Amino Acids (2003) 25: 85–94
DOI 10.1007/s00726-002-0343-3

Amino
Acids
Printed in Austria

Leucine supplementation does not enhance acute strength or running performance but affects serum amino acid concentration

H. T. Pitkänen[1], **S. S. Oja**[2,3], **H. Rusko**[4], **A. Nummela**[4], **P. V. Komi**[1], **P. Saransaari**[2], **T. Takala**[1], and **A. A. Mero**[1]

[1] Neuromuscular Research Centre, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland
[2] University of Tampere Medical School, Tampere, Finland
[3] Department of Clinical Physiology, Tampere University Hospital, Tampere, Finland
[4] KIHU – Research Institute for Olympic Sport, Jyväskylä, Finland

Received April 15, 2002
Accepted August 30, 2002
Published online January 30, 2003;

Summary. This study described the effect of leucine supplementation on serum amino acid concentration during two different exercise sessions in competitive male power athletes. The subjects performed a strength exercise session (SES; n = 16; 26 ± 4 years) or a maximal anaerobic running exercise session (MARE; n = 12; 27 ± 5 years) until exhaustion twice at a 7-day interval. The randomized subjects consumed drinks containing leucine (100 mg × kg/body weight before and during SES or 200 mg × kg/body weight before MARE) or placebo. Blood specimens taken 10 min before (B) and after (A) the sessions were analyzed for serum amino acids. In SES the concentration of leucine was distinctly higher in the leucine supplemented group than in the placebo group in both B ($p < 0.001$) and A ($p < 0.001$) samples. The leucine concentration decreased in placebo but not in the leucine supplemented group following the exercise session. Isoleucine ($p = 0.017$) and valine ($p = 0.006$) concentration decreased more in the leucine supplemented group than in placebo in A samples. In MARE the concentration of leucine was higher in the leucine supplemented group than in placebo in both B ($p < 0.001$) and A ($p < 0.001$) samples and increased ($p < 0.001$) in the supplemented group following the session. Isoleucine ($p = 0.020$) and valine ($p = 0.006$) concentration decreased in the supplemented group in A samples. There were no differences in a counter movement jump after SES or in the running performance in MARE between the leucine supplemented group and placebo. These findings indicate that consuming leucine before or before and during exercise sessions results in changes in blood amino acid concentration. However, the supplementation does not affect an acute physical performance.

Keywords: Leucine – Amino acids – Training – Nutrition – Male athletes

Introduction

The essential branched chain amino acid leucine amounts to about 4.6% of all amino acids (Takala et al., 1980), and about 65% of the body's total protein are in skeletal muscles (Goldberg et al., 1975). Leucine has many important roles: it may, for instance, regulate protein metabolism by inhibiting degradation and stimulating synthesis (Nair et al., 1992). It also supplies gluconeogenic precursors via the formation of alanine in muscle (Brooks, 1987). Leucine is an interesting substrate in muscle during exercise because it can be transaminated and oxidated to directly produce acetyl-CoA (Wagenmakers, 1998). The rate of leucine oxidation is proportional to the intensity of exercise, although it is modulated by the nutritional status (Rennie et al., 1981). The recommended dietary allowances (RDA) of leucine (14 mg/kg body weight/day) and protein (0.8 g/kg/day) are for sedentary individuals (Young and Bier, 1987) such that the intake should obviously be increased for those participating in regular physical activity.

Decreases in the concentration of amino acids have been shown to occur after intensive training when the daily protein intake is 1.3 g/kg/day (Mero et al., 1997). In that study considerable decreases were found in branched chain amino acids (BCAA) (21%) (isoleucine 21%, leucine 20% and valine 18%) during a 5-week power and endurance training period in sprinters and jumpers. In single exercise session, acute decreases in the leucine concentration have been shown to occur following both an anaerobic exercise session (5–8%) (Pitkänen et al., 2002b), an aerobic

exercise session (11%) (Blomstrand and Newsholme, 1996) and a strength exercise session (30%) (Pitkänen et al., 2002a).

In addition to its anabolic and anticatabolic effects, leucine also affects various anabolic hormones (DiPasquale, 1997) and is an important factor in the synthesis of alanine and glutamine via the glucose-alanine cycle (GAC) (Babij et al., 1983). The oxidation of leucine increases the output of alanine, which is further used for gluconeogenesis and ureagenesis. Glutamine has an important role in the regulation of the acid/base balance. Ammonia produced from glutamine helps to cope with the acid loads by combining with protons. In addition to bicarbonate and phosphate buffers, proteins are a third system to provide maintenance of the acid/base balance (DiPasquale, 1997).

Previous data of changes in blood amino acids have mostly concentrated on endurance exercises (Henriksson, 1991), while there is little data of such changes following high-intensity anaerobic and strength exercise sessions (Panton et al., 2000). Therefore, this study was carried out to determine whether leucine supplementation influences the blood amino acid profile and their responses to such exercise sessions. We hypothesised that exercise sessions with leucine supplementation could maintain a more favourable basic state for amino acids considering the successive recovery (more available amino acids for protein synthesis) following an acute short-term exercise. According to the literature, BCAA supplements help to maintain the free amino acid pool and to enhance the rate of protein synthesis (May and Buse, 1989). Secondly, we hypothesised that leucine supplementation might enhance energy yield by intensified GAC or direct oxidation in an intermittent anaerobic exercise (including short recoveries), and that this might improve acute physical performance.

