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# Modulation of whole body protein metabolism, during and after exercise, by variation of dietary protein

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O. ROOYACKERS,<sup>3</sup> A. J. M. WAGENMAKERS,<sup>3</sup> AND M. J. RENNIE<sup>1</sup>

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**Bowtell, J. L., G. P. Leese, K. Smith, P. W. Watt, A. Nevill, O. Rooyackers, A. J. M. Wagenmakers, and M. J. Rennie.** Modulation of whole body protein metabolism, during and after exercise, by variation of dietary protein. *J. Appl. Physiol.* 85(5): 1744–1752, 1998.—The aim of this study was to investigate dietary protein-induced changes in whole body leucine turnover and oxidation and in skeletal muscle branched chain 2-oxo acid dehydrogenase (BCOADH) activity, at rest and during exercise. Postabsorptive subjects received a primed constant infusion of L-[1-<sup>13</sup>C, <sup>15</sup>N]leucine for 6 h, after previous consumption of a high- (HP; 1.8 g·kg<sup>-1</sup>·day<sup>-1</sup>, *n* = 8) or a low-protein diet (LP; 0.7 g·kg<sup>-1</sup>·day<sup>-1</sup>, *n* = 8) for 7 days. The subjects were studied at rest for 2 h, during 2-h exercise at 60% maximum oxygen consumption, then again for 2 h at rest. Exercise induced a doubling of both leucine oxidation from 20 μmol·kg<sup>-1</sup>·h<sup>-1</sup> and BCOADH percent activation from 7% in all subjects. Leucine oxidation was greater before (+46%) and during (+40%, *P* < 0.05) the first hour of exercise in subjects consuming the HP rather than the LP diet, but there was no additional change in muscle BCOADH activity. The results suggest that leucine oxidation was increased by previous ingestion of an HP diet, attributable to an increase in leucine availability rather than to a stimulation of the skeletal muscle BCOADH activity.

branched chain 2-oxo acid dehydrogenase; leucine oxidation; stable isotope

THE BRANCHED CHAIN AMINO ACIDS (BCAA) leucine, isoleucine, and valine are unusual, since they are degraded largely within the skeletal muscle. The first step in the oxidative pathway is a reversible transamination catalyzed by branched chain amino acid transaminase (BCAAT); the transamination of the BCAA is coupled with the formation of glutamate, 2-oxoglutarate, acting as the amino group acceptor. The resultant branched chain 2-oxo acids are then either reaminated with the formation of BCAA or irreversibly decarboxylated. The latter reaction is catalyzed by branched chain 2-oxo acid dehydrogenase (BCOADH) and is thought to be the rate-limiting step in the oxidative pathway (21). The enzyme complex is present in active dephosphorylated and inactive phosphorylated forms, with the interconversion between the two being controlled by the relative activity of BCOADH kinase and phosphatase (11).

Moderate-intensity exercise has been shown to increase rates of amino acid oxidation (20), BCAA oxidation in particular (18, 26). Although this does not contribute significantly to increased fuel supply to the

working muscle (20), it may spare blood glucose for use by the central nervous system (8). There is also an increase in the percentage of skeletal muscle BCOADH in the active form during moderate-intensity exercise (32). The first aim of this study was to determine whether the increase in whole body leucine oxidation and the activation of skeletal muscle BCOADH occur in parallel and are of a similar magnitude. This would suggest that the increase in skeletal muscle BCOADH activity is responsible for the exercise-induced increase in leucine oxidation.

Consumption of a high-protein (HP) diet has been shown to increase leucine oxidation in people (23) and to increase the activity of the BCOADH complexes in both rat liver and muscle (3, 4), although there is little information regarding the extent of activation of BCOADH in people. Recently, van Hall et al. (31) found that BCAA supplementation resulted in an activation of skeletal muscle BCOADH at rest and that the effects of BCAA ingestion and exercise on BCOADH activation were additive. This was presumably through an inhibition of the BCOADH kinase due to the increased intracellular BCAA and branched chain 2-oxo acid concentrations (19). The second aim of this study was, therefore, to determine whether any increase in leucine oxidation induced by the HP diet, both at rest and during exercise, could be attributed to an increase in skeletal muscle BCOADH activity.

During endurance exercise in the postabsorptive state, protein synthesis is reduced and protein breakdown increased, resulting in a loss of body protein (26). In the recovery phase, a positive protein balance is reestablished through an increase in protein synthesis in excess of the elevation in protein breakdown (26). Consumption of an HP diet is associated with a reduction in protein breakdown and increased protein synthesis, at least in the fed state (23), which may be related to elevated plasma amino acid and insulin concentrations (6, 29). The final aim of this study was to determine whether consumption of an HP diet might favorably modulate protein metabolism to minimize the body protein loss concomitant with an exercise bout.

## METHODS

Subjects consumed either an HP (1.8 g protein·kg<sup>-1</sup>·day<sup>-1</sup>, *n* = 16) or a low-protein (LP) diet (0.7 g protein·kg<sup>-1</sup>·day<sup>-1</sup>, *n* = 16) for 7 days. On day 8 of this period, whole body leucine kinetics were traced before, during, and after 2-h walking on a treadmill at 60% maximal oxygen consumption ( $\dot{V}O_{2max}$ ).

**Table 1. Subject characteristics for  $[1-^{13}\text{C},^{15}\text{N}]$ leucine trials**

Dietary Protocol	Age, yr	$\dot{V}\text{O}_{2\text{max}}$ , ml·kg <sup>-1</sup> ·min <sup>-1</sup>	Weight, kg	Body Fat, %
HP	21.9 ± 0.6	45.8 ± 1.1	74.5 ± 3.4	12.4 ± 2.1
LP	22.0 ± 0.6	45.0 ± 1.2	71.9 ± 4.6	14.1 ± 2.2

Data are means ± SE ( $n = 8$  subjects).  $\dot{V}\text{O}_{2\text{max}}$ , maximal O<sub>2</sub> uptake; HP, high-protein-diet group; LP, low-protein-diet group.

