Eur J Appl Physiol (2009) 105:215–223
DOI 10.1007/s00421-008-0892-6

ORIGINAL ARTICLE

Effect of strength training session on plasma amino acid concentration following oral ingestion of leucine, BCAAs or glutamine in men

Antti Mero · Anne Leikas · Juha Knuutinen · Juha J. Hulmi · Vuokko Kovanen

Accepted: 29 September 2008 / Published online: 21 October 2008

Abstract We examined the acute effects of a 1-h strength training session (STS) on plasma amino acid concentration following orally ingestion of leucine, branched-chain amino acids (BCAAs) or glutamine in nine physically active men who participated in double-blinded and randomised experiments. The subjects took placebo, leucine, BCAAs, or glutamine capsules (50 mg/kg) in either rest (REST) or STS condition. Blood samples were taken before and at 30, 60, 90, and 120 min after the beginning of the treatment and they were assayed for plasma amino acids with HPLC. Following both leucine and BCAA ingestion the peak concentration of leucine was similar at rest (524 ± 46 and 530 ± 29 nmol/ml, respectively) and similar after STS (398 ± 43 and 387 ± 46 nmol/ml, respectively) but the rest and STS concentrations differed from each other ($P < 0.01$–0.001). The modelled polynomial data for the leucine treatment showed that the peak concentration of leucine occurred at 67 min at rest and at 90 min in STS (difference between REST and STS: $P = 0.012$). For the BCAA treatment the polynomial data showed that the peak concentration of leucine occurred at 72 min at rest and at 78 min in STS ($P = 0.067$). The peak concentration of glutamine was similar in both rest and STS condition and occurred at 60 min at rest and at 57 min in STS. In conclusion, 1-h of STS slows the increase in the peak concentration of plasma leucine similarly after oral ingestion of leucine or BCAAs but after oral ingestion of glutamine it has no slowing effect on glutamine concentration.

Keywords Strength training session · Leucine · Branched-chain amino acids · Glutamine · Insulin

A. Mero (✉) · A. Leikas · J. J. Hulmi
Department of Biology of Physical Activity,
University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland
e-mail: antti.mero@sport.jyu.fi

J. Knuutinen
Department of Chemistry,
University of Jyväskylä, Jyväskylä, Finland

V. Kovanen
Department of Health Sciences, University of Jyväskylä,
Jyväskylä, Finland

Introduction

It is currently widely studied and discussed how to use protein and amino acids before, during and after a strength training session (STS). Comparison of the maximum absorption rates of amino acids has shown that free amino acids are absorbed faster than amino acids from intact proteins (Gropper and Acosta 1991; Metges et al. 2000; Bilsborough and Mann 2006). Regarding single amino acids Kerksick et al. (2004) and Campbell et al. (2006) observed that when the subjects ingested 4 g arginine at rest after fasting for 8-h the peak blood concentration of arginine occurred at 60 min. However, we recently showed that 1-h of STS significantly delayed the peak concentration of both arginine and taurine by 35 and 23 min, respectively (Mero et al. 2008).

In order to investigate more different important amino acids and plasma amino acid concentrations we chose leucine, branched-chain amino acids (BCAAs: leucine, isoleucine and valine) and glutamine to be supplemented at rest and before STS. The BCAAs have been studied for both their anabolic and anti-catabolic effects and oxidation. In heart and skeletal muscle in vitro, increasing the concentration of the three BCAAs or leucine alone reproduces the effects of increasing the supply of all amino acids in stimulating protein synthesis and inhibiting protein degradation

(May and Buse 1989). In humans, it has been shown that leucine decreases protein degradation and that this decreased protein degradation during infusion contributed to the decrease in plasma essential amino acids (Nair et al. 1992).

Glutamine is the most abundant amino acid in human muscle and plasma and is found in relatively high levels in many human tissues. It plays fundamental physiological roles as follows: as a precursor of hepatic ureagenesis and renal ammoniagenesis, in the maintenance of the acid–base balance during acidosis, as a nitrogen precursor for the synthesis of nucleotides, as cellular fuel in certain tissue such as muscle, intestine, skin, and in the immune system, and as a direct regulator of protein synthesis and degradation (Anderson 1982; Wasa et al. 1996; Hall et al. 1996; Neu et al. 1996; Rennie et al. 1996; Castell 2003).

In order to extend our previous findings and to investigate other physiologically important amino acids we chose single leucine, a combination of BCAAs with the same amount of leucine and single glutamine. We hypothesized that STS would slow the absorption to blood of each ingested amino acid when compared to the rest condition. This was expected to be seen as a slowing increase in the peak concentration of each supplemented amino acid in the blood. Consequently, it would be interesting to see if there are real differences in plasma leucine concentration between supplementation of leucine alone or together with other BCAAs. Together with the results of leucine, BCAAs and glutamine we would have a good comparison with our earlier results (Mero et al. 2008) regarding single amino acid supplementation and plasma concentrations at rest and after STS.