Methods

Subjects

The subjects, who gave their written informed consent to take part in the present study, were competitive male power athletes (sprinters and jumpers). They were divided into a strength exercise session (SES; n = 16) group and a maximal anaerobic running exercise session (MARE; n = 12) group. The protocol was approved by the Ethical Board of the local University. The subjects were measured for their standing height, mass, fat and the sum of skin folds (Table 1). In the SES group the mean age was 26 ± 4 years, training history 9 ± 3 years and a recorded time for the 100-m sprint of 11.16 ± 0.51 s. The respective values for the MARE group were 27 ± 5 years, 10 ± 4 years and 11.24± 0.48 s.

Table 1. The physical charasteristics of the experimental groups

Physical charasteristics	SES		MARE		Difference
	Mean	SD	Mean	SD	
Height (m)	1.84	0.05	1.84	0.05	ns.
Mass (kg)	77.3	6.2	77.0	6.0	ns.
Fat (%)	9.3	1.6	9.1	1.6	ns.
Sum of skin folds (sum of 9, m)	0.054	0.006	0.052	0.006	ns.

Values are mean ± SD. P-values SES vs. MARE

Experimental design

During the two months before the experiments, each subject was initially familiarised with the training program planned by the coaches and the researchers. Nutrition and the amount and intensity of training were carefully controlled by the researchers for one week before the experiments. During the experiments the subjects had a single intensive SES or MARE session twice at the same time of the day (between 10 AM – 1 PM) in a double-blind cross-over procedure. Each subject visited the test laboratory twice and consumed, in a random order, either the leucine drink for SES (100 mg × kg/body weight; one half of the drink before and the other half of the drink in the middle of SES) or for MARE (200 mg × kg/body weight before MARE) or placebo drinks. The placebo drink was grape juice and the test drink was grape juice with leucine. Each of the subjects in the groups were allowed to drink a 500 ml of solution, which included either a leucine or placebo supplement. In SES one half of leucine (50 mg/kg) was ingested in 250 ml of solution, 50 minutes before the experiment and the other half of leucine (50 mg/kg) in 250 ml of solution in the middle of the exercise. In MARE the total amount of leucine (200 g/kg) was consumed in a 250 ml of solution 50 minutes before the experiment) and a further 250 ml of solution containing pure grape juice freely during exercise. The placebo groups ingested grape juice with the same timing and the same volumes as the leucine supplemented groups. The "wash-out time" between the sessions was 6 days (Fig. 1).

The SES exercise consisted of jumps and heavy resistance exercises during 90 min (Table 2). The speed strength of leg extensor muscles was evaluated 5 min after SES using counter movement jump (CMJ) on a contact mat (Newtest, Oulu, Finland) connected to a digital timer (±0.001 s). The timer was triggered by the lift of feet from the mat and stopped at the touch-down. The flight time during the jump was thus recorded. The rise of the centre of gravity in a jump was then calculated from the measured flight time (Komi and Bosco, 1978). MARE consisted of an unspecified number of runs, i.e. n × 20 s (intensity of 56–100% calculated afterwards from the last run and a total running time (mean ± SD) 166 ± 19 s) on a treadmill with a recovery of 100 s between the runs. The initial treadmill speed (4.08 m × s^{-1}, 4° slope) was increased by 0.38 m × s^{-1} for each consecutive run until exhaustion.

Anthropometry

Bilateral skin fold measurements were done with John Bull Skin fold calliper (British Indicators, LTD, England). Skin site readings