Four subjects (3 men and 1 woman) participated in the two trials, separated by at least 4 wk; diets were allocated by systematic rotation. A further group of eight subjects (7 men and 1 woman) participated in one trial only, being allocated by systematic rotation to one of the protocols ( $n = 4$  in each group). The subjects' characteristics are given in Table 1. An incremental exercise test on a motorized treadmill, using the criteria of Taylor et al. (28), was adopted for the measurement of  $\dot{V}\text{O}_{2\text{max}}$  by using on-line gas analysis. The study was approved by the local Tayside Ethics Committee, and all subjects gave their informed consent.

Diets were designed to supply each subject's normal diurnal energy requirement [by consideration of average values for subject's age, weight, and activity level (10), corroborated by 1-day food diary]. Diet sheets were prepared, prescribing all foods to be consumed, which provided most of the energy intake ( $82 \pm 5\%$  of  $3,152 \pm 178$  kcal) and  $0.7$  g protein·kg<sup>-1</sup>·day<sup>-1</sup>; subjects marked the sheets to show compliance. Subjects allocated to the HP diet group ( $n = 8$ ) consumed, in addition, a whey protein supplement (Maxipro, Scientific Hospital Supplies, Liverpool, UK) supplying  $1.1$  g protein·kg<sup>-1</sup>·day<sup>-1</sup>; subjects allocated to the LP diet group ( $n = 8$ ) consumed an isoenergetic peanut oil supplement (Calogen, Scientific Hospital Supplies). The supplements were prepared daily in liquid form, and the subjects were supervised as they drank them; the diets were consumed for a period of 10 days. Subjects were instructed to maintain normal activity patterns but to refrain from exercise on *day 7*, i.e., the day before the exercise trial.

After 5-day equilibration to the new protein intake, six subjects (2 LP and 4 HP) were studied after an overnight fast to determine the effect of the diet on background breath <sup>13</sup>CO<sub>2</sub> enrichment. Two basal breath samples were taken, and the subjects then walked on a treadmill at 60%  $\dot{V}\text{O}_{2\text{max}}$  for 2 h. Breath samples were taken at 20-min intervals throughout, stored in 10-ml evacuated tubes (Exetainers, Europa Scientific, Crewe, UK), and then analyzed for <sup>13</sup>CO<sub>2</sub> enrichment by using isotope ratio mass spectrometry on the Automatic Nitrogen and Carbon Analyzer (Europa Scientific).

After 7 days of dietary equilibration, subjects ( $n = 8$  in each dietary protocol) were studied during and after 2 h of treadmill exercise at 60%  $\dot{V}\text{O}_{2\text{max}}$  (Fig. 1). The subjects arrived at the laboratory after an overnight fast, and cannulas were inserted into the antecubital veins of both arms. One cannula was used for withdrawal of mixed venous blood samples and one for delivery of the leucine isotope. Basal blood and breath samples were taken, and the subjects then received a primed, constant ( $0.8$  mg/kg,  $1$  mg·kg<sup>-1</sup>·h<sup>-1</sup>) infusion of L-[1-<sup>13</sup>C,<sup>15</sup>N]leucine for 6 h. Subjects rested for the first 2 h, then walked on a treadmill at a speed and gradient designed to elicit 60% of their  $\dot{V}\text{O}_{2\text{max}}$  for 2 h, and then rested for a further 2-h period. Subjects received water during exercise ( $5$  ml·kg<sup>-1</sup>·h<sup>-1</sup>) in six equal aliquots immediately before exercise and every 20 min during exercise.

Blood and breath samples were taken every 30 min for the first and last hours of the infusion and every 20 min at all other times. In one-half of the subjects ( $n = 4$  in each dietary protocol), muscle biopsies were taken from quadriceps femoris by conchotome forceps (7). A basal biopsy was taken in the 30 min preceding exercise, and two further biopsies were taken after the first and second hours of exercise.

Blood was stored on ice until the end of the infusion period and then centrifuged at 2,500 rpm for 20 min at 4°C. The resultant plasma was stored at -70°C until the analysis could be performed. Plasma was analyzed for insulin (radioimmunoassay kit, Amersham UK), glucose, and urea (both kits from Sigma Chemical, Poole, UK). To determine labeling of <sup>13</sup>C and <sup>15</sup>N, plasma leucine and  $\alpha$ -ketoisocaproate (KIC) were analyzed as their *t*-butyldimethylsilyl derivatives (2) and

