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# Influence of ingesting a solution of branched-chain amino acids on perceived exertion during exercise

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On two occasions, seven male endurance-trained cyclists performed exhaustive exercise on a cycle ergometer in the morning after they had performed a bout of exercise the preceding evening in an attempt to lower the muscle glycogen stores. The subjects exercised at a work rate corresponding to ≈ 70% of their maximal oxygen uptake for 60 min, followed by another 20 min of maximal exercise. During exercise the subjects were given either a solution of branched-chain amino acids (BCAAs) or flavoured water (placebo). Every 10 min during exercise the subjects rated their perceived exertion and mental fatigue on two different Borg scales. During the 60 min exercise at a given work rate, the subjects' ratings of perceived exertion when they were given BCAAs were 7% lower, and their ratings of mental fatigue were 15% lower, than when they were given placebo. In addition, the performance in the colour task of Stroops' Colour Word Test performed after exercise was improved when BCAAs had been ingested during exercise, compared with the results from the placebo trial. There was no difference in the physical performance between the two trials, measured as the amount of work done during the last 20 min of exercise when the subjects performed at their maximum. The plasma concentration ratio of free tryptophan/BCAAs, which increased by 45% during exercise and by 150% 5 min after exercise in the placebo trial, remained unchanged or even decreased when BCAAs were ingested.

**Keywords** fatigue, free tryptophan, perceived exertion.

Several studies have reported that physical exercise increases the levels of monoamines, particularly 5-HT, in the brain of experimental animals (see Chaouloff 1989). In previous studies it was suggested that an increase in the rate of synthesis, and hence the level of 5-HT, in specific parts of the brain could contribute to fatigue, mainly central/mental fatigue but also physical fatigue (Blomstrand *et al*. 1988, 1989). The mechanism behind the increased level of 5-HT during sustained exercise is thought to be an increase in the plasma ratio of free (not albumin-bound) tryptophan/other large neutral amino acids (LNAAs), which occurs during sustained exercise and is very marked after exercise (Blomstrand *et al*. 1988, Davis *et al*. 1992). This concentration ratio is considered to be important since the LNAAs (including tryptophan and the branched-chain amino acids [BCAAs]) are transported into the brain by the same carrier mechanism and competition between these amino acids for entry into the brain can occur (Pardridge 1977). Studies of the effect of physical exercise on the brain level of tryptophan support the view that it is the free tryptophan concentration, rather than the total concentration, which governs the uptake of tryptophan by the brain (Chaouloff *et al*. 1986, Blomstrand *et al*. 1989).

Further support for the theory that changes in 5-HT concentration in the brain play a role in fatigue has recently been presented in three studies involving pharmacological manipulation of the 5-HT-system:

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administration of a 5-HT agonist to rats was reported to impair running performance in a dose-related manner (Bailey *et al*. 1992); administration of a 5-HT antagonist improved running performance in rats (Bailey *et al*. 1993); and administration of a 5-HT reuptake blocker to human subjects decreased the time to exhaustion during standardized exercise, compared with a control condition (Wilson & Maughan 1992).

Previous studies have shown that ingestion of a solution of BCAAs prevented the increase in the plasma ratio of free tryptophan/other LNAAs during prolonged exercise and improved mental alertness after the exercise (Blomstrand *et al*. 1991, Hassmén *et al*. 1994). However, no information is available concerning the effect of an intake of BCAAs on perceived exertion and mental fatigue during the exercise period. With regard to the effect of BCAA administration on physical performance, no effect of BCAAs could be detected during exercise of $\approx$ 30 min duration (Wagenmakers 1992, Varnier *et al*. 1994) or when BCAAs were supplied together with carbohydrates during exercise of longer duration, 80 min and 1–3 h of exhaustive exercise (Blomstrand *et al*. 1995, van Hall *et al*. 1995). However, no studies have been carried out on human subjects where BCAAs alone have been supplied during prolonged exercise.

The purpose of the present study was to investigate the effect of ingestion of BCAAs on the amount of work performed, on perceived exertion and on mental fatigue during standardized exercise in the laboratory. In an attempt to cause fatigue at an earlier stage in sustained exercise, a bout of exercise was performed in the evening prior to the experimental exercise. After the evening exercise, the subjects were not allowed to eat, and during the exercise the next morning, the subjects were supplied with either a solution of BCAAs or flavoured water.

