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Whole body leucine metabolism during and after resistance exercise in fed humans

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ABSTRACT

TARNOPOLSKY, M. A., S. A. ATKINSON, J. D. MACDOUGALL, B. B. SENOR, P. W. R. LEMON, and H. SCHWARCZ. Whole body leucine metabolism during and after resistance exercise in fed humans. *Med. Sci. Sports Exerc.*, Vol. 23, No. 3, pp. 326–333, 1991.The effects of resistance exercise upon leucine oxidation and whole body protein synthesis were studied using stable isotope methodology. L-[1-^{13}C]leucine was used as a tracer to calculate leucine oxidation and whole body protein synthesis in six healthy, fed, male athletes in response to a 1 h bout of circuit-set resistance exercise. The measurements were performed prior to, during, and for 2 h after exercise, and corrections were made for background $^{13}CO_2/^{12}CO_2$ breath enrichment and bicarbonate retention factor changes. Results demonstrated significant ($P < 0.01$) increases in the background $^{13}CO_2/^{12}CO_2$ breath enrichment at 1 and 2h after exercise and in the bicarbonate retention factor ($P < 0.01$) during exercise. At 15 min after exercise, the bicarbonate retention factor was significantly ($P < 0.05$) lower than at rest. There were no significant effects of exercise on leucine oxidation or flux, whole body protein synthesis, or the rate of appearance of endogenous leucine. We concluded that circuit-set resistance exercise did not affect the measured variables of leucine metabolism. In addition, large errors in calculating leucine oxidation and whole body protein synthesis during resistance exercise can occur if background $^{13}CO_2/^{12}CO_2$ breath enrichment and bicarbonate retention factor changes are not accounted for.

STABLE ISOTOPES, AMINO ACID OXIDATION, BICARBONATE RETENTION, WHOLE BODY PROTEIN SYNTHESIS, L-[1-^{13}C]LEUCINE

Much of the information regarding protein metabolism during exercise has been derived from nitrogen balance (8,9,20,31) and urea excretion (6,16,30,31) measurements. These methods, however, do not provide information about the effects of exercise upon the rates or regulation of amino acid oxidation/protein synthesis during and after exercise. Several studies have used stable isotope methodology (primarily L-[1-^{13}C]leucine) to investigate the effect of endurance exercise upon amino acid/protein metabolism (7,20,24,32,34). Such studies demonstrated an increase in leucine oxidation during endurance exercise (7,20,24,32,34), and two of these demonstrated a reduction in whole body protein synthesis (WBPS) (24,32).

There have been no investigations of leucine oxidation and WBPS during resistance exercise. In addition, few studies have measured leucine oxidation or WBPS for any time period following exercise (7,32,34), despite evidence that significant changes occur in both animals (3,25) and humans (5,23).

The primary long-term adaptive response to heavy resistance training is an increase in the myofibrillar protein content of skeletal muscle (17,18); yet little information is available regarding the time course changes in muscle protein synthesis (MPS) associated with a single resistance exercise bout. However, it is not yet possible to measure MPS in humans during a bout of exercise with the present method of L-[1-^{13}C]leucine incorporation into skeletal muscle (22). Because a significant, positive correlation exists between MPS and WBPS (the former accounting for 25–30% of the latter) (22), we studied leucine oxidation and WBPS changes during and following an acute resistance exercise bout as an initial attempt to assess changes in MPS.

It has been demonstrated that during exercise there are changes in the background $^{13}CO_2/^{12}CO_2$ breath enrichment (BCGND) and in the bicarbonate retention factor (c) (2,33,34) which would result in an overestimation of leucine oxidation and an underestimation of WBPS during exercise (33,34). However, these corrections have not always been employed in reported studies (7,23,32). These are the first values of leucine oxidation reported during resistance exercise, and they were corrected for measured changes in both BCGND and c.

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MATERIALS AND METHODS

Subjects

Six healthy males volunteered to participate in the study (Table 1). All were physical education students with at least 3 months of recent experience in weight training (two to five times per week). The study was conducted under the approval of the Human Ethics Committee of McMaster University, and all subjects provided written, informed consent.

Experimental Protocol

Subjects completed 3 d food records (including one weekend day) from which each individual's mean daily energy and protein intake was calculated using a computer program (ANALYZE, McMaster University). Based on this information an individual isoenergetic and isonitrogenous diet was designed for the experimental trials. During the week prior to the experimental trial, each subject had his body density determined by hydrostatic weighing, with residual volume measurements made by helium dilution.