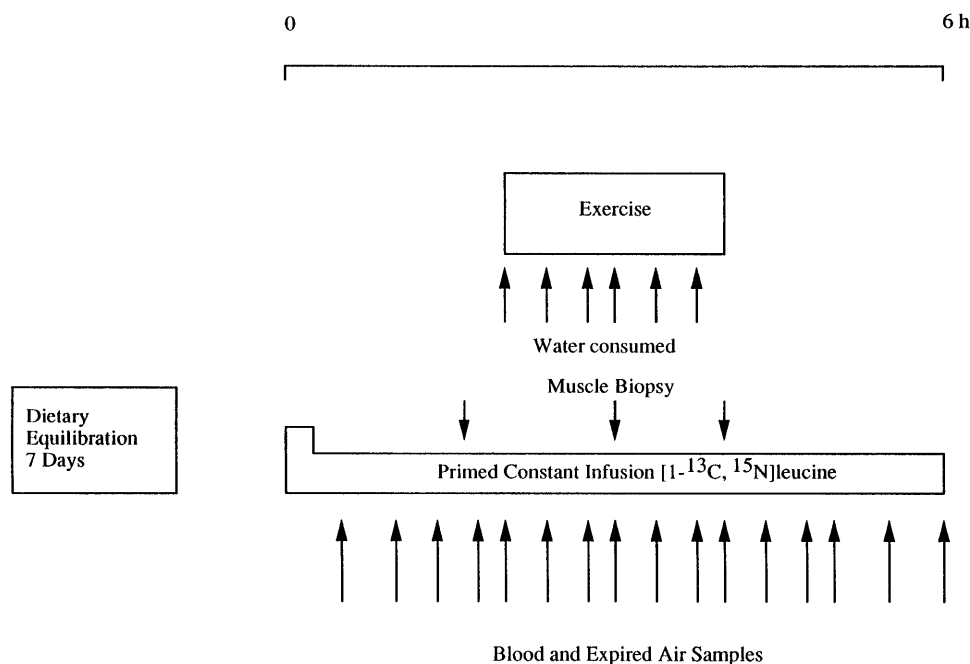


Fig. 1. Schematic of study protocol.

Table 2. Whole body leucine transamination and KIC reamination

	<i>n</i>	Preexercise	Exercise Hour 1	Exercise Hour 2	Postexercise
Leucine transamination, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$					
HP	7	118.0 ± 26.9	157.8 ± 20.8*	127.6 ± 18.2	100.0 ± 16.1
LP	8	74.4 ± 15.0	99.0 ± 12.9*	96.8 ± 12.1	83.6 ± 11.6
KIC reamination, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$					
HP	7	94.6 ± 26.4	83.0 ± 19.1	75.6 ± 17.5	74.3 ± 15.2
LP	8	58.6 ± 13.9	50.1 ± 12.2	46.4 ± 11.5	61.8 ± 11.1

Data are means ± SE; *n*, no. of subjects. KIC,  $\alpha$ -ketoisocaproate. Significantly different from preexercise values (\* $P < 0.05$ ).

*t*-butyldimethylsilyl-quinoxalinol derivatives (27), respectively, by using gas-chromatograph mass spectrometry. Leucine and KIC concentrations were obtained by using deuterated internal standards. Whole body leucine oxidation, transamination, reamination, protein breakdown, and synthesis were calculated using the model described previously (21). The factors used to account for the retention of  $^{13}\text{C}$  label in the bicarbonate pool when calculating leucine oxidation were determined in a study employing an identical experimental protocol to that used in the present study (5). Subjects received a primed constant infusion of  $\text{NaH}^{13}\text{CO}_3$ , and label recovery was determined as the ratio of the dose infused that was recovered as described previously (12). The factors thus used in the calculation of leucine oxidation were 0.77, 1.00, 0.42, and 0.48 for the preexercise period, exercise period, and the first and second hours postexercise, respectively, results that are comparable to those obtained in other studies (12). Fractional leucine transamination, i.e., the proportion of leucine nitrogen that was transaminated, was calculated as the percentage of leucine nitrogen flux that comprises transamination. Similarly, fractional oxidation of KIC, or the proportion of KIC formed that was oxidized, was calculated as the percentage of transamination that comprises oxidation.

The biopsied muscle tissue was placed in ice-cold buffer within 120 s of the cessation of exercise, to minimize any change in fractional activation, and was assayed for BCOADH activity, as described previously (32).

The breath samples were collected in 150-liter Douglas bags for 4 min at rest and 2 min during exercise and were analyzed for percent content of oxygen (Taylor Servomex, Sybron) and carbon dioxide (Grubb Parsons, Newcastle, UK). Oxygen consumption, carbon dioxide production, and respiratory quotient were calculated by standard methods. After thorough mixing, small aliquots of each breath sample were transferred into 10-ml evacuated tubes and later analyzed for  $^{13}\text{CO}_2$  enrichment as described above.

The plasma [ $^{13}\text{C}$ ]KIC enrichment, rather than plasma [ $^{13}\text{C}$ ]leucine enrichment, was used to calculate leucine carbon flux, oxidation, and incorporation into protein (22). KIC is produced intracellularly via the transamination of leucine; it has, therefore, been suggested that plasma KIC enrichment is a better predictor for intracellular leucine enrichment (34).

**Statistics.** All data are means ± SE. The Mann-Whitney *U*-test was used to compare the BCOADH data because of the small sample size. A one-way ANOVA was used to analyze the changes in background breath  $^{13}\text{CO}_2$  enrichment during exercise. A two-way ANOVA [diet HP or LP by time (preexercise, during hour 1, during hour 2, and postexercise)] was used to analyze the remaining variables. A dependent analysis (all factors within subjects) was carried out on the data derived from the four subjects who completed both trials, and an independent analysis (2 factors between and 1 factor

within subjects) was employed for the data derived from the subjects who completed only one trial each. Provided that the direction of change in the variable was the same for both analyses, the *P* values were combined by using a meta-analysis (9) (see APPENDIX for details of the method). A post hoc Tukey's test was used to locate the site of the difference where appropriate, and the *P* values were combined in the same way.

## RESULTS

In the studies in which tracer amino acid was not infused, the transition from rest to exercise did not cause a significant change in the background of expired air  $^{13}\text{CO}_2$  enrichment; the small increase that did occur was not significant compared with the expired air  $^{13}\text{CO}_2$  enrichment observed when isotope was infused. There was no significant change in background  $^{13}\text{CO}_2$  enrichment as exercise progressed.

