Free Amino Acid Pool and Muscle Protein Balance after Resistance Exercise

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[1]Neuromuscular Research Center, Department of Biology of Physical Activity, and [2]Department of Chemistry, University of Jyväskylä, Jyväskylä, FINLAND; [3]Clinical Physiology, Central Finland's Central Hospital, Jyväskylä, FINLAND; [4]Rehabilitation Center of Kankaanpää, Kankaanpää, FINLAND; and [5]Peurunka–Medical Rehabilitation Center, Laukaa, FINLAND

ABSTRACT

PITKÄNEN, H. T., T. NYKÄNEN, J. KNUUTINEN, K. LAHTI, O. KEINÄNEN, M. ALEN, P. V. KOMI, and A. A. MERO. Free Amino Acid Pool and Muscle Protein Balance after Resistance Exercise. *Med. Sci. Sports Exerc.*, Vol. 35, No. 5, pp. 784–792, 2003. **Purpose:** The aim of this study was to assess the effects of a resistance exercise session (RES) on free amino acid concentrations and protein synthesis and breakdown of the vastus lateralis (VL) muscle during recovery in male subjects. **Methods:** Both the exercise group (EG) and the control group (CG) consisted of six healthy physically active men. On the experiment day in fasting conditions, a stable isotopic tracer of L-[*ring*-$^2H_5$] phenylalanine was infused and EG started a heavy 50-min hypertrophic RES for lower extremities after 55 min of infusion. At the same time, CG was at rest. During recovery of 195 min after RES, several blood samples were drawn from the femoral artery (FA) and the femoral vein (FV) and muscle samples from the VL muscle. The enrichment was analyzed by GC/MS and leg muscle amino acid kinetics determined by three-pool compartment model between FA, FV, and VL. **Results:** During recovery at 60 min after RES, there was no difference in muscle protein synthesis or muscle protein breakdown between the groups, but at 195 min, both muscle protein synthesis ($P < 0.05$) and muscle protein breakdown ($P < 0.05$) were increased in EG compared with CG. The protein net balance was negative and similar in both groups. Simultaneously in serum concentrations, there was a decrease in leucine ($P < 0.05$) associated with an increase in aspartate ($P < 0.05$). Furthermore, the exercise-induced increase in alanine concentration decreased both in serum and muscle. **Conclusion:** In fasting conditions, protein net balance is negative and RES induces an increase in muscle protein synthesis and breakdown at 195 min but not yet at 60 min of recovery. **Key Words:** FREE AMINO ACIDS, SYNTHESIS, BREAKDOWN, NET BALANCE, STABLE ISOTOPES, EXERCISE

As a response to physical stress, amino acids are mobilized from the body's free amino acid pool (19), which is located in the plasma and in cellular spaces (28). The pool represents only 2% (approximately 200 g) of the total amino acids in the body of a 70-kg individual, and approximately half of it (approximately 100 g) exists intracellularly in skeletal muscle (28). Despite the small size of the pool, it accounts for several metabolic reactions and protein turnover (28). Some of the amino acids are used for protein synthesis, some for energy requirement of muscular activity through oxidation, and some provide substrates for gluconeogenesis (21). Exercise induces changes both in free amino acid concentrations and in protein metabolism, and these responses have been shown to be both acute and long term, lasting from several minutes to as long as several days (10). During recovery after exercise, the use of the amino acid pool is variable. The utilization of free amino acids in energy processes decreases, and their use in anabolic processes increases. Homeostatic equilibrium is restored, energy recourses are replenished, and anabolic functions are accomplished (26).

Previous studies have reported different responses in free amino acid concentrations and in muscle protein metabolism, depending on the type of exercise. Aerobic type exercise has been shown to induce marked changes in plasma and in muscle amino acid concentrations as well as in protein synthesis (4,15). Alanine output from muscle decreases rapidly after the end of exercise, thus decreasing the blood level of alanine (7,29). Carraro et al. (5) reported total intracellular free amino acid concentration to be elevated after prolonged aerobic exercise, despite the increased efflux of alanine from muscle. They also demonstrated the increase of 25% in muscle protein synthesis after walking 4 h on a treadmill (5).

The response of postexercise muscle protein metabolism to aerobic exercise differs from the response to resistance exercise. After a strength exercise session, decreases both in serum levels of EAA and total amino acids have been observed, whereas alanine, taurine, and citrulline were unchanged (18). Muscle protein synthesis has been shown to be stimulated by resistance exercise as long as the intensity

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of exercise is enough to "challenge" the muscles, but training status may play a role in the response (6,8,17,24). Muscle protein breakdown is also increased after resistance exercise but not as much as protein synthesis (2,17). The relationship between these two parameters (rate of muscle protein synthesis and muscle protein breakdown) represents the metabolic basis for muscle growth; muscle hypertrophy occurs only when a positive protein balance can be established during the recovery through an increase in protein synthesis in the excess of the elevation in protein breakdown (2). In the study of Tipton et al. (22), the responses of muscle protein metabolism were examined after the combination of endurance and resistance exercise in swimmers. The authors concluded that the combination of exercise workout provided a stimulatory effect of muscle protein synthesis but not on whole-body tissue breakdown (22).

