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Branched-chain α-amino acid chronic treatment: responses of plasma α-keto-related compounds and ammonia when used in physical exercise performance

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Summary. To examine the effects of acute branched-chain α-amino acids (BCAA) oral administration following chronic BCAA intake, a group of well trained young swimmers (n = 12) was submitted to a one month chronic BCAA treatment (0.2 g/Kg body weight per die; Leu:Val:Ileu = 2:1:1) and a physical exercise test before and after this period of treatment was carried out. The exercise tests (60 min swim) were performed in a high circulating BCAA level state which was obtained through oral BCAA administration (or placebo) just before the beginning of the exercise. The groups will be referred to as BCAA/before, BCAA/after, placebo/before, placebo/after. Blood and plasma (antecubital vein) samples were collected from the different groups at different times: on the morning of the day before the test (basal time, rest 0), the following day 30 min after an acute administration (oral dose placebo or BCAA acute treatment: Leu 4.8 g, Val 2.4 g, Ileu 2.4 g), just before the beginning of the exercise performance (time 0 min, rest 1), at the end of the exercise (time 60 min, EE) and during recovery (time 120 min, Re). Plasma ammonia levels increased significantly from rest 1 to the end of the exercise in all subjects, but it was significantly higher in BCAA treated than in placebo subjects in both the before and after chronic treatment groups (BCAA/before: from 38 ± 7 to 204 ± 65 mmol/l; placebo/before: from 36 ± 10 to 93 ± 29 mmol/l; BCAA/after: from 36 ± 9 to 171 ± 43 mmol/l; placebo/after: from 30 ± 6 to 65 ± 16 mmol/l). Plasma ammonia level increments observed before a chronic one month BCAA treatment were significantly higher than after this treatment ($p < 0.05$). Plasma alanine was at all times of the test higher before the BCAA chronic treatment than after; this difference resulted significant at rest 0, rest 1 and recovery times ($p < 0.05$). After acute BCAA administration, plasma BCAA levels increased from 618 ± 52 mmol/l to 1893 ± 284 mmol/l (p

* Name and location where the investigations were carried out.

< 0.05) from the onset of exercise and remained elevated throughout the test. Placebo and basal (rest 0) levels both before and after the chronic treatment did not demonstrate any significant differences. Plasma BCAA and BCKA levels, in the BCAA/before demonstrated significantly higher levels than placebo/before at rest 1 time (BCAA/before vs placebo/before: Leu 86 ± 27 vs 620 ± 97 mmol/l; KIC 60 ± 3 vs 87 ± 5 mmol/l, Ileu 51 ± 19 vs 359 ± 56 mmol/l, KMV 26 ± 1 vs 43 ± 2 mmol/l, Val 290 ± 79 vs 915 ± 133 mmol/l, KIV 14 ± 1 vs 24 ± 2 mmol/l). The levels after the chronic treatment maintained circa these differences in the two groups BCAA/after and placebo/after. The plasma BCAA as well as the BCKA levels of acutely treated athletes, in physical exercise, showed a different profile before and after the chronic treatment. The chronic treated BCAA/after group in fact depicted a decreasing BCKA level profile at the end of the exercise and during recovery; on the contrary, before the chronic treatments, acutely treated athletes demonstrated a tendency to increase these levels during recovery. These data seem to confirm that increased BCAA availability, before exercise, result in significantly greater plasma ammonia responses during exercise than does placebo administration; furthermore this increment is lower after chronic treatment. The interpretation of the ammonia data is difficult since the exercise type could have an influence on this phenomenon. The differences in the profile patterns of alanine, BCAA and BCKA levels seem to indicate that the chronic treatment brings about a state in which there is a better use of BCAA compounds as energy supply.

Keywords: Amino acids – Branched-chain α-amino acid (BCAA) – Branched-chain α-keto acid (BCKA) – Plasma ammonia – Physical exercise – Amino acid metabolism – Plasma acetyl-carnitine

Introduction

It is well established that exercise enhances protein metabolism, while aminoacids, deriving from protein degradation, supply a fraction of energy expenditure during physical exercise especially at the exhaustion stage. The various fields of research on amino acid metabolism during exercise have particularly concentrated on the study of branched-chain α-amino acids (BCAA), indispensable constituents in muscle. In fact skeletal muscle is the primary site of BCAA uptake where these may play a special role in nitrogen and fuel metabolism. The first step in BCAA catabolism is a reversible enzyme catalysed transamination step with the production of branched-chain α-keto acids (BCKA) followed by an irreversible oxidative decarboxylation, catalysed by the branched-chain α-keto acid dehydrogenase complex (BCKAD). The activities and concentrations of the two enzymes, involved in BCAA catabolism, are unequally distributed among the different organs and tissues. Moreover in different muscle fiber types the enzymes might also present different activities (Hageloch et al., 1990; Harper et al., 1984; Skeie et al., 1990). The BCKA decarboxylase enzyme is subject to regulation by phosphorylation and dephosphorylation. These respectively

inactive and active enzyme forms are under the influence of specific kinase and phosphatase enzymes. The activity of BCKAD complex increases with training (Wagenmakers et al., 1989), depends on the diet (Shimomura et al., 1990) and may be influenced by the type and intensity of exercise and therefore whether aerobic or anaerobic metabolism takes place. During intense prolonged exercise the contribution of carbohydrates to energy supply is varied and there is an acceleration in hepatic protein degradation resulting in compounds destined for gluconeogenesis and glucose-alanine cycle. Since the BCAA intracellular concentration increases, the keto/BCAA intracellular ratio also rises owing to the fact that the BCKAD enzyme activity varies (Harris et al., 1995). Consequently free ammonia production might also increase in the muscular cells as well as in the plasma and exercise performance deteriorates.