The tendency was for absolute rates of transamination and reamination to be higher in HP than in LP subjects throughout (Table 2). During exercise, the rate of transamination increased ( $P < 0.05$ ), and reamination tended to decrease.

Fractional transamination tended to be higher for HP than LP subjects, both at rest and during the first hour of exercise (Fig. 2). Fractional transamination was elevated throughout exercise ( $P < 0.01$ ).

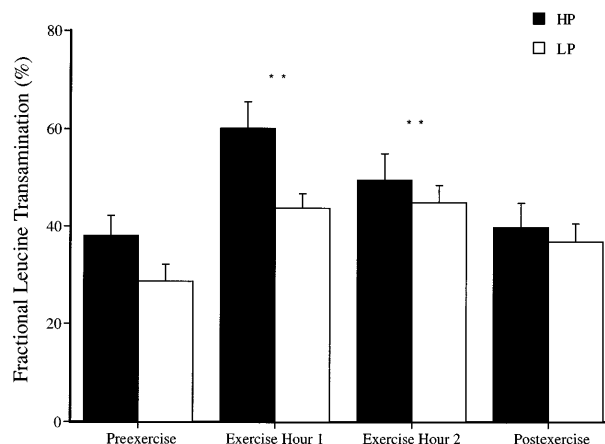


Fig. 2. Effect of dietary protein content on percentage of leucine nitrogen transaminated at rest and during exercise. HP, high-protein-diet group; LP, low-protein-diet group. Values are means ± SE ( $n = 8$  subjects). Significantly different from preexercise value (\*\* $P < 0.01$ ).

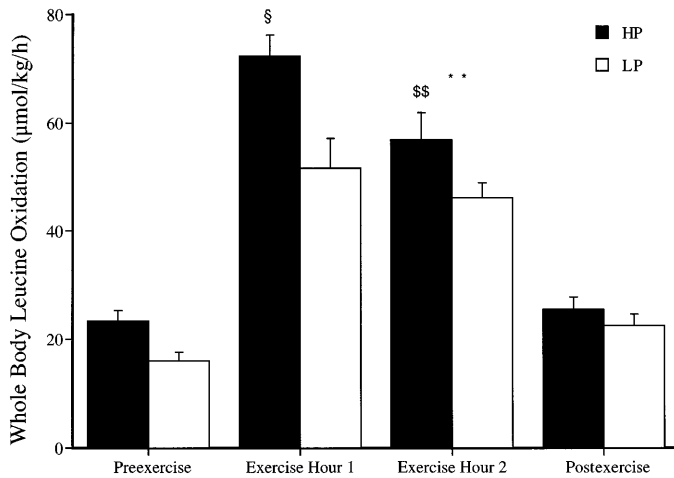


Fig. 3. Effect of dietary protein content on whole body leucine oxidation at rest and during exercise. Values are means  $\pm$  SE ( $n = 8$  subjects). HP values significantly different from LP values throughout ( $P < 0.05$ ). Significantly different from preexercise values (\*\* $P < 0.01$ ), LP values (§ $P < 0.05$ ), and exercise hour 1 values (\$\$\$ $P < 0.001$ ).

Leucine oxidation was higher for HP than LP subjects throughout (Fig. 3,  $P < 0.01$ ), and exercise induced a 216% increase (mean for 2 h) for both dietary protocols ( $P < 0.01$ ). There was an interaction between the effect of dietary protein and exercise on leucine oxidation ( $P < 0.01$ ). The leucine oxidation rate was higher for HP subjects ( $72.4 \pm 3.9$  vs.  $51.7 \pm 5.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ,  $P < 0.01$ ) during the first hour of exercise, but there was no significant difference between HP and LP subjects during the second hour of exercise. Fractional oxidation of KIC increased during the first hour of exercise for both groups ( $P < 0.05$ , Fig. 4) and tended to be higher for LP subjects throughout.

There was no significant difference between HP and LP subjects either in total activity or in the proportion of active skeletal muscle BCOADH complex at any time period. However, exercise induced a significant in-

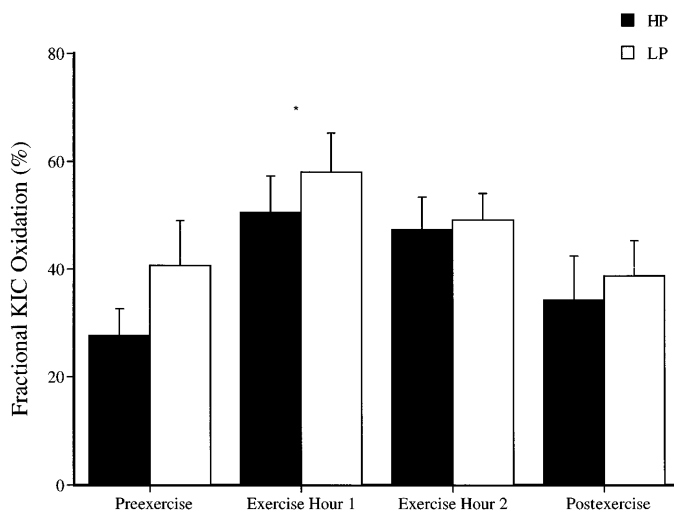


Fig. 4. Effect of dietary protein content on fractional  $\alpha$ -ketoisocaproate (KIC) oxidation at rest and during exercise. Values are means  $\pm$  SE ( $n = 8$  subjects). Significantly different from preexercise value (\* $P < 0.05$ ).