## MATERIALS AND METHODS

### *Subjects*

Eight male subjects volunteered to participate in this study. They were all endurance-trained cyclists. One subject, however, was excluded from the study since, in one of the two experiments, he had a headache, a low resting level of plasma glucose, a high resting level of plasma lactate and he showed changes during exercise in the plasma levels of glucose, alanine, glutamine and the aromatic amino acids that were in the opposite direction to those shown by all other subjects. The data are therefore presented for seven subjects. Their mean ($\pm$SE) age was $25 \pm 2.8$ years, height $180 \pm 3.1$ cm, weight $74 \pm 3.8$ kg and maximal oxygen uptake ($V\text{o}_{2\,\text{max}}$) $4.70 \pm 0.27$ L min$^{-1}$. The subjects performed ergometer cycling exercise divided into two parts: an exercise bout in the evening, followed by exhaustive exercise the next morning (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 of 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 exact number of revolutions. The subjects exercised at a pedalling rate of 80 rpm. One week before the experiment, the subjects' oxygen uptakes at three submaximal work rates were determined, together with their $V\text{o}_{2\,\text{max}}$, using the Douglas bag technique (Åstrand & 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–3 A oxygen analyser and a Beckman LB-2 carbon dioxide analyser, respectively. Based on these results, work rates corresponding to $\approx$ 70 % and 90 % of the subjects' $V\text{o}_{2\,\text{max}}$ were estimated. The respiratory exchange ratio ($R$) was calculated as the ratio of the $CO_2$ volume produced to the $O_2$ volume utilized (see Åstrand & Rodahl 1986).

### *Evening exercise*

During the two days prior to the experiment, the subjects were kept on a standardized diet (3000 kcal day$^{-1}$ – carbohydrates provided 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. They exercised on a cycle ergometer at a work rate of $246 \pm 16$ W, corresponding to $71 \pm 1.2$ % of $V\text{o}_{2\,\text{max}}$ (average heart rate $162 \pm 3$), interrupted every 10 min by 2 min intervals (except for the second interval, which lasted for 3 min, during which the oxygen uptake was measured) of exercise at a work rate of $307 \pm 16$ W, corresponding to $85 \pm 1.1$ % of their $V\text{o}_{2\,\text{max}}$ (average heart rate $174 \pm 3$). The heart rate was monitored continuously and the oxygen uptake was determined during the second period of each intensity. The duration of the whole exercise period was 73 min. This type of exercise has been reported to recruit both type I and type II fibres (Vøllestad *et al*. 1984, Vøllestad & Blom 1985). During the exercise,

the subjects were given water to drink. Following this exercise, the subjects did not eat until after the experiment the next morning. They were allowed to drink water and tea without sugar.

*Experimental procedure*

On the morning of the experiment, the subjects reported to the laboratory having fasted overnight (see above). With the subject in a supine position, a catheter was inserted into the antecubital vein and a resting blood sample was taken. A muscle biopsy specimen was taken from the lateral part of m. quadriceps, vastus lateralis, using a Weil-Blakesley conchotome (AB Wisex, Mölndal, Sweden) as described by Henriksson (1979). The sample was taken from the right vastus lateralis muscle in the first experiment and from the left one in the second experiment. The resting blood sample was taken ≈ 10 min before exercise and the muscle biopsy specimen ≈ 5 min before the start of exercise. The subjects moved from the supine position to the cycle ergometer and the exercise was started as quickly as possible. The subjects exercised for 60 min at the same work rate as the evening before (that demanding ≈ 70 % of $V\mathrm{o}_{2\,\mathrm{max}}$; see above). Thereafter, they were encouraged to perform as much work as they were able to for another 20 min, i.e. at a free pedalling rate with unchanged resistance. The amount of work performed by each subject was calculated from the work load and the number of pedal revolutions completed. 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 obtained during the last minute. Within 3–4 min of ending the exercise, a new biopsy was taken from the vastus lateralis of the same leg as used before the exercise. The post-exercise biopsy was taken at a site proximal to that of the resting biopsy sample. The oxygen uptake was measured after 17 and 47 min of exercise and the heart rate was monitored continuously during the exercise period.

Before exercise and every 10 min during exercise, the subjects were asked to rate their perceived exertion and mental fatigue on the scales described below. To assess the subjects' mental performance after exercise, they underwent the Stroop Colour and Word Test (CWT) after the end of exercise (see below). The testing was initiated within 10 min of finishing the exercise.

