Effect of branched-chain amino acid and carbohydrate supplementation on the exercise-induced change in plasma and muscle concentration of amino acids in human subjects

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Five male endurance-trained subjects performed exhaustive exercise on a cycle ergometer at a work rate corresponding to 75% of their $V_{O2\max}$ after reduction of their muscle glycogen stores. During exercise the subjects were given in random order a 6% carbohydrate solution containing 7 g L$^{-1}$ of branched-chain amino acids (BCAA), a 6% CHO solution and flavoured water. The physical performance was lowered in four of the five subjects when they were given flavoured water during exercise as compared with the two conditions when CHO was supplied. No difference in performance was found when the subjects were given CHO+BCAA or only CHO during exercise. When CHO+BCAA was supplied the plasma and muscle (vastus lateralis) concentrations of BCAA increased during exercise by 120 and 35%, respectively. In the other conditions there was no change or a slight decrease in the plasma concentrations of BCAA, but the muscle concentrations of BCAA were decreased after exercise. The plasma concentration of glutamine over the whole exercise period and 5 min after exercise was higher when CHO+BCAA were supplied during exercise compared with a supply of CHO alone or water. However, exercise caused no change in the muscle concentration of glutamine, whereas that of glutamate decreased in all three conditions. A supply of CHO+BCAA or CHO alone did not affect the exercise-induced increase in the plasma and muscle concentration of aromatic amino acids, indicating that neither BCAA nor CHO influenced the net protein degradation during exercise.

Key words: branched-chain amino acids, exercise, glutamine, skeletal muscle.

Infusion of either a mixture of BCAA or leucine alone in human subjects decreases the plasma and the muscle concentrations of several amino acids, including the aromatic amino acids (Alvestrand et al. 1990). It is suggested that these changes may be caused by a decrease in the rate of protein degradation in muscle (Louard et al. 1990, Nair et al. 1992). The BCAA are also considered to be an important source of nitrogen for the synthesis of glutamine in skeletal muscle. Thus, oral ingestion or infusion of leucine increased the rate of glutamine release from the

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resting human forelimb or forearm (Aoki et al. 1981, Abumrad et al. 1982, Elia & Livesey 1983). Although it is assumed that this increase is caused by an effect on muscle, it is possible that adipose tissue could contribute to glutamine production and release by forearm since arteriovenous differences across one depot of adipose tissue in humans indicate release of glutamine. However, it is known that adipocytes can take up rather than use glutamine (Kowalczyk et al. 1988, Keast et al. 1989), and addition of BCAA to the incubation medium is known to increase the rate of glutamine release from muscle in vivo (see below).

Despite these interesting effects, only a few studies have been done on the effects of ingestion of BCAA in relation to standardized exercise: intake of a mixture of BCAA or of leucine alone 1.5 h before exercise with reduced muscle glycogen levels had no effect on the physical performance during cycling exercise of approx. 30 min duration (Wagenmakers 1992; MacLean & Graham 1993) reported that supplementation of BCAA, 45 min before the start of exercise, elevated the plasma concentration of glutamine during exercise at 75% of maximal oxygen uptake. Ingestion of BCAA during two competitive runs, lasting for several hours, was found to prevent an increase in the concentration of the aromatic amino acids in plasma and muscle after exercise, suggesting that there was a decrease in the net rate of protein degradation during or after the exercise (Blomstrand & Newsholme 1992).

The purpose of the present study was to investigate the effect of BCAA and carbohydrate (CHO) supplementation on physical performance and amino acid changes in plasma and muscle during exercise. A solution containing a mixture of the three BCAA together with CHO or a solution containing CHO alone was given to subjects during sustained exercise and the effect on plasma and muscle concentrations of amino acids (in particular alanine, glutamine, glutamate, aromatic and branched-chain amino acids) was measured. Since the available data suggested an increased rate of metabolism of BCAA during exercise, particularly when the levels of glycogen in muscle were decreased (Wagenmakers et al. 1989) an intensive bout of exercise was carried out by the subjects, prior to the supplementation experiments, in an attempt to lower the glycogen level.

