The Clinical Pharmacology of Sport and Exercise
T. Reilly and M. Orme, editors

Branched-Chain Amino Acids and Endurance Performance

A.J.M. Wagenmakers

Department of Human Biology & Stable Isotope Research Centre, University of Maastricht, P.O. Box 616, 6200 MD Maastricht, The Netherlands

Key words: Ammonia, fatigue, glycogen phosphorylase deficiency, serotonin, tricarboxylic acid cycle.

1. INTRODUCTION

Well-trained endurance runners attempt to maintain a constant speed and an intensity between 70 and 90% of VO_2max during competitive marathons. When the muscle glycogen stores have been emptied before the finish has been reached, fatigue will occur and the speed (intensity) has to be reduced. A voluntary muscle contraction is the final step in a command chain that extends from the higher centres of the central nervous system to the actin and myosin filaments in the muscle; it involves electrical, metabolic and mechanical events. Consequently, many possible sites and mechanisms may be implicated in the fatigue process [1]. A distinction is usually made between central fatigue where the impairment is in the central nervous system and peripheral fatigue where the impairment is in the peripheral nerve or contracting muscle. Evidence has been presented that in well-motivated subjects a substantial component of fatigue can be attributed to events in the muscle leading to a loss of contractility [1,2]. Glycogen depletion, a decrease of the resting membrane potential as a consequence of potassium losses, failure of the calcium pump in the sarcoplasmic reticulum, an increase in free ADP and P_i concentration, and failure of the neuromuscular transmission have all been associated with muscle fatigue during prolonged exercise [1,2]. However, the point where the athlete has to reduce speed, often is not clear during prolonged exercise and it, therefore, cannot be excluded that central and psychological factors, such as motivation, mood and stamina, do contribute to endurance performance.

The compounds that we are dealing with in this chapter are the branched-chain amino acids (BCAA) - leucine, isoleucine and valine. Together they form three of the nine essential amino acids in man. It has been suggested that the BCAAs interfere with fatigue mechanisms both in the central nervous system and in skeletal muscle. Both proposed interactions will be described here in detail and a review will be given of performance trials and experimental research with BCAA and tryptophan supplements, that have been published to date, in order to judge 1) whether BCAA supplements influence performance and 2) whether there is experimental evidence in support of the proposed fatigue hypotheses.

2. BRANCHED-CHAIN AMINO ACIDS AS MUSCLE FUEL

After ingestion of a meal containing protein most of the amino acids are taken up and oxidised in the liver. However, the liver has a very low BCAA aminotransferase activity [3] and the BCAA largely escape from hepatic uptake and are transported to and metabolised in skeletal muscle. Skeletal muscle has an intermediate to high BCAA aminotransferase activity (reversible transamination with α-ketoglutarate as aminogroup acceptor) and a substantial activity of the branched-chain α-keto acid dehydrogenase (BCKAD) complex. The latter enzyme catalyses an irreversible oxidative decarboxylation step, a reaction which is rate-determining in BCAA oxidation in most tissues. Once decarboxylation has occurred the carbon-skeleton is lost for protein synthesis and there is no escape route left from more complete oxidation. In the late 1970's it was suggested that BCAA were the third fuel for skeletal muscle [4,5] on the basis of the measured enzyme activities and the large size of the muscle compartment (accounting for 40 to 50% of total body mass of a lean 70 kg subject). In isolated rat diaphragms the BCAA accounted for over 10% of the resting energy expenditure [4]. In the early 1980's the activity of the BCKAD-complex was shown to be regulated by a phosphorylation/dephosphorylation cycle with dephosphorylation causing inactivation. In human skeletal muscle only 4-6% of the enzyme was active at rest [6,7]. Exercise on a cycle ergometer after overnight fasting caused activation of the BCKAD-complex to about 20% active enzyme [6,7]. With stable isotope methodology ($^{13}CO_2$ production from L-[1-^{13}C]leucine) it was also shown that BCAA oxidation increased 4- to 5-fold during exercise in the overnight fasted state [8]. In the meantime it has become clear that the nutritional state of the subjects [7] and the glycogen content of the muscle [7,9] influence the activation of the BCKADH-complex by exercise. Carbohydrate loading the day before in combination with carbohydrate ingestion during exercise, as practised by endurance athletes during competition, prevented activation of the BCKADH-complex in trained subjects cycling for 2 h at 70-75% of Wmax [7]. Carbohydrate ingestion also dramatically reduces the $^{13}CO_2$ production from L-[1-^{13}C]leucine during exercise (Wagenmakers *et al.*, unpublished data). This implies that the oxidation of BCAA hardly increases during 2 h of competitive exercise at intensities of ≤ 75% VO_2max compared to rest. As the total energy expenditure increases up to 20-fold at such intensities due to increased oxidation of carbohydrates and fat, this implies that the contribution of BCAA oxidation to total energy expenditure is reduced during exercise to less than 1%. The BCAA, therefore, do not seem to play a role as a fuel during exercise of light, moderate and high intensity and from this point of view there is no reason for supplementation of BCAA during exercise.

