

Effect of strength training session on plasma amino acid concentration following oral ingestion of arginine or taurine in men

A. Mero¹, A. Leikas¹, N. Rinkinen¹, P. Huhta², J. J. Hulmi¹, H. Pitkänen³, and J. Knuutinen²

¹ Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland

² Department of Chemistry, University of Jyväskylä, Jyväskylä, Finland

³ Mykora Ltd, Kiukainen, Finland

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Summary. This study examined the acute effects of a one-hour hypertrophic strength training session (STS) on plasma amino acid concentration following oral ingestion of arginine or taurine in nine physically active men participating in a double-blind and randomised experiment. The subjects took placebo, arginine or taurine 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 assayed for plasma amino acids with HPLC. There was a significant interaction effect with STS and sample time for both arginine and taurine in the raw data ($p < 0.05$). The modelled polynomial data for the arginine treatment showed that the peak concentration of arginine occurred at 69 min at rest and at 104 min in STS, and for the taurine treatment, the peak concentration of taurine occurred at 89 min at rest and at 112 min in STS. In conclusion, one hour of hypertrophic STS slows the increase in the peak concentration of plasma arginine and taurine after oral ingestion of the respective amino acids.

Keywords: Strength training session – Amino acids – Insulin – Glucose

Introduction

The plasma concentration of a single amino acid is the result of its rate of appearance in and disappearance from plasma. The rate of amino acid appearance and disappearance are tightly regulated. The factors controlling the rate of appearance are protein or amino acid intake and tissue release. The rate of disappearance is controlled by tissue uptake and body losses such as urine and sweat. The regulation of plasma amino acid concentration involves hormones (especially insulin, glucagon, and cortisol). The peripheral availability of amino acids after protein ingestion is controlled by the liver with activation of ureagenesis with hyperprotein ingestion and repression during a hypoprotein diet.

Comparison of the maximum absorption rates of amino acids has shown that free amino acids with the same profile as casein have an absorption rate of 7–7.5 g/h whereas that of casein isolate is only 6.1 g/h (Boirie et al., 1997; Dangin et al., 2001; Bilsborough and Mann, 2006). This is consistent with other studies showing that free amino acids are absorbed faster than amino acids from intact proteins (Gropper and Acosta, 1991; Metges et al., 2000). In recent studies (Kerksick et al., 2004; Campbell et al., 2006) it has been shown without measuring absorption that when subjects ingested 4 g non-time-released arginine at rest, after fasting for 8 h, the peak blood concentration of arginine occurred at 60 min.

During intermittent high-intensity running, ingestion of carbohydrate and noncarbohydrate containing drinks has been shown to slow gastric emptying. However, the associated mechanism is still unknown (Leiper et al., 2005). It is possible that there are some mechanical factors affecting in gastric area when moving rapidly from one place to another or exercising muscles. As far as we know there are no data on exercise effect on gastric emptying and/or absorption of proteins or amino acids. Therefore, the purpose of the present study was to investigate the acute effects of a strength training session (STS) on amino acid concentrations in the blood following an oral ingestion of arginine or taurine capsules. It is nowadays very much studied and discussed how to use protein and amino acids before, during and after STS. We chose arginine, first, as a reference to the study by Campbell et al. (2006), second, because arginine is a “semi-essential” amino acid used by

all cells (Wu and Morris, 1998), i.e., it plays a critical role in cytoplasmic and nuclear protein synthesis, the biosynthesis of other amino acids, creatine synthesis, biosynthesis of nitric oxide and the urea cycle. For the purpose of comparison, the non-essential sulphur-containing amino acid taurine (which lacks the usual characteristic of an amino acid, a carboxyl group), was selected because it is not used for protein synthesis in cells and because it also has several important properties, including antioxidant effects (Kendler, 1989), antihypertensive activity (Tanabe et al., 1989) and some other features reviewed by Timbrell et al. (1995). We hypothesized that STS would slow the absorption to blood of both these amino acids when compared to the rest condition. This was expected to be seen as a slowing increase in the peak concentration of arginine and taurine in the blood.