METHODS

Subjects. Five male subjects volunteered to participate in this study. They were all endurance-trained cyclists. Their mean (± SD) age, height, weight and maximal oxygen uptake (\(\dot{V}O_{2\text{max}}\)) were 19±0.7 years, 172±1.8 cm, 63±1.1 kg and 4.36±0.13 L min⁻¹, respectively. The subjects performed ergometer cycling exercise with reduced body glycogen stores (see below). The subjects were informed about the purpose of the study and possible risks involved before giving their oral consent to participate. The study was approved by the Ethical Committee at the Karolinska Institute.

Preliminary tests. All exercise tests were performed on a mechanically braked cycle ergometer (Monark 816E) equipped with toe clips and a counter to measure the number of revolutions. The subjects exercised at a pedal rate of 80 r.p.m. One week before the experiment the subjects’ oxygen uptake at three submaximal work rates was determined together with their \(\dot{V}O_{2\text{max}}\) using the Douglas bag technique (Astrand & Rodahl 1986). Expired air was collected in Douglas bags, the volume was measured in a Tissot spirometer and the concentrations of \(O_2\) and \(CO_2\) were determined with a Beckman S-3A oxygen analyser and a Beckman LB-2 carbon dioxide analyser, respectively. Based on these results, work rates corresponding to approx. in total 70 and 90% of the subjects’ \(\dot{V}O_{2\text{max}}\) were estimated.

Reduction of the body glycogen stores. During the 2 days preceding the experiment the subjects were on a standardized diet (3000 kcal day⁻¹ – carbohydrate 57% of energy, fat 30% and protein 13%) and they were instructed not to perform any physical exercise. On the evening before the experiment the subjects performed an intensive bout of exercise. The subjects exercised on a cycle ergometer at a work rate of 228±4.9 W, corresponding to 75±0.8% of \(\dot{V}O_{2\text{max}}\) for 40 min. Thereafter, the subjects performed two 10-min bouts at a work rate of 300±8.9 W, demanding 92±2.2% of their \(\dot{V}O_{2\text{max}}\). The heart rate was monitored continuously and the oxygen uptake was determined at both exercise intensities. This type of exercise would be expected to lower the muscle glycogen content in both type I and type II fibres (Vollestad et al. 1984, Vollestad & Blom 1985). After this exercise, the subjects remained fasted until the experiment the next morning.

Experimental procedure. On the day of the experiment the subjects reported to the laboratory in the morning after fasting overnight (see above). A catheter was inserted into the antecubital vein and a resting blood sample was taken. Muscle biopsies were taken from the lateral part of the quadriceps muscle (vastus lateralis) using a Weil–Blakesley conchotome (AB Wisex, Mölndal, Sweden) according to Henriksen (1979). The resting blood sample was taken approx.
10 min before exercise and the muscle biopsies approx. 5 min before the start of exercise. The subjects then exercised on the cycle ergometer for 60 min at the same work rate as the evening before, the one demanding approx. 75% of \( V_{\text{O}_2\text{max}} \) (see above). Thereupon the subjects were encouraged to perform as much work as they were able to during another 20 min, i.e. free pedalling rate with unchanged resistance. Blood samples were taken every 20 min during the exercise and 5 min after the end of exercise. The final blood sample during exercise was taken during the last minute of exercise. The subjects moved from the cycle ergometer to supine position and as quickly as possible new biopsies were taken from the vastus lateralis. The biopsy sampling was completed within 3-4 min after the end of exercise.

Immediately before exercise and for every 15 min of exercise, the subjects were given 150 mL of either a 6% carbohydrate (CHO) solution with an addition of BCAA (7 g L\(^{-1}\) - 40% valine, 35% leucine and 25% isoleucine), a 6% CHO solution or flavoured water containing saccharine as an artificial sweetener. The oxygen uptake was measured at 17 and 47 min of exercise and the heart rate was monitored continuously during the exercise period. The drinks were given in random order and the experiment was carried out using a double blind design. The total energy supplied was for CHO + BCAA 250 kcal, for CHO 220 kcal and for flavoured water < 5 kcal. To minimize thermal stress, the temperature in the laboratory was kept at 18-20 °C and the subjects were cooled with fans during the exercise. The experiments were performed at 1-week intervals, except for one subject who performed his last experiment 3 weeks after the second one.