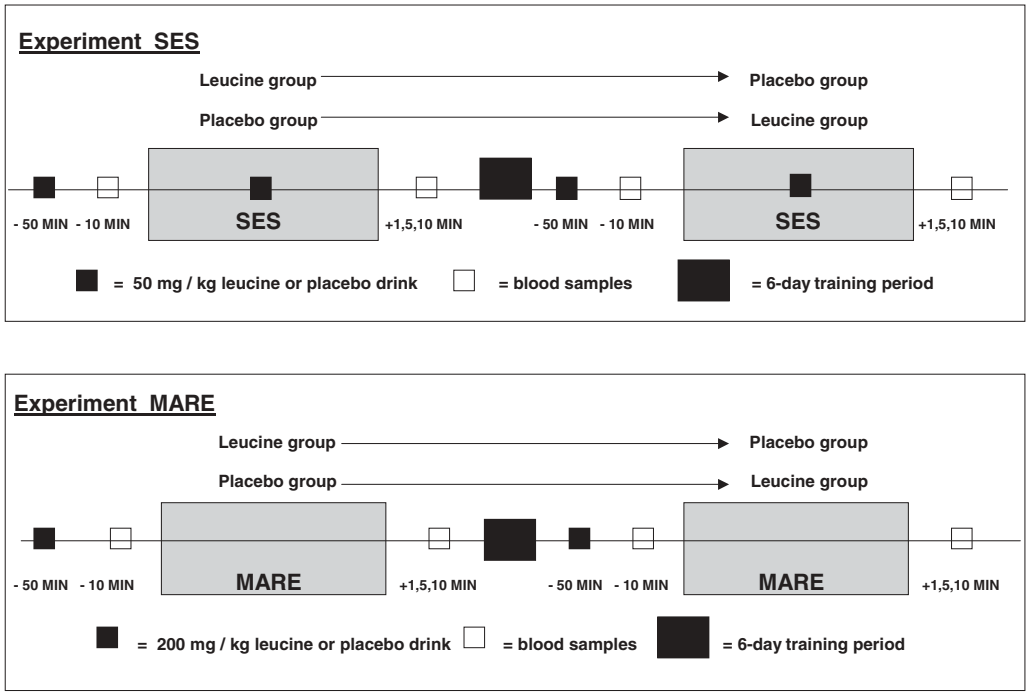

Fig. 1. Experimental procedure for SES and MARE exercise regimes

Table 2. Content of SES

Exercise order	Sets Distance	Repetitions	Recovery between sets and exercise, min
Sprint coordination/speed			
Skipping	25 m	4	2
Knee flexion	25 m	4	2
Bounding	25 m	4	2
Acceleration	25 m	4	2
Speed Strength			
Hurdle jumps	5	10	3
5 jumps	6	5	3
Heavy resistance			
Deep squat	4	10 RM	3
Calf raises	4	20 RM	3
Bench press	4	10 RM (RM = repetition maximum)	3

were taken from four skin sites (subscapula, triceps brachii, biceps brachii and supra iliaca) from the upper body and from four skin sites (calf, quadriceps femoris, hamstrings, gluteus maximus) from the lower extremities. The averaged (right and left) 8 readings together with the reading from a trunk skin site (abdomen) formed the total sum of skin folds (Durnin and Rahaman, 1967). All anthropometric measurements were carried out by a single well-trained observer (Table 1).

Training

The training took place during an indoor training season. The researchers and the coaches together planned the volume and intensity of the training program according to the principles of Mero et al. (1987). The speed work included sprint running (short sprints of 30–60 m) at the intensity level of 95–100% (from one repetition maximum 5 RM). The speed endurance work consisted of sprint running at the intensity level of 90–94% (RM) using short and long sprints ranging from 60 m to 400 m. The endurance work was aerobic training including running (distances from 100 m to 1,000 m) at the intensity level of 60–89% (RM). The jumping training included both horizontal and vertical jumps with maximal effort. The amount of jump training was calculated as take offs. The training of both SES and MARE group was similar for 6 days before each test exercise session: 5 training times, 500 m speed work, 1,500 m speed endurance work, 10,000 m endurance work, 300 jumps (take-offs) and strength training totally 14 tons.

Table 3. Percentage supply of daily energy intake from carbohydrate, fat and protein between L (leucine) and P (placebo) groups during 6 days before each test exercise session

	SES					MARE				
	L	P			Difference	L		P		Difference
	Mean	SD	Mean	SD	L vs. P	Mean	SD	Mean	SD	L vs. P
Energy (MJ)	9.8	3.6	9.7	3.2	ns.	9.2	3.0	9.2	3.2	ns.
Carbohydrate (%)	53.2	6.6	53.7	7.4	ns.	51.0	8.1	51.3	8.8	ns.
Fat (%)	31.0	5.4	31.1	6.6	ns.	32.0	7.8	31.7	6.9	ns.
Protein (%)	15.6	4.1	15.9	4.9	ns.	16.4	3.8	16.5	3.6	ns.
Protein (g/kg/body weight)	1.2	0.2	1.3	0.2	ns.	1.1	0.2	1.1	0.2	ns.

Values are mean ± SD

Nutrition

During the familiarisation phase (two months before the study), the subjects were instructed to eat according to the principles of Nutrition Recommendations for Athletes in Finland (1990). For 6 days before each experiment the subjects recorded their food and beverage intake, which was then analysed using Micro Nutrica software (version 1.0, Social Insurance Institution, Finland). The percentages of the daily supply of energy intake are presented in Table 3.

Blood collection and analysis

Five ml of blood was drawn from an antecubital vein 10 min before and 10 min after each SES and MARE (Fig. 1). The blood samples were centrifuged for 10 min at 3,500 rpm to separate cells from serum, which was immediately frozen and stored at −80°C for the amino acid analyses to be performed after two weeks. They were deproteinized with 5% sulphosalicylic acid containing L-2,4-diaminobutyrate as an internal standard, mixed with lithium citrate buffer and subjected to ion-exchange chromatography using an automatic Pharmacia LKB Alpha Plus amino acid analyzer with o-phthalaldehyde derivatisation and fluorescence detection. All samples were analyzed in duplicate and the samples from one individual were run in the same assay to minimize inter-assay variability. Intra-assay variation ranged from 1.7% to 2.8% for single amino acids. Peak blood lactate was determined enzymatically (Roche Diagnostics GmbH, Mannheim, Germany) from fingertip blood samples (50 μl) taken 1 and 5 min after the sessions.

Statistics

The data from SES and MARE were analyzed separately but exactly in the same way by an analysis of variance according to a linear model for a two-period repeated measurements cross-over design, originally presented by Wallenstein and Fisher (1977). A Wallenstein-Fisher cross-over model assumes no carry-over effects. The model included 7 fixed effects (sequence, period, treatment, time, sequence-by-time interaction, period-by-time interaction, treatment-by-time interaction), 3 random effects (subject effect for athlete, subject-by-sequence interaction, subject-by-time interaction) and an error term.