The subjects did not perform any strenuous exercise on the day prior to the study. At 0630 h, following a 12 h overnight fast, the subjects consumed a liquid diet aliquot (Ensure, Ross Laboratories, Montreal, Quebec) that was equivalent to 1/26th of each individual's mean energy intake and provided approximately 51 μmol leucine$\cdot h^{-1}$ (corrected for absorption of dietary leucine (29)). The subjects received their respective aliquots every 30 min prior to (2 h), during, and after exercise. Each subject consumed about 60% of his daily dietary intake over the 7 h experiment (Table 1).

At 0830 h, the subjects reported to the laboratory, a 22-gauge plastic catheter was inserted into a hand vein, and a baseline "arterialized" blood sample (hot box at 65 ± 5°C) and expired gas collection were completed. For isotope infusion, a second catheter was inserted into a vein of the contralateral proximal forearm in a location that was not occluded by arm bending. A priming dose of L-[1-^{13}C]leucine (1 mg$\cdot$kg^{-1}) and [^{13}C] sodium bicarbonate (0.295 mg$\cdot$kg^{-1}) (both 99% atom percent; MSD Isotopes, Pointe Claire, Quebec) was administered over 1 min, followed by a constant infusion of L-[1-^{13}C]leucine (1 mg$\cdot$kg$^{-1}\cdot$h^{-1}) for 5 h, delivered by a calibrated syringe pump (Harvard Apparatus, Boston, MA). The L-[1-^{13}C]leucine was diluted into sterile saline the day prior to infusion and the [^{13}C] sodium bicarbonate was diluted immediately prior to infusion, both under aseptic conditions, and both were sterilized by microfiltration immediately prior to infusion. Subjects 1–5 received leucine from the same batch, and all subjects received sodium bicarbonate from the same batch.

Blood samples were placed into heparinized, chilled tubes and centrifuged immediately, and the plasma was stored at −70°C until analysis. All gas samples (see below) were collected into 150 l meteorological balloons, with ventilatory volume determined on the inspired side using an on-line pneumotachometer (Validyne, Northridge, CA). Expired O_2 and CO_2 concentrations were determined within 5 min of collection with an oxygen (Rapox, Bilthoven, Holland) and carbon dioxide (Hewlett Packard, Avondale, PA) analyzer for subsequent calculations of $\dot{V}O_2$, $\dot{V}CO_2$, and RER. Duplicate gas samples were injected into 10 ml evacuated tubes (Vacutainer; Becton Dickinson, Rutherford, NJ) for subsequent cryogenic extraction of the CO_2, and $^{13}CO_2/^{12}CO_2$ was determined within 48 h using an isotope ratio mass spectrometer as described below.

At 90 (t = −30 min before exercise), 105 (t = −15), and 120 (t = 0) min after the bolus injection, gas and blood samples were taken to ensure an isotopic plateau. (Pilot data had previously determined this to occur within 60–90 min.) Immediately following the t = 0 collection, subjects completed three groups of resistance exercises on a Global Gym multistation training apparatus, with each group consisting of three sets of exercises—group A: bench press, sit-ups, leg press; group B: latissimus pulldowns, biceps curls, knee extension; group C: triceps press, military press, leg press. Subjects performed 20 sit-ups, and for all other exercises three sets of ten repetitions were performed at 70% of the

TABLE 1. Subject characteristics and daily energy and protein intakes.

	Subjects (N = 6)						
	1	2	3	4	5	6	Mean (SE)
Age (yr)	25	23	28	30	22	22	25 (1.4)
Weight (kg)	80.6	68.8	80.0	89.5	70.2	74.9	77.3 (3.1)
Height (cm)	170.5	183.0	181.5	181.0	168.5	179.5	177.3 (2.5)
Density (g$\cdot$cm^{-3})	1.052	1.075	1.074	1.082	1.083	1.076	1.074 (0.005)
Body fat (%)	20.6	10.4	10.9	7.5	7.1	10.0	11.1 (2.0)
Daily intakes:							
Protein (g$\cdot$kg^{-1})	1.0	1.3	1.4	1.9	1.2	1.5	1.4 (0.11)
Energy (kcal)	2392	2483	3228	4738	2475	3087	3067 (364)
Test day leucine intake (μmol$\cdot$kg$^{-1}\cdot$h^{-1})	39.2	47.6	53.2	70.0	43.1	50.3	50.6 (4.4)

individual's one repetition maximum (1 RM) (determined within 1 wk prior to the study). Each set was performed in approximately 30 s (1.5 s concentric; 1.5 s eccentric), with a 2 min rest between each set. Gas samples were collected for the entire second circuit of three sets in each group (approximately 7 min in duration), and blood was slowly withdrawn between 3–4 min (rest) of the respective circuit.

Upon completing the exercise, the subjects lay on a bed (without interruption of the isotope infusion), and gas/blood samples were collected at 5 (t = +5), 15 (t = +15), 60 (t = +1 h), and 120 (t = +2 h) min after exercise (Fig. 1).