Immediately before exercise and every 15 min during exercise, the subjects were given 150–200 mL of either an aqueous solution containing 7 g $L^{-1}$ of BCAAs (40 % valine, 35 % leucine and 25 % isoleucine), lemon flavour, salts, citric acid and an artificial sweetener to mask the bitter taste of the amino acids or flavoured water containing the latter ingredients in slightly different proportions. The two drinks were indistinguishable in taste. The subjects were supplied with a total of 90 mg of BCAAs (kg body wt)$^{-1}$. The drinks were given in random order and the experiment was carried out using a double-blind design. 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. Generally, two subjects exercised together and the experiments were performed at 1 week intervals, except for one subject who performed his second experiment 3 weeks after the first one. The experiments were performed from November to February, i.e. after the bicycling season. To prevent a training effect of the first experiment and to accustom the subjects to the experimental situation, they performed two exercise sessions using the same protocol as the one applied in the present study 2 and 3 weeks before the real experiment.

*Ratings of perceived exertion and mental fatigue*

The subjects were carefully instructed to rate their overall degree of perceived exertion on the 15 degree (6–20) category scale devised by Borg (1970). Ratings of mental fatigue were made on the category-ratio scale CR-10 with an absolute 0 (Borg 1982). The subjects were instructed to rate their perceived degree of mental fatigue, i.e. a rating including feelings of aversion, dejection and inertia. Contrary to the RPE scale, which is to be used exclusively for ratings of perceived exertion during physical work, the CR-10 scale can be used for ratings in a wide range of modalities including perceived stress, discomfort and annoyance (Borg 1990). The CR-10 scale was developed to 'meet the twofold demands of ratio scaling and level estimations' (Borg 1990). Based on previous research (see Hassmén 1991), the use of the CR-10 scale for ratings of mental fatigue offers possibilities for comparisons lacking in other scaling methods.

*Colour Word Test*

Stroops' Colour Word Test (Stroop 1935) was used as a measure of cognitive performance. This test has proved to be useful in a number of situations, especially when attention and speed of processing have been of interest (see MacLeod 1991). The CWT used in the present study consists of three different parts: words, colours, colour-words. The test has been described in detail in a previous study (Hassmén *et al.* 1994). The subject's assignment was to name as many words, colours and colour-words as possible

 

during 45 s for each part (i.e. the time to complete all three parts was 135 s). In the present study, the CWT was repeated five times for a total time of ≈ 15 min. In order to decrease possible training effects during the actual test sessions, subjects performed the CWT on three different occasions prior to the real test situation.

*Blood analyses*

Blood samples were collected in heparinized tubes and kept on ice until centrifuged at 3000 *g* for 10 min. The plasma was stored at −70 °C. For amino acid measurements, the plasma samples were deproteinized with 5% trichloroacetic acid (TCA, 1:5), centrifuged at 9000 × *g* for 2 min and the supernatant was stored at −70 °C until analysed. The concentration of amino acids in the supernatant was measured by reversed-phase high performance liquid chromatography (HPLC) as described by Pfeifer *et al.* (1983), with orthophthaldehyde (OPA) as the derivatizing agent. Plasma-free tryptophan was separated from albumin-bound tryptophan by an ultrafiltration method described by Bloxam *et al.* (1977), modified in the following way: 150–200 μL plasma was placed in small tubes (Millipore Ultrafree-MC) with a low-protein-binding cellulose membrane (cut-off of 5000). A small rubber top was placed on the tubes and the samples were gassed with a mixture of $O_2/CO_2$ (95/5) for 30 s. The tubes were then shaken for 10 min in an Eppendorf thermomixer and then centrifuged at 3200 *g* for 10 min. The filtrate was diluted (1:2) with 5% TCA and stored at −70 °C until analysed. The concentration of tryptophan in the ultrafiltrate was measured using the same method as that used for total tryptophan (see above).

Plasma glucose and lactate concentrations were analysed as described by Bergmeyer (1974), free fatty acids as described by Shimizu *et al.* (1979) and ammonia as described by Kun & Kearney (1974).