Methods

Subjects

Nine healthy, physically active men, who participated only in recreational non-competitive athletic activity (aerobic training including jogging, cycling, swimming 2–3 times per week and occasionally light muscle work in gym), volunteered as subjects for the study. Their (mean ± SD) age was 24 ± 3 year, mean body height 1.78 ± 0.06 m, and mean body mass 76 ± 7 kg. All the subjects were drug free, which was evaluated by interviews and questionnaires. Furthermore, none of them used supplements of amino acids, vitamins, minerals, creatine or any other supplement during the study phase which was instructed before the study and recorded from the food diaries. The protocol and the potential benefits and risks were fully explained to each subject before they signed an informed consent document. This study was approved by the local University Ethical Board.

General design

All subjects were exposed to a supplement treatment condition and a placebo treatment condition in REST or exercise (STS) conditions in a double blind, randomized, and cross-over experiment. There were eight different treatment days for all the subjects separated by 1 week of washing out period between the treatment days. The treatments were balanced so that rest and exercise were alternated for each subject but otherwise randomized. The treatment days were as follows: rest placebo (RP), exercise placebo (EP), rest leucine (RL), exercise leucine (EL), rest BCAAs (RBCAA), exercise BCAAs (EBCAA), rest glutamine (RG) and exercise glutamine (EG). Subjects were encouraged to maintain their normal recreational training program during the whole study phase and especially repeat the training similarly during the entire study phase. At least 24-h of rest was required prior to each eight treatment day. The subjects were also required to refrain from alcohol and caffeine intake for 24-h prior to the measurements. One week before the first measurement, the experimental protocol was described and familiarized to the subjects and there were anthropometry measurements and resistance load verifications for one repetition maximum (1 RM) and ten repetitions maximum (10 RM). Also anthropometric settings for each experimental exercise were determined and instructions for dietary intake were given. Timetable for the measurement day is presented in Fig. 1.

Nutritional design and supplements

The present subjects had written instructions to eat similarly three days before each measurement day. Before the beginning of the study, each subject was provided also with specific verbal and written instructions and procedures for reporting detailed dietary intake, including how to record portions by using household measures, exact brand names and preparation techniques. After the first test session the subjects were instructed to repeat the first 3 day diet during the next seven study periods. Before the measurement day the subjects fasted for 10-h before the 120 min measurement period at 8:00–10:00 a.m. After the first blood sample (Fig. 1) each subject took placebo capsules (calcium 50 mg/kg body weight) or leucine capsules (50 mg/kg body

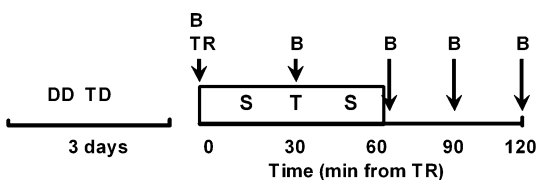

Fig. 1 Timetable for the measurement day. *DD* diet diary, *TD* training diary, *B* blood sample, *TR* treatment, *STS* strength training session

weight) or BCAAs (leucine 50 mg, isoleucine 12.5 mg and valine 12.5 mg per kg body weight) or glutamine capsules (50 mg/kg body weight) randomly with water (400 g) in either REST or STS condition before carrying out the respective measurements.

Strength training session

Strength training session (STS) started with a controlled 5 min warm-up on a rowing ergometer followed by 5 min stretching exercises. STS aimed to stimulate muscle hypertrophy (e.g. Kraemer et al. 2002) included six strength exercises: three exercises for lower limbs (leg press, leg extension, hamstring curl), two exercises for upper limbs (bench press, cable row) and one exercise for trunk (combined stomach and back muscle exercise). Each exercise was performed in three sets of 10 RM (2 min recovery). Recovery between each exercise was 3 min. The sets in exercises were performed with the maximum load possible to achieve 10 repetitions. If the subject could not achieve 10 repetitions on his own, then manual assistance was given for the remaining repetitions. If the load was too high or low, then the load was adjusted appropriately for the next set. Each subject had to drink water 200 g two times first at 24 min and then at 41 min from the beginning of STS to minimize dehydration and big changes in plasma volume.

Physical activity and dietary intake

The subjects were instructed to maintain their normal physical activity throughout the study period and to keep diaries. They reported in diaries their free-time physical activity during 5 days before the first measurement day. The subjects were then instructed to repeat physical activity similarly during each eight study period. The day before each measurement session was a rest day from physical activity. All diaries were then analyzed as times and minutes of training.

Dietary intake of the subjects was registered by food diaries for 5 days before the first measurement day. Individual food records were then returned to the subjects after the first test session to facilitate replication of their diet during the next phases of the study. All food diaries were analysed using the Micro Nutrica nutrient-analysis software (version 3.11, Social Insurance Institution of Finland).

Blood sampling and analysis

Blood samples were taken just before a supplement ingestion (0 min) and at 30, 60, 90 and 120 min after the beginning of the treatment (Fig. 1). They were taken from an antecubital vein in the sitting position. Two millilitres blood from a vein was taken in K2 EDTA tubes (Terumo Medical Co., Leuven, Belgium) for measurements of haemoglobin and haematocrit concentration with a Sysmex KX 21 N Analyzer (Sysmex Co., Kobe, Japan). The intra-assay coefficient of variation (CV) is 1.5% for haemoglobin and 2.0% for haematocrit. Plasma volume changes relative to the values from the first morning sample (0 min) were corrected with the values of haemoglobin and haematocrit (Dill and Costill 1974).