Analyses

Breath analysis. Carbon dioxide from the evacuated tubes was cryogenically extracted, and the $^{13}CO_2$ isotopic enrichment of the sample was determined using a gas-isotope ratio mass spectrometer (VG Isogas, SIRA 10, Cheshire, England) at m/z = 44/45 (27). Our intra-assay coefficient of variation (CV) was 0.46%, and the inter-assay CV was 1.03% over a range of sample enrichments from 0.002 to 0.025 atom percent excess (APE). Values are expressed as APE relative to baseline samples.

Plasma analysis. The o-quinoxalinol-trimethylsilyl derivative of α-keto isocaproic acid (α-KIC) was prepared using a modification of standard procedures (28,32). The proteins in 1 ml of plasma were precipitated using absolute ethanol (5 ml × 2) and centrifuged at 10,000 rpm × 10 min. The supernatant was dried under dry air and resuspended in 1 ml of water. To this was added 1 ml of 2% o-phenylenediamine solution (2% in 4 N HCl), and the solution was heated at 100°C × 1 h and cooled. The derivative was extracted in 2.5 ml of methylene chloride and centrifuged for 2–3 min at 3,000 rpm. The lower layer was pipetted into a clean tube and the procedure repeated. To this was added 75–100 mg of sodium sulfate, and the solution was vortexed. The upper layer was transferred to a screw-top tube and evaporated under a gentle stream of dry air. The final derivitization was performed with 75 μl of BSTFA + 1% TMCS (Pierce Chemicals, Rockford, IL) heated at 100°C × 30 min.

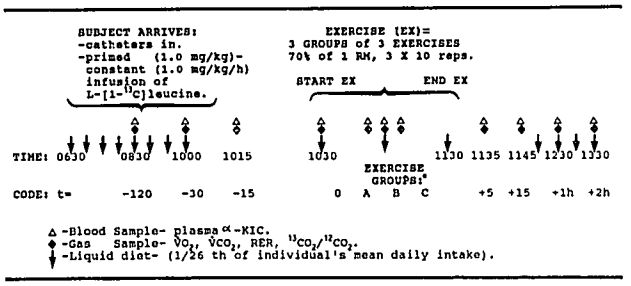

Figure 1—Study design for the experiment. Six subjects completed the trial with a primed-constant infusion of L-[1-^{13}C]leucine, four with a primed-constant infusion of [^{13}C]sodium bicarbonate (to determine c), and four with no isotope infusion (to determine background breath $^{13}CO_2/^{12}CO_2$ enrichments). Each of the three exercise groups (A, B, and C) consisted of three circuits of three resistance exercises performed at an intensity of 70% of each individual's 1 RM (see text).

A VG-Trio-2 GC/MS (VG, Cheshire, England) and a Hewlett Packard Model 5890 GC were used to determine the α-KIC enrichment in the plasma. The derivitized samples (0.3 μl) were injected into a 15 m fused silica capillary column (0.25 mm ID) (DB5 J.W. Scientific, Rancho Verda, CA) with the oven program set at an initial temperature of 120°C, ramped to 160°C at a rate of 8°C·min^{-1}, and then ramped to 290°C at a rate of 20°C·min^{-1} and held at 290°C for 3 min. Helium was used as the carrier gas (32 cm·s^{-1}). The column exited directly into the mass spectrometer ion source, and electron impact ionization was used (70 eV, trap current 170 μA, source current 1.6 mA).

We monitored the m/z 232.2/233.2 ratios by scanning the instrument over a narrow mass range (228 amu to 238 amu: cycle time 1.35 s (1.25 s scan, 0.05 s interscan)). The abundance of the ^{13}C enriched samples (233.2 amu) of α-KIC relative to the ^{12}C (nonenriched) species (232.2 amu) of α-KIC was taken from the listing of the mass spectrum acquired at the area of the chromatographic peak. This method provided an intra-assay CV of 0.77% for the raw isotope ratios of pooled, enriched plasma samples (N = 5). Thus, we analyzed each subject's results in separate batches.

Calculations

Whole body leucine kinetics. Leucine flux (Q) was calculated using the reciprocal pool model (from [^{13}C] α-KIC values) (11), at isotopic plateau, as described by others (19,32): Q = i(Ei/Ep−1), where i = L-[1-^{13}C] leucine infusion rate (μmol·kg^{-1}·h^{-1}), Ei = enrichment of the infused leucine (APE), Ep = enrichment of the plasma α-KIC (APE), and the term "−1" corrects for the contribution of the infused isotope. The reciprocal pool model assumes that the enrichment of plasma [^{13}C]α-KIC, during an infusion of L-[1-^{13}C] leucine, is more indicative of intracellular [^{13}C]leucine enrichment than is plasma [^{13}C]leucine enrichment (11).