Following BCAA transamination, the newly formed branched-chain α-keto acids (BCKA) can either be further oxidized in the muscle or released and transported to other sites, principally the liver, where oxidation will take place and this is in accordance with the different distribution of the enzymes. In fact transaminase is predominant in the muscle with respect to the liver; on the contrary decarboxylase is high in the liver and low in the muscle. The suggestion of events that could then be made is the existence of interorgan fluxes and that the BCKA are taken up by the liver and may then be utilized. A further possible fate of BCKA could be that of entering the tricarboxylic acid cycle through the succinyl-coenzyme A (Val and Ileu) or through acetyl-coenzyme A (Leu), forming oxaloacetate for gluconeogenesis. It is probable that the metabolic state and the fuel requirements could influence one of these reaction pathway steps. Under various conditions such as the degree of training, exercise intensity, glycogen depletion state and under the influence of factors such as the activation of the rate limiting step enzyme (branched-chain 2-oxo acid dehydrogenase, BC-complex), protein catabolism as well as amino acid energy supply both increase (Shimomura et al., 1990; Wagenmakers et al., 1989). Recent works confirm that during physical performance at exhaustion (Mac Lean et al., 1993) there is an increase in the BCAA transaminiation and that this phenomenon seems quite clear as for example is the case when subjects exhibit high BCAA circulating levels following an oral intake of BCAA. Muscle ammonia production during exercise is traditionally thought to originate from the PNC (purine nucleotide cycle) and from BCAA deamination. The degree of involvement of either or both of the above processes seems to depend on the duration and the intensity of the exercise as well as on energy substrate availability (Crowell et al., 1990; Graham and Mac Lean, 1992; Sewell et al., 1992). A better availability of BCAA can be obtained after oral administration since their levels in the circulation result elevated. Furthermore a chronic treatment might have an effect on previously indicated enzyme concentrations and activities, particularly when the treatment is made with exercise activity in subjects maintaining a high level of training for a long-time (Kasperek et al., 1985; Crowell et al., 1990; Wagenmakers et al., 1989).

The aim of our work was to examine the effects of an acute BCAA administration following a chronic one month BCAA treatment. The proposal was to study the BCAA and BCKA plasma level variations with physical exercise in conditions where their availability is increased, as after an acute oral administration. This was done before and after a one month chronic treatment with BCAA. The ammonia levels were measured during the test to study if, at exhaustion, they demonstrated an increase in the different conditions, in relation to the acute treatment as well as the chronic one.

Material and methods

Subjects and diet

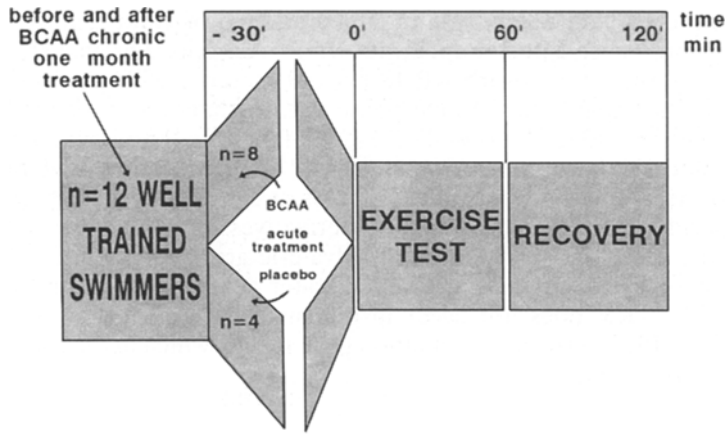
The study was performed on 12 well trained swimmers (8 females and 4 males, aged 14–17 yr.; 15.1 ± 0.9 yr.); their height varied from 164 to 179 cm (170.8 ± 5.1 cm) and their weight varied from 53 to 67 Kg (60.4 ± 5.9 Kg). Medical examination showed that the athletes were in good health during the whole study period. All swimmers, fully informed on the nature and purposes of this experiment, volunteered to participate in the study. The subjects were asked to continue with their normal diet and to consume the same foods until and including the day before each trial (about 45 Kcal/Kg per die; carbohydrates 63.5%, lipids 22%, proteins 14.5% – average values recorded of ingested food). They also maintained constant their daily training and their competition programmes. These daily training programmes aimed at a high quality level preparation; in fact about three weeks after the end of our study, all athletes participated in their national swimming competitions. Furthermore every day all performances of the athletes were monitored by their trainer. For these reasons all subjects participating in our study were in a well trained condition.