Materials and methods

Subjects

Nine healthy, physically active men who participated only in recreational non-competitive athletic activity volunteered as subjects for the study. Their (mean \pm SD) age was 25.7 ± 2.8 year, mean body height 1.80 ± 0.07 m, and mean body mass 78.4 ± 9.7 kg. All the subjects were drug-free, as ascertained by interviews and questionnaires. Furthermore, none of the subjects used supplements of amino acids, vitamins, minerals, and creatine or any other supplement during the study period instructed before the study and according to their 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 Ethics Committee of the Local University.

Experimental design

General design

All the subjects were exposed to a supplement treatment condition and a placebo treatment condition in either a REST or exercise (STS) condition in a double blind, randomized, and cross-over experiment. Half of the subjects were randomly allocated to the REST group (all three REST measurements first and then three STS measurements) and the other half to the STS group (all three STS measurements first and then three REST measurements). Amino acid and placebo treatments were fully randomized. There were six different treatment days for all the subjects separated by a one week wash-out period between the treatment days. The treatment days were as follows: rest placebo (RP), exercise placebo (EP), rest arginine (RA), exercise arginine (EA), rest taurine (RT), and exercise taurine (ET). Subjects were encouraged to maintain their normal recreational training program throughout the whole study period and especially to repeat the training similarly during the entire study phase. At least 24 h of rest was required prior to each of the six treatment days. The subjects were also required to refrain from alcohol and caffeine intake for 24 h prior to the measurements. Two weeks before the first measurement, the experimental protocol was described and familiarized to the subjects who were then prescribed a STS which mimicked the experimental protocol. One week before the first measurement the subjects attended a second session during which anthropometry measurements and resistance load verifications for the one repetition maximum (1 RM) and ten repetitions maximum (10 RM) were performed. In addition anthropometric settings

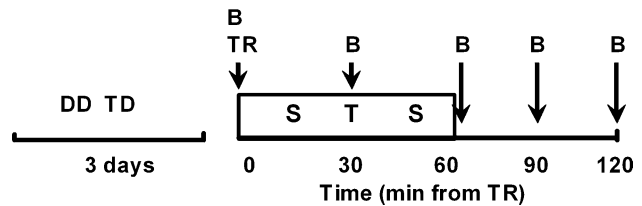


Fig. 1. Timetable for the measurement day. *DD* Diet diary, *TD* training diary, *B* blood sample, *TR* treatment, *STS* strength training session

for each experimental exercise were determined and instructions for dietary intake were given. The timetable for the measurement day is presented in Fig. 1.

Nutritional design and supplements

The present subjects were instructed in written instructions to have a similar dietary intake over the course of 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 his detailed dietary intake, including how to record portions by using household measures giving exact brand names and techniques of food preparation. After the first test session the subjects were instructed to repeat their first three day diet during the next five study periods. Before each 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 either placebo (calcium) or arginine (arginine hydrochloride) or taurine capsules (50 mg/kg body weight) randomly with water (400 ml) in either the REST or STS condition before the measurements were performed.

Strength training session

The strength training session (STS) started with a controlled 5 min warm-up on a rowing ergometer followed by 5 min of stretching exercises. The aim of the STS was to induce muscle hypertrophy, and it consisted of six strength exercises: three exercises for the lower limbs (leg press, leg extension, and hamstring curl), two exercises for the upper limbs (bench press, cable row) and one exercise for the trunk (combined stomach and back muscle exercise). Each exercise was performed in three sets of 10 RM (2 min recovery). Recovery time between each exercise was 3 min. The exercise sets were performed with the maximum load at which it was 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. In practice we had to give slight manual assistance during the last three repetitions in the second and third set for all the subjects. If the load was too high or low, then the load was adjusted appropriately for the next set. Also, subjects were advised to do every concentric component of the repetitions as explosively as possible. Each subject had to drink 200 ml of water two times first at 24 min and then at 41 min from the beginning of the STS to minimize dehydration and large fluctuations in plasma volume.