Leucine oxidation was calculated from the equation (32): total leucine oxidation = $[(IECO_2/c)/IE_{\alpha\text{-KIC}}]\cdot \dot{V}CO_2$, where $IECO_2$ = enrichment of expired CO_2 (APE), $IE_{\alpha\text{-KIC}}$ = enrichment of plasma α-KIC (APE), $\dot{V}CO_2$ = volume of carbon dioxide evolved (μmol·kg^{-1}·h^{-1}), and c = bicarbonate retention factor. Because exercise is known to affect the retention of CO_2 in the body (2,33), the bicarbonate retention factor (c) was determined for four of the six subjects 1 wk prior to the experiment using the identical experimental design with a primed-constant infusion of [^{13}C]sodium bicarbonate (prepared within 1 h of infusion to mini-

mize loss as $^{13}CO_2$), as described by Kien (15): c = $\dot{V}CO_2 \cdot$(IE bicarbonate $CO_2 \cdot F^{-1}$), where $\dot{V}CO_2$ = volume of carbon dioxide evolved ($\mu mol \cdot kg^{-1} \cdot min^{-1}$), IE bicarbonate CO_2 = isotopic enrichment of expired CO_2 at plateau (corrected for background CO_2 enrichment changes (see below)), and F = the infusion rate of [^{13}C]sodium bicarbonate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$). The individual c was used for each of four subjects, and the mean of these values was applied to the other two subjects in the calculation of leucine oxidation. In addition, four subjects were studied under identical study conditions with no isotope infusion to account for changes in background CO_2 enrichment caused by the protocol (2,33), to further correct our leucine oxidation values.

The rate of appearance of endogenous leucine (Ra end leu) was determined from total leucine flux (Q) minus dietary leucine (corrected for absorption of dietary leucine from casein hydrolysate (29)). This was used as an indicator of whole body proteolysis, and the nonoxidative portion of leucine flux was used to estimate whole body protein synthesis (19,22) according to the model where flux (Q) = synthesis (S) + oxidation (O) (19), using a tissue leucine content of 590 μmol leucine·g protein^{-1} (approximately 7.8%) (32).

Statistics

The effect of exercise upon the variables measured was determined using a one-way analysis of variance (ANOVA) (Minitab, V.7.0, State College, PA). When a significant F-value ($P < 0.05$) was observed, the location of the pairwise difference was made with the Tukey *post hoc* analysis. Data in tables, figures, and text are mean ± SE.

RESULTS

Gas exchange. There were significant ($P < 0.01$) increases for both $\dot{V}O_2$ and $\dot{V}CO_2$ during each exercise group (A, B, and C) relative to pre-exercise values (Fig. 2). The mean RER values were significantly ($P < 0.01$) greater during exercise group A relative to resting values and were significantly ($P < 0.05$) lower than pre-exercise values by 15 min after exercise (t = +15) (Fig. 2).

Background enrichment and bicarbonate retention factor. There were no effects of exercise upon the background breath $^{13}CO_2/^{12}CO_2$ isotopic enrichment during exercise ($N = 4$), yet, at 1 (t = +1 h) and 2 (t = +2 h) h after exercise, significant ($P < 0.01$) increases in enrichment were observed (Fig. 3). Prior to exercise the mean bicarbonate retention factor (c) was 0.83 ($N = 4$), which increased significantly ($P < 0.01$) to 1.72 during exercise group A and to 1.46 during exercise group B and decreased significantly ($P < 0.05$) to 0.52 by 15 min after exercise (t = +15) (Fig. 3).

