Interrelationship between Physical Activity and Branched-Chain Amino Acids[1]

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ABSTRACT Some athletes can have quite high intakes of branched-chain amino acids (BCAAs) because of their high energy and protein intakes and also because they consume protein supplements, solutions of protein hydrolysates, and free amino acids. The requirement for protein may actually be higher in endurance athletes than in sedentary individuals because some amino acids, including the BCAAs, are oxidized in increased amounts during exercise compared with rest, and they must therefore be replenished by the diet. In the late 1970s, BCAAs were suggested to be the third fuel for skeletal muscle after carbohydrate and fat. However, the majority of later studies, using various exercise and treatment designs and several forms of administration of BCAAs (infusion, oral, and with and without carbohydrates), have failed to find a performance-enhancing effect. No valid scientific evidence supports the commercial claims that orally ingested BCAAs have an anticatabolic effect during and after exercise in humans or that BCAA supplements may accelerate the repair of muscle damage after exercise. The recommended protein intakes for athletes (1.2 to 1.8 g · kg body mass^{-1} · d^{-1}) do not seem to be harmful. Acute intakes of BCAA supplements of about 10–30 g/d seem to be without ill effect. However, the suggested reasons for taking such supplements have not received much support from well-controlled scientific studies. J. Nutr. 135: 1591S–1595S, 2005.

KEY WORDS: • *exercise* • *branched-chain amino acids* • *supplements*

Among the athletic population, several groups of athletes can be identified that consume relatively large amounts of branched-chain amino acids (BCAAs). The BCAAs can be obtained from one of 4 possible sources: whole-food proteins, protein supplements, solutions of protein hydrolysates and free amino acids. The reasons why athletes can have quite high intakes of BCAAs include the following 4 conditions.

1. Higher total energy intakes and therefore higher protein intakes. The BCAAs, leucine, isoleucine, and valine, represent 3 of the 20 amino acids that are used in the formation of proteins. Thus, on average, the BCAA content of food proteins is about 15% of the total amino acid content. If we take an average man with a sedentary lifestyle, his daily energy intake will be about 10 MJ/d, and, let us say that 15% of this will come from protein. Thus, he consumes about 1500 kJ as protein, which is equivalent to about 63 g. Therefore, his intake of BCAAs is about 9.5 g. Contrast this with a Tour de France cyclist: the energy intake of these elite athletes has been measured as averaging about 25 MJ/d over a 2–3 wk period (1,2). The proportion of protein in the diet may be a little less, as much of the extra energy consumed is in the form of carbohydrate but, even so, the protein content of the diet is still about 12% of the total energy content. Thus, the elite cyclist consumes about 3000 kJ as protein, which is equivalent to 126 g, which in turn represents about 19 g of BCAA, or twice the amount of the sedentary individual. The requirement for protein may actually be higher in the endurance athlete, because some amino acids, including the BCAAs, are oxidized in increased amounts during exercise compared with rest (3).

2. Deliberate consumption of high-protein diets and protein supplements. Debate has always raged over how much dietary protein is required for optimal athletic performance, partly because muscle contains a large proportion of the protein in a human body (about 40%). Muscle also accounts for 25% to 35% of all protein turnover in the body. Both the structural proteins that make up the myofibrils and the proteins that act as enzymes within a muscle cell can change as an adaptation to exercise training. Indeed, muscle mass, muscle-protein content, and muscle-protein composition change in response to training. Interest in protein consumption is very high among amateur and professional athletes. Therefore, the fact that meat, which contains high-quality protein, is a very popular protein source for athletes (especially strength athletes) is not surprising. This preference for meat probably dates

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back to ancient Greece where athletes preparing for the Olympic games consumed large quantities of meat.

A strong belief among many athletes is that a high protein intake or certain protein or amino acid supplements increase muscle mass and strength. Despite the long history of protein use in sports, debate continues over even a simple question such as whether protein requirements are increased in athletes, and no uniform opinion exists as to what should be measured as an end point. A large intake of dietary protein is common practice in weightlifters and body builders (3). Daily protein intakes as high as 3 g/kg body mass (b.m.)[3] are not uncommon in these sports, where the aim is to develop a large muscle mass. For a 100-kg individual, this means a protein intake of 300 g/d and therefore a daily BCAA intake of about 45 g (450 mg/kg b.m.).