Physical activity and dietary intake

The subjects were instructed to maintain their normal physical activity throughout the study period. They reported in diaries their free-time physical activity during the five days before each measurement day. The subjects were instructed to repeat physical activity similarly during each of these six study periods. The diaries were then analyzed for the times and duration of training.

Dietary intake of the subjects was recorded in food diaries for the three days before each measurement day. Individual food records were

returned to the subjects after the first test session to facilitate replication of their diet during the succeeding corresponding phases of the study. The 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 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 of blood from a vein was taken into K2 EDTA tubes (Terumo Medical Co., Leuven, Belgium) for measurements of haemoglobin and haematocrit concentration which were performed with a Sysmex KX 21N Analyzer (Sysmex Co., Kobe, Japan). The intra-assay coefficient of variation (CV) for haemoglobin is 1.5% and for haematocrit 2.0%. 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 were taken into lithium-heparin tubes (Terumo Medical Co., Leuven, Belgium) for measurement of lactate and glucose, performed with a Nova Biomedical STAT Profile PhOX Plus L Analyzer (Nova Biomedical, Waltham, MA, USA). The intra-assay CV for lactate is 3.0% and for glucose 5.0%. 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[®] 1000 (DPC, Los Angeles, USA). The sensitivity of the assay for insulin is 2 mIU/l and the 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) (Waters 501 pumps, Waters 717 autosampler and Zorbax C₁₈ column). The 18 essential amino acids and two internal standards (β -Abc and Nor-Valine) were detected by a Perkin Elmer LS-4 fluorescent detector using the wavelengths 338 nm (excitation) and 455 nm (emission). One hundred microlitre of internal standard solution was added to the plasma sample (50 μl) and acetonitrile (100 μl) was used to precipitate the proteins. Seven hundred and fifty microlitre of distilled deionized water was added and the resulting sample was vortexed and allowed to stand on an ice bath for 1 h. Two hundred microlitre of the sample was transferred to an ultraspin centrifuge filter and centrifuged. The clear mixture was transferred to a HPLC vial, derivatized with OPA derivatizing solution and analyzed by a waters HPLC system using gradient two-buffer elution. The concentration of arginine and taurine were analyzed twice in two subjects (six treatments) and the coefficient of variation (CV) for arginine was 2.2% and for taurine 2.4%.

Statistics

All data were analyzed using the SPSS for Windows (release 11.01) statistical software package (SPSS, Chicago, Ill., USA). Amino acid data were log-transformed where appropriate to stabilize the variance and covariance matrices prior to analysis. The Sphericity of the data was checked before the *F*-test and, if needed, corrected with a Greenhouse-Geisser or Huynh-Feldt estimator. The effects of sample time and the supplement used were assessed by a general linear model (GLM) two-way analysis of variance (treatment \times sample time) with repeated measures in both factors. When a significant difference in treatments or in sample times was detected, then a least significant difference (LSD) post hoc test was performed to locate the pairwise differences. When a significant interaction or tendency to interaction between treatment (arginine and taurine) and sample time was found, the effect of sample time was modelled by the second order polynomial function (curve fitting) separately for rest and training periods.

Results

Physical activity and dietary intake

There were no differences in physical activity during the five days before each measurement day between the six study periods. The average five-day physical activity in all six study periods consisted of two bouts of low intensity aerobic training with a total duration of 126 min.

The subjects ate similarly in each study phase and the average daily macronutrient intake across the three-day periods was as follows (mean \pm SD): energy 2448 \pm 438 kcal, protein 107 \pm 26 g, carbohydrate 301 \pm 54 g, and fat 83 \pm 25 g.

Blood haemoglobin, haematocrit, plasma volume, and blood lactate

Both haemoglobin and haematocrit decreased ($p < 0.05$ –0.001) in all the rest conditions (RP, RA, and RT) during the first 30 min but then recovered to the pre-values. Whereas in the STS conditions (EP, EA, and ET) strong ($p < 0.05$ –0.001) increases were observed in haemoglobin and haematocrit both at 30 and at 60 min until the concentrations recovered to the pre-STS values at 90 min and 120 min. In the rest conditions during 120 min plasma volume showed non-significant increases (0.8–5.9%) whereas in the STS conditions it showed strong ($p < 0.05$ –0.001) decreases (9.1–12.7%). The greatest decreases were observed at 30 min or 60 min.