Before the analysis of variance was performed, the consistency of data from SES and MARE was checked with the assumptions of equality of group variances. Moreover, the multivariate normality assumption of errors was assessed by diagnostic methods. Some variables were transformed (log-transformation: sum of EAAs, sum of BCAAs, isoleucine, leucine, valine) before final analyses, because of violations in those assumptions. So, the results of statistical tests (p-values) of these variables can be based on transformed values, even though the represented means and standard errors (SE) were estimated for original values. All the analyses were performed by means of SAS System, using the MIXED (1996), UNIVARIATE (1990) and GPLOT (1991) procedures.

Results

SES

Treatment (leucine supplementation: 100 mg × kg/body weight) had an effect on serum values for leucine ($p = 0.004$), isoleucine ($p = 0.017$), valine ($p = 0.001$) and taurine ($p = 0.037$). The amino acid concentrations in the leucine group (L) and in the placebo group (P) of SES are shown in Table 4. The concentration of leucine ($p = 0.005$) and branched chain amino acids (BCAAs) decreased ($p = 0.033$) with placebo but not with leucine supplementation (Table 4). The total amino acids (TAAs) and essential amino acids (EAAs) decreased significantly in both groups (Table 4). The concentration of leucine, BCAAs and EAAs were greater in the leucine supplemented group than in placebo in the blood specimens taken both before (B; $p < 0.001$) and after (A; $p < 0.001$) the exercises. Interactions between treatment and exercise were as follows: leucine ($p < 0.001$), isoleucine ($p = 0.017$), valine ($p = 0.006$), and arginine ($p = 0.020$) (Fig. 2).

There were no significant differences in peak blood lactate (2.5 ± 0.4 mmol/L in L and 2.4 ± 0.8 mmol/L in P) or in CMJ (0.55 ± 0.03 m in L and 0.56 ± 0.03 m in P) following SES.

Table 4. Serum amino acid concentrations (μmol/L) before and after SES

	Leucine group (L)					Placebo group (P)				
	Before		After		Difference	Before		After		Difference
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
Total amino acids	2,932	140	2,633	140	$p = 0.020$	2,820	137	2,431	137	$p = 0.003$
Branched chain amino acids	591	37	502	37	$p = 0.064$	430	36	329	36	$p = 0.033$
Essential amino acids	1,142	60	961	60	$p = 0.011$	979	59	785	59	$p = 0.006$
Non-essential amino acids	1,788	90	1,673	90	$p = 0.077$	1,841	88	1,646	88	$p = 0.006$
Leucine	318	24	354	23	$p = 0.266$	139	22	97	22	$p = 0.005$
Isoleucine	64	6	25	6	$p < 0.001$	69	5	48	5	$p = 0.002$
Valine	209	15	122	15	$p < 0.001$	222	14	184	14	$p = 0.006$
Histidine	96	5	86	4	$p = 0.014$	92	4	80	4	$p = 0.002$
Lysine	176	13	147	13	$p = 0.003$	176	12	139	12	$p < 0.001$
Methionine	29	2	22	2	$p < 0.001$	29	2	24	2	$p = 0.002$
Phenylalanine	63	4	48	3	$p < 0.001$	61	3	50	3	$p < 0.001$
Threonine	125	8	105	8	$p = 0.001$	132	7	113	7	$p = 0.002$
Tryptophan	59	5	51	5	$p = 0.090$	60	4	50	4	$p = 0.036$
Taurine	73	6	70	6	$p = 0.477$	71	6	58	6	$p = 0.001$
Aspartate	17	1	12	1	$p < 0.001$	17	1	11	1	$p < 0.001$
Serine	99	6	82	6	$p = 0.001$	103	6	86	6	$p = 0.001$
Asparagine	99	10	74	10	$p = 0.010$	99	9	74	9	$p = 0.010$
Glutamine	569	33	552	33	$p = 0.482$	578	33	517	33	$p = 0.021$
Glutamate	45	3	37	3	$p = 0.004$	47	3	37	3	$p = 0.002$
Glycine	203	12	178	12	$p = 0.005$	210	12	188	12	$p = 0.009$
Alanine	371	32	401	32	$p = 0.170$	419	31	433	31	$p = 0.490$
Citrulline	29	2	30	2	$p = 0.929$	28	2	26	2	$p = 0.272$
Tyrosine	79	5	63	5	$p < 0.001$	77	5	66	5	$p = 0.004$
Ornithine	112	7	93	7	$p = 0.003$	102	7	79	7	$p < 0.001$
Arginine	95	5	82	4	$p = 0.003$	92	4	72	4	$p < 0.001$

Values are mean ± SE

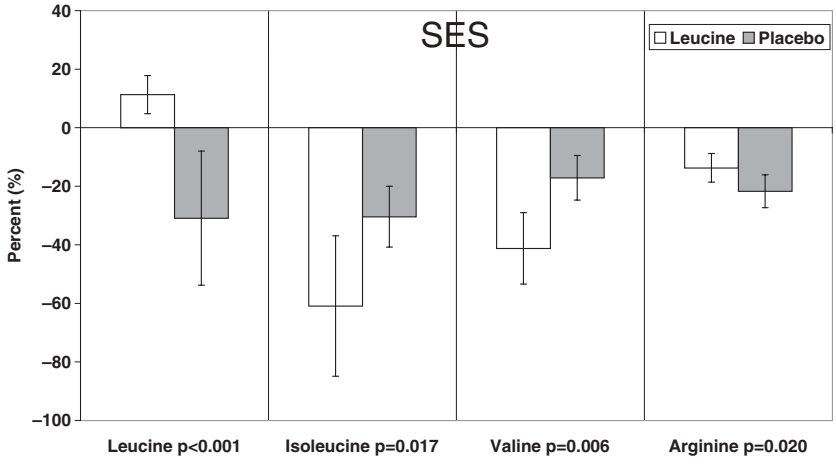

Fig. 2. Relative changes in concentration of leucine, isoleucine, valine and arginine in both groups following SES (before – after comparison between groups, mean ± SE)