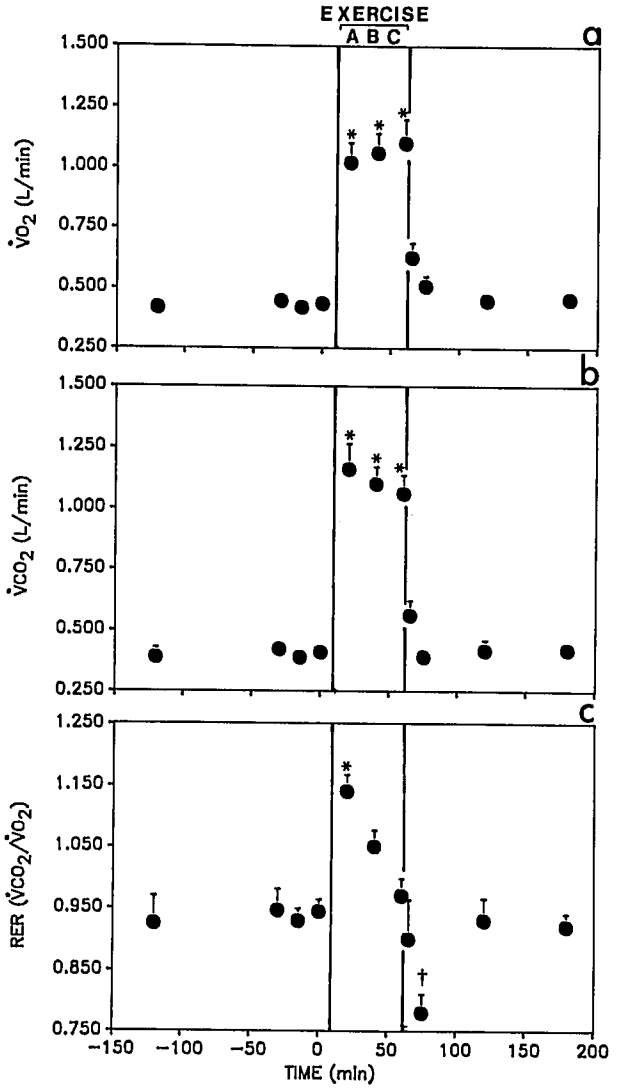

Figure 2—Volume of oxygen production ($\dot{V}O_2$) (Panel a), volume of carbon dioxide production ($\dot{V}CO_2$) (Panel b), and respiratory exchange ratio (RER) (Panel c) before, during (exercise groups A, B, and C; see text), and after resistance exercise. *$P < 0.01$ compared with mean pre-exercise values. †$P < 0.05$ compared with mean pre-exercise values.

Breath and plasma α-KIC isotopic enrichments. Plateau isotopic enrichments were achieved in both breath CO_2 and plasma α-KIC within 90 min of infusion (Fig. 4). A lower breath CO_2 enrichment plateau was achieved during exercise groups A, B, and C and at time +5 and +15 min after exercise. This latter plateau was significantly different ($P < 0.01$) from that attained before (t = −30, −15, 0) and at time +1 h and +2 h after exercise (Fig. 4). An α-KIC isotopic enrichment plateau was maintained throughout the study protocol (Fig. 4).

Calculations of leucine flux and oxidation; estimations of whole body protein synthesis (WBPS) and proteolysis. We did not find an effect of resistance

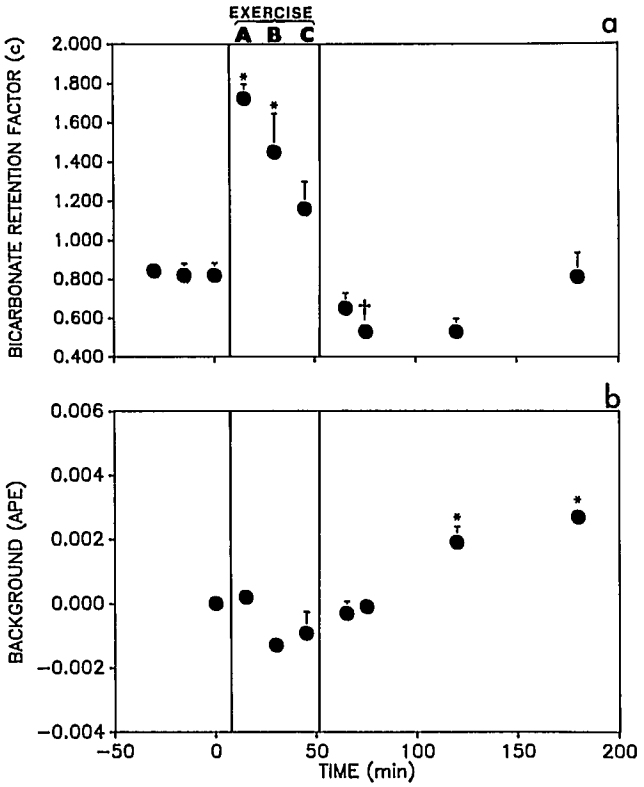

Figure 3—Bicarbonate retention factor (c) determined by primed-constant infusion of [^{13}C]sodium bicarbonate (N = 4 subjects) (Panel a). Background breath $^{13}CO_2/^{12}CO_2$ isotopic enrichment with no isotope infusion (N = 4 subjects). Values are atompercent excess (APE) vs pre-exercise value (Panel b). *$P < 0.01$ compared with mean pre-exercise values. †$P < 0.05$ compared with mean pre-exercise values.

