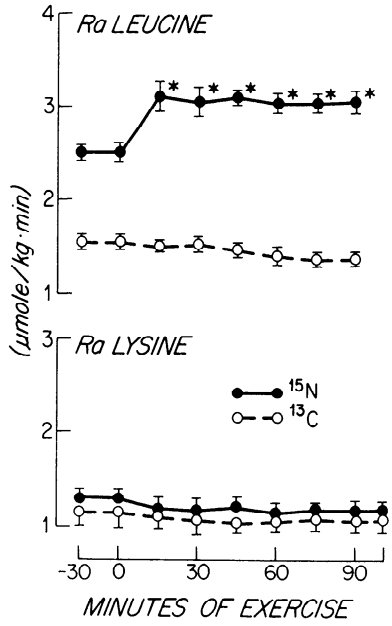

FIG. 4. Rates of appearance (R_a) of plasma leucine and lysine as determined with ^{15}N (●—●) or ^{13}C (○—○) tracer. [^{13}C]leucine data are from Ref. 23.

TABLE 1. *Protein synthesis and catabolism calculated with different tracers*

	[α-^{15}N]Lysine	[1-^{13}C]Lysine	[1-^{13}C]Leucine*
Rest			
S	0.162 ± 0.009	0.161 ± 0.010	0.128 ± 0.0047
C	0.180 ± 0.013	0.183 ± 0.009	0.168 ± 0.007
Exercise			
S	0.134 ± 0.007†	0.135 ± 0.019†	0.066 ± 0.0077†
C	0.153 ± 0.006†	0.171 ± 0.031	0.204 ± 0.067

Values are means ± SE. Units are g protein·kg^{-1}·h^{-1}. S, synthesis; C, catabolism. *From Ref. 23. †Significantly different from corresponding rest value, $P < 0.05$.

of plasma urea increased linearly. At the start of exercise the enrichment of leucine dropped significantly to a new lower plateau value (Fig. 5). Over the first 40 min of exercise there was diminution in the rate of increase in urea enrichment, presumably a reflection of the decline in precursor ([^{15}N]leucine) enrichment in plasma. From 40 min to the end of exercise, the enrichment of urea resumed its linear increase at a rate that was comparable to the rate of increase during rest (Fig. 5).

The plasma leucine flux significantly increased during exercise when measured with ^{15}N, which is in contrast to our previous findings using [^{13}C]leucine (23; Fig. 4). Also, the rate of flux as determined with [^{15}N]leucine was significantly greater than the ^{13}C flux, indicating a significant rate of transamination-reamination interaction between leucine and α-KIC, which increased during exercise.

As with the other isotopes, an isotopic plateau was achieved by 90 min of rest when [2,3-^{13}C]alanine was infused. During exercise there was an approximate doubling in the rate of transfer of leucine N to alanine as well as a doubling in the rate of appearance of alanine in plasma (Fig. 6).

Figure 7 displays schematically the values (expressed in μmol·kg^{-1}·min^{-1}) for certain amino acid interactions that we have been able to derive from this experiment. The contribution of protein catabolism to the leucine and alanine pools was based on the [^{15}N]lysine determination of the overall rate of catabolism and the assumed values of 570 μmol leucine /g protein and 670 μmol alanine/g protein (15). At rest, 13.6% of N for newly synthesized alanine was derived from leucine, and in exercise the contribution of leucine N to alanine increased in an absolute sense and also as a percent of the newly synthesized alanine (to 17.6%; Fig. 6). In exercise, both the rate of transamination of leucine and the rate of reamination of α-KIC were increased, but the increase in transam-