3. Consumption of protein hydrolysates or mixtures of essential amino acids during and after exercise. Protein hydrolysates are produced from purified protein sources (e.g., casein) by heating with acid or, more usually, by addition of proteolytic enzymes followed by purification procedures. Such hydrolysates contain peptides of which up to about 40% may be dipeptides and tripeptides. Consumption of amino acids as dipeptides and tripeptides results in faster absorption into the blood stream compared with the ingestion of whole proteins or single amino acids. This is a desirable characteristic for athletes who wish to maximize amino acid delivery to the muscles, although whether this has a practical effect of improving muscle protein synthesis, accretion of muscle mass, or improved recovery from exercise has not yet been established. Nevertheless, this possibility remains attractive to consumers, and there is some strong recent evidence that carbohydrates consumed together with protein hydrolysates with added leucine and phenylalanine promote higher insulin secretion than can be achieved with carbohydrate intake alone (4). The potential advantages of this effect are that it may *a*) further promote muscle glucose uptake and stimulate muscle glycogen synthesis and so both increase the stores of this important fuel before exercise and enhance its restoration in the recovery phase after exercise and *b*) stimulate muscle amino acid uptake and protein synthesis during recovery from exercise.

4. Consumption of drinks containing BCAA. Although this practice is not common, because few drinks containing significant amounts of BCAAs are commercially available, there are some studies that have suggested that BCAA intake during prolonged exercise may enhance endurance performance by delaying the onset of central fatigue. The 3 BCAAs are classed as essential amino acids because they cannot be synthesized in the body. Yet, they are oxidized during exercise, and they must, therefore, be replenished by the diet. In the late 1970s, BCAAs were suggested to be the third fuel for skeletal muscle after carbohydrate and fat (5). BCAAs are sometimes supplied to athletes in energy drinks to provide extra fuel. Claims have also been made that BCAA supplementation can reduce net protein breakdown in muscle during exercise and reduce fatigue and enhance performance via effects on the brain.

BCAAs as a fuel for exercise

Although early studies suggested that BCAAs can act as a fuel during exercise in addition to carbohydrate and fat, it has been shown that the activities of the enzymes involved in the oxidation of BCAAs are too low to allow a major contribution of BCAAs to energy expenditure (6,7). Detailed studies with a ^{13}C-labeled BCAA (^{13}C-leucine) showed that the oxidation of BCAAs only increases 2- to 3-fold during exercise, whereas the oxidation of carbohydrate and fat increases 10- to 20-fold (8,9). Also, carbohydrate ingestion during exercise can prevent the increase in BCAA oxidation. BCAAs, therefore, do not seem to play a major role as a fuel during exercise, and from this point of view, the supplementation of BCAAs during exercise is unnecessary (10).

BCAAs and protein turnover

The claims that BCAAs reduce protein breakdown were initially based on early in vitro studies, which showed that adding BCAAs to an incubation or perfusion medium stimulated tissue protein synthesis and inhibited protein degradation. Several in vivo studies in healthy individuals (11–13) failed to confirm the positive effect on protein balance that had been observed in vitro. However, several studies in recent years have inferred an anabolic effect of leucine or the BCAAs on muscle protein breakdown and a stimulatory effect on muscle protein synthesis [see the article by Dwight Matthews (14) in this supplement for a review]. Very recent work suggests that leucine itself, not its metabolites, acts as a signal to stimulate protein synthesis (15). Furthermore, it has just been reported that coingestion of protein and leucine with carbohydrate stimulates muscle protein synthesis and optimizes whole-body protein balance when compared with the intake of carbohydrate only after a 45-min bout of resistance exercise (16). Thus, evidence is accumulating now that supports the commercial claims that orally ingested BCAAs have an anticatabolic effect during and after exercise. It is possible that BCAA supplements could also accelerate the repair of muscle damage after exercise, though evidence for this is not yet available (10).

BCAAs and central fatigue

The "central fatigue hypothesis," which is illustrated in **Figure 1**, was proposed in 1987 as an important mechanism contributing to the development of fatigue during prolonged exercise (17). This hypothesis predicts that during exercise, FFAs are mobilized from adipose tissue and are transported via the blood to the muscles to serve as fuel. Because the rate of mobilization is greater than the rate of uptake by the muscle, the blood FFA concentration increases. Both FFAs and the amino acid tryptophan bind to albumin and compete for the same binding sites. Tryptophan is prevented from binding to albumin by the increasing FFA concentration, and, therefore, the free tryptophan (fTRP) concentration and the fTRP: BCAA ratio in the blood rises. Experimental studies in humans have confirmed that these events occur. The central fatigue hypothesis predicts that the increase in the fTRP: BCAA ratio results in an increased fTRP transport across the blood–brain barrier, because BCAA and fTRP compete for carrier-mediated entry into the central nervous system by the large neutral amino acid (LNAA) transporter (18,19). Once taken up, the conversion of tryptophan to serotonin [5-hydroxytryptamine (5-HT)] occurs and leads to a local increase of this neurotransmitter (19). Serotonin plays a role in the onset of sleep and is a determinant of mood and aggression. Therefore, the increase in serotoninergic activity might subsequently lead to central fatigue, forcing athletes to stop exercise or reduce the exercise intensity. The assumption that increased fTRP uptake leads to increased serotonin synthesis