3. THE CENTRAL FATIGUE HYPOTHESIS

In 1987 Newsholme and colleagues [10] launched the 'central fatigue hypothesis' as an important mechanism contributing to the development of fatigue during prolonged moderate intensity exercise. During exercise free fatty acids (FFA) are mobilised from adipose tissue and via the blood transported to muscle to serve as fuel. As a consequence the blood FFA concentration will increase. Both FFA and the amino acid tryptophan bind to albumin and compete for the same binding sites. Tryptophan will be disposed from binding to albumin by the increasing FFA concentration and, therefore, the free tryptophan concentration in the blood

will rise. Simultaneously, the increased oxidation of BCAA in muscle [6,7] will lead to a decrease of the sum concentration of the BCAA in the blood and the free tryptophan/BCAA ratio, therefore, will increase substantially. The increase in this ratio would lead to increased tryptophan transport across the blood-brain barrier, since BCAA and tryptophan compete for carrier-mediated entry into the central nervous system by the large neutral amino acid (LNAA) transporter [11-13]. Once taken up, conversion of tryptophan to serotonin (or 5-hydroxytryptamine) would occur and lead to a local increase of this neurotransmitter [11,12]. This increase indeed has been found in certain brain areas in the rat [12], but it has not been established whether it also occurs in man. According to the 'central fatigue hypothesis' the increase in serotoninergic activity would subsequently lead to central fatigue, forcing athletes to stop exercise or reduce running or cycling speed. In neurobiology it has been established that serotonin plays a role in the onset of sleep and that it is a determinant of mood and aggression. It is uncertain, though, that it also could play a role in the experience of fatigue during prolonged exercise as suggested in the 'central fatigue hypothesis'. One of the implications of the 'central fatigue hypothesis' is that ingestion of BCAA could reduce the exercise-induced increase of brain tryptophan uptake and thus delay fatigue and give athletes the ability to push on for a more prolonged period even when peripheral fatigue mechanisms come into operation. In other words BCAA would be ergogenic. Another implication is that ingestion of tryptophan prior to exercise would reduce time to exhaustion.

4. INTERACTION OF THE BCAA-AMINOTRANSFERASE REACTION WITH THE TRICARBOXYLIC ACID (TCA-) CYCLE IN MUSCLE.

The rate of ATP turnover in skeletal muscle among others is determined by the carbon-flux in the TCA-cycle. One possibility for achieving an increase in TCA-cycle activity going from rest to exercise (to meet the increased energy demand of exercise) is to increase the concentration of the TCA-cycle intermediates in muscle such that more substrate is available for the individual enzymatic reactions. This increase in concentration has indeed been observed for the most abundant TCA-cycle intermediates early during exercise [14,15] and is achieved by rapid conversion of the muscle glutamate pool into α-ketoglutarate [16]. The reaction used to achieve that increase is the alanine aminotransferase reaction: glutamate + pyruvate <--> α-ketoglutarate + alanine [16]. The alanine aminotransferase reaction is a near equilibrium reaction. This implies that the increase in muscle pyruvate concentration which occurs at the start of exercise due to an acceleration of glycolysis will automatically lead to production of alanine and α-ketoglutarate and consumption of glutamate. After the early increase of the concentration of TCA-cycle intermediates Sahlin *et al.* [15] observed a subsequent gradual decrease in human subjects exercising until exhaustion at 75% VO_2max.