Five millilitres of blood was taken in lithium-heparin tubes (Terumo Medical Co., Leuven, Belgium) for measurement of lactate and glucose with a Nova Biomedical STAT Profile PhOX Plus L Analyzer (Nova Biomedical, Waltham, MA, USA). The intra-assay CV is 3.0% for lactate and 5.0% for glucose. For the determination of serum insulin, seven millilitres of blood were taken. Serum samples were kept frozen at −80°C until assayed. Serum insulin concentrations were analyzed by an immunometric chemiluminescence method with Immulite® 1,000 (DPC, Los Angeles, USA). The sensitivity of the assay for insulin is 2 mIU/L and CV 3.4%.

Concentrations of free amino acids in plasma were determined applying the procedure of Pfeifer et al. (1983) by reversed phase high performance liquid chromatography (RPHPLC). The HPLC system included Quaternary Gradient Pump unit, PU-2089 Plus, Intelligent AutosamplerAS-2057 Plus, and Intelligent Fluoresence Detector, FP-2020 by Jasco. Data processing software was Jasco Chrompass. Zorbax C_{18} column (3.0 mm × 150 mm × 3.5 µm) was from Agilent Technologies. Flow rate was 0.5 ml/min and injection volume 10 µl. β-Abc and Nor-Valine were used as internal standards. 100 µl of internal standard solution was added to the plasma sample (50 µl) and acetonitrile (100 µl) was used for precipitating the proteins to recover free amino acids. 750 µl of distilled deionized water was added, vortexed and allowed to stand on ice bath for 1-h. A 200 µl aliquot of the sample was centrifuged and the clear supernatant was used to prepare the fluorescent OPA derivatives for the individual amino acids (Schwarz 2005; Fekkes 1996). Detection wavelengths were 338 nm for excitation and 455 nm for emission.

Statistical analysis

All data were analyzed using the SPSS for Windows (release 12.01) statistical software package (SPSS, Chicago, Il, USA). Amino acid data were log transformed where appropriate to stabilize the variance and covariance matrices before analysis. Sphericity of the data was checked before the F-test and corrected with Greenhouse-Geisser or Huynh-Feldt estimator if needed. The effects of sample time, exercise or rest and supplement used were assessed by a general linear model (GLM) analysis of variance. When a significant difference in treatments or in sample times was detected, then a LSD (least significant

difference) post hoc test was performed to locate the pairwise differences. When a significant interaction or tendency to interaction between treatment and sample time was found (leucine, BCAAs, glutamine), the effect of sample time was modeled by polynomial function (curve fitting) separately for rest and exercise periods. The goodness of the fit was tested with the r^2 value.

Results

Physical activity and dietary intake

There were no differences in physical activity during the 4 days before each measurement day between the eight study periods. The last day before the measurement was a rest day. The average 4 days physical activity of all eight study periods consisted of two bouts of low intensity aerobic training with a total duration of 114 min. The subjects ate similarly in each study phase and the average daily macronutrient intake was as follows (mean ± SD): energy 2,767 ± 659 kcal, protein 145 ± 49 g, carbohydrate 328 ± 85 g, and fat 89 ± 24 g.

Plasma volume and blood lactate

Plasma volume increased by 3.4–5.5% (ns – $P < 0.01$) in the rest conditions during 120 min whereas in the STS conditions there were strong ($P < 0.001$) decreases (9.7–11.3%). The greatest decreases were observed at 30 or 60 min.

Blood lactate concentrations increased strongly ($P < 0.001$), as expected in the hypertrophic STS conditions. The peak values occurred in the middle of STS at 30 min and were (mean ± SD) 11.7 ± 1.2 mmol/l (EP), 13.6 ± 1.1 mmol/l (EL), 10.9 ± 0.9 mmol/l (EBCAA) and 10.2 ± 0.5 mmol/l (EG).

Amino acid concentrations in the placebo rest condition

In the placebo rest condition nonsignificant decreases were observed, during 2-h after fasting 10-h, in the concentration of total amino acids (3%), essential amino acids (4%), branched-chain amino acids (BCAAs 9%), and non-essential amino acids (2%).

Effect of leucine ingestion on amino acid concentrations

Leucine concentration

Significant interaction effects were observed for leucine: in STS and sample time ($P < 0.05$), and treatment and sample time ($P < 0.001$). The concentration of leucine in plasma increased significantly ($P < 0.05$–0.001) following leucine ingestion in both rest and STS conditions while in the placebo the concentrations remained stable at rest but decreased in STS ($P < 0.05$–0.001) (Fig. 2a). The peak concentration in the rest condition following leucine supplementation was 524 ± 46 nmol/ml and occurred at 60 min whereas in the STS condition it was 398 ± 43 nmol/ml and occurred at 90 min. The concentrations at 60 min after leucine ingestion between RL and EL differed significantly ($P < 0.001$). Figure 2b presents the modelled polynomial data for leucine in the rest and STS conditions and shows a difference ($P = 0.012$; STS and sample time interaction) between REST and STS. The peak concentration of leucine occurred at 67 min at rest and at 90 min in the STS condition.