Several studies have examined the response of muscle protein metabolism to exercise, but the data have been collected primarily in response to endurance exercise. To our knowledge, research in the area of resistance exercise and its effects on amino acid concentrations and on net muscle protein balance simultaneously is limited. Studies to date have not addressed the potential relationship between the arteriovenous (a-v) and intracellular concentrations of amino acids and muscle protein kinetics after resistance exercise. The role of plasma and muscle amino acid concentrations in the early recovery is interesting, as amino acid availability plays an important role in the control of muscle protein kinetics. Increased blood flow, increased amino acid delivery to the muscle, and increased amino acid transport increase the rate of muscle protein synthesis (1,2,23) up to 24 h (4). In our earlier study, we have concentrated on the changes in serum amino acid concentrations during different kinds of anaerobic exercise, and decreases were demonstrated in the levels of several amino acids after running and strength exercise sessions (18). In the present study, we aimed to quantify the amino acid concentrations during the first 3 h of recovery. We chose the resistance exercise session on the basis of our previous results, as the observed decreases in all amino acid concentrations were greater in strength exercise session than in running exercise sessions. In addition, it has been shown (2) that resistance exercise has a profound effect both on muscle protein synthesis and muscle protein breakdown.

Therefore, the purpose of this study was to determine the amino acid profile in arterial and venous blood and in vastus lateralis (VL) muscle after a resistance exercise session (RES) in physically active males. We aimed also to assess whether a RES can induce changes in protein kinetics of VL muscle during recovery by using a three-compartment model (1).

MATERIAL AND METHODS

Subjects. Six volunteer subjects of resistance exercise group (EG; age, 25.9 (mean) ± 5.1 (SD) yr; weight, 86.8 ± 21.7 kg; height, 182.5 ± 8.4 cm; body mass index (BMI), 26.1 ± 2.9 kg·m^{-2}; leg volume, 8587 ± 675 mL) and control group (CG; age, 26.2 ± 4.7 yr; weight, 81.3 ± 9.2 kg; height 181.3 ± 3.2 cm; BMI, 24.9 ± 1.7 kg·m^{-2}; leg volume, 8457 ± 643 mL) consisted of healthy, physically active men (noncompetitive athletes). They took part in fitness (conditioning) training 3–5× wk^{-1}. The fitness training included warm-up on a bicycle ergometer (10 min), circuit training using own body (15 min), resistance training with strength machines (15 min), and stretching and recovery training on a bicycle ergometer (5–10 min) per training time. Leg volume was estimated using the anthropometric approach of Jones and Pearson (11). The study protocol, purpose, and possible risks were explained to each subject before their written consent was obtained. This study was approved by both the University Ethical Board and the Hospital Ethical Board.

TABLE 1. Content of resistance exercise session (RES).

Exercise Order	Sets	Repetitions	Recovery between Sets (min)	Recovery between Exercises (min)
Maximal isometric leg extension	3	1	1	2
Deep squat	2	10	2	2
Hip extension	2	10	1.5	2
Maximal isometric leg extension	3	1	1	2
One leg press	2	10	0.5	2
Deep squat	2	10	2	2
Hip extension	2	10	1.5	2
One leg press	2	10	0.5	2
Maximal isometric leg extension	3	1	1	2

Experimental design. All subjects (EG; $N = 6$ and CG; $N = 6$) were randomized and instructed to avoid heavy physical activities during last 5 d before the measurements, and exercise was forbidden 1 d before the test. In addition, they recorded their training during 2 wk before the measurements. Their food and beverage intake, which were recorded during 5 d before the test day, were then analyzed using the Micro Nutrica software (version 3.0, Social Insurance Institution, Turku, Finland). EG subjects completed a heavy hypertrophic RES of 50 min for lower extremities (Table 1). At the same time, CG was at rest. All subjects were allowed only to drink water (1500 mL in EG and 1000 mL in CG) during the measurement day (Fig. 1). Total fasting time was 15 ± 3 h including overnight fasting time of 10 ± 3 h and total experiment time of 5 h. Two weeks before the infusion study, each subject was familiarized with the exercise protocol, and his one repetition maximum (1 RM; the maximum weight that can be lifted in one repetition) and 10 repetition maximum were measured to design RES (Table 1). Experimental design is presented in Figure 1.

Data collection protocol. The protocol was planned to study only one subject (either EG or CG) per day. The subjects fasted overnight and reported to the laboratory in the morning, where their body mass and height were measured. An 18-gauge polyethylene catheter was inserted into a left antecubital vein (AV) for basal blood samples. Thereafter, a primed, continuous infusion of L-[*ring*-$^2H_5$] phenylalanine was started and maintained throughout the measurements. The prime was 2 μmol·kg^{-1} and the infusion rate