**Plasma analyses.** Blood samples were collected in heparinized tubes and kept on ice until centrifuged at 3600 r.p.m. for 10 min. The plasma was stored at -70 °C. For amino acid measurements, the plasma samples were deproteinized with 5% trichloroacetic acid (1:5), centrifuged at 9000 g for 2 min and the supernatant was stored at -70 °C. The concentration of amino acids in the supernatant was measured by reversed-phase high performance liquid chromatography (HPLC) according to Pfeifer (1983), with orthophthaldehyde (OPA) as the derivatizing agent. Plasma glucose and lactate concentrations were analysed according to Bergmeyer (1974).

**Muscle analyses.** (a) **Histochemical analyses.** The biopsy specimen was mounted in an embedding medium (Tissue-Tek® II, O.C.T. Compound) and frozen in isopentane cooled to its freezing point in liquid nitrogen. Serial cross-sections (10 μm thick) were cut in a cryostat at -20 °C and stained for myofibrillar ATPase at pH 9.4 (Padykula & Herman 1955) after preincubation at pH 10.3 (Brooke & Kaiser 1969) in order to identify type I and II fibres. Serial sections (16 μm thick) were stained for glycogen using the periodic acid-Schiff (PAS) reaction (Pearse 1961). The fibres on the PAS stained cross-sections were evaluated by the same person and classified as high, medium or low. The PAS stain was then compared with the corresponding ATPase stain to identify the fibre types. A biopsy sample for histochemistry was taken at eight of the 15 occasions before exercise and 12 occasions after exercise.

(b) **Biochemical analyses.** The muscle biopsies were immediately frozen in liquid nitrogen. For amino acid analysis, the frozen muscle specimen was weighed and homogenized in 10 volumes of 5% trichloroacetic acid (TCA) using a ground glass homogenizer. The weight of the frozen biopsies varied between 20 and 90 mg. The TCA-homogenate was centrifuged at 9000 g for 2 min and the supernatant was stored at -70 °C until analysed. Amino acid concentrations were measured by the same method as for the plasma samples (see above). Muscle glycogen was determined after KOH digestion according to Leighton et al. (1989). Measurements were carried out both on the TCA supernatant and on the pellet from the TCA-homogenates. The muscle glycogen concentration is given as the sum of these measurements.

**Statistics.** The data were analysed with Student's t-test to identify differences between pre- and post-exercise values in the muscle biopsies, or with a one-way repeated measures analysis of variance (ANOVA) to evaluate changes in plasma concentrations during the exercise period. A one-way ANOVA was also applied to identify a possible treatment effect. However, since the data on plasma concentrations are dependent over time an overall comparison for the whole exercise period was made, i.e. an estimation of the area under the time-concentration curve was compared for each amino acid, glucose and lactate in the three conditions. To avoid an effect of the pre-exercise level, all plasma concentrations during exercise have been related to the pre-exercise level of each metabolite in each condition. When a significant treatment effect was indicated, pair-wise contrasts were used to determine where the significance occurred.

Differences were regarded as statistically significant at a probability level of \( P < 0.05 \). All values are presented as means ± SE of means.

**RESULTS**

**Performance and cardiorespiratory parameters.**

There was no difference in the oxygen uptake, heart rate and respiratory quotient during exercise between the three conditions (Table 1). The physical performance was lowered in four of the five subjects when they were given flavoured water during exercise as compared with the two
conditions when CHO was supplied. No difference in performance was found between the two latter conditions, i.e. when the subjects were given CHO + BCAA or only CHO during exercise (Table 1).