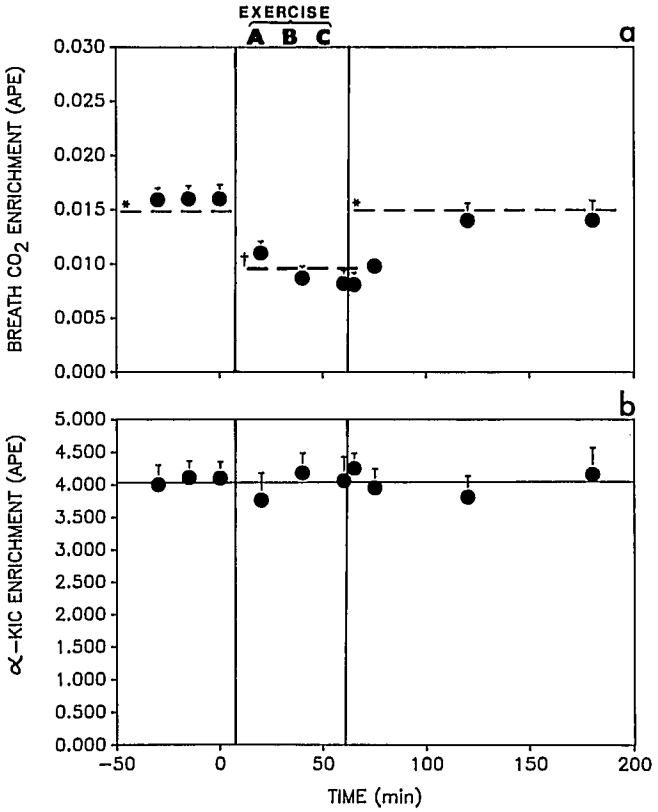

Figure 4—Breath CO_2 enrichment (corrected for background and c changes) (Panel a) and plasma α-KIC enrichment changes (Panel b) during the L-[1-^{13}C]leucine infusion protocol. All points joined with the *thin line* (*) are not significantly different from each other but are significantly different ($P < 0.01$) from those joined with the *thicker line* (†).

exercise upon leucine oxidation or WBPS during or for up to 2 h after exercise (Fig. 5). In addition, we did not find that resistance exercise altered leucine flux or the rate of appearance of endogenous leucine (Ra end leu) (Fig. 6).

DISCUSSION

To our knowledge, the present data represent the first published report of leucine metabolism, bicarbonate kinetics, and background $^{13}CO_2$ enrichments during and after resistance exercise. Resistance exercise did not affect any of the measured indices of leucine metabolism, yet there were highly significant effects of exercise upon the bicarbonate retention factor (c) during exercise and upon the background $^{13}CO_2$ enrichments after exercise.

The small decrease in background $^{13}CO_2$ enrichment that we observed at the start of exercise is consistent with the results of studies using endurance exercise (2,32). The subsequent progressive rise in breath $^{13}CO_2$ enrichment with continued exercise has also been observed by others (2). However, at 1–2 h post-exercise, background breath $^{13}CO_2$ enrichment values have not been previously reported.

It is known that the $^{13}CO_2$ enrichment adjusts much more slowly during exercise than do changes in $\dot{V}O_2$ and $\dot{V}CO_2$ (2), which likely reflects the time delay for a $^{13}CO_2$ molecule to pass through one or all of the pools of bicarbonate in the body (12). Thus, the increased $^{13}CO_2$ enrichment at 1 and 2 h post-exercise may indicate an increased carbohydrate oxidation (greater ^{13}C enrichment vs fats (26)) during and not necessarily after exercise (2).

At rest, the mean bicarbonate retention factor was 0.83, which is consistent with the value of 0.82 found in a recent study of fed, healthy, young male subjects (10). It is also very close to the accepted value of 0.81 that has commonly been used to calculate leucine oxidation in studies where c was not determined directly (21,24,32). The finding of a large increase in c at the onset of exercise with a subsequent gradual decrease is consistent with the results obtained by Wolfe et al. (34) during endurance exercise. We considered this increase to be indicative of an increased flux of $^{13}CO_2$ through body bicarbonate pools consequent to exercise (1,2)