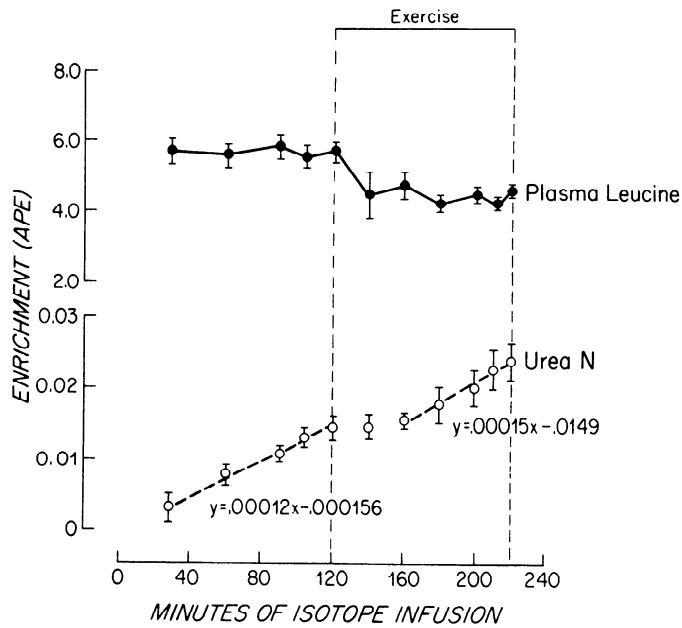

FIG. 5. Plasma enrichment of [^{15}N]leucine and urea N during primed-constant infusion of [^{15}N]leucine at (R_a) rate of 10 μmol/min, prime = 600 μmol.

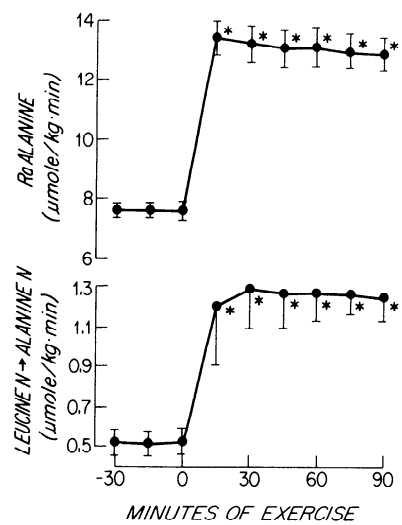

FIG. 6. Rate of appearance (R_a) of alanine at rest and during exercise as determined by the primed-constant infusion of [2,3-^{13}C]alanine. Rate of transfer of leucine N to alanine was calculated as described in METHODS.

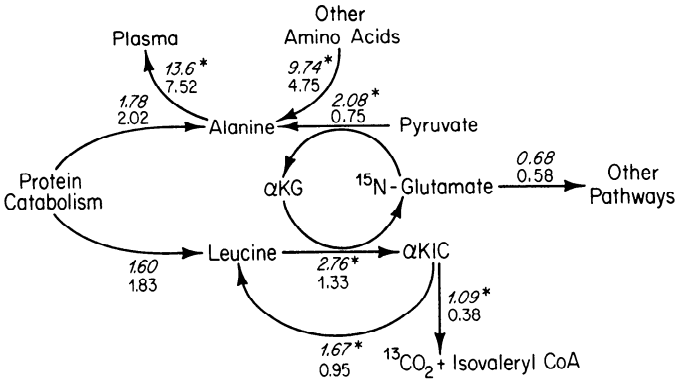

FIG. 7. Rates of amino acid interactions at rest and in exercise (*italics*) as calculated from data presented in this paper and Ref. 23. Units are $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

ination exceeded the rate of reamination, thus accounting for the increased transfer of leucine N to alanine (Fig. 4). Despite the more than doubling of the rate of transamination of leucine to glutamate, there was no significant increase in the rate of subsequent transfer of that N to pathways other than that to alanine. In addition to the increased transfer of N from leucine to alanine in exercise, other amino acids also contributed to the synthesis of alanine as well, leading to an increased release of 6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ of alanine in exercise. Since we have confirmed by both [^{15}N]urea infusion (23) as well as the incorporation of ^{15}N from precursors that urea production was not increased in this exercise study, the ultimate fate of the extra N released in alanine during exercise is not clear. Expansion of the alanine pool could account for only a trivial fraction of the increased alanine output. Further studies are required to address this issue specifically.

DISCUSSION

The effect of exercise on the rate of net protein catabolism has been investigated for at least 100 yr without a clear resolution of the problem. Attention has primarily focused on the rate of formation of urea as the primary final common pathway of amino acid catabolism. However, urea excretion data obtained in exercise have been inconclusive, probably due to the fact that the rates of urea production and urea excretion are not equal (21). In exercise, the loss of urea through sweat can be considerable (e.g., Ref. 17) but difficult to measure, and altered kidney function in exercise makes urea excretion data difficult to interpret (5). For these reasons we used an isotopic technique that was validated both theoretically and experimentally (24) to measure the rate of urea production during the rest and light exercise lasting 105 min. We found that urea production was not affected in exercise, leading to the conclusion that the rate of net protein catabolism was not changed. However, in that study we simultaneously used another approach to the determination of net protein catabolism that relied on a different rationale involving the infusion of [1-^{13}C]leucine and the determination of leucine flux and α–KIC oxidation (25). In contrast to the urea data, the leucine data suggested a marked increase in net protein catabolism, thus leading to the present series of studies designed attempt to reconcile this apparent discrepancy.