MARE

Treatment (leucine supplementation: 200 mg × kg/body weight) had an effect on leucine ($p < 0.001$), BCAAs ($p = 0.005$) and EAAs ($p = 0.009$). The amino acid concentrations in the leucine supplemented group and placebo of MARE are shown in Table 5. The concentration of leucine, BCAAs and EAAs was greater in leucine supplemented group than in placebo in both B and A samples as follows: leucine (B: $p < 0.001$, A: $p < 0.001$), BCAAs (B: $p = 0.003$, A: $p = 0.001$), EAAs (B: $p = 0.007$, A:

Table 5. Serum amino acid concentrations (μmol/L) before and after MARE

	Leucine group (L)					Placebo group (P)				
	Before		After		Difference	Before		After		Difference
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
Total amino acids	3,560	305	3,734	305	$p = 0.120$	3,221	305	3,482	306	$p = 0.031$
Branched chain amino acids	837	93	934	93	$p = 0.019$	484	93	494	93	$p = 0.660$
Essential amino acids	1,404	142	1,457	142	$p = 0.340$	1,044	142	1,037	142	$p = 0.890$
Non-essential amino acids	2,156	177	2,277	177	$p = 0.120$	2,177	177	2,417	178	$p = 0.006$
Leucine	528	79	676	79	$p < 0.001$	181	79	191	79	$p = 0.790$
Isoleucine	65	7	49	7	$p = 0.006$	64	7	66	7	$p = 0.700$
Valine	245	21	210	21	$p = 0.008$	240	21	238	21	$p = 0.900$
Histidine	101	9	107	9	$p = 0.120$	98	9	110	9	$p = 0.003$
Lysine	244	36	251	36	$p = 0.300$	241	36	253	36	$p = 0.110$
Methionine	30	2	28	2	$p = 0.230$	28	2	29	2	$p = 0.610$
Phenylalanine	68	9	60	9	$p = 0.054$	63	9	62	9	$p = 0.890$
Threonine	134	10	119	10	$p = 0.004$	140	10	135	10	$p = 0.300$
Tryptophan	92	9	64	9	$p = 0.002$	88	9	64	9	$p = 0.007$
Taurine	93	11	105	11	$p = 0.270$	97	11	131	11	$p = 0.027$
Aspartate	19	2	19	2	$p = 0.920$	19	2	21	2	$p = 0.046$
Serine	107	9	95	9	$p = 0.017$	111	9	109	9	$p = 0.590$
Asparagine	88	8	75	8	$p = 0.003$	95	8	88	8	$p = 0.063$
Glutamine	666	55	702	55	$p = 0.120$	654	55	707	56	$p = 0.028$
Glutamate	70	6	82	6	$p = 0.022$	67	6	81	6	$p = 0.006$
Glycine	236	22	215	22	$p = 0.016$	238	22	236	22	$p = 0.820$
Alanine	429	40	526	40	$p = 0.002$	462	40	579	40	$p < 0.001$
Citrulline	37	5	39	5	$p = 0.046$	41	5	43	5	$p = 0.230$
Tyrosine	84	11	79	11	$p = 0.150$	89	11	88	11	$p = 0.820$
Ornithine	121	10	117	10	$p = 0.310$	120	10	114	10	$p = 0.120$
Arginine	104	10	116	10	$p = 0.070$	87	10	107	10	$p = 0.006$

Values are mean ± SE

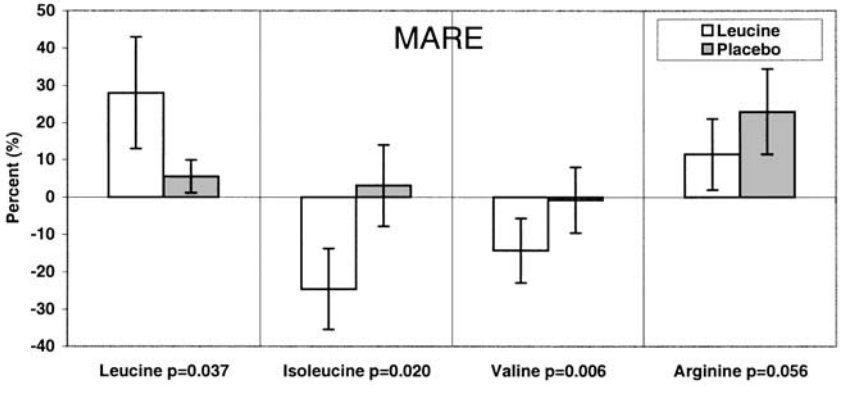

Fig. 3. Relative changes in concentration of leucine, isoleucine, valine and arginine in both groups following MARE (before – after comparison between groups, mean ± SE)

$p = 0.007$). Following MARE the concentration of leucine increased only in the leucine supplemented group (28%; $p < 0.001$). Other significant changes are shown in Table 5. Interactions between treatment and exercise were as follows: leucine ($p = 0.037$), isoleucine ($p = 0.020$), valine ($p = 0.006$) and arginine ($p = 0.056$) (Fig. 3). In addition, there was a similar significant exercise-induced increase ($p = 0.002$ in supplemented and $p < 0.001$ in non-supplemented) in alanine following MARE.