Treatment

The swimmers were studied in June for the first test and in July for the second, following one month of chronic BCAA treatment 0.2 g/Kg die (Friliver Leu = 12%, Ileu = 6%, Val = 6% W/W; 4 Kcal/g kindly supplied by Bracco Industria Chimica spa Milan, Italy). Each athlete assumed about 6–8 sachets of BCAA powder per day; the amino acid quantity in each sachet was Leu 1.2 g, Ileu 0.6 g, Val 0.6 g. The doses were taken by the swimmers themselves as desired, although the team physician did suggest that they should be taken during their meals (two-three sachets). This BCAA treatment was adhered to by all athletes. The acute treatment wich took place only on the day of the test, consisted of a single dose oral administration of 4 sachets of BCAA or of 4 sachets of placebo. The powder, dissolved in water, was assumed in the morning, 30 minutes before the start of the exercise trial. An outline scheme (Scheme 1), describing the experimental protocol, follows.

Physical exercise test

The first and second tests followed the same scheme described as follows. The day before the test the athletes reported to the laboratory after an overnight fast and a first blood sample was drawn (rest 0). Owing to the large amount of blood to be drawn, we decided that the basal laboratory study should take place the day before the test was made. The next day the swimmers reported to the swimming pool after a second overnight fast, and three hours after the ingestion of a 650 Kcal breakfast (this breakfast was the same for all



Scheme 1

subjects and its composition was carbohydrates 74.5%, lipids 15%, proteins 9.5%). The objective for giving this breakfast was to avoid having the subject exercising in a state of an overnight fast. This period of fast, in fact, would have been too long since the exercise started in the morning at about eleven o'clock. The subjects were randomly assigned one of two acute treatments: placebo or BCAA (four sachets). The placebo or BCAA powder was administered in a single dose (dissolved in 300–400 ml tap water) at 11.00 a.m. A second blood sample was obtained after 30 min just before the exercise started (rest 1). The muscular exercise test began with a light warm up (5 min) after which the athletes swam for 40 min at about 70% of their own maximum performance (this level was defined by the trainer taking into consideration the maximum swimming speed performance of each subject); 15 min before the end all athletes were invited to increase the performance load until exhaustion. A third blood sample was obtained at the end of the exercise (end of exercise – 60 minutes after the start of the exercise text). Blood samples were taken within 1–3 minutes from the end of the exercise. Sixty minutes after the end of the physical performance a fourth blood sample was obtained (Recovery).

In July a second test was carried out after a month of chronic treatment. In this case the athletes interrupted their intake of BCAA forty-eight hours before the beginning of the test. Maintaining the same work load in all trials of both June and July tests was difficult, so each athlete during his performances was timed by the trainer and swimming speed was controlled. In this way the comparison between the athletes and between the trials could become more profitable.

Analytical methods

Approximatly fifteen ml of blood sample was collected from an antecubital vein, at rest 0 (basal), rest 1 (before start of exercise), at end of exercise and during recovery time. Suitable plasma aliquots were immediately transported in the laboratory (in ice-water bath) and ammonia enzymatic endpoint assay was performed within one hour using Sigma Ammonia kit (171-C) and Beckman DU-65 spectrophotometer. Other plasma sample aliquots, immediately obtained by centrifugation, were frozen. Blood glucose, lactate, β-OH-butyrate, free fatty acids (FFA), plasma carnitine and acetyl-carnitine were all determined by enzymatic methods as previously described and the assays were performed within 60–90 days (De Palo et al., 1993). After collection, the samples were immediatly centrifuged and stored in an ice bath, with the centrifuge and the refrigerator being at the edge of the swimming pool.

Plasma amino acids were assayed following a modified Godbel HPLC method as previously described (De Palo et al., 1993; Godel et al., 1984). Plasma branched-chain

keto acids were assayed following a modified method of Hara (Hara et al., 1985). The HPLC and fluorescence detector apparatus were as described previously; the column was Glass two-100X3 mm Lichrosorb RP-18 packed material with particle size 7 mm, purchased from Chrompack, the mobile phase was methanol-acetonitrile-25 mmol/l phosphate buffer pH 6.5 (32.5%–20%–47.5%), the flow rate 0.5 ml/min. The derivatization procedure used DDB solution (5 mmol/l – 1,2 diamino 4,5 methylene dioxybenzene dihydrochloride, Sigma Chemical) and 5 ml plasma samples were the same ones collected for amino acids analysis. The standard derivatives of α-ketovaleric acid (KIV), α-ketoisocaproic acid (KIC), α-keto-β-methylvaleric acid (KMV) and α-ketovaleric acid (KV) were prepared separately in distilled water at a concentration of 4 mmol/ml and stored at −20°C. KV does not occur in human plasma so it was used as an internal standard. The HPLC performance method gave results which agreed well with the above mentioned Hara method.