The bicarbonate recovery study led to the seemingly impossible conclusion that during exercise a steady state existed in which more $^{13}CO_2$ was excreted than infused. This observation was explained by a second study in which exercise was performed but no isotope was infused. In that study we found that the change in endogenous substrate metabolism in exercise was sufficient to induce a significant increase in the enrichment of expired CO_2 (26); this is because during exercise carbohydrate is oxidized at a faster rate, relative to fat, than at rest, and the naturally occurring enrichment of carbon in carbohydrate is higher than in fat (12). For the purpose of correction of ^{13}C oxidation data, therefore, the bicarbonate recovery data were corrected for the change in natural enrichment that occurred when no isotope was infused. Consequently, at rest we corrected the oxidation data by dividing by 0.78 and in exercise we divided the oxidation data by 1.06. The values for leucine oxidation in exercise obtained earlier were lowered when these correction factors were used, but the increase in leucine oxidation during exercise was still highly significant ($P < 0.001$).

The results from the lysine infusion experiments revealed that leucine metabolism in exercise is not representative of the metabolism of all essential amino acids. Not only was the increase in lysine oxidation in exercise small compared with the increase in leucine oxidation, but also the relationship between the [^{15}N] and [^{13}C]-lysine flux data was different than the corresponding [^{13}C] and [^{15}N]leucine flux data. The disparity between the response of leucine metabolism and lysine metabolism suggests that any model relying on the use of an essential amino acid tracer to determine the rates of whole-body protein synthesis and catabolism in exercise will not be reliable if it is assumed that the changes in metabolism of that amino acid are representative of changes in the other essential amino acids. Our results emphasize the need to test more than one amino acid in any circumstance in which rates of whole-body protein synthesis and catabolism are calculated. When more than one amino acid is used as well as different tracers of the same amino acid ([α-^{15}N]- and [1-^{13}C]lysine), and similar data are obtained, such as we found at rest (Table 1), the validity of the data is strengthened. When different tracers yield different absolute data as well as different directions of change, as we saw in the calculation of the rate of protein catabolism during exercise (Table 1), the failure of the model is identified.

Whereas our experiment raises doubt as to the validity of the estimation of the rate of net protein catabolism using a single essential amino acid tracer, our results strengthen the use of the direct isotopic measurement of urea production as an index of net protein catabolism. The technique we used previously to measure urea production involved the GCMS analysis of urea enrichment. Because of the limitations in sensitivity of the GCMS technique, it was possible that, if there was an increased rate of incorporation of N from only a few amino acids during exercise, including leucine, the resulting change in urea enrichment from the resting value might have

been too small to measure. However, by taking advantage of the greater sensitivity of the isotope ratio mass spectrometer, in this study we have confirmed that neither leucine N nor lysine N was transferred to urea at an increased rate in light exercise. Since the $[^{15}N]_2$urea technique (23) determines the rate of appearance (as opposed to excretion) of urea, increased losses of urea in the sweat or changes in urea concentration in plasma do not complicate the interpretation of the data. Furthermore, not only have we shown that the technique can immediately detect changes in urea appearance due to urea infusion (23), but we have recently shown that rapid changes in urea production induced physiologically can be detected by our method. We found that urea production was significantly reduced within 60 min after the start of an exogenous glucose infusion at the rate of 4 $mg \cdot kg^{-1} \cdot min^{-1}$ in normal volunteers (19). For these reasons we feel that the original conclusion we based on the urea production data [that protein catabolism is not increased in light exercise (25)] is correct. Since the rate of alanine release from muscle has been shown to be proportional to the intensity of exercise (8), it is possible that with more strenuous exercise the rate of net protein catabolism would increase. Also, the work of Rennie et al. (18) suggests that even at a moderate work load protein catabolism may be increased if interval of work was extended to close to 4 h.