*Muscle glycogen*

Muscle biopsy specimens were immediately frozen in liquid nitrogen and stored at −70 °C. The muscle specimen was weighed and homogenized in 10 volumes of 5% TCA, using a ground glass homogenizer. The homogenate was centrifuged at 9000 × *g* for 2 min and the supernatant and the pellet were stored at −70 °C. The muscle glycogen concentration was measured both on the supernatant and on the pellet from the TCA homogenate using the method described by Leighton *et al.* (1989). The total glycogen concentration is given as the sum of these measurements.

The effect of ingestion of BCAAs on amino acid levels in muscle and on changes in their concentration in plasma is reported elsewhere (Blomstrand *et al.* 1996).

*Statistics*

A one-way repeated measures analysis of variance (ANOVA) was applied to evaluate changes in perceived exertion, mental fatigue and plasma concentrations during the exercise period. Since the data for each variable are dependent over time, differences between the two trials have been evaluated by comparing the areas under the time/RPE, time/CR-10 and time/concentration curves (Campbell & Machin 1990). Students' *t*-test for paired observations was then applied to identify differences between trials, and also to evaluate differences between pre-exercise and post-exercise levels of muscle glycogen. Wilcoxon's signed rank test was used when there was an obvious skewed distribution of the data, as was the case for the differences in the CWT. A probability level of $P < 0.05$ was employed due to the relatively small number of subjects. Values in the text are means ± SE of means.

## RESULTS

*Cardiorespiratory data and physical performance*

No differences were found in oxygen uptake, heart rate or respiratory exchange ratio (*R*) between the two trials during the first 60 min of exercise when the subjects exercised at a given work rate (Table 1). For oxygen uptake and *R* value, the mean of the measurements at 17 and 47 min of exercise are given, since no differences in these variables were found at the given points in time. There was no difference in physical performance, measured as the amount of work done during the last 20 min of exercise when the subjects exercised at their maximum. Three of the subjects improved their performance when BCAAs were taken, whereas four of the subjects performed equally well in the two trials (Table 1).

*Perceived overall exertion and mental fatigue*

The ratings of perceived exertion (RPE) increased during exercise in both trials. The statistical analysis of the areas under the time/RPE curves revealed a difference between the two situations: the area under the placebo curve was greater than the area under the BCAA curve when a comparison was made for the time-span 0–60 min, i.e. when the work rate was the

**Table 1** Individual values for oxygen uptake ($\dot{V}o_2$), respiratory exchange ratio ($R$), average heart rate (HR) during the first 60 min of exercise and amount of work done under the two conditions. For the $\dot{V}o_2$ and the $R$ value, means of the measurements at 17 and 47 min of exercise are given. During the exercise period the subjects were given a mixture of the three BCAA in an aqueous solution or a placebo consisting of flavoured water

| Subject | intake | $\dot{V}o_2$ (L min$^{-1}$) | $R$ value | Mean HR 0–60 min | Work done (kJ) 0–60 min | Work done (kJ) 60–80 min |
|---|---|---|---|---|---|---|
| 1 | BCAA | 2.98 | 0.83 | 166 | 726 | 176 |
| | Placebo | 2.97 | 0.84 | 160 | 690 | 176 |
| 2 | BCAA | 3.31 | 0.87 | 161 | 809 | 286 |
| | Placebo | 3.32 | 0.85 | 155 | 801 | 288 |
| 3 | BCAA | 4.36 | 0.87 | 155 | 1184 | 379 |
| | Placebo | 4.35 | 0.86 | 149 | 1179 | 380 |
| 4 | BCAA | 2.98 | 0.76 | 168 | 736 | 248 |
| | Placebo | 3.00 | 0.78 | 174 | 725 | 199 |
| 5 | BCAA | 3.57 | 0.86 | 163 | 937 | 319 |
| | Placebo | 3.46 | 0.86 | 163 | 937 | 254 |
| 6 | BCAA | 3.56 | 0.80 | 165 | 979 | 271 |
| | Placebo | 3.67 | 0.78 | 161 | 976 | 275 |
| 7 | BCAA | 3.22 | 0.84 | 175 | 790 | 245 |
| | Placebo | 3.16 | 0.87 | 181 | 782 | 179 |
| Mean | BCAA | 3.43 | 0.83 | 165 | 880 | 275 |
| | Placebo | 3.42 | 0.83 | 163 | 870 | 250 |