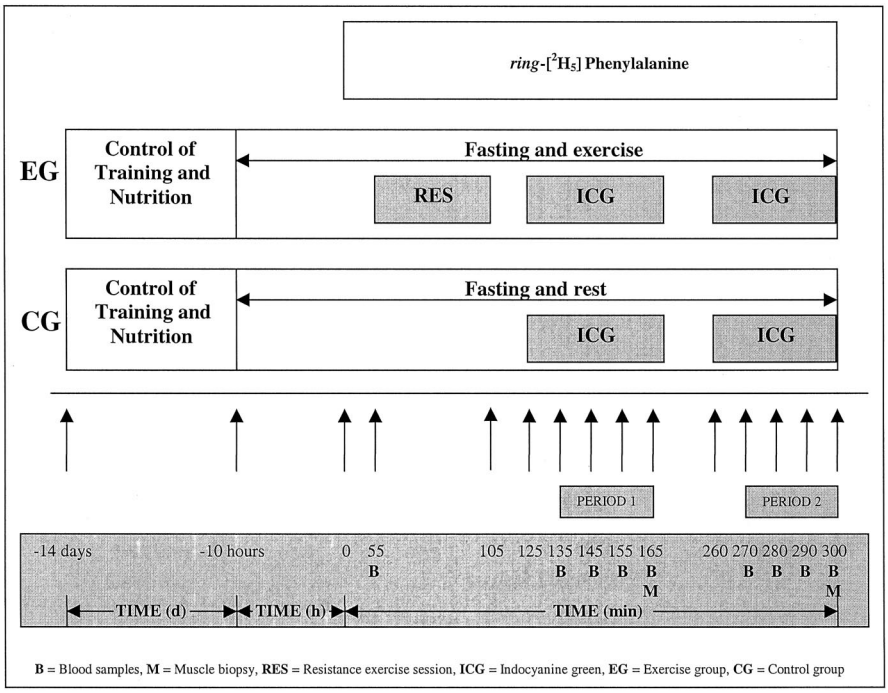

FIGURE 1—Study design. Each subject was studied on one occasion after an overnight fast. The subjects were divided either in a resistance exercise group (exercise group, EG) or a rest group (control group, CG). Familiarization period was 14 d, and the fasting period was started 10 h before experiment. Infusion of ring-$^2H_5$-phenylalanine was started at 0 min. Blood samples (B) were taken at 55 min from an antecubital vein (AV), at 135, 145, 155, 165 min (period 1) and at 270, 280, 290, 300 min (period 2) from femoral artery (FA) and femoral vein (FV). The muscle biopsies were taken at 165 and 300 min from vastus lateralis muscle. The leg blood flow was measured at 125–165 min (period 1) and at 260–300 min (period 2) during a continuous infusion of indocyanine green (ICG).

was 0.05 μmol·kg^{-1}·min^{-1}. At 125 min, polyethylene catheters (20-gauge) were inserted into the right femoral artery (FA) and femoral vein (FV), as well as an 18-gauge catheter was inserted into a right AV for drawing blood samples. The arterial catheter was used for the infusion of indocyanine green (ICG) for measuring blood flow (Fig. 1).

Collection of blood and muscle samples and blood flow. The ICG infusion (0.5 mg·mL^{-1}; 60 mL·h^{-1}) was started 10 min before the first blood sample during periods 1 and 2. In period 1, the blood samples for isotopic measurements and blood flow were drawn at 135, 145, 155, and 165 min (30, 40, 50, and 60 min after RES) after the initiation of L-[*ring*-$^2H_5$] phenylalanine infusion. In period 2, the blood samples for isotopic measurements were taken 270, 280, 290, and 300 min (165, 175, 185, and 195 min after RES) after the initiation of infusion. For serum free amino acid concentration analysis blood samples were drawn 135, 165, 270, and 300 min after the initiation of infusion and analyzed by high performance liquid chromatography (HPLC). Blood flow samples were simultaneously drawn from an FV and a right AV. The ICG infusion was briefly halted to allow sampling from the FA for isotopic measurements. Blood for amino acid concentrations and enrichments was placed into preweighed tubes containing 2-mL sulfosalicylic acid and known amount of internal standard ($^{13}C_6$ phenylalanine; 50 μmol·L^{-1}). Samples were mixed carefully and stored in ice. Blood flow samples were stored at room temperature until analysis. After the last blood sample (165 min), a muscle biopsy was taken for isotopic measurements and for analysis of free amino acid concentration from the VL muscle under local anesthesia. With the use of sterile technique, the skin and subcutaneous tissue were anesthetized, and a 6- to 7-mm incision was made. A 4-mm biopsy needle was advanced 3–5 cm into the muscle with the closed cutting window. The cutting cylinder was opened and closed two to four times and a sample of 30–50 mg was obtained. Visible fat and connective tissue were removed and the samples were rinsed with ice-cold saline before storing into tubes in liquid nitrogen. The second muscle biopsy was taken after the last blood sample (~300 min after the initiation of L-[*ring*-$^2H_5$] phenylalanine infusion).

Analysis of enrichment and concentration of phenylalanine. Enrichment and concentration of phenylalanine in whole blood were measured by gas chromatography-mass spectrometry (GC/MS; Hewlett Packard Agilent 5973N, GC 6890 Plus+, Palo Alto, CA). To determine the enrichment of infused amino acid in whole blood, the tertiary-butyl dimethylsilyl derivative was made. Isotopic enrichments were expressed as a tracer-to-tracee ratio. Concentration of phenylalanine was determined with an internal standard ($^{13}C_6$ phenylalanine; Cambridge Isotope Laboratories, Andover, MA) solution as previously described (1–3). In addition, detected masses were m/z 336 (natural phenylalanine), m/z 341 (infused $^2H_5$-phenylalanine), and m/z 342 ($^{13}C_6$ phenylalanine in internal standard). Because the tube weight and the amount of blood were known, the blood amino acid concentration was determined from the internal standard enrichment on the basis of the amount of blood and internal standard (200 μL of 50 μmol·L^{-1} $^{13}C_6$ phenylalanine) added. Appropriate corrections were made for any spectra that overlapped, contributing to the tracer-to-tracee ratio (30).

