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Plasma lactate, GH and GH-binding protein levels in exercise following BCAA supplementation in athletes

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Summary. Branched chain amino acids (BCAA) stimulate protein synthesis, and growth hormone (GH) is a mediator in this process. A pre-exercise BCAA ingestion increases muscle BCAA uptake and use. Therefore after one month of chronic BCAA treatment ($0.2\,g\,kg^{-1}$ of body weight), the effects of a pre-exercise oral supplementation of BCAA (9.64 g) on the plasma lactate (La) were examined in triathletes, before and after 60 min of physical exercise (75% of VO_2max). The plasma levels of GH (pGH) and of growth hormone binding protein (pGHBP) were also studied. The end-exercise La of each athlete was higher than basal. Furthermore, after the chronic BCAA treatment, these end-exercise levels were lower than before this treatment ($8.6 \pm 0.8\,mmol\,L^{-1}$ after vs $12.8 \pm 1.0\,mmol\,L^{-1}$ before treatment; $p < 0.05$ [mean ± std. err.]). The end-exercise pGH of each athlete was higher than basal ($p < 0.05$). Furthermore, after the chronic treatment, this end-exercise pGH was higher (but not significantly, $p = 0.08$) than before this treatment ($12.2 \pm 2.0\,ng\,mL^{-1}$ before vs $33.8 \pm 13.6\,ng\,mL^{-1}$ after treatment). The end-exercise pGHBP was higher than basal ($p < 0.05$); and after the BCAA chronic treatment, this end-exercise pGHBP was $738 \pm 85\,pmol\,L^{-1}$ before vs $1691 \pm 555\,pmol\,L^{-1}$ after. pGH/pGHBP ratio was unchanged in each athlete and between the groups, but a tendency to increase was observed at end-exercise.

The lower La at the end of an intense muscular exercise may reflect an improvement of BCAA use, due to the BCAA chronic treatment. The chronic BCAA effects on pGH and pGHBP might suggest an improvement of muscle activity through protein synthesis.

Keywords: Amino acids – Growth hormone binding protein (GHBP) – Branched-chain amino acids (BCAA) – Physical exercise – Lactic acid

Introduction

Increased BCAA metabolism in the muscle can lead to a decrease of branched chain amino acids (BCAA) plasma concentration (Lehmann et al., 1996). In fact the increased oxidation of BCAA, during exercise and in particular with prolonged and intense physical activity, has been described (Blomstrand et al., 1991; De Palo et al., 1993; Lehmann et al., 1995). Furthermore, when physical performance exhausts, glycogen defects (Vukovich et al., 1994) and protein breakdown increases to produce free amino acids for glyconeogenesis or for direct fuel supply (particularly BCAA). Recently G. van Hall (1996), in agreement with other authors (Wagenmakers et al., 1989, 1991; Mittleman et al., 1998), have demonstrated that a pre-exercise BCAA ingestion causes an increased muscle BCAA uptake. A higher intramuscular BCAA concentration and activation of branched chain α-keto acid dehydrogenase complex (BCKADH), the regulatory enzyme in the oxidative BCAA pathway, were also demonstrated. In fact, besides the extent of the BCKADH activation, there appears to be a relation to whether the subjects are metabolising aerobically or anaerobically. Wagenmakers et al. (1991) observe that the largest BCKADH activation is seen when lactate production is early during exercise, that is when energy demand in the muscle rises and oxygen delivery is poor so that anaerobic glycolysis causes lactic acid accumulation in the blood. It is well established that the alanine release increases during exercise, moreover Mac Lean et al. (1994, 1996) measured a greater amount of alanine, during exercise, in BCAA trial than in control trial. This alanine production, involving the addition of the amino group to pyruvate, could be linked to lactate particularly when exhaustion exercise is carried out.