Table 3. Skeletal muscle BCOADH activity

	Preexercise	Exercise Hour 1	Exercise Hour 2
<b>HP</b>			
Total activity, $\text{nmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$	$5.6 \pm 0.9$	$5.3 \pm 0.2$	$4.2 \pm 0.3$
%Activation	$6.7 \pm 2.1$	$10.6 \pm 2.0^*$	$14.8 \pm 3.0^*$
<b>LP</b>			
Total activity, $\text{nmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$	$5.5 \pm 0.4$	$4.9 \pm 0.6$	$5.9 \pm 0.4$
%Activation	$7.9 \pm 0.8$	$21.8 \pm 5.1^*$	$20.5 \pm 5.8^*$

Data are means  $\pm$  SE;  $n = 4$  subjects. BCOADH, branched chain 2-oxo acid dehydrogenase. Significantly different from preexercise values (\* $P < 0.05$ ).

crease in the activation of the complex for both groups ( $P < 0.05$ , Table 3).

Plasma leucine concentration tended to be higher throughout the experimental protocol in subjects previously consuming an HP diet (Fig. 5), but this difference did not attain statistical significance. Plasma leucine concentration was 15 and 8% lower during the second than during the first hour of exercise for HP and LP subjects, respectively ( $P < 0.05$ ); this decrease coincided with a 20% ( $P < 0.01$ ) and a 10% fall in leucine oxidation for HP and LP subjects, respectively.

Plasma KIC concentration was higher for HP than for LP subjects throughout the 6-h protocol ( $P < 0.05$ , Fig. 6). Plasma KIC concentration was 15 and 11% lower during the second than during the first hour of exercise for HP and LP subjects, respectively ( $P < 0.05$ ).

Plasma glucose concentrations were not different between the dietary protocols; there was a small decrease during the second hour of exercise for both groups, from 4.8 to 4.4 mM. Plasma urea concentration was higher for HP than for LP subjects ( $P < 0.01$ ) at all time points ( $5.2 \pm 0.1$  vs.  $3.1 \pm 0.2$  mM at rest before exercise), but there was no increase during the exercise

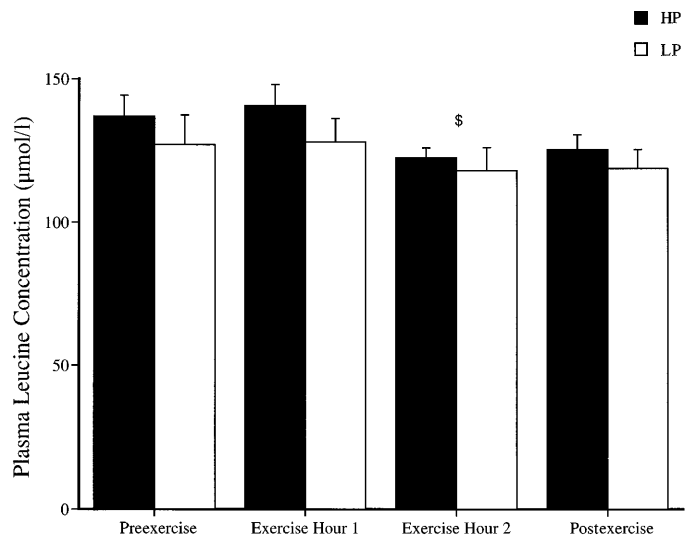


Fig. 5. Effect of dietary protein content on plasma leucine concentration at rest and during exercise. Values are means  $\pm$  SE ( $n = 8$  subjects). Significantly different from exercise hour 1 value (§ $P < 0.05$ ).

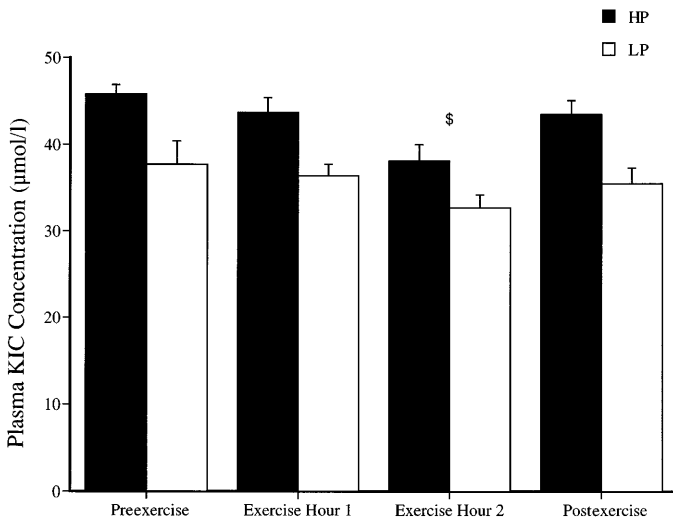


Fig. 6. Effect of dietary protein content on plasma KIC concentration at rest and during exercise. Values are means  $\pm$  SE ( $n = 8$  subjects). HP values significantly different from LP values throughout. Significantly different from exercise hour 1 value ( $^{\$}P < 0.05$ ).

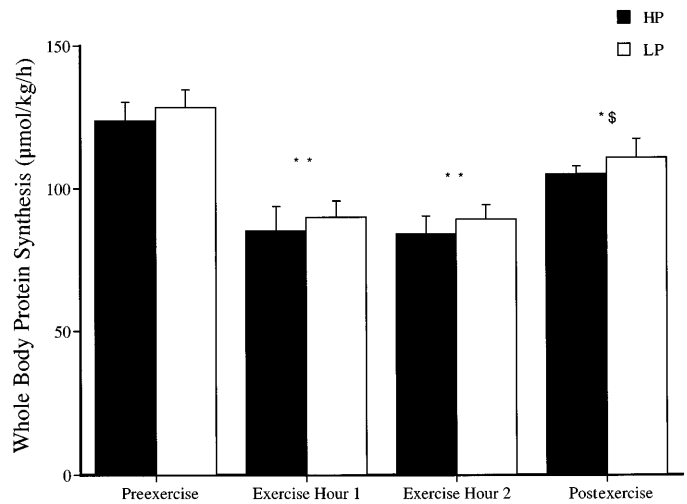


Fig. 8. Effect of dietary protein content on whole body protein synthesis at rest and during exercise. Values are means  $\pm$  SE ( $n = 8$  subjects). Significantly different from preexercise value ( $*P < 0.05$ ,  $**P < 0.01$ ) and significantly different from exercise hour 1 value ( $^{\$}P < 0.05$ ).