Analysis of muscle samples. Muscle tissue samples were analyzed for intracellular amino acid enrichments. On thawing, the tissue was weighed and the protein was precipitated with 0.8 mL of 14% perchloroacetic acid. The tissue was then homogenized and centrifuged, and the su-

pernatant was collected. This procedure was repeated once, and the collected supernatant was processed like blood samples.

Analysis of leg blood flow. Leg blood flow was determined from blood samples collected during a continuous infusion of ICG (12). The blood flow was determined by spectrophotometrically measuring the ICG concentration in serum from the FV and the peripheral vein as described previously (1,12). Leg plasma flow was calculated from steady-state values of dye concentration and converted to blood flow with the hematocrit (1). Serum from the blood samples was analyzed in a spectrophotometer with absorbance set at 805 nm.

Calculations. The three-compartment model of leg muscle amino acid kinetics has been described previously by Biolo et al. (1,2). The use of this model allows determining the rate of utilization of phenylalanine for muscle protein synthesis and appearance from breakdown because phenylalanine is neither oxidized nor synthesized in muscle. The blood values for blood flow, blood and muscle phenylalanine concentrations, and enrichments are the averages of the sampling periods (period 1: 135, 145, 155, 165 and period 2: 270, 280, 290, 300 min). Muscle net balance was determined by calculating the difference between muscle protein synthesis and muscle protein breakdown.

Model assumption. The calculation of intracellular phenylalanine utilization (protein synthesis) and appearance (protein breakdown) assumes that there is no *de novo* trace production or oxidation in the leg. Net balance and the muscle biopsy data assume that the muscle accounts for the leg metabolism of amino acids. It is also assumed that the tissue enrichment and amino acid concentrations are representative of the intracellular space and that the intracellular free amino acid pool is homogenous. It is also assumed that the free amino acid pool is the precursor for protein synthesis. The detailed model assumption has been described earlier (e.g., 1).

Analysis of serum free amino acid concentrations in blood. Concentrations of free amino acids in serum were determined applying the procedure of Pfeifer et al. (16) by reversed phase high performance liquid chromatography (RPHPLC) (Waters 501 pumps, Waters 717 autosampler and Zorbax C_{18} column). The 18 amino acids and two internal standards (β-Abc and Nor-Valine) were detected by Perkin Elmer LS-4 fluorescent detector using wavelengths 338 nm (exitation) and 455 nm (emission); 100 μL of internal standard solution was added to the serum sample (50 μL) and acetonitrile (100 μL) was used to precipitate the proteins; 750 μL of distilled deionized water was added, and the resulted sample was vortexed and allowed to stand on ice bath for 1 h. The 200 μL of the sample was transferred to the ultraspin centrifuge filter and centrifuged. The clear mixture was transferred to the HPLC vial, derivatized with orto-pthaldehyde derivatizing solution and analyzed by Waters HPLC system using gradient two-buffer elution.

Analysis of free amino acid concentrations in muscle. Concentrations of free amino acids in muscle were analyzed using HPLC equipped with fluorescent detector similarly to blood samples (see above). Samples were prepared by taking at least 10 mg of muscle and adding 400 μL of 5% perchloric acid and 100 μL of internal standard solution. After standing on an ice bath for 1 h, the samples were ground with pestles and centrifuged. Supernatants were transferred to clean tubes, and pH of the supernatants were adjusted to 5–7 by using potassium hydroxide. After spinning, 200 μL of the mixtures was transferred to the ultraspin centrifuge filter and centrifuged. The residual matters were removed and the solutions were ready for the HPLC analysis.

Statistical analysis. Data are expressed as means $\pm$ SEM or SD. The main effect of group and the interactions were assessed by MANOVA. With MANOVA, it can be constructed a statistical model corresponding to arrangements of the experiment. The dependent variables are TAA, EAA, NEAA, BCAA, and single amino acids, whereas the independent variables are vessels (FA, FV), group, and sample (time period 1: 135–165 min and time period 2: 270–300 min). If differences were detected, then a Bonferroni *post hoc* test was used to determine pair-wise differences. Also, residual examinations were made by diagnostic methods to make sure that the assumptions of statistical analysis were valid. Relationships between blood and muscle amino acids were determined using Pearson correlation coefficients. In addition, an unpaired Student's *t*-test was used to determine differences between groups for physical activity, nutrition, free amino acids, leg blood flow, protein synthesis, protein breakdown, and protein net balance between groups. Statistical significance was set at $P < 0.05$. All the analyses were done using the SPSS 10.1. for Windows software package.

RESULTS

Free time physical activity and nutrition. There were no statistical differences between the EG and the CG during 2 wk before the study day either in number of free time physical activity (7.8 $\pm$ 5.6 and 8.7 $\pm$ 2.7 times, respectively) or in total hours of physical activity (9.5 $\pm$ 5.7 and 12.3 $\pm$ 5.8 h, respectively). The nutrient intake was similar in the groups during the 5-d period (Table 2).

Main effect of group and interactions in serum free amino acids. The results revealed that compared with CG, EG had significantly ($P < 0.05$) higher concentration in aspartate in both vessels. On the other hand, the concentration in leucine was lower ($P < 0.05$) in EG than in CG, respectively (Table 3). Furthermore, the concentration of alanine was significantly higher ($P < 0.01$) in EG than in CG during period 1 in both vessels. In addition, the concentrations of asparagine ($P < 0.001$) and threonine ($P < 0.05$) were higher in FV than in FA in CG. However, methionine ($P < 0.05$) concentration was higher in FV than in FA in both groups.