The main purposes of the investigation of BCAA supplementation effects both on performance and on fuel metabolism during exercise has been presented above; a further aspect to consider is the relation between physical exercise and growth hormone (GH) circulating levels. In fact GH can have its effects directly (metabolic pathways and particularly lipolysis) or indirectly through activation of other hormones (and in particular insulin-like growth factors) stimulating the protein synthesis.

Furthermore GH may play a role in body adaptation to anabolic mechanism stimulation. Even if the effects of exercise in increasing the circulating levels of growth hormone (GH) are known (Nevill et al., 1996), the effects of these GH variations are not entirely clear. It can be marked that GH increases as a stress hormone or as a hormone satisfying to body request synthesis of molecules, particularly of proteins. Both these effects can be involved with different mechanisms, times and intensities, therefore in relation to various factors, like type of exercise (intensity, duration, etc) and body condition (training state, electrolyte balance, body mass, fuel availability, etc.). GH involvement in amino acid turnover throughout proteolysis, amino acid uptake and proteosynthesis or amino acid catabolism has been proposed.

The study on the GH role could be improved not only by analysing the circulating growth hormone levels, but also by measuring the growth hormone

binding protein (GHBP), which is considered to reflect the tissue GH receptor concentration (Hocquette et al., 1990; De Vos et al., 1992). In fact GH and this circulating binding protein, which originates from the external domain of the GH receptor, might be linked to the effects of physical exercise on protein metabolism and in particular on protein synthesis. Both hypothesised GH mechanisms, namely growth stimulus such as stress and lipolytic activity, could be in relation to target tissues, considering that the main tissues where GHBP originates are liver and adipose tissue (Baumann 1994, 1996).

BCAA can be a stimulus to protein synthesis also through GH action and that a chronic treatment might yield an improvement in the use of BCAA also as fuel compounds during physical exercise. Over these preliminary remarks, the aim of the present study was to evaluate the improvement of energy fuel utilisation during muscular exercise, when BCAA availability increases. In particular the effects of a pre-exercise BCAA oral supplementation after a BCAA chronic treatment were examined and the level variations of plasma lactate, growth hormone (GH) and its binding protein (GHBP) were investigated.

Methods

Subjects

The Ethics Committee at the Medical Faculty of the University of Padua approved the experimental protocol and consent was obtained from eleven healthy male athletes who were informed of the purposes and risks of the study.

The triathletes were aged 25–49 years (mean ± standard error, 32.8 ± 2.1 years), weighed 65.5–81.0 kg (71.9 ± 1.7 kg) and measured 168.5–185.5 cm in height (175.3 ± 1.6 cm). All athletes during the trial followed a controlled balanced diet.

Six members of the group of eleven well-trained triathletes underwent one month of chronic oral treatment of BCAA while the remaining five served as placebo subjects.

Treatment

The subjects followed a standardised diet of well-known protein, lipid and carbohydrate content (carbohydrates 55%, lipids 30%, proteins 15%; ~ 3,500 kcal per day). The protein content in the diet was 1.2 g kg^{-1} of body weight; the BCAA, when ingested during the treatment, substituted part of this protein content, in this manner the quantity of protein was maintained constant.

Table 1. Body masses of the athletes

Type	Body weight (kg) Mean ± std. Err.
treated 1st test	75.0 ± 2.1
treated 2nd test	75.2 ± 2.1
placebo 1st test	68.2 ± 1.5
placebo 2nd test	68.0 ± 1.6

Values are means ± standard error.

The BCAA chronic treatment consisted in 0.2 g Kg^{-1} of body mass per day of a BCAA powder in sachet (Leu = 1.17 g, Ileu = 0.63 g, Val = 0.61 g) dissolved in tap water and taken before each main meal (morning, midday and evening). The placebo consisted in calcium casein (2.15 g per sachet) so that the total quantity of protein intake was maintained the same as the BCAA trial.