Our group [7,17] has hypothesized that increased oxidation of the BCAA plays an important role in that subsequent decrease of the concentration of the TCA-cycle intermediates. The branched-chain α-keto acid dehydrogenase is increasingly activated during prolonged exercise leading to glycogen depletion [6,7,9] and an increase in oxidation by definition will increase the flux through the BCAA aminotransferase step. In the case of leucine this reaction will put a net carbon drain on the TCA-cycle as the carbon-skeleton of leucine is oxidised to 3 acetyl-CoA molecules and the aminotransferase step takes away α-ketoglutarate: leucine + α-ketoglutarate <--> 3 acetyl-CoA + glutamate. Increased oxidation

of valine and isoleucine will not lead to net removal of TCA-cycle intermediates as the carbon skeleton of valine is oxidised to succinyl-CoA and that of isoleucine to both succinyl-CoA and acetyl-CoA. Net removal of α-ketoglutarate via leucine transamination can be compensated by the alanine aminotransferase reaction (see above) as long as muscle glycogen is available and the muscle pyruvate concentration is kept high. However, as activation of the BCKAD-complex is highest in glycogen depleted muscle this mechanism eventually is expected to lead to a decrease in the concentration of TCA-cycle intermediates, a reduced TCA-cycle activity, a reduction of the ATP turnover rates which via increases in free ADP and P_i will offset the known muscle fatigue mechanisms.

The BCAA are rapidly extracted by the leg muscles after their oral ingestion [9] and this is accompanied by activation of the BCKADH-complex at rest and increased activation during exercise [9]. This could imply that the indicated carbon-drain on the TCA-cycle is larger after BCAA ingestion and that BCAA ingestion by this mechanism causes premature fatigue during prolonged exercise leading to glycogen depletion.

This implies that two interactions have been suggested in the literature by which BCAA may interact with metabolism in both central nervous system and in muscle. One of the interactions predicts that BCAA supplementation is ergogenic, while the other predicts that it will lead to premature fatigue. In the next section the performance trials will be reviewed to judge whether there is experimental evidence in support of these interactions or whether the proposed hypotheses have only theoretical rather than practical relevance.

5. EFFECT OF BCAA AND TRYPTOPHAN INGESTION/INFUSION ON ENDURANCE PERFORMANCE

The effect of BCAA ingestion on physical performance was investigated for the first time in a field test by Blomstrand *et al.* [18]. Male subjects (193) were studied during a marathon in Stockholm. Subjects were randomly (without matching) divided into an experimental group receiving 16 g of BCAA in plain water during the race and the placebo group receiving flavoured water. The subjects additionally had ad libitum access to carbohydrate (CHO)-containing drinks. No difference was observed in the marathon time of the two groups. However, when the original subject group was divided into groups of fast and slower runners, then a small significant reduction in marathon time was observed in the slower runners only. Retrospectively this first study has been the one and only study claiming a positive effect of BCAA ingestion during exercise. However, three main criticisms can be raised against its design: 1) in a performance test investigating a potentially ergogenic effect subjects in the two groups should have been matched for previous performance; 2) CHO intake and nutritional status should have been controlled and matched in the two groups; and 3) division of subjects in a group of fast and slower runners, taking an arbitrary marathon time as selection criterion is not in accordance with accepted statistical methods. Each of these points may have biased the data obtained in [18].

Verger *et al.* [19] failed to find an effect of intragastric BCAA supplementation on time to exhaustion in rats running on a treadmill in comparison with a "water control".

Varnier *et al.* [20] investigated 6 moderately trained subjects after glycogen depleting exercise followed by overnight fasting. Subjects were investigated the following morning during graded incremental exercise to exhaustion and received an intravenous infusion of BCAA (260

mg/kg/h for 70 min) or saline only. No significant differences were observed between the tests in total work performed.