Statistical analyses

Treatment and time effects were analysed using the ANOVA test. The study was carried out to verify the effects of chronic treatment in exercise performance, so we decided that the acutely treated group should be more numerous than the placebo control group. In effect previous work did not show clear significant plasma BCAA differences in control subjects (De Palo et al., 1993). To estimate the effect of the chronic treatment in the same acute treated group a paired Student's t-test was used. Significance was accepted at $p < 0.05$. All values, in tables and figures, are indicated as means ± standard deviation. In the Tables the significant differences demonstrated by statistical analysis were marked in the following way: when the groups (treated-placebo) were different but the test time was the same then the significant differences were marked in capitals (A, B,...); when the differences were calculated in the same group (treated-placebo) but the test times were different then significant differences were marked in small letters (a, b,...).

Results

Tables 1 and 2 demonstrate the blood and plasma measurements carried out at rest 0, rest 1, at the end of the exercise and during recovery time. No differences were observed between the reported levels and those from the literature which are well known (Mac Lean, 1991). As the number of control subjects were very low in some cases, the degree of freedom for statistical analysis was insufficient. Despite this, for the sake of completeness, these data are also reported and they are discussed only when the difference is clearly evident.

In particular, plasma lactate was increased at the end of the physical performance in all cases, that is, in acutely treated athletes, in placebo treated athletes, as well as before and after chronic treatment. The tendency was for blood glucose levels (data not reported) to decrease but there were no significant variations, before and after the treatment in the treated and control group of athletes.

Plasma branched-chain amino acid levels (Tables 3 and 4) were, as expected, significantly higher in acutely treated athletes at the beginning of the exercise (rest 1) before as well as after chronic treatment and these levels tended to continue to be different from those in the placebo group throughout the test.

Table 1. Concentrations of substrates in blood and plasma before chronic treatment: basal, after acute BCAA treatment at different times of the test

Time	FFA μmol/l	Lactate mmol/l	β-OH-Butirate mmol/l	Alanine μmol/l	Carnitine μmol/l	Acetyl-Carnitine μmol/l
Rest 0						
PL	582 ± 154	1.74 ± 0.25	0.06 ± 0.04	423 ± 29	33 ± 3	6.7 ± 1.6
BCAA	472 ± 150 (c)	1.71 ± 0.11	0.02 ± 0.02	465 ± 37	39 ± 4	7.1 ± 1.8
Rest 1						
PL	366 ± 110 (a)	2.06 ± 0.50 (a)	0.02 ± 0.03 (a)	408 ± 98 (b)	34 ± 3	9.9 ± 2.7 (a)
BCAA	206 ± 65 (b,c)	1.70 ± 0.33 (c)	0.07 ± 0.03 (b)	490 ± 36 (c)	40 ± 6	7.4 ± 1.4 (b,c)
End exercise						
PL	571 ± 340	5.34 ± 1.52 (a,b)	0.10 ± 0.05	591 ± 66 (a,b)	34 ± 5	14.4 ± 2.8
BCAA	497 ± 182 (b)	5.32 ± 2.59 (c,d)	0.15 ± 0.06 (b)	550 ± 94	36 ± 4	14.3 ± 5.9 (b)
Recovery						
PL	743 ± 290 (a,A)	1.60 ± 0.31 (b)	0.19 ± 0.16 (a,A)	326 ± 77 (a,A)	30 ± 4	15.4 ± 1.2 (a)
BCAA	398 ± 155 (A)	1.88 ± 0.92 (d)	0.08 ± 0.03 (A)	591 ± 84 (c,A)	36 ± 3	12.0 ± 4.4 (c)

means ± SD; Placebo (*PL*), n = 4; Treated (*BCAA*), n = 8. ANOVA test: significant ($p < 0.05$) differences in the same treatment groups: *a*, *b*, *c*, and between different, treatments, different groups and same time: *A*.

Table 2. Concentrations of substrates in blood and plasma after chronic treatment: basal, after acute BCAA treatment at different times of the test

Time	FFA μmol/l	Lactate mmol/l	β-OH-Butirate mmol/l	Alanine μmol/l	Carnitine μmol/l	Acetyl-Carnitine μmol/l
Rest 0						
PL	432 ± 96	2.74 ± 0.39	0.04 ± 0.03	238 ± 19	25 ± 3	4.9 ± 1.7
BCAA	428 ± 244	2.87 ± 0.51	0.07 ± 0.04	288 ± 83	33 ± 9	5.2 ± 2.0
Rest 1						
PL	375 ± 23 (a)	2.80 ± 0.06	0.03 ± 0.02 (a)	370 ± 62 (a)	29 ± 9 (a,b)	5.4 ± 1.9 (a,b)
BCAA	247 ± 78 (c)	3.06 ± 0.39 (a)	0.07 ± 0.02	379 ± 50 (b)	37 ± 9 (c,d)	3.5 ± 1.5 (c,d)
End exercise						
PL	456 ± 173 (b)	4.39 ± 0.92	0.09 ± 0.01 (b)	367 ± 50	28 ± 9 (a)	13.0 ± 2.4 (a)
BCAA	370 ± 82	6.43 ± 3.11 (a,b)	0.12 ± 0.06	447 ± 122 (c)	35 ± 4 (c)	9.9 ± 2.4 (c)
Recovery						
PL	951 ± 212 (a,b,A)	2.48 ± 0.08	0.34 ± 0.09 (a,b,A)	237 ± 12 (a)	25 ± 6 (b,A)	13.6 ± 1.2 (b,A)
BCAA	500 ± 204 (c,A)	3.26 ± 1.30 (b)	0.09 ± 0.07 (A)	285 ± 54 (b,c)	32 ± 7 (d,A)	7.7 ± 1.8 (d,A)