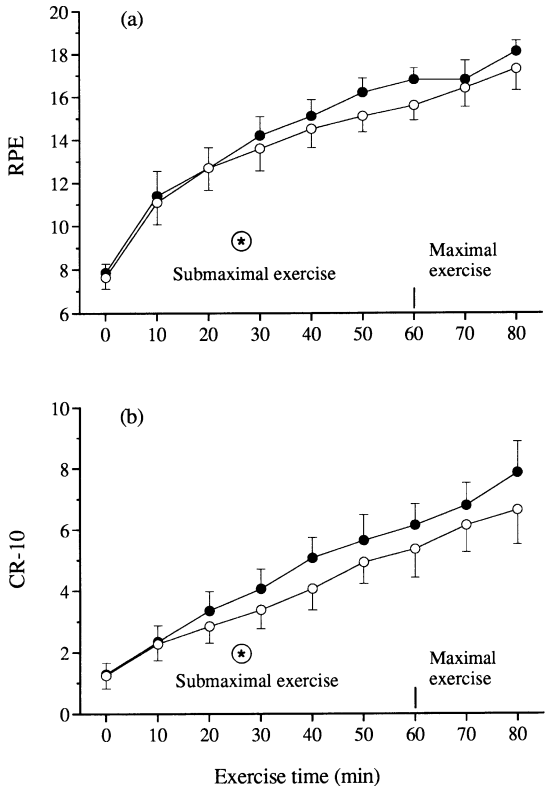


**Figure 1** Ratings of (a) perceived exertion (RPE) and (b) mental fatigue (CR-10) during exercise. The subjects were supplied with a mixture of the three BCAAs in an aqueous solution (○) or a placebo (●) consisting of flavoured water during the exercise period. Data are presented as means ± SE for seven subjects. ⊛ indicates a difference ($P < 0.05$) between the trials over the exercise period 0–60 min.

same. This shows that the subjects rated their overall exertion as higher during the placebo trial than during the BCAA trial (Fig. 1a).

**Table 2** Scores obtained in the Colour Word Test (CWT) after exhaustive exercise. During the exercise period the subjects were given a mixture of the three BCAA in an aqueous solution or a placebo consisting of flavoured water. Data are means, with ranges in parentheses, for seven subjects

| | Colour Word Test | | |
|---|---|---|---|
| Intake | Words | Colours | Colour-words |
| BCAA | 150 (118–191) | 83.2 (74–97)* | 62.6 (50–75) |
| Placebo | 148 (108–204) | 78.9 (62–97) | 61.1 (50–76) |

* $P < 0.05$ for BCAA vs. placebo.

The ratings of mental fatigue increased during exercise in both trials. Furthermore, the ratings of mental fatigue differed between the two situations for the time-span 0–60 min. When evaluated by comparing the areas under the time/CR-10 curves, there was a higher degree of mental fatigue during the placebo trial than during the BCAAs trial (Fig. 1b).

During the last 20 min of exercise, when the subjects were encouraged to perform at their maximum, no differences in the ratings of perceived exertion and mental fatigue were detected, although there was a tendency towards a higher rating in the placebo trial.

### *Stroop's Colour Word Test*

The results of the CWT are shown in Table 2. In the colour task, the subjects performed better when BCAAs were supplied during exercise than when water was supplied. For the word and colour-word tasks, no difference was detected between the two trials.