Effect of BCAA ingestion on amino acid concentrations

Leucine concentration

Significant interaction effects were observed for leucine: in STS and sample time ($P < 0.05$), in treatment and sample time ($P < 0.001$) and contrast to leucine ingestion in STS and treatment ($P < 0.05$). The concentration of plasma leu-

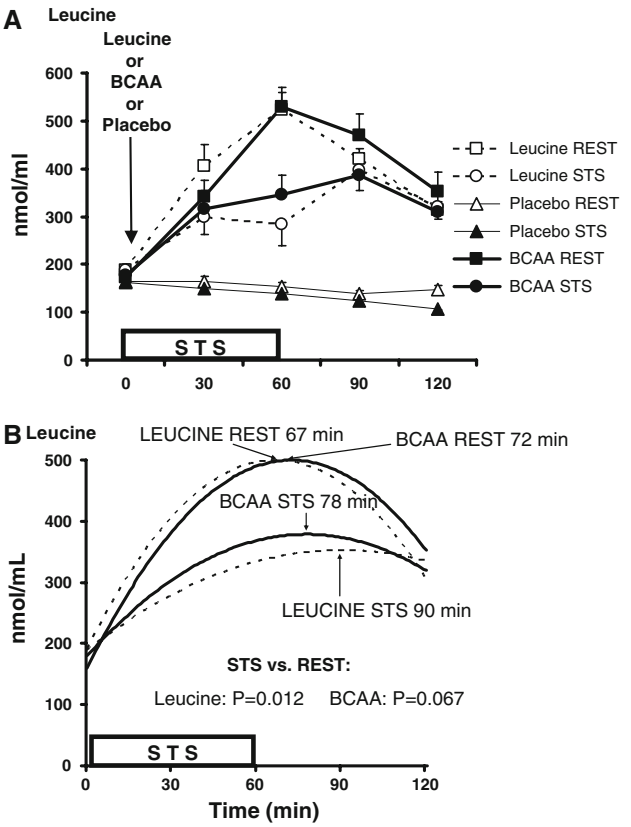

Fig. 2 Leucine concentration after leucine and BCAA treatment: **a** raw data (mean ± SE) and **b** modelled polynomial data. Goodness of fit values were as follows: leucine rest $r^2 = 0.59$ ($P < 0.001$), leucine STS $r^2 = 0.25$ ($P < 0.01$), BCAA rest $r^2 = 0.59$ ($P < 0.001$), BCAA STS $r^2 = 0.31$ ($P < 0.001$). *STS* strength training session. Significances are mentioned in the *result text*

cine increased significantly ($P < 0.01$–0.001) following BCAA ingestion in both rest and STS conditions, but in the placebo the concentrations remained stable at rest but decreased in STS ($P < 0.01$–0.001) (Fig. 2a). The peak concentration of leucine in the rest condition following BCAA supplementation was 530 ± 29 nmol/ml and occurred at 60 min whereas in the STS condition the peak concentration was 387 ± 46 nmol/ml and occurred at 90 min. The peak leucine concentrations between RBCAA and EBCAA differed significantly ($P < 0.01$). At rest the peak leucine concentration increased to threefold but in STS only to twofold. Figure 2b presents a modelled polynomial data for leucine in the rest and STS conditions and it shows that there was a trend for significance ($P = 0.067$) between REST and STS. The peak concentration of leucine occurred at 72 min at rest and at 78 min in the STS condition.

Isoleucine concentration

Significant interaction effect was observed for isoleucine in treatment and sample time ($P < 0.001$). The concentration of plasma isoleucine increased significantly ($P < 0.01$–0.001) following BCAA ingestion at rest but not in STS (Fig. 3a). In the placebo the concentrations remained stable at rest but decreased in STS ($P < 0.05$–0.01). The peak isoleucine concentration in the rest condition following BCAA supplementation was 155 ± 10 nmol/ml and occurred at 60 min whereas in the STS condition the peak concentration (102 ± 9 nmol/ml) and occurred at 30 min. The peak concentrations between RBCAA and EBCAA differed significantly ($P < 0.01$).

Valine concentration

Significant interaction effects were observed for valine: in STS and sample time ($P < 0.05$) and treatment and sample time ($P < 0.001$). The concentration of plasma valine increased significantly ($P < 0.01$–0.001) following BCAA ingestion at rest but not in STS (Fig. 3b). In the placebo the concentrations remained stable at rest but decreased in STS ($P < 0.05$–0.01). The peak valine concentration in the rest condition following BCAA supplementation was 443 ± 17 nmol/ml and occurred at 60 min whereas in the STS condition the peak concentration was 348 ± 17 nmol/ml and occurred at 30 min. The peak valine concentration between RBCAA and EBCAA differed significantly ($P < 0.01$).