means ± SD; Placebo(*PL*) n = 3; Treated (*BCAA*) n = 9. ANOVA test: significant differences ($p < 0.05$) in the same groups are indicated by: *a*, *b*, *c*, *d*, and between groups, same time, by: *A*.

Table 3. Concentrations of BCAA in plasma before chronic treatment: basal, after acute BCAA treatment at different times of the test

Time	Leucine μmol/l	Valine μmol/l	Isoleucine μmol/l
Rest 0			
PL	156 ± 7	377 ± 29	72 ± 2
BCAA	153 ± 21	385 ± 33	79 ± 9
Rest 1			
PL	86 ± 27 (A)	290 ± 79 (A)	51 ± 19 (A)
BCAA	620 ± 97 (a,b,A)	915 ± 133 (a,A)	359 ± 56 (a,b,A)
End exercise			
PL	115 ± 15 (B)	293 ± 45 (B)	64 ± 15 (B)
BCAA	371 ± 63 (a,c,B)	719 ± 105 (a,b,B)	193 ± 29 (a,B)
Recovery			
PL	105 ± 36 (C)	266 ± 91 (C)	58 ± 13 (C)
BCAA	507 ± 116 (b,c,C)	833 ± 132 (b,C)	220 ± 58 (b,C)

means ± SD; Placebo (*PL*) n = 4; Treated (*BCAA*) n = 8.
ANOVA test: statistical significant ($p < 0.05$) differences in the same groups are indicated by: *a*, *b*, *c*, and between groups, same times, by: *A*, *B*, *C*.

Table 4. Concentrations of BCAA in plasma after chronic treatment: basal, after acute BCAA treatment at different times of the test

Time	Leucine μmol/l	Valine μmol/l	Isoleucine μmol/l
Rest 0			
PL	93 ± 2	223 ± 32	53 ± 3
BCAA	103 ± 7	244 ± 16	55 ± 7
Rest 1			
PL	98 ± 7 (A)	237 ± 31 (A)	78 ± 13 (A)
BCAA	568 ± 53 (a,b,A)	610 ± 115 (a,A)	306 ± 34 (a,b,A)
End exercise			
PL	78 ± 22 (B)	199 ± 28 (B)	57 ± 2 (B)
BCAA	325 ± 83 (a,c,B)	563 ± 115 (b,B)	192 ± 44 (a,c,B)
Recovery			
PL	67 ± 9 (b,C)	163 ± 21 (C)	55 ± 4
BCAA	121 ± 22	312 ± 32 (a,b,c)	84 ± 14 (b,c)

means ± SD; Placebo (*PL*) n = 3; Treated (*BCAA*) n = 9.
ANOVA test: statistical significant ($p < 0.05$) differences in the same groups are indicated by: *a*, *b*, *c*, and between groups, same times, by: *A*, *B*, *C*.

At the end of the exercise plasma ammonia levels were significantly higher in the BCAA treated athletes (n = 8) than in placebo (n = 4) and this difference was observed before (end exercise – acutely treated: 204 ± 65 vs placebo: 93 ± 29 mmol/l) as well as after chronic treatment (end exercise – acutely treated: 171 ± 43 vs placebo: 65 ± 16 mmol/l) (Figs. 1 and 2).

In the same group (n = 7), after an acute treatment with BCAA the ammonia levels reached, at the end of the exercise, were found to be significantly lower ($p < 0.05$, t-Student paired data test) after than before in the

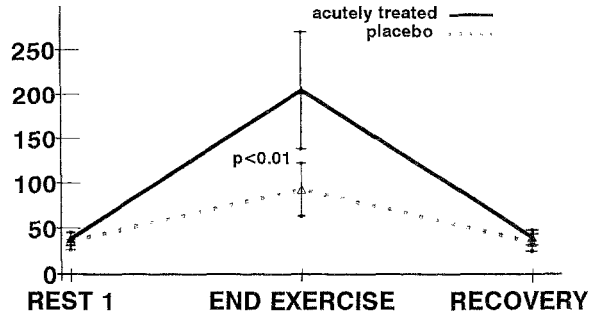


Fig. 1. Plasma ammonia concentrations before chronic treatment, after acute BCAA or placebo treatment at different times of the test