The BCAA chronic treatment was stopped 24 hours before starting the exercise test. 30 min before the start of the exercise, the triathletes, ingested an oral acute dose of BCAA (9.64 g of which Leu 4.68 g, Ileu 2.52 g and Val 2.44 g).

Experimental protocol

The athletes were randomly divided and assigned to the placebo (n = 5) or the BCAA (n ± 6) trial. None of the athletes knew which group he had been assigned to.

Two days before the exercise test, each athlete, underwent an exercise bout to measure VO_2max; afterwards he performed one exercise test before and one after the one month of chronic treatment. The athletes performed the exercise bouts after four hours of fasting, late in the morning, 24 h after having stopped the BCAA (or placebo) supplementation. An acute dose of BCAA (four sachets) was given to each athlete 30 min prior to the start of the exercise test. Venous blood samples were drawn at different times before the acute dose (−30′), at the start of the exercise (0′), 15 min before the exhaustion performance (45′), at the end of the performance (60′) and during recovery time (90′ and 120′).

Exercise test

The exercise test consisted of a cyclergometer performance lasting 60 minutes. The exercise was carried out at 75% of their VO_2max and for the last 15 min the workload was increased by 50 watt every two minutes and the athletes were encouraged to continue to increase their performance until exhaustion, which was reached on average after about 10–15 minutes.

Analytical methods

The blood samples were drawn from each athlete before, during, at the end and after the exercise test. A REA (Radiant Energy Attenuation) using TDX Abbot was used for the plasma lactate analysis. The plasma GH analysis was carried out in duplicate using an immunoradiometric immunoassay kit (Immunotech) and this GH plasma analysis measured the whole circulating GH. The plasma bound GH fraction levels were measured using an HPLC method separation after incubation with ^{125}I-GH following a modified method (De Palo et al., 1990). The free fractions were calculated as the total minus bound. The plasma GHBP levels were measured, assuming that the association constant of the binding reaction was the same for all athletes (Baumann, 1995; Roelen et al., 1997).

Calculations and statistics

Calculation of the values of the concentrations of different biochemical molecules was carried out tracing specific standard curves obtained using suitable standard samples. All data are means ± standard error. t-Student's test for dependent and independent samples was used to determine the statistical significance of changes in circulating levels, in body masses and in ratios. The Kruskall-Wallis non-parametric ANOVA test was also carried out. In both cases the level of significance was set at $p < 0.05$; indication is given

in the text only when this significance was verified using parametric and non-parametric tests.

Results

The plasma lactate concentrations of all the athletes, at the end of exercise, were higher than basal (Fig. 1). The lactate levels demonstrated the same pattern in both trials, but it was lower in the BCAA trial than in the placebo. The levels changed from ($1.6 \pm 0.3\,mmol\,L^{-1}$ basal, $1.6 \pm 0.2\,mmol\,L^{-1}$ basal) to ($8.6 \pm 0.8\,mmol\,L^{-1}$ end of exercise, $10.4 \pm 0.7\,mmol\,L^{-1}$ end of exercise) in treated and placebo groups respectively. The patterns (Fig. 1) demonstrated that, at the end of exercise, plasma lactate levels were significantly lower after the one month treatment than before (8.6 ± 0.8 vs $12.8 \pm 1.4\,mmol\,L^{-1}$; $p < 0.05$) in BCAA trial. On the contrary, at the end of exercise, the lactate