Running velocity and peak blood lactate at the end of MARE were similar in the supplemented and

non-supplemented groups (7.66 ± 0.28 m/s, 20.7 ± 2.2 mmol/L and 7.63 ± 0.29 m/s, 20.7 ± 2.4 mmol/L, respectively).

Discussion

The major finding of this study was that leucine supplementation, before and during (SES) or before (MARE) exercise sessions decreased the serum concentration of isoleucine and valine following SES and MARE. In addition, leucine supplementation seemed to have a sparing effect on taurine during SES. However, the supplementation did not affect peak blood lactate and physical performance (explosive strength following SES and running time in MARE) following exercise sessions when the daily protein intake was 1.1–1.3 g/kg body weight.

In our previous study (Mero et al., 1997) the subjects ingested leucine 50 mg/kg/body weight per day during a 10-week training period, which appeared to prevent a decrease in the serum leucine concentration during intensive training. In the present study we aimed to quantify whether leucine supplementation results in acute changes in amino acid concentration during short-term exercise sessions. In addition, we tried to alter such responses by using larger doses of leucine and by changing the ingestion time which was now either before (MARE) or both before and during (SES) the exercise session. In SES, when the leucine supplements (100 mg/kg body weight) were ingested in two doses, serum leucine was not significantly altered, while there was a decrease of 30% following the session in the placebo group. In MARE, the leucine supplement (200 mg/kg body weight) was consumed in one dose, and the concentration of leucine increased by 28% following the session. Rapid leucine absorption seems to result in an increase in the serum leucine concentration and to diminish the concentration of valine and isoleucine. The supplementation also seems to prevent the decrease in the concentration of glutamine during SES, which may be important because glutamine has a role in the immune system and is the amino acid that has the biggest relative proportion of total protein in humans (Hargreaves and Snow, 2001, Parry-Billings et al., 1990). In addition, the concentration of arginine is better maintained after SES due to leucine supplementation, which may be of great importance, because arginine has both anabolic and anticatabolic effects, being useful for increasing lean body mass and strength (Elam, 1988). Arginine is also a precursor of creatine and has a role as a cellular energy source (DiPasquale, 1997).

In SES, the leucine supplement was consumed before and during the exercise in order to keep the intake high and regular throughout the loading. Protein synthesis is depressed due to exercise (DiPasquale, 1997) and the leucine intake during exercise increases the free amino acid pool for protein synthesis in the recovery phase. This was also seen in higher concentrations of BCAAs both before and after exercise in the supplemented group. Leucine was ingested also during the exercise session, when the concentration of leucine may have remained high even after the session. Thus, there were more substrates for protein synthesis during recovery immediately after SES. The remarkably strong decrease in the concentration of total amino acids, observed following SES, must have affected the recovery phase after exercise. An increase in amino acids available for protein synthesis and the possible increase in the rate of the synthesis, due to supplementation, might be of importance during intermitted loading (as SES and MARE) both during the recovery phase and after exercise. Anthony et al. (1999) have previously demonstrated that orally administered leucine could stimulate muscle protein synthesis after exercise, independently of increased plasma insulin. Volpi et al. (1998, 1999) have also investigated the response of amino acid supplementation and protein synthesis, and found that muscle protein anabolism can be stimulated by increased availability of exogenous oral amino acids both in the elderly and the young. Our study revealed that following the exercise session the increase in leucine (11% in SES and 28% in MARE) concentration was accompanied by strong decreases in the concentrations of other BCAAs: valine (42% in SES and 14% in MARE) and isoleucine (61% in SES and 25% in MARE) in the leucine supplemented group. Previously Oxender et al. (1963) had stated that the decreased levels of valine and isoleucine following leucine supplementation might be due to changes in the affinity for these three amino acids by their common transporter. Nair et al. (1992) demonstrated that leucine infusion caused decreases in plasma concentrations of essential amino acids, but they concluded that leucine decreased protein degradation. Shinnick and Harper (1977) have also suggested that the decreased concentrations of valine and isoleucine may be the result of either decreased protein

degradation or increased protein synthesis, possibly mediated by insulin. Our results are in agreement with these previous studies, but since the transport system or protein kinetics was not estimated, the explanation for the changes in the amino acid concentrations remains unclear.

One important finding in the present study was that leucine treatment seemed to inhibit the decrease in taurine concentration during SES, but not during MARE. This may result from the different timing of the leucine supplement during experiments or the longer duration or more intense nature of SES than MARE. Taurine is a non-essential amino acid, which has antioxidant and protective properties. Taurine is also reported to have a role in the modulation of calcium levels and osmoregulation (DiPasquale, 1997). In addition, taurine has been indicated to be a stimulator of GH-secretion (Ikuyama et al., 1988). Such a large decrease in taurine concentration may lead to pathological changes and toxic damage in cells (DiPasquale, 1997). Since taurine has many important physiological effects in the human body, leucine supplements may play a marked role in the programming of strength exercise.