Fig. 2. Plasma ammonia concentrations after chronic treatment, after acute BCAA treatment at different times of the test

chronically treated subjects (end exercise – before 212 ± 65 vs after 172 ± 46mmol/l). This was not so in the placebo group. Plasma alanine levels increased at the end of the exercise following an acute dose as well as in placebo athletes both before and after chronic treatment. This could be in relation to an increased release of alanine from muscle. During the recovery phase, after an acute dose of BCAA, a significant difference was observed with higher values before than after chronic treatment. Moreover the whole profile of alanine plasma levels was clearly lower after the chronic treatment than it was before (Fig. 7). FFA plasma levels before and after the chronic treatment both demonstrated that in the case of the placebo the levels tended to increase, as expected, at the end of the exercise as well as during the recovery phase. However following an acute dose of BCAA it was found that during recovery there was a tendency for the FFA levels not to alter appreciably (Tables 1 and 2). The displayed levels of plasma BCKA were significantly higher in athletes following an acute dose of BCAA with respect to placebo at rest 1 and the difference was also observed at other successive times as well. During the recovery phase, the BCKA plasma levels demonstrated a tendency towards a decrease after chronic treatment (Table 5 and Fig. 5).

Regarding leucine in particular and its keto analogue (KIC) the levels were found to be increased following an acute oral dose of BCAA with respect to placebo (Tables 6 and 7). Furthermore at the end of the exercise the

leucine levels tended to decrease significantly and following an analysis of the single profiles we verified that there was a significant decrease in the leucine levels at rest 1 with respect to end of exercise and the result was the same before as well as after the chronic treatment (before chronic treatment, Rest 1: 620 ± 97 vs End exercise: 371 ± 63 mmol/l $p < 0.05$; after chronic treatment, Rest 1: 568 ± 53 vs End exercise: 325 ± 83 mmol/l $p < 0.05$). KIC levels followed a similar pattern as well and the statistical analysis confirmed the above observed tendency for KIC to decrease following an acute dose before chronic treatment (rest 1: 87 ± 5 vs end exercise: 68 ± 2 mmol/l $p < 0.05$) as

Table 5. BCKA plasma levels before chronic treatment, after acute BCAA or placebo treatment at different times of the test

Time	KIC μmol/l	KMV μmol/l	KIV μmol/l
Rest 0			
PL	59 ± 4	26 ± 1	14 ± 1
BCAA	59 ± 3	26 ± 2	15 ± 1
Rest 1			
PL	60 ± 3 (A)	26 ± 1 (A)	14 ± 1 (A)
BCAA	87 ± 5 (a,A)	43 ± 2 (a,A)	24 ± 2 (a,A)
End exercise			
PL	55 ± 2 (B)	22 ± 1 (B)	11 ± 1 (B)
BCAA	68 ± 2 (a,b,B)	35 ± 2 (a,b,B)	19 ± 1 (a,b,B)
Recovery			
PL	64 ± 2 (C)	25 ± 1 (C)	13 ± 1 (C)
BCAA	104 ± 8 (b,C)	40 ± 2 (b,C)	23 ± 2 (b,C)

means ± SD; Placebo (*PL*) n = 4; Treated (*BCAA*) n = 8.
ANOVA test: significant ($p < 0.05$) differences in the same treatment groups: *a*, *b*, *c*, and between different treatment, different groups and same times: *A*, *B*, *C*.

Table 6. BCKA plasma levels after chronic treatment, after acute BCAA or placebo treatment at different times of the test

Time	KIC μmol/l	KMV μmol/l	KIV μmol/l
Rest 0			
PL	71 ± 2	25 ± 2	13 ± 1
BCAA	70 ± 6	24 ± 2	15 ± 1
Rest 1			
PL	73 ± 2 (A)	25 ± 1 (A)	14 ± 1 (A)
BCAA	137 ± 11 (a,A)	45 ± 5 (a,A)	25 ± 2 (a,A)
End exercise			
PL	59 ± 6 (B)	22 ± 1 (B)	11 ± 5 (B)
BCAA	98 ± 4 (a,b,B)	33 ± 1 (a,b,B)	19 ± 1 (a,b,B)
Recovery			
PL	66 ± 1 (C)	19 ± 1 (C)	9 ± 1 (C)
BCAA	109 ± 3 (b,C)	25 ± 2 (b,C)	15 ± 1 (b,C)

means ± SD; Placebo (*PL*) n = 3; Treated (*BCAA*) n = 9.
ANOVA test: significant ($p < 0.05$) differences in the same treatment groups: *a*, *b*, and between different treatments, different groups and same times: *A*, *B*, *C*.

well as after chronic treatment (rest 1: 137 ± 11 vs end exercise: 98 ± 4 mmol/l $p < 0.05$).