Our [α-^{15}N]lysine technique for estimating net protein catabolism agreed with the conclusion that net protein catabolism was not stimulated in exercise. The synthesis and catabolism calculations for both isotopes of lysine relied on the determination of the rates of lysine flux and lysine catabolism. Because the α-N of lysine does not participate to any known extent in transamination reactions, the flux rates as determined by the two tracers were similar at rest and in exercise. For the same reason the rates of lysine decarboxylation and deamination should also have been similar, which was the case at rest. The similarity of the results from the two means of determining the rate of lysine catabolism strengthens the validity of each approach for achieving a rapid equilibrium in end-product enrichment (in the one case priming the bicarbonate pool with $NaH^{13}CO_3$ and in the other case priming the urea pool with $[^{15}N]_2$urea). However, the two techniques revealed significantly different values for lysine catabolism in exercise. If one value was in error, it seems likely that the $^{13}CO_2$ data resulted in an overestimation of the true rate of oxidation. The only enrichment that changed in the lysine experiment was that of the expired CO_2 and perhaps it had not reached a true plateau in exercise. Nonetheless, the increase in lysine oxidation was small as compared with the increase in leucine oxidation.

Although our amino acid data do not enable a clear-cut extrapolation to rates of whole-body protein turnover, certain aspects of the response to exercise have been clarified (Fig. 7). At rest, we have confirmed previous observations that there is a significant rate of interconversion of leucine and α-KIC (16) and that leucine N is an important source of alanine N (11). In exercise, the rate of interconversion of leucine and α-KIC increased markedly as did the rate of decarboxylation of α-KIC. Consequently, there was also a significant increase in the rate of transfer of leucine N to glutamate. Rather than being released from muscle in glutamate or other amino acids, all of the additional leucine N transferred to glutamate appeared in the plasma in alanine. This observation is consistent with the observation that during exercise alanine is the only amino acid released from muscle and it supports the concept of an enhanced activity of the "glucose-alanine" cycle in exercise (7). The increased rate of alanine release in exercise was due not only to an increase incorporation of leucine N but also to an increased rate of transfer of N from other amino acids. This conclusion is based on the difference between the increase in alanine flux in exercise and the sum of the alanine released from protein catabolism and the alanine derived from leucine N. Although the precise value of protein catabolism is in doubt for reasons cited above, the maximal range of possible values for catabolism could not affect the conclusion regarding alanine synthesis.

The results of these studies do not explain the fact that urea production did not increase in exercise even though there was an increased rate of transfer of N via alanine to the liver. Perhaps in exercise pathways existed whereby the alanine N is quantitatively reincorporated into the amino acid rather than into urea. The sensitivity of the analytical procedure we used for the determination of amino acid enrichment in this study precluded the identification of such pathways. If such pathways do exist, then there would be a redistribution of N from certain essential amino acids (such as leucine) to nonessential amino acids. In that case, even though urea production might not be increased in exercise, the dietary requirement for certain essential amino acids might increase with chronic exercise training. At the present time this possibility remains speculative.

In summary, these studies have confirmed our earlier observation that decarboxylation of leucine is increased in light exercise but urea production is not. On the basis of ^{15}N incorporation studies, we feel it is likely that the urea production rate accurately reflects the rate of net protein catabolism. Amino-acid kinetics in exercise indicate an increased rate of various transamination reactions, but the relationship between the altered amino-acid kinetics and net protein catabolism on the one hand and the rate of production of urea on the other hand is not clear.

The authors thank Drs. Lou Stevenson and Richard Goodenough for helping to perform the infusions, Knut Greulich for technical assistance, and Portia Wongwajarachot for manuscript preparation.

This study was supported by National Institutes of Health Grants GM-00455-05 and GM-21700-08.

Received 27 December 1982; accepted in final form 3 August 1983.

REFERENCES

1. ADAMS, R. F. Determination of amino acid profiles in biological samples by gas chromatography. *J. Chromatogr.* 57: 189–212, 1974.

2. AHLBORG, G., P. FELIG, L. HAGENFELDT, et al. Substrate turnover during prolonged exercise in man: splanchnic and leg metab-