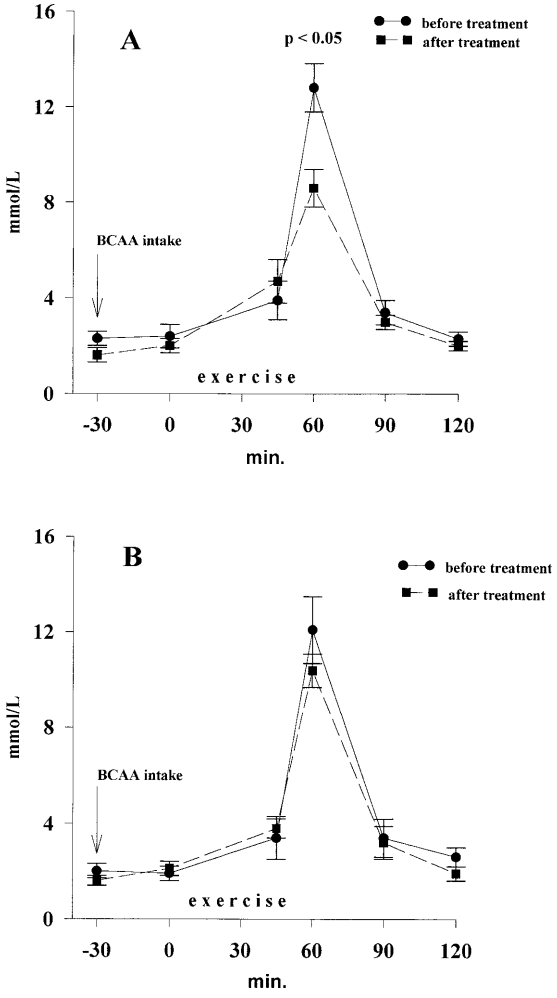

Fig. 1. Plasma lactate concentrations before and after one-month of BCAA (**A**) and placebo (**B**) chronic treatment. Values (means ± standard error) were measured before (−30′ and 0′), during (45′), at the end (60′) and after (90′ and 120′) the exercise test

Table 2. Plasma GH levels and GH bound/GH free ratio and GHBP plasma concentrations (mean ± std. err.) before (1st) and after (2nd) the chronic treatment

	Type	Basal	End of exercise	Recovery
hGH ng mL^{-1}	treated 1st test	4.16 ± 1.50	12.33 ± 2.00 #	1.98 ± 0.36
	treated 2nd test	2.05 ± 1.22	33.84 ± 13.60	1.88 ± 0.49
	placebo 1st test	1.87 ± 1.37	15.73 ± 3.25 #	2.85 ± 0.61
	placebo 2nd test	1.45 ± 1.09	14.19 ± 3.86 #	3.79 ± 1.53
GHBP pmol L^{-1}	treated 1st test	305 ± 26	738 ± 85 #	296 ± 14
	treated 2nd test	306 ± 21	1,691 ± 556 #	271 ± 38
	placebo 1st test	281 ± 49	820 ± 116 #	300 ± 29
	placebo 2nd test	311 ± 40	787 ± 136 #	322 ± 29
Bound/free	treated 1st test	0.258 ± 0.014	0.277 ± 0.012	0.276 ± 0.013
	treated 2nd test	0.281 ± 0.014	0.257 ± 0.023	0.252 ± 0.037
	placebo 1st test	0.261 ± 0.092	0.240 ± 0.013	0.271 ± 0.019
	placebo 2nd test	0.258 ± 0.041	0.277 ± 0.017	0.276 ± 0.021
GH/GHBP (10^{-3})	treated 1st test	0.67 ± 0.20	0.75 ± 0.09	0.29 ± 0.06
	treated 2nd test	0.32 ± 0.19	1.32 ± 1.13	0.42 ± 0.16
	placebo 1st test	0.25 ± 0.17	0.83 ± 0.07 #	0.42 ± 0.08
	placebo 2nd test	0.18 ± 0.12	0.76 ± 0.09 #	0.51 ± 0.17

Values are means ± standard error; statistical significance $p < 0.05$. # Significant difference from basal, same group same trial.

concentration of the placebo trial was not significantly different (10.3 ± 0.7 vs 12.1 ± 1.4 mmol L^{-1}).