TABLE 2. Daily nutrient intake of the exercise group (EG) and the control group (CG) during 5 d before experiment.

	EG		CG		Difference
	Mean	SD	Mean	SD	(EG vs CG)
Energy (MJ)	10.8	2.3	9.8	1.2	NS
Protein					
g	120.2	28.2	95.0	19.9	NS
g·kg^{-1} body weight	1.4	0.4	1.2	0.2	NS
%	19.0	2.7	17.1	5.9	NS
Fat					
g	72.2	28.0	71.8	14.4	NS
g·kg^{-1} body weight	0.9	0.4	0.9	0.2	NS
%	25.7	8.7	27.3	2.7	NS
Carbohydrate					
g	346.7	105.6	315.0	49.4	NS
g·kg^{-1} body weight	4.1	1.3	4.0	0.9	NS
%	54.4	10.5	54.0	3.5	NS

Data are means ± SD; Values are average intakes per day; %, percent of total energy.

Serum free amino acid concentrations in AV, FA, and FV. There were no significant differences between the groups in fasting free amino acid concentrations in AV (55 min before study period; Table 4). The concentration of methionine was higher ($P < 0.05$) in EG than in CG at 30 min of recovery. At 195 min of recovery, the sum of free BCAA in FV was significantly ($P < 0.01$) lower in EG than in CG (Table 4). There was a significantly higher concentration of alanine at 30 min ($P < 0.01$) and 60 min of recovery ($P < 0.05$) in FA, and at 30 min of recovery ($P < 0.01$) in FV in EG than in CG (Table 5).

Main effect of group, interactions and free amino acid concentrations in muscle. There was a significantly higher ($P < 0.001$) concentration of alanine in EG than in CG after RES at 60 min of recovery (Table 5). All results for alanine and for the sums of free amino acids are presented in Tables 4 and 5. In addition, a significant ($P < 0.05$) difference between 60 min and 195 min of recovery (interaction in group × sample: time effect) was observed for alanine.

Correlation coefficients between serum amino acid and muscle amino acid concentrations. Only low and nonsignificant correlation coefficients were observed in serum amino acid concentrations between blood (mean values of four serum samples per period) and muscle.

Leg blood flow. Mean blood flow in leg was significantly ($P < 0.01$) higher in EG during both periods (period 1: 6.10 ± 0.48 mL·min^{-1}·100 mL^{-1} leg volume and period 2: 5.58 ± 0.18 mL·min^{-1}·100 mL^{-1} leg volume) than in CG (period 1: 3.62 ± 0.17 mL·min^{-1}·100 mL^{-1} leg volume and period 2: 3.96 ± 0.22 mL·min^{-1}·100 mL^{-1} leg volume).

Protein synthesis, breakdown, and net balance. The only significant difference was observed during period 2 when protein synthesis and protein breakdown were significantly ($P < 0.05$) greater in EG compared with CG (Fig. 2). Net balance was negative during both periods.

DISCUSSION

Main results. One of the main results of this study was the increase of muscle protein synthesis (21%) and protein breakdown (17%) at 195 min after RES but not yet at 60 min compared with resting conditions. In fasting conditions, however, net muscle protein balance was negative in both recovery phases. During the 195-min recovery, the serum concentration (mean value of 30, 60, 165, and 195 min) of aspartate was greater in EG than in CG in both FA and FV, but the serum concentration of leucine was greater in CG than in EG in both vessels. The BCAA concentration in FV was also greater in CG than in EG at 195 min during recovery. The concentration of alanine was greater in EG than in CG at 30 min after RES in both vessels but at 60 min only in FA. In muscle, the concentration of alanine was greater in EG than in CG at 60 min but not at 195 min after RES.

Muscle protein synthesis, breakdown, and net balance. The most important finding of this study was that at 60 min during recovery after RES both protein synthesis and protein breakdown increased only slightly (insignificantly), but at 195 min a strong increase was observed in both parameters compared with the resting values. The increase in protein breakdown possibly occurred in order to provide amino acids in the fasting conditions for muscle protein synthesis. In the later recovery, the catabolic responses may take place in the most active muscle fibers that have not contributed to the mobilization of protein resources during intense exercise. Biolo et al. (2) have examined untrained men 3 h after a heavy resistance exercise and found an increase of 100% in muscle protein synthesis and an increase (50%) in muscle protein degradation after physical exercise. In the present study, protein synthesis increased significantly by 21% and the protein breakdown by 17% in the second period after the recovery of 195 min after RES. The mean values of protein synthesis and protein breakdown in our study may underestimate the real effect of exercise on these parameters, as the deviation in the amount of protein synthesis and breakdown was remarkable among the EG subjects especially in the second period, possibly due to different individual response to the exercise workload. This assumption is supported by the report of Phillips et al. (17), who have examined both trained and untrained subjects and found that resistance training attenuates the exercise induced increase in muscle protein turnover. In the present study, the observed increase in absolute protein synthesis was low, although RES must have provided challenge for the muscles of the subjects in EG. The training before experiment was performed at low intensity and the rate of repetitions were only half from those of the study day. Thus, in order to give challenge for the muscles, the exercise session during experiment included 50% more repetitions than during pretraining. Also, the intensity was greater because the subjects were told to lift the load as quickly as possible in the study. Because RES was performed very effectively, we must consider the possibility of the challenge for the muscles. According to studies in rats (8) and humans (6), muscle protein synthesis can be also inhibited by extreme exercise. After resistance exercise, protein synthesis has been reported to rebound for 48 h, whereas protein degradation remains slightly elevated, resulting in positive net balance only if amino acid availability

TABLE 3. Mean concentrations of serum free amino acids (μmol·L^{-1}) in femoral artery (FA) and vein (FV) between EG and CG during recovery.