period for either group. Postabsorptive resting plasma insulin concentrations tended to be higher for HP subjects ( $19.2 \pm 2.0$  vs.  $11.4 \pm 0.7$   $\mu\text{U/ml}$ ), but during exercise insulin levels fell for the HP group, whereas those of LP subjects remained constant, so there was no difference during exercise ( $14.2 \pm 2.2$  vs.  $11.8 \pm 1.3$   $\mu\text{U/ml}$ , mean for 2 h) or during the period after exercise ( $14.7 \pm 2.3$  vs.  $12.5 \pm 1.4$   $\mu\text{U/ml}$ ).

Neither leucine carbon flux (whole body protein breakdown) nor nonoxidative leucine disposal (whole body protein synthesis) was different between HP and LP subjects (Figs. 7 and 8). Whole body protein synthesis decreased during exercise ( $P < 0.01$ ) and increased during the first 2 h of recovery ( $P < 0.05$ ) but remained suppressed relative to basal values ( $P < 0.05$ ).

Total leucine nitrogen flux tended to be higher for HP than for LP subjects throughout (Fig. 9), and during

exercise it tended to decrease, particularly for HP subjects.

DISCUSSION

In the group of subjects who undertook exercise with no tracer amino acid infusion, the background breath  $^{13}\text{CO}_2$  enrichment was not altered by the transition from rest to exercise. This is despite an increase in carbohydrate and decrease in fat oxidation, which has been shown to occur (1), suggesting that the subjects' endogenous carbohydrate fuel stores were not  $^{13}\text{C}$  enriched. These findings, which are in close agreement with those of Wagenmakers et al. (33), suggest that it is not necessary to conduct background  $^{13}\text{CO}_2$  measurements for  $^{13}\text{C}$ -tracer studies conducted in Europe, where the diet tends to be relatively unenriched in  $^{13}\text{C}$ -labeled carbohydrates.

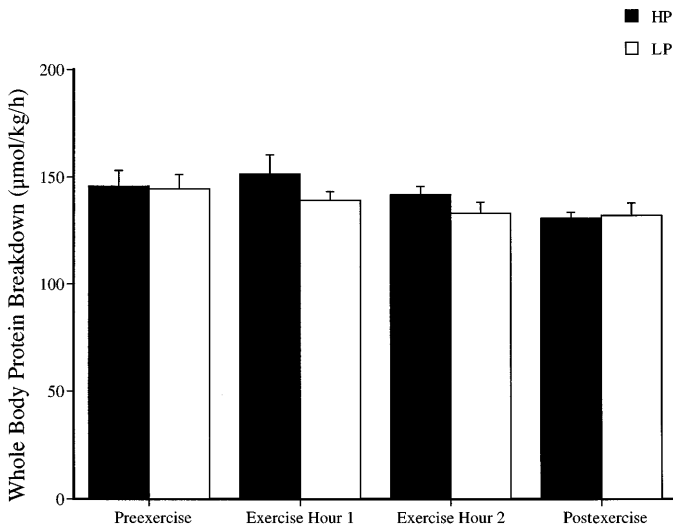


Fig. 7. Effect of dietary protein content on whole body protein breakdown at rest and during exercise. Values are means  $\pm$  SE ( $n = 8$  subjects).

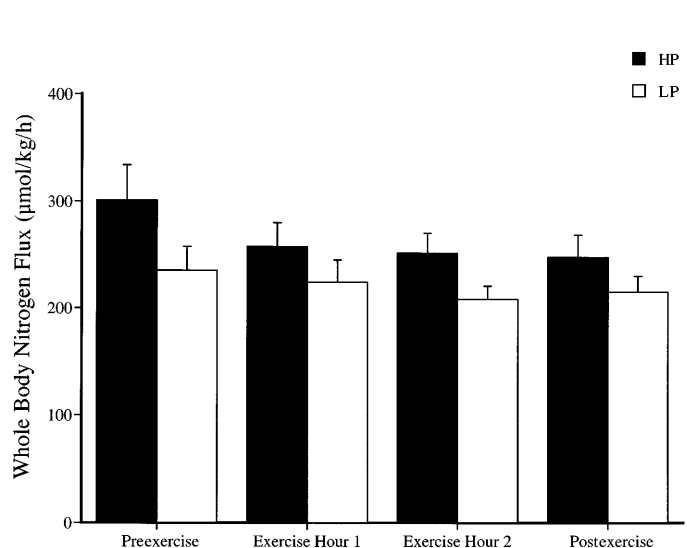


Fig. 9. Effect of dietary protein content on leucine nitrogen flux at rest and during exercise. Values are means  $\pm$  SE ( $n = 8$  subjects).

A 7-day dietary-equilibration period was used for subjects to adapt to the new dietary protein content. Quevedo et al. (25) found that nitrogen balance had stabilized 5 days after reducing dietary protein intake from 1.82 to 0.77 g protein·kg<sup>-1</sup>·day<sup>-1</sup>. In the same study, there was no significant change in either leucine oxidation or leucine balance between *days 7* and *14* of a dietary-control period when dietary protein intake was reduced from 1.89 to 0.77 g protein·kg<sup>-1</sup>·day<sup>-1</sup>. It is unlikely, therefore, that a longer period of adaptation would have produced qualitatively different results, and only small additional quantitative changes might be expected.