Growth hormone circulating levels at the end of the physical exercise were significantly higher than basal (Table 2). The plasma GH concentration patterns were the same in both trials. The patterns of the two BCAA trials demonstrated that, at the end of exercise, the concentrations were higher (also if not significantly) after the one-month treatment than before (33.84 ± 13.60 ng mL^{-1} before vs 12.33 ± 2.00 ng mL^{-1} after). GHBP circulating levels at the end of physical exercise were higher than basal as well as during recovery time (Table 2). The plasma GHBP concentration patterns were the same in both trials. But, also if not significantly, the end of exercise concentrations were higher after the one-month treatment than before (1,691 ± 556 pmol L^{-1} after vs 738 ± 85 pmol L^{-1} before).

Table 2 shows the circulating levels of GH and GHBP. Bound/free and GH/GHBP ratios are also demonstrated: no significant differences in former ratios were observed at any time; in the latter the measured differences (statistically not significant) suggest a tendency to increase the ratio at the end of exercise in all groups of athletes.

Discussion

The purpose of the present research was to investigate the effect of one month of BCAA supplementation on physical exercise when BCAA circulating

levels were raised by ingestion in well trained triathletes. The main findings were that the plasma lactate levels resulted lower, at the end of the exercise test, than before the one-month of BCAA supplementation treatment. At the end of the exercise plasma GH and GHBP increments (but statistically not significant $p = 0.14$ and $p = 0.7$ respectively) were also observed. A single dose of BCAA was ingested 30 minutes before the start of exercise. In agreement with the results of previous investigations it can be argued that BCAA circulating levels were artificially elevated by this ingestion so that the exercise bouts were carried out in an elevated BCAA availability for energy supply (De Palo et al., 1996; MacLean et al., 1996).

The amino acid ingestion, carried out immediately before the exercise bout, as used by Suminski et al. (1997) might have caused a weak availability of branched aminoacids in the skeletal muscle (Smith and Rennie, 1990; van Hall et al., 1996). In fact a different kinetic of circulating drugs can be suggested when the exercise starts immediately after the intake or thirty minutes after. Furthermore Calders and co-workers (1997) observed an increase in the plasma BCAA concentrations in trained rats five minutes after intra-peritoneal injection. Calders did not observe a difference, in the BCAA with respect to placebo, in the increased lactic acid concentration in rats after 30 min of sub-maximal exercise and after exercise until exhaustion. Vukovich et al. (1997) did not observe any influence of BCAA supplementation on blood lactate response. His protocol used graded short duration exercises and seven days of supplementation (untrained condition) but following 6 weeks of supplementation and training (anaerobic and aerobic combined), muscle lactate concentration was significantly reduced, but treated and control subjects did not demonstrate significant differences in muscle and blood lactate levels. Nevertheless the subjects did not have high circulating BCAA levels during the exercise test because the subjects had not ingested a BCAA dose at the start of the exercise bouts.

The key role of alanine in amino acid output from muscle and uptake by the liver is well known. The protein breakdown, resulting in BCAA degradation, increases during intense exercise with a subsequent alanine release increment (Consoli et al., 1990; Lemon, 1995; MacLean et al., 1996), in fact transamination of pyruvate forms alanine transferring the α-amino group and this reaction involves glutamate and α-ketoglutarate. In agreement with our previous observations (De Palo et al., 1993, 1996) MacLean et al. (1996) demonstrated a significantly greater amount of alanine during exercise in a BCAA trial than in a control trial.

The well known major alanine formation capacity in BCAA trial (De Palo et al., 1996; MacLean et al., 1996) suggested a large use of pyruvate both for alanine and acetyl-CoA production, the latter to participate in the citric acid cycle. Nevertheless a relation with the blood lactate pattern might exist; in fact in the present work the trial before the BCAA chronic treatment demonstrated significantly higher ($p < 0.05$) lactate levels than after (Fig. 1). MacLean et al. (1996) also observed that the lactate release and its arterial concentrations were all lower in the BCAA trial than in the control trial. Previous literature observations show that the branched-chain oxo acid dehy-

drogenase (BCOADH), the rate limiting enzyme in the catabolism of BCAA, inactive in skeletal muscle, is activated during exercise and by elevated BCAA intramuscular levels (van Hall et al., 1996; Jackman et al., 1997).