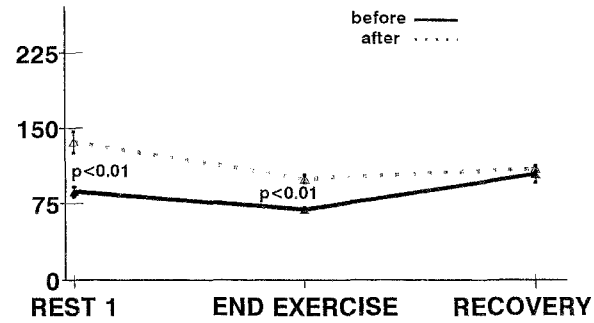


Fig. 3. Plasma α-ketoisocaproic acid (KIC) concentrations before and after chronic treatment in the same subjects, after acute BCAA treatment at different times of the test

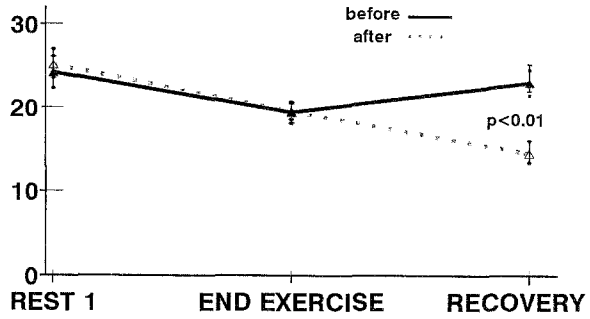


Fig. 4. Plasma α-ketoisovaleric acid (KIV) concentrations before and after chronic treatment in the same subjects, after acute BCAA treatment at different times of the test

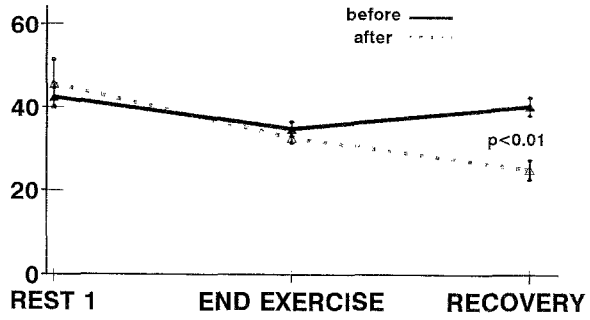


Fig. 5. Plasma α-keto-β-methylvaleric acid (KMV) concentrations before and after chronic treatment in the same subjects, after acute BCAA treatment at different times of the test

Discussion

In the present study plasma ammonia concentrations following acute oral ingestion of BCAA increased significantly during and after physical exercise, compared with levels after oral placebo (Figs. 1 and 2). It is already well

established that physical exercise has an influence on ammonia levels particularly with certain types of exercise, such as long term sporting activities (reaching exhaustion). Various biochemical processes of different nature, such as the purine nucleotide cycle and amino acid catabolism, contribute to this increase of ammonia levels. In the present study it is found that, at the end of the exercise, the ammonia levels are increased following an acute oral dose of BCAA. However the increment observed before a one month chronic BCAA treatment was significantly higher than after. The present results are in accordance with the results of Mac Lean et al. who also demonstrated significantly higher plasma ammonia levels following BCAA administration as compared to placebo (Mac Lean and Graham, 1993). In comparing prolonged exercise and short performance to exhaustion the ammonia seems to derive mainly from the amino acid catabolism rather than the purine nucleotide cycle (PNC) and this is more salient in the former rather than in the latter case (Graham and Mac Lean, 1992; Wagenmakers et al., 1990). Plasma BCAA levels, at rest and during exercise, following BCAA acute oral ingestion, were found to be increased, as expected, demonstrating a better availability of these compounds (Table 7).

It is quite difficult to explain why the ammonia level increment resulted significantly less after the chronic treatment. An hypothesis might be that an increased energy request, during prolonged exercise in which the last part of the performance was carried to exhaustion, as in our protocol, may cause an increase BCAA oxidation with an increased production of ammonia. When a well trained organism gets used to a chronic intake of BCAA, the enzyme limiting step BCKA dehydrogenase, an enzyme present in an active or inactive dephosphorylated or phosphorylated form, might be present in increased amounts in its active form (Kasperek et al., 1985; Shimomura et al., 1990;

Table 7. Plasma BCAA and BCKA concentrations before and after chronic treatment in the same subjects, after acute BCAA treatment at different times of the test

Time	BCAA before μmol/l	BCAA after μmol/l	BCKA before μmol/l	BCKA after μmol/l
Rest 0				
PL	605 ± 36	369 ± 27	99 ± 4	109 ± 3
BCAA	618 ± 52 (a)	402 ± 20 (a)	100 ± 4 (a)	108 ± 6 (a)
Rest 1				
PL	427 ± 116 (A)	412 ± 47 (A)	101 ± 4 (A)	112 ± 3 (A)
BCAA	1893 ± 284 (a,b,c,A)	1483 ± 121 (a,b,c,A)	154 ± 5 (a,b,A)	207 ± 16 (a,b,c,A)
End exercise				
PL	472 ± 56 (B)	334 ± 50 (B)	90 ± 2 (B)	107 ± 12 (B)
BCAA	1284 ± 185 (b,d,B)	1081 ± 238 (b,d,B)	123 ± 2 (b,c,B)	151 ± 3 (b,B)
Recovery				
PL	429 ± 140 (C)	286 ± 28	103 ± 4 (C)	154 ± 86
BCAA	1559 ± 288 (c,d,C)	516 ± 60 (c,d)	168 ± 8 (c,C)	149 ± 3 (c)

means ± SD; Placebo (*PL*) n = 4 before, n = 3 after; Treated (*BCAA*) n = 8 before, n = 9 after. ANOVA test: statistical significant ($p < 0.05$) differences in the same group are indicated by *a*, *b*, *c*, *d*, and between groups, same times, by *A*, *B*, *C*.