Amino Acid and Vessel	EG				CG				Difference between the Groups during Recovery
	135	165	270	300	135	165	270	300	
Alanine									
FA	207 ± 17	171 ± 13	101 ± 10	100 ± 11	118 ± 17	119 ± 13	99 ± 10	104 ± 11	NS
FV	218 ± 15	196 ± 16	133 ± 11	126 ± 16	151 ± 15	154 ± 16	133 ± 11	149 ± 16	NS
Arginine									
FA	45 ± 6	49 ± 6	40 ± 6	55 ± 6	61 ± 6	64 ± 6	61 ± 6	59 ± 6	NS
FV	53 ± 7	49 ± 7	47 ± 5	58 ± 9	68 ± 7	66 ± 7	60 ± 5	66 ± 9	NS
Asparagine									
FA	22 ± 2	21 ± 2	19 ± 2	19 ± 2	22 ± 2	24 ± 2	22 ± 2	20 ± 2	NS
FV	25 ± 2	24 ± 2	20 ± 2	18 ± 1	28 ± 2	27 ± 2	24 ± 2	26 ± 1	NS
Aspartate									
FA	13 ± 2	9 ± 1	8 ± 2	11 ± 3	5 ± 2	6 ± 1	5 ± 2	6 ± 3	$P < 0.05$
FV	10 ± 2	11 ± 1	10 ± 2	9 ± 2	5 ± 2	5 ± 1	5 ± 2	6 ± 2	$P < 0.05$
Glutamate									
FA	34 ± 6	32 ± 6	29 ± 4	26 ± 3	28 ± 6	27 ± 6	27 ± 4	24 ± 3	NS
FV	21 ± 5	16 ± 4	13 ± 1	13 ± 2	15 ± 5	14 ± 4	12 ± 1	13 ± 2	NS
Glutamine									
FA	284 ± 25	274 ± 21	280 ± 23	285 ± 20	256 ± 25	284 ± 21	252 ± 23	269 ± 20	NS
FV	285 ± 29	289 ± 28	292 ± 26	310 ± 31	250 ± 29	272 ± 28	259 ± 26	278 ± 31	NS
Glycine									
FA	106 ± 13	82 ± 8	72 ± 7	89 ± 7	90 ± 13	85 ± 8	84 ± 7	81 ± 7	NS
FV	104 ± 20	94 ± 11	86 ± 10	105 ± 13	100 ± 20	100 ± 11	100 ± 10	101 ± 13	NS
Histidine									
FA	46 ± 4	50 ± 7	53 ± 10	43 ± 5	55 ± 4	48 ± 7	43 ± 10	42 ± 5	NS
FV	42 ± 5	47 ± 5	45 ± 4	47 ± 7	53 ± 5	53 ± 5	42 ± 4	47 ± 7	NS
Isoleucine									
FA	27 ± 2	27 ± 2	26 ± 1	26 ± 1	30 ± 2	29 ± 2	28 ± 1	28 ± 1	NS
FV	31 ± 2	27 ± 2	26 ± 1	25 ± 1	32 ± 2	31 ± 2	26 ± 1	31 ± 1	NS
Leucine									
FA	60 ± 5	55 ± 3	59 ± 4	63 ± 4	70 ± 5	71 ± 3	71 ± 4	71 ± 4	$P < 0.05$
FV	60 ± 5	54 ± 4	61 ± 5	62 ± 4	72 ± 5	74 ± 4	70 ± 5	76 ± 4	$P < 0.05$
Lysine									
FA	115 ± 10	111 ± 9	101 ± 9	98 ± 9	122 ± 10	116 ± 9	102 ± 9	92 ± 9	NS
FV	122 ± 10	119 ± 10	105 ± 11	104 ± 13	130 ± 10	122 ± 10	106 ± 11	110 ± 13	NS
Methionine									
FA	17 ± 1	13 ± 1	12 ± 1	13 ± 2	13 ± 1	14 ± 1	12 ± 1	12 ± 2	NS
FV	17 ± 1	15 ± 1	14 ± 1	15 ± 2	15 ± 1	16 ± 1	13 ± 1	16 ± 2	NS
Phenylalanine									
FA	37 ± 3	37 ± 3	38 ± 3	35 ± 2	33 ± 3	35 ± 3	34 ± 3	33 ± 2	NS
FV	40 ± 3	38 ± 3	35 ± 3	33 ± 3	36 ± 3	36 ± 3	34 ± 3	37 ± 3	NS
Serine									
FA	52 ± 6	49 ± 4	46 ± 6	49 ± 5	58 ± 6	58 ± 4	57 ± 6	52 ± 5	NS
FV	49 ± 6	48 ± 5	45 ± 4	47 ± 6	55 ± 6	55 ± 5	52 ± 4	54 ± 6	NS
Threonine									
FA	50 ± 5	52 ± 5	45 ± 5	41 ± 5	50 ± 5	46 ± 5	45 ± 5	42 ± 5	NS
FV	49 ± 5	52 ± 5	45 ± 5	42 ± 6	56 ± 5	54 ± 5	45 ± 5	50 ± 6	NS
Tryptophan									
FA	32 ± 2	32 ± 1	28 ± 2	26 ± 2	30 ± 2	29 ± 1	27 ± 2	25 ± 2	NS
FV	34 ± 1	33 ± 2	27 ± 2	26 ± 2	31 ± 1	29 ± 2	26 ± 2	28 ± 2	NS
Tyrosine									
FA	51 ± 8	34 ± 4	35 ± 6	39 ± 5	33 ± 8	33 ± 4	35 ± 6	33 ± 5	NS
FV	47 ± 11	39 ± 4	38 ± 8	50 ± 9	35 ± 11	34 ± 4	41 ± 8	44 ± 9	NS
Valine									
FA	116 ± 9	114 ± 8	114 ± 8	106 ± 7	119 ± 9	124 ± 8	119 ± 8	118 ± 7	NS
FV	123 ± 9	114 ± 8	108 ± 8	97 ± 7	126 ± 9	122 ± 8	112 ± 8	124 ± 7	NS