Blood lactate concentrations increased strongly ($p < 0.001$), as expected in the hypertrophic STS conditions but were similar in all the treatment conditions. The peak values occurred at the end of STS (60 min) and were (mean \pm SD) 12.1 \pm 0.9 mmol/l (EP), 12.5 \pm 1.5 mmol/l (EA), and 12.7 \pm 0.5 mmol/l (ET).

Amino acid concentrations in the placebo rest condition

In the placebo rest condition non-significant decreases were observed, during the two hours after fasting for 10 h, in the concentration of total amino acids (4%), essential amino acids (6%), branched-chain amino acids (BCAAs; 7%), and non-essential amino acids (2%).

Effect of arginine ingestion on amino acid concentrations

Arginine concentration

Significant interaction effects were observed for arginine in STS and sample time ($p < 0.05$) and treatment and

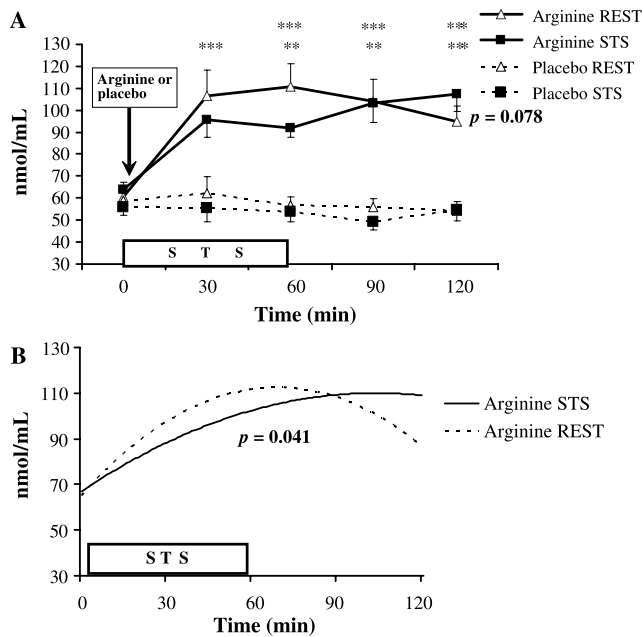


Fig. 2. Arginine concentration: (A) raw data (mean \pm SE) and (B) modelled polynomial data. STS Strength training session

sample time ($p < 0.001$). The concentration of plasma arginine increased significantly ($p < 0.01$ – 0.001) following arginine ingestion in both the rest and STS conditions, while the placebo concentrations remained stable (Fig. 2A). The peak concentration in the rest condition following arginine supplementation was 111 ± 11 nmol/ml and occurred at 60 min whereas in the STS condition the peak concentration was 107 ± 8 nmol/ml and occurred at 120 min. The concentrations at 120 min after arginine ingestion (107 ± 8 nmol/ml in the STS condition and 95 ± 7 nmol/ml in the rest condition) tended to differ ($p = 0.078$). Figure 2B presents the modelled polynomial data for arginine in the rest and STS conditions and shows a difference ($p < 0.041$) between REST and STS, the peak concentration of arginine occurring at 69 min at rest and at 104 min in the STS condition.

Concentrations of other amino acids

After ingestion of arginine there were no differences between the treatments in the concentrations of the other amino acids.

Effect of taurine ingestion on amino acid concentrations

Taurine concentration

Significant interaction effects were observed also for taurine: in STS and sample time ($p < 0.05$) and treatment and