Effect of glutamine ingestion on amino acid concentrations

Glutamine concentration

Significant interaction effect was observed for glutamine in treatment and sample time ($P < 0.05$). The concentration of

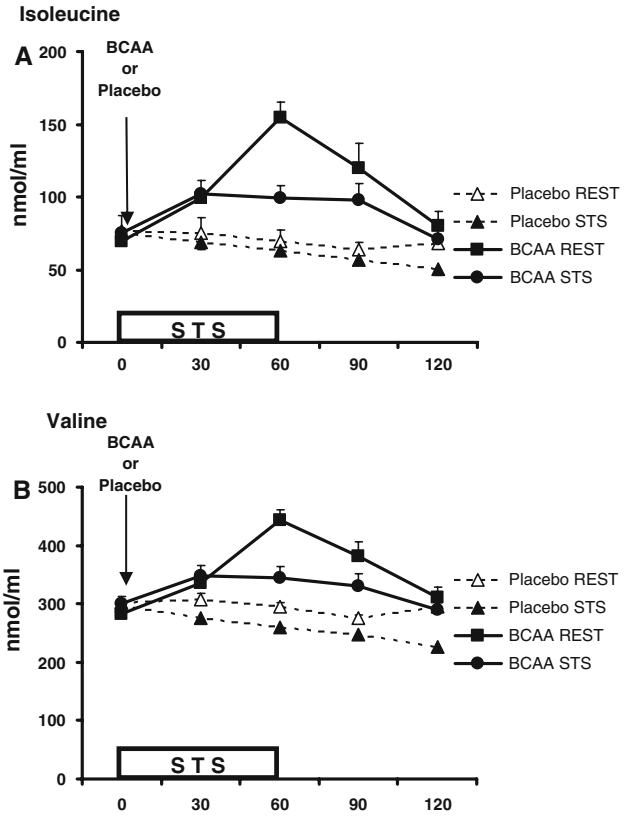

Fig. 3 Isoleucine **a** and valine **b** concentration after BCAA treatment: raw data (mean ± SE), *STS* strength training session. Significances are mentioned in the *result text*

plasma glutamine increased significantly ($P < 0.05$–0.01) following glutamine ingestion in both rest and STS conditions but in the placebo the concentrations remained stable (Fig. 4). The peak concentration in the rest condition following glutamine supplementation was 740 ± 41 nmol/ml and in the STS condition 771 ± 30 nmol/ml and both occurring at 30 min. The modelled polynomial data for glutamine to explain variation in the STS condition was not significant ($r^2 = 0.11$; $P = 0.10$) and also slight in the rest condition ($r^2 = 0.15$, $P = 0.03$).

Effect of STS on amino acid concentrations

STS induced strong decreases in BCAAs in EP ($P < 0.05$, −28%) and in EG ($P < 0.001$, −26%) during 2-h but increases in EL (ns, 18%) and in EBCAA ($P < 0.05$, 48%). The concentration of alanine increased strongly in the STS conditions at 30, 60, and 90 min. The peak values occurred at 60 min and the increases were 51% for EP ($P < 0.001$), 46% for EL ($P < 0.001$), 71% for EBCAA ($P = 0.001$) and 64% for EG ($P < 0.001$). The strong increase in alanine in the STS conditions achieved also increases ($P < 0.05$–0.001) in the concentration of in the sum of all non-essential amino acids at 30, 60 and 90 min.

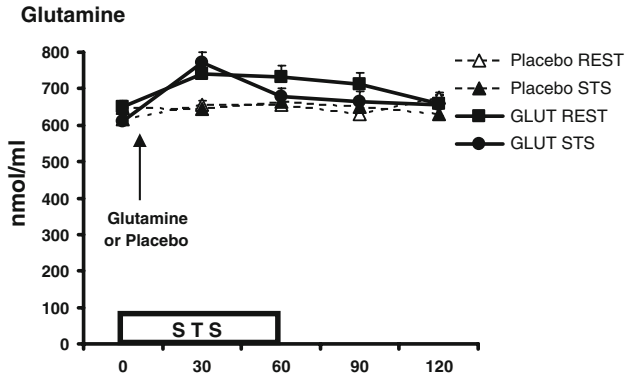

Fig. 4 Glutamine concentration: raw data (mean ± SE). *STS* strength training session. Significances are mentioned in the *result text*

Blood glucose and serum insulin

Blood glucose was stable in RP and RG but decreased ($P < 0.01$) at 120 min in RL and RBCAA treatments. In all the STS conditions (EP, EL, EBCAA, EG) there were non-significant increases at 60 or 90 min but at 120 min there were strong decreases ($P < 0.01$–0.001) compared with the pre-values except EG in which there was no change.

Serum insulin decreased during 2-h in all rest conditions but significantly only in RP ($P < 0.001$) and RL ($P < 0.01$) at 120 min. In all the STS conditions the peak values occurred at 60 or 90 min and were greater ($P < 0.01$) than the pre-values. Thereafter serum insulin decreased to the pre-values.

Discussion

Major results

In the present study, STS strongly affected the concentration of leucine in the leucine and BCAA condition compared to the resting situation. Leucine concentrations were similar following ingestion of leucine alone or with other BCAAs both at rest and after STS, however, the peak concentrations were lower after STS. The modelled polynomial data for the leucine treatment showed that the peak concentration of leucine occurred at 67 min at rest and was delayed by 23 min in STS. For the BCAA treatment the polynomial data showed that the peak concentration of leucine occurred at 72 min at rest and delayed by 6 min in STS. On the other hand, following glutamine ingestion the peak concentration of glutamine was similar in both rest and STS conditions and occurred at 60 min in the rest condition and at 57 min in STS.

STS conditions and subjects

The exercise STS model was similar as in our earlier study (Mero et al. 2008) where we investigated effects of STS with arginine and taurine. The hypertrophic whole body STS increased blood lactate by 10–13 mmol/l, which is similar as we had earlier and typical for this kind of exercise (e.g. Kraemer et al. 1990). The subjects had only slight experience with this kind of heavy STS and could perform in bench press 1RM about their own body weight (75–80 kg) and full squat about 80–110 kg.