Data are means ± SEM.
Period 1; samples of 135 and 165 min.
Period 2; samples of 270 and 300 min.

is increased (21). These findings are in line with our results, in which net balance remained negative in the fasting conditions both in CG but also in EG when low availability of free amino acids was further diminished after exercise.

Free amino acid concentrations in serum. The response of alanine to exercise was similar as expected according to the literature. EG showed higher concentrations of alanine (43%) in FA than CG during recovery of 30 min. The observed postexercise increase in alanine was associated with the lower venous concentration of asparagine in EG which may due to the role as a donor of the amino radical in the formation of alanine (7). After exercise, the splanchnic uptake of gluconeogenic substrates has been shown to increase (25), and because alanine is utilized in hepatic gluconeogenesis (9), the concentration of alanine decreases. Thus, the difference between EG and CG was almost disappeared at 60 min of recovery. The changes in the concentration of threonine were similar as in asparagine, as both of these amino acids had higher concentrations in FV than in FA and, moreover, insignificantly lower in EG than in CG. Threonine is one of the glucogenic amino acids that are released from muscles during fasting (20). The

TABLE 4. Concentrations of free amino acids in the antecubital vein (AV), femoral artery (FA), and femoral vein (FV), and in muscle of the exercise group (EG) and control group (CG).

	EG				CG			
	AV (μmol·L^{-1})	FA (μmol·L^{-1})	FV (μmol·L^{-1})	Muscle (μmol·kg^{-1})	AV (μmol·L^{-1})	FA (μmol·L^{-1})	FV (μmol·L^{-1})	Muscle (μmol·kg^{-1})
Total amino acids								
55 min	1319 ± 64				1357 ± 64			
135 min		1318 ± 69	1328 ± 74			1195 ± 69	1259 ± 74	
165 min		1211 ± 57	1264 ± 63	78 ± 6		1209 ± 57	1262 ± 63	68 ± 6
270 min		1104 ± 70	1148 ± 65			1122 ± 70	1159 ± 65	
300 min		1123 ± 63	1186 ± 92	78 ± 8		1110 ± 63	1256 ± 92	62 ± 9
Essential amino acids								
55 min	644 ± 36				650 ± 36			
135 min		549 ± 35	571 ± 34			584 ± 35	620 ± 36	
165 min		540 ± 31	548 ± 31	12 ± 2		575 ± 31	602 ± 31	13 ± 2
270 min		515 ± 38	512 ± 31			541 ± 38	534 ± 31	
300 min		505 ± 30	508 ± 37	12 ± 1		522 ± 30	586 ± 37	11 ± 1
Nonessential amino acids								
55 min	675 ± 34				707 ± 34			
135 min		769 ± 52	758 ± 59			611 ± 52	639 ± 59	
165 min		671 ± 34	716 ± 49	65 ± 5		635 ± 34	660 ± 49	55 ± 5
270 min		589 ± 45	636 ± 43			580 ± 45	625 ± 43	
300 min		617 ± 44	678 ± 63	66 ± 7		588 ± 44	670 ± 63	51 ± 8
Branched chain amino acids								
55 min	254 ± 14				237 ± 14			
135 min		203 ± 15	214 ± 14			219 ± 15	230 ± 14	
165 min		196 ± 12	195 ± 12	2.6 ± 0.3		224 ± 12	226 ± 12	2.9 ± 0.3
270 min		199 ± 11	195 ± 12			218 ± 11	209 ± 12	
300 min		195 ± 12	184 ± 9**	2.4 ± 0.3		217 ± 12	230 ± 9**	2.7 ± 0.4

Data are means ± SEM.
Significant difference between EG and CG (** $P < 0.01$).