[3] Abbreviations used: 5-HT, 5-hydroxytryptamine; b.m., body mass; fTRP, free tryptophan; LNAA, large neutral amino acid.

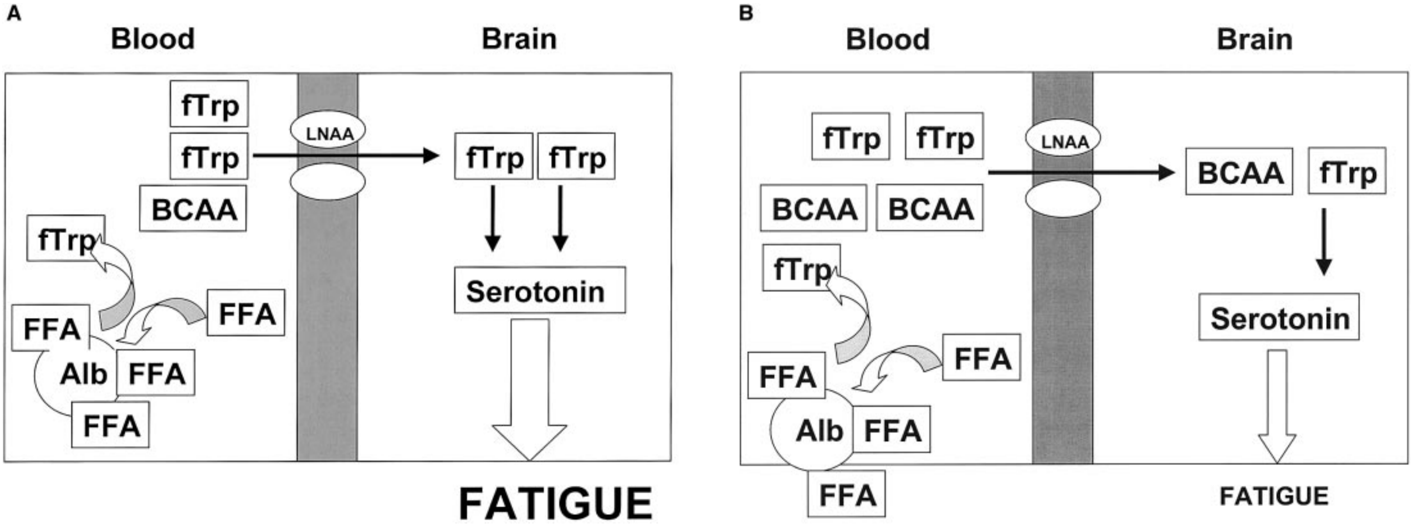

FIGURE 1 Central fatigue hypothesis. *A*) The central fatigue hypothesis proposes that during exercise FFAs are mobilized from adipose tissue and are transported via the blood to the muscles to serve as fuel. Because the rate of mobilization is greater than the rate of uptake by the muscle the circulating FFA concentration increases. Both FFA and the fTRP bind to albumin and compete for the same binding sites. fTRP is displaced from binding to albumin by the increasing FFA concentration and therefore the fTRP concentration and the fTRP:BCAA ratio in the blood rises. Experimental studies in humans have confirmed that these events occur. The central fatigue hypothesis predicts that the increase in this ratio results in an increased fTRP transport across the blood–brain barrier, because BCAAs and fTRP compete for carrier-mediated entry into the central nervous system by the LNAA transporter. Once taken up, the conversion of fTRP to serotonin (5-HT) occurs and leads to a local increase of this neurotransmitter. It has been well established that serotonin plays a role in the onset of sleep and that it is a determinant of mood and aggression. It was therefore hypothesized that the increase in serotoninergic activity subsequently leads to central fatigue, forcing athletes to stop exercise or to reduce the exercise intensity. *B*) The involvement of plasma fTRP and BCAAs in the central fatigue hypothesis also predicts that ingestion of BCAAs will raise the plasma BCAA concentration and hence reduce transport of fTRP into the brain. Subsequent reduced formation of serotonin may alleviate sensations of fatigue and hence improve endurance exercise performance.

and activity of serotoninergic pathways (i.e., increased synaptic serotonin release) is a rather large "leap of faith."