In all STS conditions blood glucose levels slightly increased and achieved the peak value either at 60 or 90 min and thereafter decreased. STS was carried out after 10-h fasting, which leads to an increase in exercise-induced hepatic glucose output (e.g. McArdle et al. 2007) as only placebo and water or amino acids and water was given to the subjects. The elevated blood glucose levels within the pancreas then probably directly stimulated the release of serum insulin. The peak serum insulin levels in STS were observed at the same time point as the peak blood glucose level. This elevation of insulin, in turn, probably induced glucose entry into cells and therefore strongly lowered blood glucose levels at 120 min. The subjects drank 400 ml water at the beginning of each treatment. In the rest conditions this induced increases (3.4–5.5%) in plasma volume over the succeeding 2-h. In spite of the additional 400 ml water during the intensive exercise, the plasma volume decreased by 9.7–11.3%, especially at 30 and 60 min, due to sweating and other metabolic processes in body. The plasma volume changes are similar as in our earlier study (Mero et al. 2008) and somewhat smaller than the earlier results in corresponding resistance exercise (e.g. Collins et al. 1986; Ploutz-Snyder et al. 1995; Durham et al. 2004), mainly owing to the intake of 800 ml water (400 ml before and 400 ml during STS).

Decreases in BCAAs occurred during two hours in the placebo and glutamine conditions after STS, which result is similar to our earlier study (Mero et al. 2008) and observed also by Pitkänen et al. (2002) 10 min after a combined maximal and explosive strength training session with a blood lactate level of 2.5 ± 0.4 mmol/l. The decrease may be explained by need of BCAAs for energy and protein synthesis (Pitkänen et al. 2003).

Leucine ingestion

The physically active subjects but not athletes ate on average 145 g protein daily which is 1.91 g/kg body weight. It is clearly more than the recommended RDA value of 0.8 g/kg body weight (DRI 2005) and in fact it is in the range value of strength athletes reported 1.5–2.0 g/kg body weight (Lemon 2000; Hoffman et al. 2004; Campbell et al.

2007). The measurements were carried out in 10-h fast conditions. In the rest placebo conditions there was a slight (3%) decrease in the concentration of all the amino acids during the 2-h follow-up period and, correspondingly a 9% decrease in BCAAs and 8% in leucine. This indicates that a long fasting period over night leads to a continuous but slight decrease in amino acid concentration. The ingestion of 4 g leucine at rest induced a 3.1 fold increase in the peak leucine concentration (524 ± 46 nmol/ml) at 60 min. The amount of 4 g leucine is similar to that contained in about 230 g of beef (USDA 2006). With leucine treatment the intensive whole body STS delayed the peak concentration by ~23 min, the peak value being 24% smaller in STS than in REST. The smaller concentration of leucine in STS after leucine or also BCAA treatment suggests that leucine is probably used as energy through oxidation and to stimulate the protein synthesis and anticatabolic processes (May and Buse 1989; Norton and Layman 2006). These speculations are strengthened by the observation that the effect of amino acids on protein synthesis can be noticed 30 min after supplementation and the peak rate of protein synthesis occurs between 60 and 90 min (Rennie et al. 2002, 2004). After exercise, recovery of muscle protein synthesis requires dietary protein, BCAAs or leucine alone to increase tissue levels of leucine in order to activate of the protein kinase mammalian target of rapamycin (mTOR) pathway (Norton and Layman 2006). Effect of leucine on mTOR is synergistic with an allowing role of insulin via the phosphoinositol 3-kinase signalling pathway and consequently insulin and leucine coordinate protein synthesis with physiological state and dietary intake (Norton and Layman 2006). The impact of a delayed peak leucine concentration is difficult to speculate but it may suggest that the activation of protein synthesis is delayed by some minutes compared to the ingestion at resting state.

The delayed peak plasma concentration of leucine following STS after ingesting it alone in combination with isoleucine and valine confirms our earlier result with arginine and taurine (Mero et al. 2008); Leiper et al. (2005) showed that intermittent high-intensity running slowed gastric emptying of drinks containing carbohydrate and non-carbohydrate. The authors speculated that some mechanical factors may affect gastric area when moving rapidly from one place to another. In both of our two studies the subjects performed movements during resistance exercises which means that the gastric area together with the contents of the stomach was in rapid motion. Because the gastric region after ingestion of a single amino acid was moving during STS although not so much as in running, there may be some delay in the emptying process. Another possible explanation may be blood flow. During eating, the blood flow to the gastric area is increased, but when doing exercise the effect is opposite (e.g. McKirnan et al. 1991). During intensive exercise, blood flow through the exercising skeletal muscles can be up to 20 times greater than through the resting muscles (McArdle et al. 2007), and less blood is distributed to the gastric region. In the present studies we did not measure blood flow, but it can be speculated that during STS the blood flow to the gastric region was diminished, which in consequence delayed the transport of a single amino acid into the blood.