Although the exercise types of this study were mainly anaerobic, there are also aerobic contributions to the total energy production, for example during short recoveries. During these short-term recoveries within the session, leucine supplementation could result in extra fuel via direct oxidation in muscle or through the intensified alanine production with GAC. However, in the present study, leucine supplementation did not improve performance, which was measured as the outcome measurements of CMJ or peak blood lactate. This may be interpreted that there are no sufficient changes in aerobic energy production with extra leucine. Secondly, despite the fact that exercise induced increase in alanine production, the exercise period might be too short to utilise the extra fuel via GAC.

On the other hand, we must consider the possible long-term effects of leucine supplementation on performance. Tipton and Wolfe (2001) have stated that post-exercise metabolic processes stimulate protein synthesis when the availability of amino acids is increased and that muscle hypertrophy is possible if muscle protein synthesis exceeds muscle protein breakdown. Thus the increased leucine availability through the supplementation can enhance the performance in the long-term recovery. In addition, there is some evidence that BCAA ingestion may reduce mental fatigue during exercise and enhance post-exercise cognitive performance (Blomstrand, 2001).

In MARE, the large dose of leucine was consumed before exercise in order to keep the availability high from the beginning of the exercise. The supplementation resulted in an increase in leucine (28%) but in a decrease in valine (14%) and isoleucine (25%) concentration. The strong rise in alanine (23% in supplemented and 25% in non-supplemented) seems to reflect similar GAC during MARE in both treatments. In TAAs, no increases were seen in the leucine supplemented group but a strong increase in the placebo group was due to an exercise session, whereas in SES strong decreases were observed following the session. This may be explained partly by a greater protein degradation in an energy demanding situation, as in MARE. The increase in the free amino acid pool in MARE might have affected the buffer capacity, but there was neither any difference in the peak lactate between the groups nor any improvement in performance.

The possible shifts in plasma volume following exercise sessions must be taken into consideration even though they were not measured or corrected in this study. Previous data have shown that running at approximately 75% VO_2 max results in a 5% to 10% decrease in plasma volume (Wilmore and Costill, 1994). In addition, Collins et al. (1986, 1989) have investigated the changes of plasma volume among weight lifters and observed the plasma volume decreases of 7.7–14.3% during weight lifting. We assumed that the 500 ml solution that the subjects consumed during experiments would have been sufficient to compensate for plasma loss. But if the subjects of the present study had suffered from dehydration, the amino acid concentrations following the exercise sessions would have been higher in both groups. Thus the real increases e.g. in leucine and alanine would have been smaller and the real decreases in valine and isoleucine would have been greater than presented in our results. On the other hand, the changes in concentrations of the amino acids have been compared between leucine and placebo groups in both experiments. Consequently, the plasma volume shifts would have been similar and thus would influence the results to a comparable effect.

Earlier investigators (Hood and Terjung, 1990, Wagenmakers, 1998) have suggested that the

recommended dietary allowances (RDA) for leucine should be increased from 14 to 45 mg/kg body weight/day in sedentary individuals. Consequently, the RDA for leucine should be increased in people who are regularly undertaking physical exercise. Assuming that the leucine content of protein is 4.6% of all amino acids (Takala et al., 1980), the proposed higher RDA for leucine could be met by an average extra protein intake of 0.5 g/kg body weight/day. Thus, viewed solely from the point of view of leucine, the recommended protein intake of 0.8 g/kg body weight/day for adult sedentary men is inadequate to meet the leucine requirement, depending on the type of protein habitually ingested. For active individuals, a protein intake of 1.2 to 1.7 g/kg body weight/day appears to be generally adequate (Lemon, 1998). Consequently, this means that the RDA for leucine in active individuals should be increased from the recommended values for sedentary people.

In conclusion, the present investigation shows that leucine supplementation exerts significant effects upon the serum amino acid profile following both a strength exercise session and a maximal anaerobic running exercise session. However, there are no effects on an acute physical performance.

Acknowledgements

The authors thank Mr Hannu Tuuri for help in statistical analysis.

References

Anthony JC, Anthony TG, Layman DK (1999) Leucine supplementation enhances skeletal muscle recovery in rats following exercise. J Nutr 129: 1102–1106

Babij P, Matthews SM, Rennie MJ (1983) Changes in blood ammonia, lactate and amino acids in relation to workload during bicycle ergometer exercise in man. Eur J Appl Physiol 50: 405–411

Blomstrand E (2001) Amino acids and central fatigue. Amino Acids 20: 25–34

Blomstrand E, Ek S, Newsholme EA (1996) Influence of ingesting a solution of branched-chain amino acids on plasma and muscle concentrations of amino acids during prolonged submaximal exercise. Nutrition 12: 485–490

Brooks GA (1987) Amino acid and protein metabolism during exercise and recovery. Med Sci Sports Exerc 19: S150–S156

Collins MA, Hill DW, Cuerton KJ, DeMello JJ (1986) Plasma volume change during heavy-resistance weight lifting. Eur J Appl Physiol 55: 44–48

Collins MA, Cuerton KJ, Hill DW, Ray CA (1989) Relation of plasma volume change to intensity of weight lifting. Med Sci Sports Exerc 21: 178–185

Di Pasquale M (1997) Amino acids and proteins for the athlete. Boca Raton, Florida, CRC Press, pp 30–145

Durnin JVGA, Rahaman MM (1967) The assesment of the amount of fat in the human body from the measurement of skinfold thickness. Br J Nutr 21: 681–688

Elam RP (1988) Morphological changes in adult males from resistance exercise and amino acid supplementation. J Sports Med Phys Fitness 28: 35–39