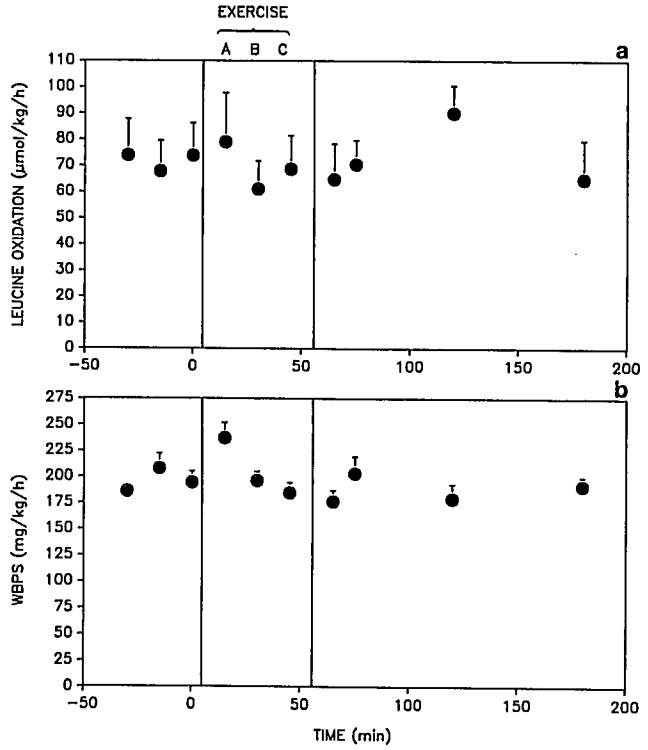

Figure 5—Leucine oxidation values (corrected for background enrichment and c changes) (Panel a). Whole body protein synthesis (WBPS) values determined from the nonoxidative portion of leucine flux (Panel b).

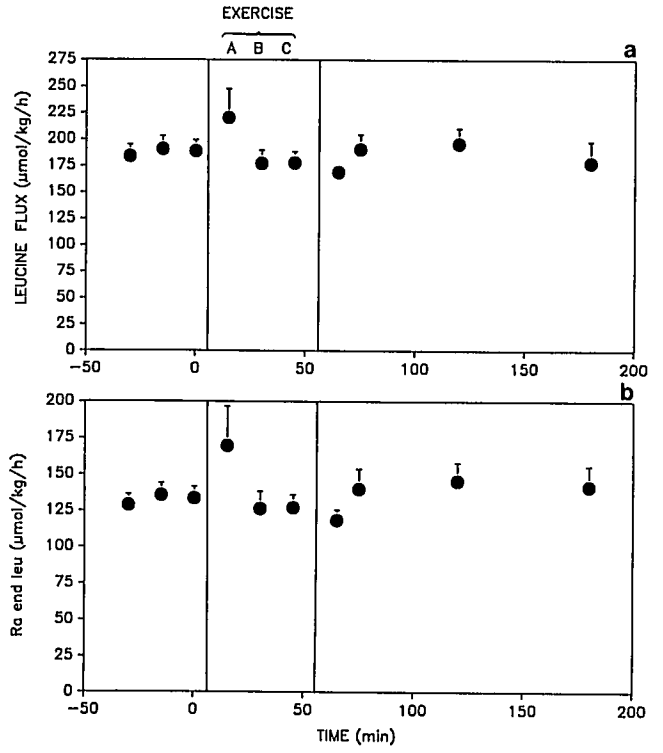

Figure 6—Leucine flux (Panel a) and the rate of appearance of endogenous leucine (an indicator of proteolysis) (Panel b).

rather than of increased oxidation of carbohydrates as suggested by others (34). If increased c values were indicative of increased carbohydrate oxidation, it would be expected that breath $^{13}CO_2$ enrichment would also increase at the same time, which did not occur.

The significant effects of exercise upon background enrichment and c clearly demonstrate the need to perform preliminary studies to quantitate these variables in any study using stable isotopes in order to determine substrate oxidation during an altered metabolic state such as exercise (34).

Contrary to previous studies involving endurance exercise (7,24,32,34), leucine oxidation was not increased during resistance exercise. The calculation of leucine oxidation in the present study has taken into account measured changes in background breath isotopic enrichment and in the bicarbonate retention factor. Studies which have neglected to measure the effects of their study protocol upon these variables and to correct their oxidation values may have overestimated leucine oxidation during (7,24,32) and underestimated it following exercise. If changes in c were not determined in the present study, the leucine oxidation values would have been overestimated by 107% and 76% during exercise groups A and B, respectively, and would have been underestimated by 37% at 15 min after exercise. This would also lead to a bias in favor of underestimating WBPS during and overestimating WBPS after exercise because WBPS is estimated from the nonoxidative portion of leucine flux (19,32).

During the infusion of L-[1-^{13}C]leucine, our pre-exercise, corrected (for background and c changes) breath CO_2 enrichments had reached an isotopic plateau which decreased to a new plateau during exercise groups A, B, and C and at times +5 and +15 min. These results were nearly identical to those reported by Wolfe et al. (32) for subjects performing endurance exercise at about 30% $\dot{V}O_{2max}$ and demonstrated much less variance. We were also able to establish an isotopic plateau in α-KIC enrichment for the duration of the experiment, which is another of the prerequisites to using the steady-state equations (described above in Results) for the calculation of leucine oxidation (32).