Blomstrand and colleagues [21] also investigated performance in the laboratory in five male endurance-trained subjects during exhaustive exercise on a cycle ergometer at a work-rate corresponding to 75% of VO_2max after reduction of their muscle glycogen stores. During exercise the subjects were given in random order a 6% CHO solution containing 7 g/l of BCAA, a 6% CHO solution and flavoured water. The positive effect of the field test was not confirmed in this controlled laboratory study as no difference in performance was seen when the subjects were given CHO + BCAA or only CHO.

Madsen and colleagues [22] recently investigated performance in 9 trained cyclists in a 100 km time trial in the laboratory. Subjects used their own bike at a freely chosen power output, simulating field conditions, and were studied while ingesting flavoured water only (placebo), a 5% carbohydrate solution (66 g/h) and carbohydrates (66 g/h) plus BCAA (6.8 g/h). There was no difference between treatments in the time needed to finish the 100 km.

As the 'central fatigue hypothesis' has two implications (BCAA ingestion would improve performance and tryptophan ingestion would reduce time to exhaustion) Van Hall and colleagues [23] designed an experiment in which both aspects of the 'central fatigue hypothesis' were investigated. Ten endurance-trained male athletes were studied during cycle exercise at 70-75% of Wmax, while ingesting, in random order and double-blind, drinks that contained 6% sucrose (control) or 6% sucrose supplemented with 1) tryptophan (3 g/l), 2) a low dose of BCAA (6g/l; comparable to ref [18]) and 3) a high dose of BCAA (18 g/l). These treatments greatly increased the plasma concentration of the respective amino acids to values well outside the normal physiological range. As the concentration of all amino acids competing for transport by the large neutral amino acid carrier were measured, Van Hall and colleagues [23] were able to calculate the rate of unidirectional influx of circulating plasma tryptophan into the brain using kinetic parameters of transport of human brain capillaries reported by Hargreaves and Pardridge [24].

These calculations showed that the BCAA gifts only reduced tryptophan transport at exhaustion by 8-12%, while tryptophan ingestion caused an increase of 600 to 1900% (depending on the use of free or total tryptophan concentration in the calculations). Despite these massive differences in tryptophan transport, the time to exhaustion was not different between the four treatments. Van Hall and colleagues, therefore, concluded that manipulation of tryptophan supply to the brain by ingestion of BCAA and tryptophan in drinks containing CHO either did not change the serotonin concentration in relevant local areas in the brain or that a change in serotoninergic activity during prolonged exercise contributed little to mechanisms of fatigue. Two earlier studies [25,26] had investigated the effect of lower doses of tryptophan. Segura and Ventura [25] in fact reported that 1.2 g of L-tryptophan supplementation taken in 300 mg doses over a 24-hour period before exercise increased total exercise time by 49% in 12 subjects who were running at 80% of maximal oxygen uptake (in full contradiction to the 'central fatigue hypothesis'). The results of this study were questioned by Stensrud *et al.* [26]. They studied 49 well trained males in a randomized double-blind placebo experiment. Subjects in the tryptophan group (n=24) and placebo group (n=25) were matched for performance (maximal oxygen uptake, "anaerobic threshold" and speed during an all-out run). Tryptophan ingestion (again 1.2 g over a 24 h period prior to the run) had no effect on running performance, when subjects ran until exhaustion at a speed corresponding to 100% of their VO_2max.

Wagenmakers *et al.* [17] investigated the effect of BCAA ingestion (20 g) 30 min prior to graded incremental exercise in two patients with McArdle's disease (muscle glycogen phosphorylase deficiency). Ingestion of BCAA reduced the Wmax that was reached during the tests by about 20% and heart rate and perceived exertion were higher at the same workload. Ingestion of the keto acids of the BCAA (without the amino group) improved performance in the patients [17]. Ingestion of BCAA increased the production of ammonia by muscle and plasma ammonia concentration during exercise both in these patients [17] and in healthy controls [22,23,27,28]. Wagenmakers [27] also observed a higher heart rate after BCAA ingestion in glycogen depleted subjects exercising to exhaustion at 75% Wmax.