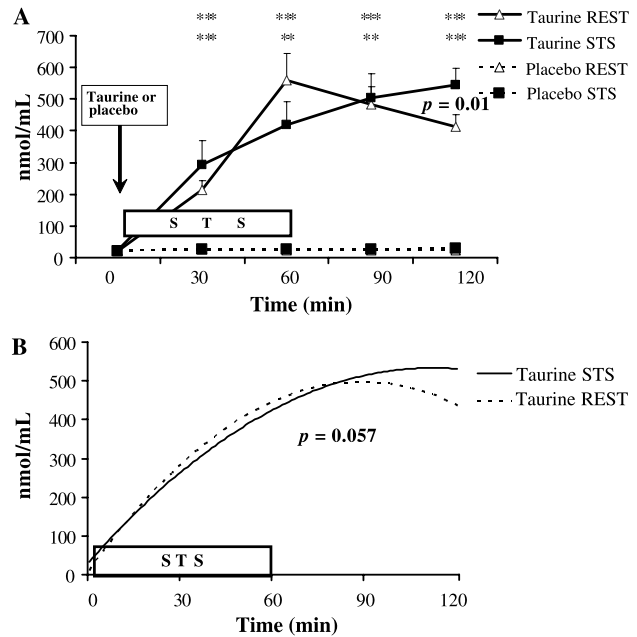


Fig. 3. Taurine concentration: (A) raw data (mean \pm SE) and (B) modelled polynomial data. STS Strength training session

sample time ($p < 0.001$). The concentration of plasma taurine increased significantly ($p < 0.01$ – 0.001) following taurine supplementation in both the rest and STS conditions, but the placebo concentrations remained stable and low (Fig. 3A). The peak concentration in the rest condition following taurine supplementation was 558 ± 87 nmol/ml and occurred at 60 min whereas in the STS condition the peak concentration (545 ± 52 nmol/ml) occurred at 120 min. The concentrations at 120 min (545 ± 52 nmol/ml in the STS condition and 414 ± 36 nmol/ml in the rest condition) differed significantly ($p < 0.05$). Figure 3B presents the modelled polynomial data for taurine in the rest and STS conditions and shows that there was a strong significant trend ($p < 0.057$) between STS and REST and the peak concentration of taurine, occurring at 89 min at rest and at 112 min in the STS condition.

Concentrations of other amino acids

After ingestion of taurine there were no differences between the treatments in the concentrations of the other amino acids.

Effect of STS on amino acid concentrations

In all the STS conditions BCAAs showed a similar decrease throughout the follow-up period. The peak decreases at 120 min were 21% for EP ($p < 0.01$), 25% for EA ($p < 0.01$), and 25% for ET ($p < 0.001$). The concen-

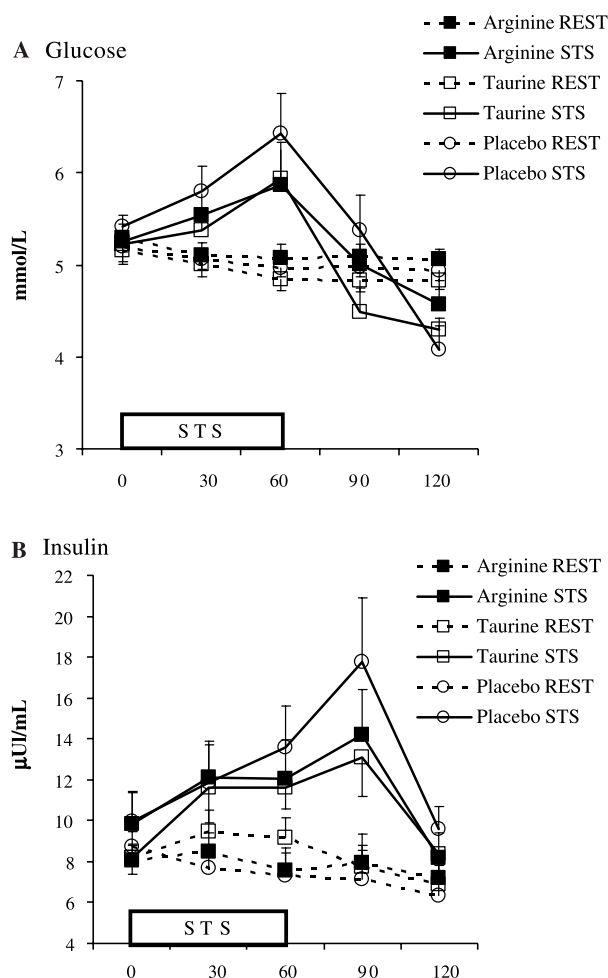


Fig. 4. Blood glucose concentration (A) (mean \pm SE). STS Strength training session. Serum insulin concentration (B) (mean \pm SE). STS Strength training session. For significances see results in text

tration of alanine increased strongly in the STS conditions. The peak values occurred at 60 min and the increases were 49% for EP ($p < 0.01$), 49% for EA ($p < 0.001$), and 42% for ET ($p < 0.001$).