smaller increase in threonine concentration in EG than in CG in FV may result from the increased gluconeogenesis due to both fasting and exercise. Methionine is a glycogenic essential amino acid, which is released from muscle during fasting (20) and which has a limiting role in the maintenance of body protein and nitrogen balance under conditions of greater protein turnover (13). In this study, the concentration of methionine remained at the same level through the periods at rest, whereas the significantly higher arterial and venous levels seen postexercise decreased to values similar to CG at 195 min of recovery after RES. The base level of BCAA, measured from antecubital vein before exercise, was similar in EG and CG. The BCAA concentration remained lower in EG than in CG throughout the recovery, but the difference between the groups was only significant at 195 min of recovery after RES, when the concentration was 20% lower in EG than in CG in FV. The release from other tissues into blood may have been reduced and/or the blood free amino acids may have been utilized in energy metabolism after RES in the fasting conditions, which confirms the previous study (14) suggesting that increased muscle protein metabolism results in decrease of blood BCAA. The results also indicated that the concentration of BCAA leucine decreased, whereas that of aspartate increased in EG during recovery compared with CG. Aspartate plays an important role in purine nucleotide cycle in maintaining the pool of adenosine triphosphate (ATP) in muscle (27). The observed inverse relationship between these two amino acids may reflect the degradation of leucine and the consequent synthesis of aspartate. BCAA degradation in muscle results in formation of glutamate, which can further provide the amino group with oxaloacetate to synthesize aspartate (27). It seems that the changes in serum amino acid concentrations after an intensive RES may be associated also with the energy demands.

Free amino acid concentrations in muscle. Alanine is one of the major carriers of nitrogen among body tissues and is constantly released from skeletal muscle into the bloodstream (1). Alanine efflux increases in fasting and during exercise (7), which was confirmed in this study. Our results indicated that there was a significantly higher concentration of muscle alanine in EG than in CG after RES. During recovery total amino acids remained high, whereas alanine decreased by 30%. When the muscle biopsies and

TABLE 5. Concentrations of free alanine in antecubital vein (AV), femoral artery (FA), and femoral vein (FV), and in muscle of the exercise group (EG) and control group (CG).

	EG				CG			
	AV (μmol·L^{-1})	FA (μmol·L^{-1})	FV (μmol·L^{-1})	Muscle (μmol·kg^{-1})	AV (μmol·L^{-1})	FA (μmol·L^{-1})	FV (μmol·L^{-1})	Muscle (μmol·kg^{-1})
Alanine								
55 min	146 ± 12				170 ± 12			
135 min		207 ± 17**	218 ± 15**			118 ± 17**	151 ± 15**	
165 min		171 ± 13*	196 ± 16	20 ± 1***		119 ± 13*	154 ± 16	12 ± 1***
270 min		101 ± 10	133 ± 11			99 ± 10	133 ± 11	
300 min		100 ± 11	126 ± 16	14 ± 2		104 ± 11	149 ± 16	11 ± 2

Data are means ± SEM.
Significant difference between EG and CG (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

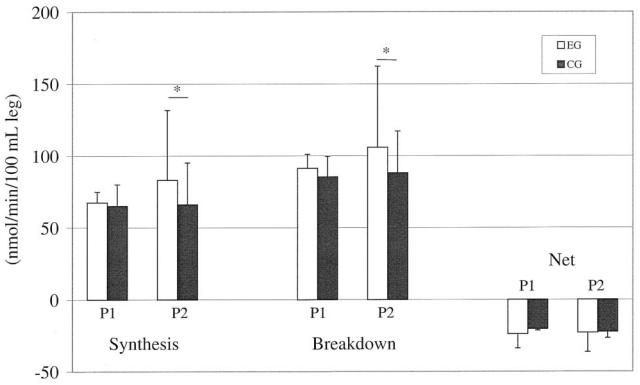

FIGURE 2—Comparison of the response of muscle protein synthesis, muscle protein breakdown, and net protein balance in the exercise group (EG) and the control group (CG) during period 1 (P1) and period 2 (P2). Values are mean ± SE. A significant difference (*$P < 0.05$) between EG and CG.

blood samples of 60 and 195 min during recovery were compared, the decrease of alanine concentration was observed to occur more slowly in muscle. There was seen a difference between the groups in alanine, which showed a 40% higher concentration in EG than CG at 60 min of recovery but only 21% at 195 min, respectively. Our results indicate that the differences in the concentrations of free amino acids in muscle between CG and EG are scant, except in alanine, which increased significantly after RES. The fasting conditions (at least 12 h before the first samples) in both groups may partly explain the differences via the decreased availability of the free amino acids. In addition, no correlative relationships between muscle and blood amino acid concentrations were seen.

Leg blood flow. As expected, according to the literature, the leg blood flow was increased in the exercise group during both periods of recovery. The basal mean values (period 1: 3.62 and period 2: 3.96 $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ leg volume) in CG and the postexercise mean values in EG (period 1: 6.10 and period 2: and 5.58 $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ leg volume) were in line with the results of Biolo et al. (2), who reported the basal leg blood flow to be at rest 3 $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ leg volume and after the bout of exercise to increase to 5–6 $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ leg volume in normal adults. Increased blood flow affects muscle protein metabolism by increasing transport of amino acids into cells and consequently stimulating protein synthesis (3). Amino acid availability has also been shown to be an important factor in the regulation of muscle protein metabolism (e.g., 3). In this study, the increased blood flow may have enhanced free amino acid availability for protein synthesis in EG. The leg blood flow does not alone explain the increased protein metabolism at the end of recovery because the flow slightly decreased during the second period. Other things such as increased amino acid availability through breakdown at the end of recovery or increased blood flow wash out effect on amino acids in the beginning of the recovery may have affected protein metabolism.

In summary, this study provides evidence that RES induces a significant increase in muscle protein synthesis and muscle protein breakdown at 195 min but not yet at 60 min during recovery in fasting conditions. However, negative protein net balance is not changed.

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