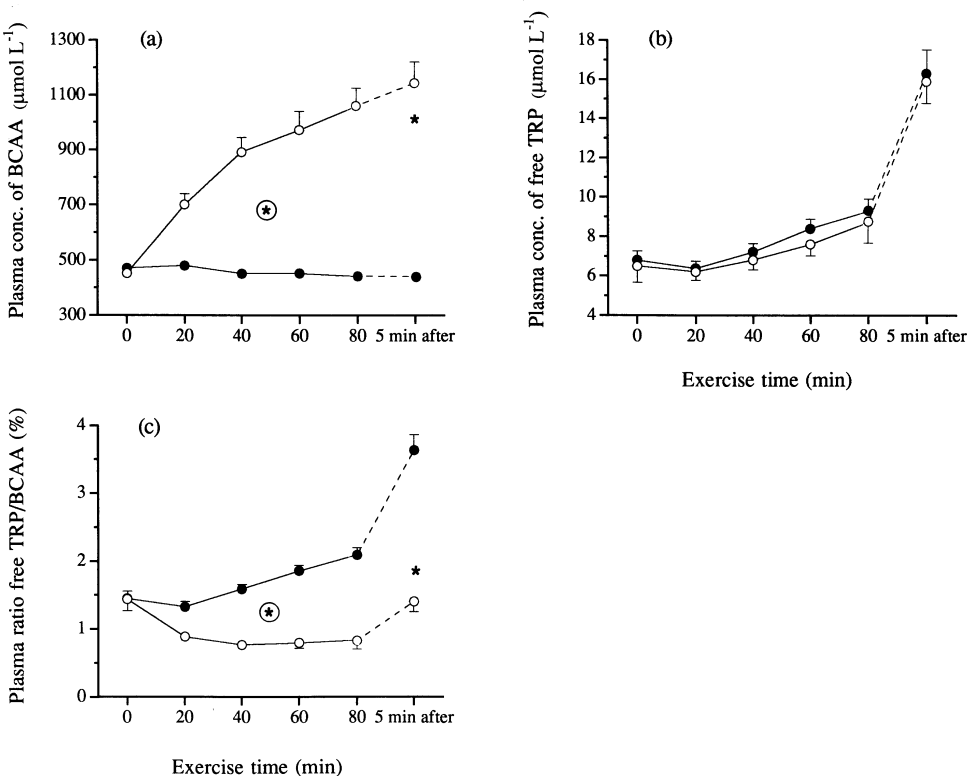


**Figure 2** Plasma concentrations of branched-chain amino acids (BCAA, valine + isoleucine + leucine), free tryptophan (TRP) and the plasma ratio of free TRP/BCAA during and 5 min after exercise. For a description of details, see legend to Figure 1. ⊛ and * indicate a difference ($P < 0.05$) between the trials over the whole exercise period and 5 min after exercise, respectively.

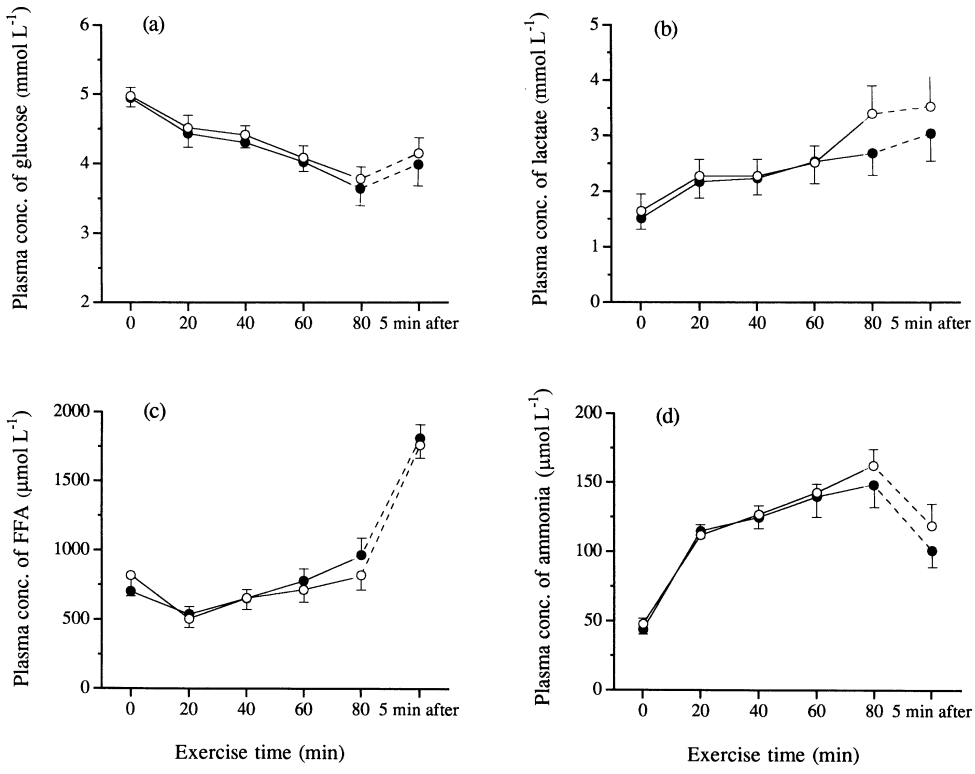


**Figure 3** Plasma concentrations of glucose, lactate, free fatty acids (FFA) and ammonia during and 5 min after exercise. For a description of details, see legend to Figure 1.