The findings of a study by Phillips et al. (24) suggested that there are gender differences in leucine kinetics. Male and female subjects demonstrated qualitatively similar responses to exercise, but male subjects oxidized more leucine at rest and during exercise (24). However, for 10 days before the exercise-induced changes in leucine metabolism and nitrogen balance were investigated, subjects were supplied foods with a protein content approximating the Canadian recommended nutrient intake for protein. Unfortunately, the measured protein intake was different for the male (0.94 g protein·kg<sup>-1</sup>·day<sup>-1</sup>) and female subjects (0.80 g protein·kg<sup>-1</sup>·day<sup>-1</sup>). Dietary protein intake modifies leucine metabolism, and leucine oxidation is particularly sensitive. Under the circumstances, given the fact that no gender differences in protein metabolism have ever been reported previously, the results of Phillips et al. (24) cannot be regarded as conclusive. In the present study, data from male and female subjects were pooled; data generated by the female subjects were both qualitatively and quantitatively similar to those from the male subjects.

The findings of a twofold increase in both leucine oxidation and fractional activation of skeletal muscle BCOADH during exercise are in accordance with many papers by other authors (16, 18, 26, 32). Leucine oxidation was higher for subjects who had previously consumed an HP diet, but this was not accompanied by a higher skeletal muscle total or fractional BCOADH activity. In rats, consumption of a 50% casein diet for 2 wk resulted in an increase in postabsorptive skeletal muscle BCOADH activity from 2% (on a 25% casein diet) to 7%, and the postprandial activation increased from 20 to 40% (3). In people, BCAA ingestion caused a threefold activation of the BCOADH complex at rest, and this was additive to exercise-induced activation (30). This is presumably due to the elevation in muscle BCAA concentration and, hence, KIC concentration. BCOADH kinase, which causes phosphorylation and, hence, inactivation of the BCOADH complex, is inhibited by KIC; thus an increase in muscle KIC concentration will tend to cause an activation of BCOADH. The effect of dietary protein on skeletal muscle BCOADH activity appears to be more pronounced in the fed state, when BCAA concentration tends to be elevated. The studies reported in this paper were carried out in postabsorptive people, so any differences in BCOADH activity may be too small to detect and therefore of

minimal importance in explaining the greater leucine oxidation for HP than for LP postabsorptive subjects.

In people, ~60% of total body BCOADH complex is in muscle and 30% in liver (17), so it is possible that differences in the activation of the liver enzyme, which was not measured, may account for the greater leucine oxidation in subjects on the HP diet. In rats, in which 70% of total BCOADH is in the liver (17), the liver complex is only 40–45% active during consumption of an LP diet but is fully activated by consumption of an adequate protein diet (4). If activation of the liver complex also occurs in people, then the higher whole body leucine oxidation observed for HP subjects at rest may be due to a greater rate of KIC decarboxylation in the liver. However, during exercise, it would be expected that a reduction in the contribution of hepatic to whole body leucine oxidation would occur because of the reduced blood flow to the splanchnic bed and the increase in blood flow to the exercising muscle. This would tend to alter the relationship between leucine oxidation rates in HP and LP subjects if altered rates of hepatic leucine oxidation were the source of the difference. However, the excess oxidation remained constant during the first hour of exercise (at ~40%), which suggests that the difference was not due to altered liver BCOADH activity.

Previous studies have found a positive correlation between plasma leucine and KIC concentrations and leucine oxidation (6). One possible mechanism for this positive relationship between leucine concentration and oxidation, discussed previously, is a KIC-induced inhibition of BCOADH kinase (19) and thus activation of the BCOADH complex. In the present study, the relationship was not so simple, since there was an exercise-induced activation of BCOADH, apparently independent of changes in plasma leucine concentration. During the second hour of exercise, when leucine oxidation had decreased in HP subjects, plasma leucine and KIC concentrations were also reduced. However, there was no difference in BCOADH activation, despite the difference in plasma leucine and KIC concentrations between subjects who had previously consumed diets with different protein contents. This tends to eliminate the possibility of an allosteric inhibition of BCOADH kinase, due to the higher plasma KIC concentrations, causing the increased leucine oxidation in HP subjects.

Hutson and Rannels (15) found that incubation of mitochondria with ATP resulted in increased leucine oxidation; they proposed that the resultant increase in mitochondrial pH facilitated transport of KIC into the mitochondria, since branched chain keto acid transport is achieved by proton symport. This would suggest that in some physiological situations mitochondrial KIC concentration may be rate limiting for leucine oxidation. During exercise, lactic acid production (catalyzed by lactate dehydrogenase, which is situated in the cytosol of the cell) may cause a decrease in cytosolic pH. This will tend to increase the proton gradient between cytosol and mitochondria and should, therefore, facilitate mitochondrial KIC transport, which may contrib-

ute to the exercise-induced increase in leucine oxidation. Indeed, metabolic acidosis has been found to increase BCAA oxidation in rats, through both increased transamination and an activation of BCOADH (22). BCAAT has a Michaelis constant ( $K_m$ ) of 0.4–0.8 mM (11), which is two to six times higher than the likely muscle concentrations; therefore, since transamination is an equilibrium reaction, an increase in leucine availability should result in increased KIC formation. Unfortunately, we did not measure intracellular pH in the studies reported here. However, plasma lactate concentration was not significantly elevated during exercise, and it is difficult to envisage a situation where a decrease in cytosolic pH during exercise might occur without a concomitant rise in plasma lactate concentration.