Goldberg AL, Etlinger JD, Goldspink DF, Jablecki C (1975) Mechanism of work-induced hypertrophy of skeletal muscle. Med Sci Sports 7: 185–198

Hargreaves M, Snow R (2001) Amino acids and endurance exercise. Int J Sport Nutr Exerc Metab 11: 133–145

Henriksson J (1991) Effect of exercise on amino acid concentrations in skeletal muscle and plasma. J Exp Biol 160: 149–165

Hood DA, Terjung RL (1990) Amino acid metabolism during exercise and following endurance training. Sports Med 9: 23–35

Ikuyama S, Okajima T, Kato KI, Ibayashi H (1988) Effect of taurine on growth hormone and prolactin secretion in rats. Possible interaction with opioid peptidergic system. Life Sci 43: 807–812

Komi PV, Bosco C (1978) Utilization of stored elastic energy in leg extensor muscles by men and women. Med Sci Sports 10: 261–265

Lemon PWR (1998) Effects of exercise on dietary protein requirements. Int J Sport Nutr 8: 426–447

May ME, Buse MG (1989) Effects of branched-chain amino acids on protein turnover. Diabetes Metab Rev 5: 227–245

Mero A, Peltola E, Saarela J (1987) Speed and speed endurance training (in Finnish). Gummerus, Jyväskylä, pp 55–116

Mero A, Pitkänen H, Oja SS, Komi PV, Pöntinen P, Takala T (1997) Leucine supplementation and serum amino acids, testosterone, cortical and growth hormone in male power athletes during training. J Sports Med Phys Fitness 37: 137–145

Nair KS, Schwartz RG, Welle S (1992) Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. Am J Physiol 263: E928–E934

Nutrition Recommendations in Athletes in Finland (in Finnish) (1990) Printer center, Helsinki, pp 5–64

Oxender DL, Christensen HN (1963) Distinct mediating system for the transport of neutral amino acids by the Ehrlich cell. J Biol Chem 238: 3686–3699

Panton LB, Rathmacher JA, Baier S, Nissen S (2000) Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate during resistance training. Nutrition 16: 734–739

Parry-Billings E, Blomstrand E, McAndrew N, Newsholme EA (1990) A communicational link between skeletal muscle, brain, and cells of the immune system. Int J Sports Med 11:S122–S128

Pitkänen HT, Mero A, Oja SS, Komi PV, Pöntinen PJ, Saransaari P, Takala T (2002a) Serum amino acid responses to three different exercise sessions in male power athletes. J Sports Med Phys Fitness 42: 472–480

Pitkänen HT, Mero A, Oja SS, Komi PV, Rusko H, Nummela A, Saransaari P, Takala T (2002b) Effects of training on the exercise-induced changes in serum amino acids and hormones. J Strength Cond Res 16: 390–398

Rennie MJ, Halliday D, Davies CTM, Edwards RHT, Krywawych DJ, Millward DJ, Matthews DE (1981) Exercise induced increase in leucine oxidation in man and the effect of glucose. In: Walser M, Williamson JR (eds) Metabolism and clinical implications of branched chain amino acid ketoacids. Elsvier/North Holland, Amsterdam, pp 361–366

SAS Institute Inc., SAS Procedures Guide, Version 6, 3rd edn., Cary, NC (1990): SAS Institute Inc., ISBN 1-55544-378-8, p 705

SAS Institute Inc., SAS System for Statistical Graphics, First Edition, Cary, NC (1991) SAS Institute Inc., ISBN 1-55544-441-5, p 697

SAS Institute Inc., SAS/STAT Software (1996) Changes and enhancements through release 6.11, Cary, NC: *SAS* Institute Inc., ISBN 1-55544-274-9, p 1104

Shinnick FL, Harper AE (1977) Effects of branched-chain amino acid antagonism in the rat on tissue amino acid and keto acid concentrations. J Nutr 107: 887–895

Takala T, Hiltunen K, Hassinen E (1980) The mechanism of ammonia production and the effect of mechanical work load on proteolysis and amino acid catabolism in isolated perfused rat heart. Biochem J 192: 285–295

Tipton KD, Wolfe RR (2001) Exercise, protein metabolism, and muscle growth. Int J Sport Nutr Exerc Metab 11: 109–132

Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR (1998) Exogenous amino acids stimulate net muscle protein synthesis in the elderly. J Clin Invest 101: 2000–2007

Volpi E, Mittendorfer B, Wolf SE, Wolfe RR (1999) Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. Am J Physiol 277: E513–E520

Wagenmakers AJ (1998) Muscle amino acid metabolism at rest and during exercise: role in human physiology and metabolism. Exerc Sport Sci Rev 26: 287–314

Wallenstein S, Fisher AC (1977) The analysis of the two-period repeated measurements crossover design with application to clinical trials. Biometrics 33: 261–269

Wilmore JH, Costill DL (1994) Physiology of sport and exercise. Human Kinetics, Champaign IL, USA, pp 139–141

Young VR, Bier DM (1987) A kinetic approach to the determination of human amino acid requirements. Nutr Rev 45: 289–298

Authors' address: Antti A. Mero, Neuromuscular Research Centre, Department of Biology of Physical Activity, University of Jyväskylä, P.O. Box 35, 40351 Jyväskylä, Finland,
E-mail: mero@maila.jyu.fi