**Plasma concentrations of amino acids**

There was no change in the plasma concentration of valine and a slight decrease in that of isoleucine and leucine (11 and 14%, respectively) when the subjects were given CHO or flavoured water during the exercise, whereas, not surprisingly, the concentrations of all three of the BCAA were significantly increased when the subjects were given BCAA (Fig. 1a).

The concentration of alanine increased significantly during the whole exercise period in the two conditions when CHO was supplied and was approx. 80% higher at the end of exercise compared with before exercise. When water was taken during exercise, the alanine concentration increased during the first 40 min, after which the concentration levelled off (Fig. 1b).

The concentration of glutamine increased significantly when the subjects were given CHO + BCAA during exercise, whereas it remained approximately constant when the subjects were given CHO alone or flavoured water. Thus, the concentration over the whole exercise period was higher when CHO + BCAA were supplied during exercise compared with a supply of CHO alone or water (Fig. 1c). Five min after exercise the concentration was still higher when BCAA had been taken during the exercise period than in the other two conditions (Fig. 1c).

The concentrations of glutamate (Fig. 1d) and the aromatic amino acids, tyrosine, phenylalanine and tryptophan (the concentration changes for tyrosine only are shown in Fig. 1e), increased significantly in all three conditions. The concentrations of taurine followed a similar pattern as for the aromatic amino acids, although the average increase during exercise was approx. 50% in all three conditions. For the remaining amino acids that were measured (serine, histidine, glycine, threonine, arginine, methionine, lysine), there was either no change or a small increase (less than 20%) in their plasma concentrations during exercise. This small increase in plasma concentration is probably a reflection of a decrease in plasma volume during exercise, which has been reported to be approx. 6% during a similar type of exercise (Montain & Coyle 1992).

For most of the amino acids, there was a statistically significant decrease in the plasma concentration during recovery so that levels were lower 5 min after exercise compared with the concentration at the end of exercise, with the exceptions of the BCAA, tyrosine, taurine and glutamate, these concentrations remained approximately unchanged or increased during recovery.

**Plasma concentrations of glucose and lactate**

The concentrations of glucose during the whole exercise period and at 5 min after exercise were higher when CHO was supplied, either with or without BCAA, than when flavoured water was supplied (Fig. 2a).

The concentration of lactate increased significantly in all three conditions during the
Amino acid changes during exercise

Fig. 1. Plasma concentrations of the branched-chain amino acids (BCAA), alanine, glutamine, glutamate and tyrosine during and 5 min after ergometer exercise. The subjects were supplied CHO + BCAA (■), CHO (□) and flavoured water (▲) during exercise. Values are means ± SE of means for five subjects. * and ** indicate a difference (P < 0.05) between the conditions over the whole exercise period and 5 min after exercise, respectively. △ indicates P < 0.05 for the value 5 min post-exercise vs. the value at the end of exercise (80 min).
Fig. 2. Changes in plasma concentrations of glucose and lactate during and 5 min after ergometer exercise in three different situations. For explanation of symbols, see Fig. 1.

exercise period (Fig. 2b). At the end of exercise the concentration was higher in the two situations in which CHO was supplied than when water was supplied, although the difference was statistically significant only for CHO vs. water.

Concentrations of amino acids in the muscle

There was no difference in the concentration of amino acids before exercise between the three conditions (Table 2). The concentrations of BCAA were increased after exercise when the mixture of BCAA + CHO was supplied during exercise. When CHO or water was supplied there was a decrease in the concentration of leucine after exercise in both conditions and a decrease in isoleucine when water was supplied during exercise (Table 2).

The post-exercise concentration of alanine was increased in the two situations when CHO was supplied during exercise; it increased 27% (which did not reach statistical significance) when CHO + BCAA were taken and 39% when only CHO was taken during exercise. When flavoured water was taken during exercise there was no difference between the values before and after exercise (Table 2).

The concentration of glutamine was not different before and after exercise in any of the three situations, whereas that of glutamate was decreased by 35–45% after exercise in all three situations (Table 2).

Exercise caused an increase in the concentrations of the aromatic amino acids, tyrosine and phenylalanine, in all three conditions; however, the increase was statistically significant only when CHO was supplied during exercise (Table 2).