The central fatigue hypothesis also predicts that ingestion of BCAAs will raise the plasma BCAA concentration and, hence, reduce transport of fTRP into the brain. Subsequent reduced formation of serotonin may alleviate sensations of fatigue and in turn improve endurance exercise performance.

Effects of BCAA ingestion on exercise performance

The effect of BCAA ingestion on physical performance was investigated for the first time in a field test by Blomstrand et al. (20). A total of 193 male subjects were studied during a marathon in Stockholm. The subjects were randomly divided into an experimental group receiving BCAAs in plain water and a placebo group receiving flavored water. The subjects also had free access to carbohydrate-containing drinks. No difference was observed in the marathon time of the 2 groups. However, when the original subject group was divided into faster and slower runners, a significant reduction in marathon time was observed in subjects given BCAAs in the slower runners only. This study has since been criticized for its design and statistical analysis. For example, fluid and carbohydrate ingestion were not controlled during the race, subjects receiving BCAAs were not matched to controls in terms of previous performance, and the retrospective division of subjects into groups relating to their performance times in the race has been criticized as statistically invalid.

A study that examined the effect of BCAA ingestion during exercise in the heat (ambient temperature of 34°C) has provided some further evidence in support of these early findings (21). A 14% increase in the capacity to perform relatively low intensity exercise (40% $\dot{V}O_{2max}$) was reported after BCAA supplementation compared with placebo. No difference in peripheral markers of fatigue was reported between the BCAAs and placebo treatments and the BCAA supplementation (which began 1 h before the start of exercise) resulted in a 2- to 3-fold reduction in the plasma ratio of fTRP to BCAAs. The capacity to perform prolonged exercise is reduced at high ambient temperatures, and this premature fatigue is not adequately explained by peripheral mechanisms. Indeed, there is now some convincing evidence that central fatigue plays an important role in limiting exercise capacity in the heat (22). However, 2 recent studies that have examined the effects of BCAA supplementation (~20 g BCAA consumed before and during exercise) on cycling exercise capacity

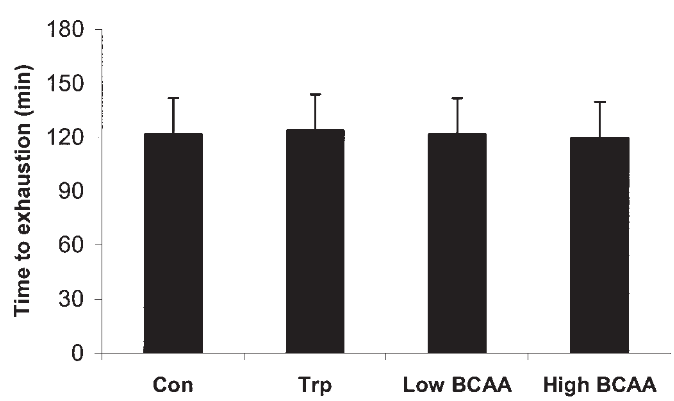

FIGURE 2 Time to exhaustion during cycling at 75–80% $\dot{V}O_{2max}$. No effect is seen with drinks containing tryptophan (3 g/L), a small dose of BCAAs (6 g/L), or a large dose of BCAAs (18 g/L) compared with the control drink. Data from van Hall et al. (27).

in the heat at 2 different exercise intensities (60% $\dot{V}O_{2max}$ at 35°C and 50% $\dot{V}O_{2max}$ at 30°C) failed to find any effect on exercise capacity or ratings of perceived exertion (23,24).

Indeed, the majority of studies, using various exercise and treatment designs and several forms of administration of BCAAs (infusion, oral, and with and without carbohydrates), have failed to find a performance-enhancing effect (25–29). Van Hall et al. (27) studied time-trial performance in trained cyclists consuming carbohydrate during exercise with and without BCAAs. A high and a low dose of BCAAs were given, but no differences were seen in time-trial performance (see **Fig. 2**).