olism of glucose, free fatty acids, and amino acids. *J. Clin. Invest.* 53: 1080–1090, 1974.
3. ALLSOP, J. R., R. R. WOLFE, AND J. F. BURKE. Tracer-priming the bicarbonate pool. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 45: 137–140, 1978.
4. BERGMEYER, H. U. *Methods of Enzymatic Analysis.* New York: Academic, 1974, p. 1791–1794.
5. CASTENFORS, J. Renal function during exercise. *Acta Physiol. Scand. Suppl.* 293: 1–40, 1967.
6. DIETZ, W. H., M. H. WOLFE, AND R. R. WOLFE. A method for the rapid determination of protein turnover. *Metabolism* 31: 749–754, 1982.
7. FELIG, P. The glucose-alanine cycle. *Metab. Clin. Exp.* 22: 179–205, 1973.
8. FELIG, P., AND J. WAHREN. Amino acid metabolism in exercising man. *J. Clin. Invest.* 50: 2703–2709, 1971.
9. FOLIN, D., AND R. D. BELL. Applications of a new reagent for the separation of ammonia. *J. Biol. Chem.* 29: 329–335, 1917.
10. HAWK, P. B., B. L. OSER, AND N. H. SUMMERSON. *Practical Physiological Chemistry* (13th ed.). New York: McGraw-Hill, 1954.
11. HAYMOND, M. W., AND J. M. MILES. Branched chain amino acids as a major source of alanine nitrogen in man. *Diabetes* 31: 86–89, 1982.
12. JACOBSON, B. S., B. N. SMITH, S. EPSTEIN, AND G. C. LATIES. The prevalence of carbon^{-13} in respiratory carbon dioxide as an indicator of the type of endogenous substrate. *J. Gen. Physiol.* 55: 1–17, 1970.
13. MATHEWS, D. E., M. J. RENNIE, D. HALLIDAY, K. J. MILLWARD, G. C. CLUGSTON, R. H. T. EDWARDS, AND D. M. BIER. Modeling leucine metabolism in man. In: *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids*, edited by M. Walser and J. R. Williamson. New York: Elsevier/North-Holland, 1981, p. 313–316.
14. MCGUIRE, E. A. H., J. H. HELDERMAN, J. D. TOBIN, R. ANDRES, AND M. BERGMAN. Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J. Appl. Physiol.* 41: 565–573, 1976.
15. MUNRO, H. N., AND A. FLECK. Analysis of tissues and body fluids for nitrogenous constituents. In: *Mammalian Protein Metabolism*, edited by H. N. Munro. New York: Academic, 1969, vol. 3, p. 508.
16. NISSEN, S., AND M. W. HAYMOND. Effects of fasting on flux and interconversion of leucine and α-ketoisocaproate in vivo. *Am. J. Physiol.* 241 (*Endocrinol. Metab.* 4): E72–E75, 1981.
17. REFSUM, H. E., R. GJESSING, AND S. B. STROMME. Changes in plasma amino acid distribution and urine amino acids excretion during prolonged heavy exercise. *Scand. J. Clin. Lab. Invest.* 39: 407–413, 1979.
18. RENNIE, M. J., R. H. T. EDWARDS, S. KRYWAWYCH, C. T. M. DAVIES, D. HALLIDAY, J. C. WATERLOW, AND D. J. MILLWARD. Effect of exercise on protein tumors in man. *Clin. Sci.* 61: 627–639, 1981.
19. ROYLE, G. T., J. A. MOLNAR, M. H. WOLFE, R. R. WOLFE, AND J. F. BURKE. Urea, glucose, and alanine kinetics in man: effects of glucose infusion. *Clin. Sci.* 62: 553–556, 1982.
20. STEELE, R. Influence of glucose leading and of injected insulin on hepatic glucose output. *Ann. NY Acad. Sci.* 82: 420–430, 1959.
21. WALSER, M., AND L. BODENLOS. Urea metabolism in man. *J. Clin. Invest.* 38: 1617, 1959.
22. WATERLOW, J. C. Lysine turnover in men measured by intravenous infusion of L-[U-^{14}C]-lysine. *Clin. Sci.* 33: 507–515, 1967.
23. WOLFE, R. R. Measurement of urea kinetics in vivo by means of a constant tracer infusion of di-^{15}N-urea. *Am. J. Physiol.* 240 (*Endocrinol. Metab.* 3): E428–E434, 1981.
24. WOLFE, R. R., J. R. ALLSOP, AND J. F. BURKE. Glucose metabolism in man: response to intravenous glucose infusion. *Metabolism* 28: 210–220, 1979.
25. 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.: Respirat. Environ. Exercise Physiol.* 52: 458–466, 1982.
26. WOLFE, R. R., J. H. F. SHAW, E. R. NADEL, AND M. H. WOLFE. Effect of substrate intake and physiological state on background $^{13}CO_2$ enrichment. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 56: 230–234, 1984.
27. ZUNTZ, N. Uber die Bedentung der verschiedener Nahr-Stoffe als Erzeuger der Muskeldraft. *Pfluegers Arch.* 83: 557–582, 1900.