BCAA ingestion

The ingestion of 6 g BCAAs (4 g leucine, 1 g isoleucine and 1 g valine) at rest induced a 3.0-fold increase in the peak leucine concentration (530 ± 29 nmol/ml) at 60 min. The intensive whole body STS delayed the peak concentration of leucine by 30 min in the raw data but only ~6 min in the polynomial data, the peak value being 27% smaller in STS than in REST. These results show that the concentration of leucine is similar at rest and in STS if you take leucine alone or with isoleucine and valine. The only small difference is in the delay of the peak concentration in STS where the possible synergistic role of isoleucine and valine can be seen. At 60 min both isoleucine and valine concentrations were strongly lower in STS compared to the rest condition. The peak values of isoleucine and valine occurred at 30 min in STS but the concentration differences between 30, 60 and 90 min are small being almost in a plateau. Consequently it may show that also isoleucine and valine are used in energy production and/or anabolic processes during STS. This might have changed the polynomial curve slightly compared to leucine treatment. Ingestion of BCAAs increases their concentrations in plasma and this may reduce the uptake of tryptophan by the brain and also 5-HT synthesis and thereby delay fatigue (Blomstrand 2006). In human subjects with the ingestion of BCAAs before endurance performance the ratings of perceived exertion and mental fatigue reduced and in some situations physical performance improved (Blomstrand 2006). Consequently, the BCAA supplementation before STS and fatigue needs more research.

Glutamine ingestion

The ingestion of 4 g glutamine increased the peak concentration of glutamine by 14% at rest and by 26% in STS and it occurred in both conditions at 30 min. Consequently, it can be concluded that with 4 g glutamine treatment glutamine concentration behaves similarly at rest and in STS. This result is different if we compare to the results of other single amino acids leucine (arginine and taurine; Mero et al. 2008) in our studies. The reason why STS does not affect glutamine but affects leucine as well as arginine and taurine (Mero et al. 2008) is unknown but may be related to

different metabolism of glutamine. According to the literature (Antonio et al. 2002) glutamine has no effect on weight lifting performance and consequently supplementation with glutamine before STS is not important. On the other hand, glutamine has many tasks (e.g. Castell 2003) in human body in exercise but these tasks are probably not dependent on timing of supplementation with glutamine and extra glutamine may not be needed as an additional supplement in healthy people.

Methodological concerns

In this study the blood samples have been taken from an arm vein and it is a representation of the net processes of amino acid appearance into blood and disappearance into tissues. Therefore, it is difficult to conclude if exercise was slowing absorption of amino acids from the gut because there was not direct sampling from the portal vein. If we consider that exercise strongly increases the uptake of amino acids by the muscle (e.g. Biolo et al. 1995), it is likely that the delay in leucine concentration after STS was at least in part the result of leucine or the whole BCAAs being taken up by exercised muscle for the synthesis of muscle proteins or for energy. This would result in a better match between the delivery of amino acids from the gut to the uptake of amino acids by muscle which would ultimately result in a reduction in venous blood amino acid concentration. This is supported by the fact that peak concentrations of BCAAs were smaller after STS than at rest and that glutamine appearance was not altered with exercise. Glutamine is not limiting for exercise-induced muscle protein synthesis and this may be the reason for observed result. However, in our last article, also taurine showed delayed concentration in blood after its ingestion before STS (Mero et al. 2008). Taurine is not used for muscle protein synthesis and this fact argues against the uptake of amino acids for protein synthesis being the reason for the slowing up of the blood amino acid concentrations during STS.

Conclusions

It is concluded that orally ingestion of leucine alone or together with isoleucine and valine has the same effect on plasma leucine concentration and 1-h whole body STS slows the increase in the peak concentration of plasma leucine by 6–23 min. STS does not affect plasma glutamine after oral ingestion of glutamine.

Acknowledgments The authors thank Risto Puurtinen, Kaisa-Leena Tulla, Tarja Lyytinen and Aila Ollikainen for their help in data collection and Hannu Tuuri for help in statistical analysis.

References

Anderson KE (1982) Hormones and liver function: peptide hormones and catecholamines. In: Schiff L, Schiff ER (eds) Diseases of the liver. Lippincott, Philadelphia

Antonio J, Sanders MS, Kalman D, Woodgate D, Street C (2002) The effects of high-dose glutamine ingestion on weight lifting performance. J Str Cond Res 16:157–160. doi:10.1519/1533-4287(2002)016<0157:TEOHDG>2.0.CO;2

Bilsborough S, Mann N (2006) A review of issues of dietary protein intake in humans. Int J Sport Nutr Exerc Metab 16:129–152

Biolo G, Maggi SP, Williams BD et al (1995) Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. Am J Physiol Endocrinol Metab 268:E514–E520

Blomstrand E (2006) A role for branched-chain amino acids in reducing central fatigue. J Nutr 136:544S–547S

Campbell B, Kreider RB, Ziegenfuss T et al (2007) International society of sports nutrition position stand: protein and exercise. J Int Soc Sports Nutr 4:8. doi:10.1186/1550-2783-4-8

Campbell B, Roberts MS, Kerksick C et al (2006) Pharmacokinetics, safety, and effects on exercise performance of L-arginine-ketoglutarate in trained adult men. Nutrition 22:872–881. doi:10.1016/j.nut.2006.06.003

Castell LM (2003) Glutamine supplementation in vitro and in vivo, in exercise and in immunodepression. Sports Med 33:323–345. doi:10.2165/00007256-200333050-00001