6. CONCLUSIONS

The conclusion after review of the performance studies is that neither BCAA ingestion nor tryptophan ingestion has an effect on endurance performance in healthy subjects. This implies that the performance studies do not provide experimental support for the 'central fatigue hypothesis' of Newsholme and colleagues nor for the proposed interaction of BCAA with the TCA-cycle in skeletal muscle proposed by our own group. The data obtained in the patients with McArdle's disease may contain the message that BCAA ingestion may be disadvantageous at muscle level via the proposed mechanism in conditions where there is no co-ingestion of CHO and where the glycogen stores of the body have simultaneously been emptied. As ammonia has been suggested to lead to central fatigue and loss of motor-coordination [29] great care also seems to be indicated with the use of BCAA supplements in sport activities where performance depends on motor-coordination (e.g. tennis and soccer). As there are no positive effects of BCAA supplementation on performance, athletes are advised not to use BCAA supplements.

REFERENCES

1. R.H. Fitts. Physiol. Rev., 74 (1994) 49.
2. B. Bigland-Ritchie, J.J. Woods. Muscle Nerve, 11 (1984) 691.
3. A.J.M. Wagenmakers, P.B. Soeters. In: L.A. Cynober, ed. Amino Acid Metabolism and Therapy in Health and Nutritional Disease. New York: CRC Press (1995) 67.
4. A.L. Goldberg, T.W. Chang. Federation Proc., 37 (1978) 2301.
5. F.L. Shinnick, A.E. Harper. Biochim. Biophys. Acta, 437 (1976) 477.
6. A.J.M. Wagenmakers, J.H. Brookes, J.H. Coakley, T. Reilly, R.H.T. Edwards. Eur. J. Appl. Physiol., 59 (1989) 159.
7. A.J.M. Wagenmakers, E.J. Beckers, F. Brouns, H. Kuipers, P.B. Soeters, G.J. van der Vusse, W.H.M. Saris. Am. J. Physiol., 260 (1991) E883.
8. J. Knapik, C. Meredith, B. Jones, R. Fielding, V. Young, W. Evans. J. Appl. Physiol., 70 (1991) 43.
9. G. van Hall, D.A. MacLean, B. Saltin, A.J.M. Wagenmakers. J. Physiol., 494 (1996) 899.
10. E.A. Newsholme, I.N. Acworth, E. Blomstrand. In: G. Benzi, ed. Advances in Myochemistry. London: John Libby Eurotex (1987) 127.

11. P.J. Knott, G. Curzon. Nature, 239 (1972) 452.
12. F. Chaouloff, J.L. Elghozi, Y. Guezennec, D. Laude. Br. J. Pharmacol., 86 (198) 33.
13. F. Chaouloff, G.A. Kennett, B. Serrurrier, D. Merino, G. Curzon. J. Neurochem., 46 (1986) 1647.
14. B. Essen, L Kaijser. J. Physiol., 281 (1978) 499.
15. K. Sahlin, A. Katz, S. Broberg. Am. J. Physiol., 269 (1990) C834.
16. G. van Hall, B. Saltin, G.J. van der Vusse, K. Söderlund, A.J.M. Wagenmakers. J. Physiol., 489 (1995) 251.
17. A.J.M. Wagenmakers, J.H. Coakley, R.H.T. Edwards. Int. J. Sports Med., 11 (1990) S101.
18. E. Blomstrand, P. Hassmén, B. Ekblom, E.A. Newsholme. Eur. J. Appl. Physiol., 63 (1991) 83.
19. Ph. Verger, P. Aymard, L. Cynober, G. Anton, R. Luigi. Physiol. Behaviour, 55 (1994) 523.
20. M. Varnier, P. Sarto, D. Martines, L. Lora, F. Carmignoto, G.P. Leese, R. Naccarato. Eur. J. Appl. Physiol., 69 (1994) 26.
21. E. Blomstrand, S. Andersson, P. Hassmén, B. Ekblom, E.A. Newsholme. Acta Physiol. Scand., 153 (1995) 87.
22. K. Madsen, D.A. MacLean, B. Kiens, D. Christensen. J. Appl. Physiol., (1996) In Press.
23. G. van Hall, J.S.H. Raaymakers, W.H.M. Saris, A.J.M. Wagenmakers. J. Physiol., 486 (1995) 789.
24. K.M. Hargreaves, W.M. Pardridge. J. Biol. Chem., 263 (1988) 19392.
25. R. Segura, J.L. Ventura. Int. J. Sports Med., 9 (1988) 301.
26 T. Stensrud, F. Ingjer, H. Holm, S.B. Strømme. Int. J. Sports Med., 13 (1992) 481.
27. A.J.M. Wagenmakers. In: P. Marconnet, P.V. Komi, B. Saltin, O.M. Sejersted, eds. Muscle Fatigue Mechanisms in Exercise and Training. Basel: Karger, Med. Sport Sci. Series volume 34 (1992) 69.
28. D.A. MacLean, T.E. Graham, B. Saltin. J. Physiol., 493 (1996) 909.
29. E.W. Banister, B.J.C. Cameron. Int. J. Sports Med., 11 (1990) S129.