Blood glucose and serum insulin

Blood glucose (Fig. 4A) was stable in the RP, RA, and RT treatments. In all three STS conditions (EP, EA, and ET) slight increases ($p < 0.05$ – 0.10) were observed at 60 min where the peak values occurred. At 120 min there were strong decreases ($p < 0.01$ – 0.001) compared with the pre-values.

Serum insulin (Fig. 4B) was stable in RA and RT but decreased in RP at 60 min ($p < 0.05$), 90 min ($p < 0.05$), and 120 min ($p < 0.01$). In all the STS conditions the peak values occurred at 90 min and were greater ($p < 0.05$ – 0.01) than the pre-values.

Discussion

Main results

In this study STS significantly affected the concentrations of arginine and taurine in the supplement condition compared to the resting situation. The modelled polynomial data showed that when supplementing with arginine the peak concentration of arginine occurred at 69 min at rest and at 104 min in the STS condition. Essentially, the same trend was observed with the taurine supplement, when the peak concentration of taurine occurred at 89 min at rest and at 112 min in the STS condition.

STS conditions

The hypertrophic whole body STS increased blood lactate by more than 12 mmol/l, which is typical for this kind of exercise (e.g., Kraemer et al., 1990). In all STS conditions blood glucose levels increased and achieved the peak value at 60 min. 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 one amino acid and water were given to the subjects. The elevated blood glucose levels within the pancreas then directly stimulated the release of serum insulin. The peak serum insulin levels were observed 30 min after (at 90 min) the peak blood glucose level. This elevation of insulin, in turn, induced glucose entry into cells and 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 achieved slight non-significant increases (0.8–5.9%) in plasma volume over the succeeding two hours and significant decreases in haemoglobin and haematocrit concentrations at 30 min. In spite of the additional 400 ml water during the intensive exercise, the plasma volume decreased by 3.5–12.7%, especially at 30 and 60 min, partly due to sweating. At the same time haemoglobin and haematocrit increased because of lowered plasma levels. The plasma volume decreases are 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 during STS.

Decreases in BCAAs occurred at 60 min after STS, which is similar to the results observed 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 an

increased need of BCAAs for energy and protein synthesis (Pitkänen et al., 2003). In the combined maximal and explosive exercise the authors did not observe increases in the alanine concentration. In the present study alanine increased strongly. One explanation for this may be the blood lactate level, which was over 12 mmol/l. Such a high exercise-induced lactate level may activate the glucose-alanine cycle. This is supported by the results from sprint running exercises where peak blood lactate levels ranged from 13 to 16 mmol/l and alanine increased strongly (Pitkänen et al., 2002).

Arginine ingestion

In the present study the physically active subjects ate on average 107 g protein daily which is 1.36 g/kg body weight and more than the recommended RDA value of 0.80 g/kg body weight (DRI, 2005). The measurements were carried out following 10 h fasting, and in the placebo conditions there was a slight (4%) decrease in the concentration of all the amino acids during the 2 h follow-up period and, correspondingly a 7% decrease in arginine. This indicates that a long fasting period overnight leads to a continuous but slight decrease in amino acid concentration. The ingestion of 4 g arginine at rest induced a 45% increase in the peak arginine concentration (111 ± 11 nmol/ml) at 60 min which is consistent with the result (139 ± 33 nmol/ml at 60 min; mean \pm SD) of Kerkick et al. (2004). This increase is in the range reported in subjects during daytime hours when they were ingesting a "normal" diet (Tangphao et al., 1999). The amount of 4 g arginine is similar to that contained in about 300 g of beef (First Data Bank, 1998). Here the intensive whole body STS delayed the peak concentration by ~ 35 min, the peak value being similar in REST and STS. As Leiper et al. (2005) showed, intermittent high-intensity running slowed gastric emptying of drinks containing carbohydrate and noncarbohydrate. The authors speculated that some mechanical factors may affect gastric area when moving rapidly from one place to another. In the present study the subjects performed rapid movements during the resistance exercises with the result that the gastric area, together with the contents of the stomach, were in rapid motion. Control of gastric emptying is complex, with possible mediators including gastrointestinal peptides (Green et al., 1988; McCallum, 1989) and reflex pathways involving extrinsic neurones (Forster et al., 1991). Because of the movement of the gastric area during STS this could have delayed the emptying process, slowing arginine absorption. Another possible explanation may be blood flow.