In the present work a significant lactate circulating level difference was measured comparing the end of exercise levels before and after the chronic BCAA supplementation. It can be speculated that the lower lactate level at the end of exercise, presumably released by the working muscle, may depend on the activation of this enzyme after chronic treatment. It can be hypothesised that it was related to a lower accumulation of pyruvate (and therefore of lactate) because of its conversion into alanine (Gibala et al., 1997).

It can be mentioned that alanine aminotransferase reaction is the major anaplerotic process in TCA cycle during exercise, in fact at the onset of exercise there is an accumulation of muscle pyruvate. When physical exercise is prolonged, the carbon skeleton, of the accumulated pyruvate, continues to be converted and to enter into TCA cycle; if its conversion in acetyl-CoA lessens, a reduction to lactate happens. A wider discussion of this topic is beyond the aim of the present paper, furthermore more researches are necessary before the mentioned mechanisms can be explained and described.

An eventual accumulation of substrates in the TCA cycle cannot be observed possibly because α-ketoglutarate, through transaminase, might follow the glutamate pathway. This hypothesis might justify a lessening of circulating lactate.

Moreover in agreement with Wagenmakers' observations (Wagenmaker's et al., 1989), the BCOADH activation can be postulated also in this work, favouring the BCAA muscle oxidation. A connection with Calders et al. (1997) could exist so that it might be that the mechanism responsible for the present finding is that the availability of BCAA lessens lactate.

In any case, in agreement also with other authors, the main suggestion that may be proposed in relation to the lower lactate levels after chronic/acute BCAA availability is an increased BCAA oxidation. The consequence might be an increased fuel availability and also an increased subtraction of pyruvate, consequently of lactate, that might form alanine through transamination using amino groups provided by amino acids and finally a reduced flux of lactate from the muscle to the blood. Also a variation in liver-muscle substrate exchange might be hypothesised but present results are unable to give any indication.

The GHBP levels demonstrated variations in the different analysed conditions. But these observations suggested that the chronic treatment might be able to influence the concentration of the GHBP, but this influence seems lesser than in GH levels. This last observation may be easier to notice taking into account the lower variations measured by the ratios.

Physical exercise is a potent natural stimulus for GH release, but the mechanism of this production is not known. Different actions, in relation to its function as a stress hormone or as a growth-promoting hormone can be hypothesised. Perhaps the mechanisms may be related to production of IGF-I by the liver, or to metabolism control in other districts such as in the adipose tissue.

By analysing the GHBP circulating levels, the eventual effect of exercise and chronic treatment can be studied, since the GHBP is strictly related to the GH receptor concentration. For the first time, it can be noted that present work showed a significant GHBP increase with physical exercise ($p < 0.05$); present results could also suggest an increased increment after the BCAA chronic treatment. These results agree with Roelen et al. (1997); in fact this authors recently observed that plasma GHBP levels were increased in healthy young subjects who followed a training programme.

In conclusion, taking into account that a BCAA oral supplementation leads to an increase in their plasma concentration within 20–30 min, and therefore that, at the start of the exercise test, the BCAA circulating levels are increased:

1) BCAA utilisation may induce an energy store compound saving process and the lower plasma lactate level, after intense muscular exercise, may reflect the improvement of energy use, due to the BCAA chronic treatment. BCAA treatment might improve muscular oxidation, including the lactate, which is better metabolised or less formed in the muscle, with less accumulation in the circulation;

2) the effects of chronic BCAA treatment on GH, GHBP might suggest an improvement of muscle oxidation capacity. Considering also the unchanged bound/free GH ratio, this improvement may be related to anabolic mechanisms of adaptation causing muscle growth or it might be correlated with mechanisms of adaptation at cellular level, such as enzyme activation.

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