Wagenmakers et al., 1989) suggesting that its activation could have been enhanced by the treatment. Each time the oxidation of BCAA in the cell reaches completion, the energy requests can be in part satisfied by the BCAA sources themselves and therefore there could be a reduced release of ketoacids in the circulation, as outlined in Fig. 6, in agreement with the Bier D. M. scheme (Bier, 1989).

We can observe that plasma alanine (Fig. 7), a major gluconeogenetic precursor, was at all times of the test higher before the BCAA chronic treatment than after; it may be that BCAA intake could help in sustaining exercise by causing a reduction in glycogen depletion (Bigard et al., 1993). The idea can be put forward that glycogen depletion leads to a demand of gluconeogenetic compounds, such as alanine, deriving outside the liver. Therefore large amounts of alanine force the liver into its gluconeogenic activity (Jungans et al., 1992). Blood circulating ammonia level increments measured after the oral chronic treatment are found to be lower than expected and the rate of gluconeogenesis from alanine seems in accordance with this finding. It is difficult to interpret these results. However, we should take into account that with exercise, liver gluconeogenesis does not only depend on the substrates presented to the liver, but also on an increased efficiency of glucose precursor utilisation (Brokman, 1987) by the liver itself, as well as an

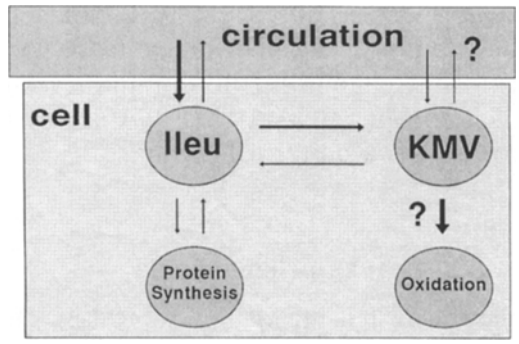


Fig. 6. Outline of BCAA and BCKA cell and extra cellular compartment exchange

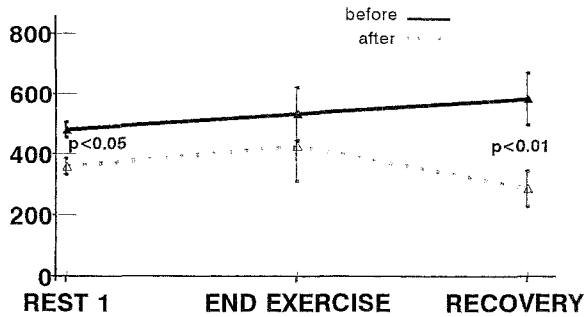


Fig. 7. Plasma alanine concentrations before and after chronic treatment in the same subjects, after acute BCAA treatment at different times of the test

increased efficiency on the part of the muscle in the utilization of fuel substrates. The present hypothesis can be further confirmed by the fact that BCKA levels, after a chronic treatment, tend to decrease throughout recovery (Figs. 4, 5). This seems to be in agreement with the idea that BCKAD enzyme complex is more active after treatment and the catalysed step is irreversible so oxidation in this way is favoured. Furthermore BCKA high circulating levels, particularly KIC, could inhibit BCKAD kinase, the enzyme responsible for the inactivation of enzyme complex by phosphorylation. A second hypothesis could be related to the body composition as well as to the level of training (Hageloch et al., 1990). A suggestion could be that body mass might be influenced by a chronic BCAA treatment. But these latter aspects as well need further investigation and have not been studied here. The duration and the type of exercise too should be well examined together with the dietary intake. In fact Tarnopolski and co-workers (Tarnopolski et al., 1992) observed no effects of varying protein intake on the indices of lean body mass for strength athletes or for sedentary subjects. However protein requirement for athletes was greater than for sedentary individuals. In regard to leucine, and its keto-acid, the observation that after chronic treatment the basal levels resulted higher is not easily explained. In any case the special role occupied by leucine in amino acid metabolism may be responsible for this and so the leucine turnover and non-oxidative utilisation may contribute to this phenomenon (Lamont et al., 1990).

In conclusion the present results seem to confirm that an increase in BCAA availability before physical performance leads to an increase in their oxidation with a significant change in the ammonia levels during exercise. Moreover the use of a chronic BCAA rich diet seems to suggest that the BCAA oxidation process has a better follow up.

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