The higher intracellular leucine concentration expected in HP subjects should tend to increase mitochondrial KIC concentration both via an increase in muscle leucine uptake [via system L, which is a rapidly equilibrating low-affinity, high-capacity transporter (13)] and by increased transamination. The mitochondrial KIC concentration is thought to be 20–25  $\mu$ M (14), and the  $K_m$  of the BCOADH complex is 15–50  $\mu$ M (11), so any increase in mitochondrial KIC concentration is likely to result in a proportionally equivalent increase in leucine oxidation. It seems likely, therefore, that the greater leucine supply in HP subjects is sufficient to stimulate flux through the oxidative pathway. An activation of the BCOADH complex is not a prerequisite for such an increase in leucine oxidation.

The transamination and reamination data provide further evidence that the leucine supply and, therefore, delivery of KIC to the mitochondrial BCOADH complex was rate limiting. The proportion of KIC formed that was oxidized tended to be higher for LP than for HP subjects throughout and was stimulated during exercise for both subject groups. This is similar to the pattern of change in the activity state of the skeletal muscle BCOADH complex, which would be expected, since the rate-limiting decarboxylation of KIC is catalyzed by this enzyme complex. However, at rest and during the first hour of exercise, fractional transamination of leucine tended to be higher for HP than for LP subjects at a time when measured leucine oxidation was also higher for HP subjects. This suggests that the higher intracellular leucine concentration predicted for HP subjects drives transamination at a more rapid rate, and because the BCOADH complex is working at or below its  $K_m$  (15–50  $\mu$ M), the increase in KIC formation stimulated flux through the oxidative pathway. This is supported by the observation that in the second hour of exercise, when plasma leucine concentration, which is an indicator of intracellular leucine concentration (14), was decreased for HP subjects, there was also a fall in leucine transamination and oxidation.

Fractional transamination was increased during exercise for both subject groups. Leucine transamination is an equilibrium reaction, due to the high  $K_m$  of the BCAAT complex; therefore, the most likely explanation

for the increase in the proportion of leucine transaminated is a right shift in the reaction equilibrium related to exercise-induced changes in muscle metabolite concentrations. During the first 10 min of endurance exercise, muscle glutamate concentration falls by 70% (30), possibly because of increased flux through the alanine aminotransferase pathway, resulting in the formation of 2-oxoglutarate and alanine. This fall in muscle glutamate concentration will tend to favor the transamination of leucine and 2-oxoglutarate with the production of KIC and glutamate.

The exercise bout did not alter whole body protein breakdown, but protein synthesis was suppressed by 31%. The literature suggests (18) that endurance exercise in the postabsorptive state has a negligible effect on whole body protein breakdown. In the 2-h recovery period, protein breakdown was not altered, but protein synthesis remained suppressed. Previous research suggests (26) that both protein breakdown and protein synthesis are elevated during recovery from exercise, with protein synthesis being stimulated to a greater extent than protein breakdown, so that a positive nitrogen balance is reestablished. However, the changes that occur are dependent on exercise intensity and duration; in this study, it appears that the subjects were in a catabolic state, at least for the first 2 h after exercise.

There was no difference observed in either whole body protein breakdown or protein synthesis between HP and LP subjects in the postabsorptive state, despite the higher plasma insulin and leucine concentrations in HP subjects; these factors would have been expected to suppress protein breakdown and increase protein synthesis (29). It may be that the differences in these variables between the HP and LP subjects were insufficient to elicit changes in protein breakdown and synthesis. This is consistent with the literature (23), where differences in protein turnover due to variation of dietary protein were evident in the fed state but, over the range examined in this study, were not significant in the postabsorptive state (23).

In conclusion, exercise of a moderate intensity resulted in a twofold increase in leucine oxidation, a 31% reduction in whole body protein synthesis, and no change in protein breakdown. There was also a twofold activation of the skeletal muscle BCOADH complex, which may account for the increase in leucine oxidation. At rest in the postabsorptive state, previous consumption of an HP diet resulted in increased leucine oxidation, and this difference remained constant for the first hour of exercise. This could not be attributed to differential activation of the skeletal muscle BCOADH complex but may be due to increased substrate delivery, since the complex is working at or below its  $K_m$  in physiological conditions. These results tend to confirm the link between activation state of skeletal muscle BCOADH complex and leucine oxidation during exercise but suggest that, in the postabsorptive state, the stimulation of leucine oxidation while an HP diet is consumed cannot be attributed to an activation of the BCOADH complex, at least not in skeletal muscle.



## APPENDIX

*Combination of P Values From Separate Analyses Using Meta Analysis*

The *P* values obtained from the two-way ANOVA analysis of dependent and independent data were combined by using meta analysis, provided that the direction of change in the variable was the same for both analyses. The technique employs the criteria that the sum of the  $\chi^2$  values for the separate analyses must fit a  $\chi^2$  distribution to achieve significance (8). Where

$$\Sigma \chi^2 = -2 \cdot [\ln(\text{independent-analysis } P \text{ value}) + \ln(\text{dependent-analysis } P \text{ value})]$$

There are four degrees of freedom when *P* values from two analyses are combined, and the 5% value is 9.488, whereas the 1% value is 13.28. *P* values were only combined if the direction of change in the variable was the same for both analyses. An example of the analysis of the leucine oxidation data follows (Table A1).

Table A1.

	Independent- Analysis P Value	Dependent- Analysis P Value	$\Sigma \chi^2$	Combined P Value
Protein main effect	0.0525	0.0657	11.34	<0.05
Time main effect	<0.0001	<0.0001	59.87	<0.01
Protein/time interaction	0.0014	0.1970	16.39	<0.01

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