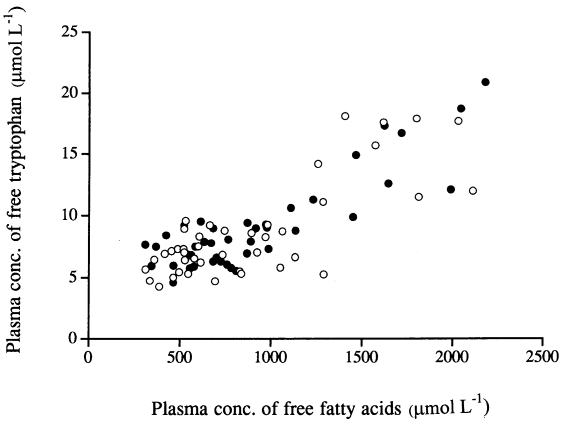


**Figure 4** Correlation between the concentration of free tryptophan and free fatty acids in plasma. For a description of details, see legend to Figure 1.

*Changes in plasma concentrations*

*Amino acids*. The concentration of BCAAs increased when these amino acids were ingested during exercise, while there was no change in their concentration when the placebo was ingested (Fig. 2a). The concentration of free tryptophan increased during exercise by similar amounts under both conditions (Fig. 2b). When flavoured water was ingested, the ratio of free tryptophan/BCAAs increased by 45% during exercise, but 5 min after exercise the ratio was 150% above that before exercise. In contrast, when BCAAs were ingested during exercise there was a decrease or no change in this ratio during, and 5 min after, exercise (Fig. 2c). Similar changes were obtained in the plasma ratio of free tryptophan/other LNAAs (BCAAs, tyrosine, phenylalanine, histidine, methionine). In the placebo trial, this ratio was increased by 40% at 80 min of exercise and by 150% 5 min after exercise, whereas there was a decrease or no change in this ratio when BCAAs were ingested. Values for tyrosine, phenylalanine, histidine and methionine are given elsewhere (Blomstrand *et al.* 1996).

*Glucose*, *lactate*, *FFA and ammonia*. Figure 3 shows the changes in plasma concentration of glucose, lactate, FFA and ammonia during exercise. The glucose concentration decreased, whereas the FFA and ammonia concentrations increased during exercise in both trials. The only difference noted between the two conditions was for the lactate concentration, which increased in the BCAA trial while there was no significant change during exercise in the placebo trial. During the 5 min recovery period, there was a marked increase in the concentration of FFA, as was the case with free tryptophan. Figure 4 shows the correlation between the concentration of free tryptophan and FFA in the two trials ($r = 0.78$, $P < 0.01$ in the BCAAs trial, and $r = 0.87$, $P < 0.01$ in the placebo trial).

*Muscle glycogen*. There was a more pronounced decrease in muscle glycogen concentration in the placebo trial than in the BCAA trial. The concentration of glycogen decreased from $86 \pm 5.8$ to $77 \pm 6.5$ mmol kg$^{-1}$ ($P > 0.05$) during exercise when BCAAs were supplied, and from $78 \pm 5.1$ to $50 \pm 4.4$ mmol kg$^{-1}$ ($P < 0.05$) when water was supplied. There was no significant difference in the resting levels of glycogen between the two trials.

## DISCUSSION

The major finding in the present study is that an intake of BCAAs decreases the perceived exertion and mental fatigue during exercise at a given work rate. However, when the subjects exercised at their maximum, no significant effect of BCAAs could be detected. The RPE and CR-10 scales that were applied to measure the subjects' perceived exertion and mental fatigue have proved to be valid and reliable instruments for obtaining estimates of subjective symptoms and have been frequently used during different types of exercise (see Hassmén 1991). In addition, the performance in the colour task of the CWT was improved after the exercise when BCAAs had been ingested. In previous studies, the CWT has been presented to subjects before and after a competitive event. Under these conditions, ingestion of BCAAs during exercise improved the performance in all three parts of the test, while there was no effect when subjects ingested the placebo (Blomstrand *et al.* 1991, Hassmén *et al.* 1994). The procedure followed in the present study differs from that followed in previous studies in that the CWT was performed only after exercise. It is possible that the word and colour-word tasks are more susceptible to practice effects than the colour task, which makes it more difficult to detect a small difference, or that each task tests different aspects of cognitive function and only one is influenced in the present experiment. This effect of BCAAs suggests that their ingestion during intense exercise has a central effect.