Muscle glycogen

Exercise caused a small, but not statistically significant decrease in the total concentration of glycogen, which was similar in the three conditions. During exercise the concentration decreased from 114 ± 10 to 100 ± 9.7, from 118 ± 16 to 97 ± 15 and from 102 ± 17 to 82 ± 11 mmol kg⁻¹ muscle with CHO + BCAA, CHO and flavoured water, respectively. However, the results of the PAS-stains of the biopsy samples obtained after exercise show that there is a selective emptying of the type I fibres during this form of exercise. Approx. 40% of the type I fibres were classified as low in glycogen content, whereas only 6% of the type II fibres were classified as low in glycogen content (Table 3).

DISCUSSION

When a mixture of the three BCAA + CHO are taken during standardized exercise no difference in physical performance (measured by the amount of work performed during 80 min of exercise) was found compared with an intake of CHO alone. However, exercise performance was decreased in four of the five subjects when only water was taken; the subject who performed equally well when given water was able to maintain his plasma glucose level during exercise in contrast to the rest of the subjects in whom the plasma glucose level fell during the last part of the exercise. A fall in the blood glucose level
Amino acid changes during exercise

Table 3. Percentage of high, medium and low-intensity PAS-staining of fibres in biopsy samples taken before and after ergometer cycling exercise. Values are means \( \pm \) SE of means for the number of samples given in parenthesis.

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Condition</th>
<th>High</th>
<th>Medium</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Pre-ex. (8)</td>
<td>57±15</td>
<td>34±11</td>
<td>8±4</td>
</tr>
<tr>
<td></td>
<td>Post-ex. (12)</td>
<td>21±9</td>
<td>42±9</td>
<td>37±10</td>
</tr>
<tr>
<td>Type II</td>
<td>Pre-ex. (8)</td>
<td>78±10</td>
<td>22±9</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>Post-ex. (12)</td>
<td>61±8</td>
<td>33±7</td>
<td>6±1</td>
</tr>
</tbody>
</table>

is likely to contribute to the impairment of physical performance when water is supplied during exercise (Coyle et al. 1983, 1986, Widrick et al. 1993).

The intake of BCAA during exercise resulted in an increase in the level of these amino acids in both plasma and muscle, which was to be expected on the basis of previous studies in which BCAA had been given either orally or intravenously to healthy subjects (Alvestrand et al. 1990, Blomstrand & Newsholme 1992). When CHO or water was taken during exercise, the plasma concentration of BCAA remained unchanged or decreased slightly, whereas the concentration in the muscle was decreased after exercise. These changes are somewhat different from the results reported for sustained exercise performed after ingestion of a high-carbohydrate or a mixed diet for 2.5 days: in this situation there was no difference in the muscle concentrations of BCAA before or after sustained exercise (McLean et al. 1991).

Exercise caused the plasma and muscle level of the aromatic amino acids, tyrosine and phenylalanine, to increase regardless of whether BCAA and/or CHO were taken during exercise. This indicates that there is an increased net protein degradation during exercise that is not influenced by an intake of BCAA and/or CHO. This finding contrasts with that of a previous study which showed that an intake of BCAA during exercise prevented the exercise-induced increase in the plasma and muscle level of aromatic amino acids (Blomstrand & Newsholme 1992). However in this study the plasma and muscle samples were taken up to 45 and 90 min, respectively, after the termination of exercise; it is therefore tempting to speculate that the BCAA
stimulated the net rate of protein synthesis during this longer recovery phase so that the levels of some of the essential amino acids, including the aromatic amino acids, would fall in the muscle. It is, therefore, interesting to note that Alvestrand et al. (1990) showed that infusion of leucine, in a resting condition, decreased both the intracellular and plasma levels of tyrosine and phenylalanine despite the fact that the rate of exchange of these amino acids across the leg was not changed.