The calculation of total leucine oxidation from plasma α-KIC enrichment (reciprocal pool model (11)) is superior to values obtained using plasma leucine enrichment, for it has been shown that leucine oxidation values during exercise are underestimated when derived from plasma leucine enrichment (32). In addition, it has been demonstrated that the enrichment of plasma α-KIC is more indicative of intracellular leucine enrichment than is plasma leucine enrichment (11).

Using L-[1-^{13}C]leucine as a tracer, it has been found that endurance exercise at approximately 30% $\dot{V}O_{2max}$ resulted in a 371% increase in total leucine oxidation

(32). In a later publication, Wolfe et al. (34) determined the c value for a group of subjects under identical exercise conditions and found that, when applied to their original results (32), leucine oxidation values were actually lower by about 36%, yet the increased oxidation values were still highly significant ($P < 0.001$) (34).

Although we did not measure $\dot{V}O_{2max}$ in this study, the peak mean value attained by the subjects for all three exercise groups combined (1.12 $l \cdot min^{-1}$) is similar to that obtained in another study of circuit resistance training of about the same intensity (23) and would represent about 30–35% $\dot{V}O_{2max}$. The intermittent nature of the resistance exercise protocol in this study (30 s exercise-2 min rest) may have contributed to the differences in leucine oxidation that we observed compared with endurance exercise performed at a similar oxygen uptake (32). During endurance exercise, the activity of branched-chain 2-oxo acid dehydrogenase increases (BCOAD) (13), which increases leucine oxidation, and this enzyme activity is inversely correlated with adenosine triphosphate levels (13). The 2 min rest period that we have used in the present study would have allowed for a repletion of the high energy phosphate pool, which would decrease BCOAD activity and, subsequently, decrease leucine oxidation.

In addition, we have previously demonstrated, using nitrogen balance techniques, that endurance athletes require about 67% more dietary protein, yet resistance athletes require only 12% more protein than sedentary controls (31). An increased urea excretion for male endurance athletes (30,31) indirectly supports the hypothesis that increased amino acid oxidation is responsible for the increased protein requirements of this group, while the small increase in requirements for resistance athletes likely results from increased muscle protein accretion (17,18) and not increased amino acid oxidation (31). The higher state of training of our subjects compared with those of other studies (24,32) may also have contributed to our finding of no increase in leucine oxidation for the progressive improvement in nitrogen balance demonstrated for subjects during training (9) and may involve an adaptive reduction in leucine oxidation. Finally, our subjects were fed a regular, defined-formula diet, whereas other studies have examined subjects in the fasted state (32,34), and it is known that oral feeding (21) increases leucine flux and oxidation. Therefore, feeding *per se* may have had a predominant effect upon leucine oxidation that masked an effect of exercise. For up to 2 h after exercise, we found no changes in leucine oxidation; however, leucine oxidation is decreased between 2–3 h after endurance exercise (5). It has been proposed that the decrease in leucine oxidation after endurance exercise is due to increased levels of free fatty acids and/or β-hydroxybutyrate (5), neither of which were likely to be elevated to the same level by the present study protocol (14).

In exercising animals, muscle protein synthesis (MPS) is reduced, and it is increased at about 1–2 h after exercise (3,25). Because resistance exercise results in increased muscle mass (17,18), it would have been desirable to directly measure MPS during and after the exercise bout. However, the present method of determining MPS from muscle biopsy samples using a primed-constant infusion of L-[1-^{13}C]leucine requires at least 4 h of constant isotope infusion to determine MPS with precision (22). Clearly, this method would not be applicable during exercise due to the time requirement, but studies are presently underway in our laboratory to examine the effect of resistance exercise on MPS 2–8 h after completion of exercise.

The fact that we did not find an effect of exercise upon WBPS may relate to one of several factors. First, since MPS represents only 25–30% of WBPS (22), changes in MPS consequent to exercise may have gone undetected; second, WBPS may have increased at some time point beyond 2 h post-exercise, for it is known that satellite cell proliferation occurs at about 2–3 d post-resistance-type exercise (4); third, the measurement of WBPS during exercise may not be accurate based upon different protein oxidation results obtained using [^{13}C]lysine vs L-[1-^{13}C]leucine as tracers (34); and fourth, MPS for exercised and nonexercised muscle and splanchnic protein synthesis may each change in opposite directions after exercise (5,24), which may render WBPS unchanged.

It is not possible to definitively determine the effects of resistance exercise upon MPS until direct measurements are made. Further studies are needed to determine the effects of resistance exercise upon WBPS beyond 2 h post-exercise, and multiple tracer studies need to be employed to determine the validity of using L-[1-^{13}C]leucine to measure WBPS after exercise. Any study using stable isotopes to measure substrate oxidation during exercise must quantitate the effects of the protocol upon BCGND and c.

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