The concentration of free tryptophan increased during the later part of the exercise under both conditions. However, when the BCAAs were ingested, the plasma concentration ratio of free tryptophan/BCAAs was maintained at pre-exercise levels or even decreased, which would be expected to prevent an increase in the brain 5-HT level. Consequently, the lower ratings of perceived exertion and mental fatigue during exercise at a given work rate after ingestion of BCAAs (Figs 1a, b) is consistent with the hypothesis given at the beginning of this paper. No significant

 

effect of BCAAs was found on the physical performance during the last 20 min of exercise when the subjects performed at their maximum. This is in agreement with results from earlier studies where human subjects exercised for ≈ 30–40 min after reduction of their muscle glycogen stores (Wagenmakers 1992, Varnier *et al.* 1994). In these studies, administration of BCAAs either orally or intravenously 70–90 min before intensive exercise did not affect the physical performance, measured as exercise time to exhaustion or total work done during graded incremental exercise. When BCAAs were added to a carbohydrate solution and given to subjects during prolonged exercise, no additional effect on physical performance could be detected as a result of the BCAA intake (Blomstrand *et al.* 1995, van Hall *et al.* 1995). However, it is important to note that an intake of carbohydrates has been reported to depress and delay the increase in the concentration of free tryptophan in plasma during exercise (Davis *et al.* 1992), and ingestion of carbohydrates might therefore delay the development of not only the physical fatigue but also the central/mental fatigue. The observed increase in plasma concentration of free tryptophan is most likely attributed to the increase in the plasma level of FFA. The latter are also transported bound to albumin in plasma, and an increase in their concentration will release part of the tryptophan from albumin (Curzon *et al.* 1973). In support of this view, there was a very good correlation between the plasma concentration of free tryptophan and FFA under both exercise condidions, which is in agreement with earlier studies (Blomstrand *et al.* 1988, Davis *et al.* 1992).

In the present study, there was a more pronounced decrease in muscle glycogen concentration during exercise when the subjects ingested flavoured water than when they ingested BCAAs. This might indicate that BCAAs have a sparing effect on muscle glycogen degradation, at least when the exercise starts with reduced glycogen levels. However, the observed rise in plasma lactate during exercise when BCAAs were ingested, which was not found in the placebo trial, does not suggest a smaller glycogen degradation in the BCAA trial. Additional studies need to be done to elucidate further the effect of BCAAs on rates of glycogen degradation in muscle during exercise. The decrease in muscle glycogen concentration during exercise seemed suprisingly small. However, the amount of carbohydrates oxidized during exercise, calculated from an oxygen uptake of 3.4 L $min^{-1}$ and an *R* value of 0.83 (indicating that 50 % of the energy is derived from carbohydrates) would be about 165 g. With the assumptions that the glucose uptake by the active muscles is 0.75–0.9 g $min^{-1}$ (Ahlborg 1974, unpublished observations with reduced muscle glycogen stores), that the brain and inactive muscles utilize 10 g during the exercise period and that the active muscle mass is 15 kg, a degradation of 83–95 g of muscle glycogen or ≈ 30–35 mmol (kg muscle)$^{-1}$ could be expected. This is in fact similar to the decrease observed in the placebo trial.

Based on the observation that an intake of BCAAs has a detrimental effect on physical performance in McArdle's patients due to increased production of ammonia, it has been suggested that this would also be the case with healthy individuals in a glycogen-depleted state (Wagenmakers 1992). In the same study, elevated levels of ammonia were found after exhaustive exercise when subjects were given 30 g of either a mixture of BCAAs or leucine alone 1.5 h before the exercise, after a heavy exercise bout which was used to lower muscle glycogen, but no effect on performance was found. In the present study, no difference in the plasma concentration of ammonia was found between the two conditions, i.e. an increased plasma level of BCAAs did not augment the exercise-induced increase in ammonia concentration (Fig. 3). This finding is different from those presented in other studies (Wagenmakers 1992, McLean & Graham 1993, van Hall *et al.* 1995) in which an intake of BCAAs before exercise caused a more pronounced increase in the plasma concentration of ammonia during exercise. However, the exercise protocol in these studies differed from the one in the present study, and, furthermore, in one study (Wagenmakers 1992) the amount of BCAAs that was supplied to the subjects was four to five times greater than in the present study.

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