During eating, the blood flow to the gastric area is increased, but when undertaking 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 area. In the present study we did not measure blood flow, but it can be speculated that during STS the blood flow to the gastric area was diminished, which in consequence would have delayed the transport of arginine into the blood.

Taurine ingestion

In the rest placebo conditions there was a non-significant decrease (8%) in the concentration of taurine, which is parallel with the changes in the other amino acid concentrations. The ingestion of 4 g taurine at rest led to a 25-fold increase (from 22 ± 1 to 558 ± 87 nmol/ml) in the peak taurine concentration, which occurred at 60 min. This is a much a higher concentration than that observed with arginine. Plasma taurine concentrations at rest for healthy human adults have been reported to vary strongly because of sampling and analytical techniques (e.g., Trautwein and Hayes, 1990). Use of the HPLC technique with OPA, as in the present study, has shown fasted values of 44 ± 9 nmol/ml (mean \pm SD) (Trautwein and Hayes, 1990). Also plasma taurine responds rapidly to dietary supplementation in humans (Trautwein and Hayes, 1995), as was also seen in our results. The intensive whole body STS delayed the peak in taurine concentration by 23 min, although the peak value was similar to that in the rest condition. Possible variables explaining the delay are mechanical factors and diminished blood flow during STS. Consequently, the model proposed for taurine, both at rest and in STS, is very much similar to that for arginine. For both amino acids the rate of appearance is the sum of intake and release by tissues, mainly the muscles, and the rate of disappearance is the sum of two processes, tissue uptake and body losses. However, the tissues do not take up taurine for protein synthesis, and therefore the difference between REST and STS was slightly smaller than with arginine.

The results for taurine and also for arginine are presented without correction for plasma volume changes. The plasma volume decreased during STS and then 30–60 min after STS it increased slightly compared with the REST condition. If amino acid concentrations are corrected with the plasma volume changes, the amino acid concentrations in the STS situation during STS (at 30 min

and 60 min) would be even lower and after STS (at 90 min and 120 min) on average slightly higher. Thus, the correction would increase the differences between STS and REST at 90 and 120 min and therefore further emphasizes the main results shown.

Glucose and insulin

Arginine or taurine ingestion had no effects on glucose concentration. In the placebo rest condition insulin decreased at 60–120 min, decrease being hindered by arginine and taurine supplementation. For arginine this does not confirm the recent result by Cannon et al. (2002), who showed that orally administered arginine in an amount of 1 mmol/kg lean body mass (about 10 g in a typical 70 kg young man) increased plasma arginine by 64% but did not stimulate insulin secretion. Earlier studies (e.g., Floyd et al., 1966; Palmer et al., 1975) have shown that arginine given intravenously increases the circulating insulin concentration. Oral taurine supplementation has also been shown earlier to have no effect on insulin secretion or sensitivity in humans (Brons et al., 2004). In spite of our new finding at rest, in the STS conditions no differences in insulin concentration between the different treatments were observed.

Conclusion

It is concluded that one hour of hypertrophic whole body STS slows the increase in the peak concentration of plasma arginine by 35 min and taurine by 23 min after oral ingestion of these amino acids.

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- Authors' address:** Antti Mero, Department of Biology of Physical Activity, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland, Fax: +358-14-2602071, E-mail: antti.mero@sport.jyu.fi