Another important finding was that the intake of BCAA caused a pronounced increase in the plasma level of glutamine and also maintained glutamine at the pre-exercise level for at least 5 min after the exercise. This finding was to be expected from the results of earlier studies (Aoki et al. 1981, Abumrad et al. 1982); it was found that the rate of glutamine release from the resting human forearm muscle was increased after the intake or infusion of leucine. Furthermore, ingestion of BCAA before exercise augmented the increase in the plasma concentration of glutamine during exercise (MacLean & Graham 1993). These findings suggest either that BCAA increase the rate of release of glutamine from muscle or decrease the rate of glutamine utilization (e.g. by enterocytes or lymphocytes). The former explanation is the more likely one since it has been shown that a supply of BCAA increases the rate of release of glutamine from skeletal muscle in vitro (e.g. Ruderman & Berger 1974, Garber et al. 1976, Parry-Billings 1989, Newsholme & Parry-Billings 1990). It has been suggested that this effect on release of glutamine is caused by a direct stimulation of the transport process that causes the efflux of glutamine from muscle rather than a stimulation of glutamine synthesis (Parry-Billings 1989, Newsholme & Parry-Billings 1990). Although there was no change in the level of glutamine within the muscle after exercise (Table 2) the level of glutamate was decreased. This is in agreement with the results of Henriksson (1991), while Sahlin et al. (1990) found a decrease in both glutamine and glutamate concentration in the muscle during sustained exercise to fatigue. In the present study the resting levels of glutamine were lower than was reported earlier (e.g. Sahlin et al. 1990, Henriksson 1991). The reason for this is unclear, although the protocol applied in the present study with an evening exercise bout followed by an overnight fast is different from what has been done before.

During exercise the plasma level of alanine increased in all three conditions. This was to be expected from the results of earlier studies showing an increased rate of alanine release from muscle during exercise (Felig & Wahren 1971, Aghborg et al. 1974, Sahlin et al. 1990). In the latter study, it was reported that exercise also caused an increase in the level of alanine in muscle, whereas, in the present study, the level of alanine in the muscle was elevated only after exercise when CHO was supplied during exercise. However, during low-intensity exercise, corresponding to 50% of the subjects' maximal oxygen uptake, a transient increase in the muscle concentration of alanine early in exercise was reported and at the end of exercise the concentration had returned to the resting level (Henriksson 1991).

The relatively high glycogen concentrations found in the muscle samples before exercise were unexpected since the subjects performed 1 h of relatively heavy exercise on the evening before the experiment. One explanation for this might be that the subjects had a very high level of muscle glycogen when they started the exercise the previous evening, since they were highly trained endurance athletes who are known to have an increased ability to store glycogen in their muscles (Jansson & Kaijser 1987). However, it is also possible that there has been a resynthesis of muscle glycogen during the night, i.e. during the 13 h between the evening exercise and the start of the morning exercise. Furthermore, the decrease in total muscle glycogen during the experimental exercise was small, so that the content of glycogen at the end of the exercise is still high. The respiratory quotient was 0.81-0.83, and was similar in all three conditions, indicating that only approx. 50% of the energy was derived from the breakdown of glycogen. This could explain the relatively small decrease in total muscle glycogen during exercise of approx. 20 mmol kg^-1. However, the results of the PAS-stains of the biopsies obtained after exercise show that the type I-fibres were low in glycogen, whereas most of the type II-fibres contained high or medium amounts of glycogen. This suggests that these subjects were not able to recruit their type II-fibres to any great extent, despite the fact that the type I-fibres were low in glycogen and presumably affected by fatigue.
In conclusion, when CHO + BCAA or CHO alone were supplied during standardized exercise there was no difference in physical performance, whereas the performance was lowered in four of the five subjects when they were supplied flavoured water during exercise. In all three conditions exercise induced an increase in the plasma and muscle concentration of the aromatic amino acids indicating net protein degradation during exercise independent of whether BCAA and/or CHO were taken. When CHO + BCAA was supplied during exercise the plasma concentration of glutamine was higher throughout the exercise period and 5 min after exercise as compared with a supply of CHO alone or water.

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