Discussion: Branched-Chain Amino Acids And Endurance Performance

M. Orme:
The amino acid tryptophan has been used for some time as a treatment for depression because its metabolite, 5-hydroxy tryptamine (5-HT) is an important neurotransmitter amine. However tryptophan has been largely abandoned because of a lack of efficacy. In some of the early studies, before the days of comprehensive Ethics committees, concentrations of 5-HT were measured in the cerebrospinal fluid (CSF). However little correlation could be found between CSF concentrations of 5-HT and the dose of tryptophan or the blood level of tryptophan. This helps to confirm your data in the exercise field.

A.J.M. Wagenmakers:
So what you are suggesting really is that an increase or decrease of tryptophan intake or blood tryptophan concentration will not lead to a change in serotoninergic activity in athletes during exercise.

M. Orme:
There is some doubt as to whether the CSF concentrations of 5-HT as measured from a lumbar puncture sample will correlate with CSF concentrations elsewhere in the brain. However with this proviso in the field of depression, it ties in well with your negative effects of exercise.

T.E. Graham:
I would like to mention that Marty Gabela from our lab has recently looked at the individual TCA cycle intermediates and it is very clear that in all exercise situations every intermediate has its own response and you can not predict them from any other intermediate. Alpha-ketoglutarate falls with exercise continuously, but that does not necessarily mean that it is limiting. I think that your own data with branched-chain amino acid supplementation would suggest that it is not impeding exercise endurance.

A.J.M. Wagenmakers:
Why do you make that last comment?

T.E. Graham:
Well, you give branch-chain amino acid supplements and exercise them to exhaustion and they are not exhausting more quickly and yet you might expect them to have a greater drain of the alpha-ketoglutarate.

A.J.M. Wagenmakers:
I think that there are various interactions between the metabolism of branched-chain amino acids and the TCA-cycle. In extreme situations, where there is no access to glycogen, such as in patients with muscle glycogen phosphorylase deficiency, supplementation with BCAA leads to premature fatigue and an increased heart rate. This warns me that we should be careful with the conclusion that BCAA supplementation does not impede endurance exercise in athletes in competitive field events. Glycogen levels reached during competitive exercise

in the field are much lower than ever can be reached in the exercise laboratory. In our laboratory tests performed in subjects after glycogen lowering exercise BCAA supplementation increased plasma ammonia concentration and heart rate, but it had no effect on time to exhaustion. In conditions where there is ample carbohydrate available, such as in the tests where we gave a mixture of BCAA and glucose, supplementation only increased plasma ammonia concentration and had no effect on heart rate and time to exhaustion. The alanine aminotransferase reaction may be used in that case to regenerate α-ketoglutarate and prevent a carbon drain on the TCA cycle. So I feel that the last words on the carbon-drain of α-ketoglutarate by the BCAA aminotransferase reaction have not been spoken, but I agree with you in that we should have more data on the muscle concentration of TCA cycle intermediates, so I am glad that your lab has measured some.