Collins MA, Hill DW, Cureton KJ et al (1986) Plasma volume change during heavy-resistance weight lifting. Eur J Appl Physiol 55:44–48. doi:10.1007/BF00422891

Dill DB, Costill DLJ (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 37:247–248

Durham WJ, Miller SL, Yeckel CW et al (2004) Leg glucose and protein metabolism during an acute bout of resistance exercise in humans. J Appl Physiol 97:1379–1386. doi:10.1152/japplphysiol.00635.2003

Fekkes D (1996) State-of-the-art high-performance liquid chromatographic analysis of amino acids in physiological samples. J Chromatogr B Analyt Technol Biomed Life Sci 682:3–22. doi:10.1016/0378-4347(96)00057-6

Gropper SS, Acosta PB (1991) Effect of simultaneous ingestion of L-amino acids and whole protein on plasma amino acid and urea nitrogen concentrations in humans. J Parenter Enter Nutr 15:48–53. doi:10.1177/014860719101500148

Hall JC, Heel K, McCauley R (1996) Glutamine. Br J Surg 83(3):305–312. doi:10.1002/bjs.1800830306

Hoffman JR, Falvo MJ (2004) Protein—which is best? J Sport Sci Med 3:118–130

Kerksick C, Campbell B, Taylor L et al (2004) Pharmokinetic profile of time released and non-time released oral arginine. Sport Nutr Rev J 1:1–14

Kraemer WJ, Marchitelli L, Gordon SE et al (1990) Hormonal and growth factor responses to heavy resistance exercise protocols. J Appl Physiol 69:1442–1450

Kraemer WJ, Häkkinen K (eds) (2002) Strength training for sport. An IOC medical commission publication, Blackwell Science, Oxford

Leiper JB, Nicholas CV, Ali A et al (2005) The effect of intermittent high-intensity running on gastric emptying of fluids in man. Med Sci Sports Exerc 37:240–247. doi:10.1249/01.MSS.0000152730.74596.50

Lemon PW (2000) Beyond the zone: protein needs of active individuals. J Am Coll Nutr 19:513S–521S

May ME, Buse MG (1989) Effects of branched-chain amino acids on protein turnover. Diabetes Metab Rev 5(3):227–245

McArdle WD, Katch FI, Katch VL (eds) (2007) Exercise physiology, energy, nutrition and human performance. Lippincott Williams & Wilkins, Maryland

McKirnan MD, Gray CG, White FC (1991) Effects of feeding on muscle blood flow during prolonged exercise in miniature swine. J Appl Physiol 70:1097–1104

Mero A, Leikas A, Rinkinen N et al (2008) Effect of strength training session on plasma amino acid concentration following oral ingestion of arginine or taurine in men. Amino Acids 35:99–106. doi:10.1007/s00726-007-0619-8

Metges CC, el-Khoury AE, Selvaraj AB et al (2000) Kinetics of L-[1–13C]leucine when ingested with free amino acids, unlabelled or intrinsically labelled casein. Am J Physiol Endocrinol Metab 278:1000–1009

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(5 Pt 1):E928–E934

Neu J, Shenoy V, Chakrabarti R (1996) Glutamine nutrition and metabolism: where do we go from here? FASEB J 10(8):829–837

Norton LE, Layman DK (2006) Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. J Nutr 136:533S–S537

Pfeifer R, Karol R, Korpi J et al (1983) Practical application of HPLC to amino acid analysis. Am Lab 15:78–87

Pitkänen H, Mero A, Oja SS et al (2002) Serum amino acid responses to three different exercise sessions in male power athletes. J Sports Med Phys Fitness 42:472–480

Pitkänen H, Nykänen T, Knuutinen J et al (2003) Free amino acid pool and muscle protein balance after resistance exercise. Med Sci Sports Exerc 35:784–794. doi:10.1249/01.MSS.0000064934.51751.F9

Ploutz-Snyder LL, Convertino VA, Dudley GA (1995) Resistance exercise-induced fluid shifts: change in active muscle size and plasma volume. Am J Physiol Regul Integr Comp Physiol 269:536–543

Rennie MJ, Ahmed A, Khogali SE et al (1996) Glutamine metabolism and transport in skeletal muscle and heart and their clinical relevance. J Nutr 126(4(Suppl.)):1142S–1149S

Rennie MJ, Bohe J, Wolfe RR (2002) Latency, duration and dose–response relationships of amino acid effects on human muscle protein synthesis. J Nutr 132:3225S–3227S

Rennie MJ, Wackerhage H, Spangenburg EE et al (2004) Control of the size of the human muscle mass. Annu Rev Physiol 66:799–828. doi:10.1146/annurev.physiol.66.052102.134444

Schwarz EL, Roberts WL, Pasquali M (2005) Analysis of plasma amino acids by HPLC with photodiode array and fluorescence detection. Clin Chim Acta 354:83–90. doi:10.1016/j.cccn.2004.11.016

USDA (2006) National Nutrient Database for Standard Reference, Release 19

Wasa M, Bode BP, Abcouwer SF et al (1996) Glutamine as a regulator of DNA and protein biosynthesis in human solid tumor cell lines. Ann Surg 224(2):189–197. doi:10.1097/00000658-199608000-00012