If the central fatigue hypothesis is correct and the ingestion of BCAAs reduces the exercise-induced increase of brain fTRP uptake and thereby delays fatigue, the opposite must also be true, that is, ingestion of tryptophan before exercise should reduce the time to exhaustion. A few studies have included supplemental tryptophan in human subjects before or during exercise (27,30) and from these studies the conclusion must be drawn that tryptophan has no effects on exercise performance.

The effect of chronic administration of BCAAs on exercise performance has also been examined (31). After 2 wk of BCAA supplementation (16 g/d), performance of a 40-km cycling time trial in temperate ambient conditions was improved by 12% compared with placebo. However, the data from this study are still not published as a full paper, precluding any definitive conclusions on those results. The influence of chronic BCAA supplementation on exercise performance warrants further investigation.

BCAAs and immune responses to exercise

Prolonged strenuous exercise is associated with a temporary immunodepression that affects macrophages, neutrophils, and lymphocytes (32,33). The mechanisms involved are not fully established and appear to be multifactorial, including hormonal actions (e.g., of catecholamines and cortisol), inhibition of macrophage and T-cell cytokine production, altered heat shock protein expression, and a fall in the plasma concentration of glutamine (34). BCAAs are nitrogen donors for glutamine synthesis, and some studies have evaluated the effectiveness of BCAA supplements during exercise to maintain the plasma glutamine concentration and to modify immune responses to exercise. One recent study showed that BCAA supplementation (6 g/d) for 2–4 wk and a 3 g dose 30 min before a long distance run or a triathlon race prevented the 24% fall in the plasma glutamine concentration observed in the placebo group and also modified the immune response to exercise (35). These authors reported that BCAA supplementation did not affect the lymphocyte proliferative response to mitogens before exercise but did prevent the 40% fall in lymphocyte proliferation observed after exercise in the placebo group. Furthermore, blood mononuclear cells obtained from athletes in the placebo group after exercise presented a reduction in the production of several cytokines, including TNF-α, INF-γ, IL-1, and IL-4 compared with before exercise. BCAA supplementation restored the production of TNF-α and IL-1 and increased that of IFN-γ. However, athletes given BCAA supplements presented an even greater reduction in IL-4 production after exercise. However, there were flaws in the experimental design and statistical analysis of the data in this study, and the results need to be confirmed in more controlled studies. Because several previous studies have indicated that glutamine supplementation during exercise does not prevent the exercise-induced fall in lymphocyte proliferation (36,37), these findings must be viewed with some caution.

Do high intakes of protein or amino acids cause health risks for athletes?

Excessive protein intake (>3 g · kg b.m.$^{-1}$ · d^{-1}) may have various negative effects, including kidney damage, increased blood lipoprotein levels (which has been associated with arteriosclerosis), and dehydration. The latter may occur as a result of increased nitrogen excretion in urine, which results in increased urinary volume and dehydration. Athletes consuming a high-protein diet therefore must increase their fluid intake to prevent dehydration. The recommended protein intakes for athletes (1.2 g · kg b.m.$^{-1}$ · d^{-1} to 1.8 g · kg b.m.$^{-1}$ · d^{-1}) and up to ~2 g · kg b.m. $^{-1}$ · d^{-1} (3) do not seem to be harmful. Acute intakes of BCAA supplements of about 10–30 g/d seem to be without ill effect. However, intake of individual amino acids has no added nutritional value compared with the intake of proteins containing these amino acids.

Despite the lack of strong evidence for the efficacy of BCAA supplements, athletes continue to use them. However, normal food alternatives are available and are almost certainly cheaper. For example, a typical BCAA supplement sold in tablet form contains 100 mg valine, 50 mg isoleucine, and 100 mg leucine. A chicken breast (100 g) contains ~470 mg valine, 375 g isoleucine, and 656 mg leucine, the equivalent of about 7 BCAA tablets. One quarter of a cup of peanuts (60 g) contains even more BCAA and is equivalent to 11 tablets.

What do studies on athletes tell us about the safe upper limits of BCAA intake?

The studies on protein and BCAA supplementation that have been conducted on physically active humans indicate that a rather large dietary excess of the 3 BCAAs is well tolerated when consumed in diets containing surfeit amounts of protein. Ingestion of BCAAs in the diet up to 450 mg · kg b.m.$^{-1}$ · d^{-1}, which is a little over 3 times the estimated average requirement, appears to cause no adverse effects in healthy adults. Furthermore, the ingestion of acute doses of BCAA supplements containing all 3 BCAAs appears to be well tolerated by adults in amounts up to 450 mg/kg b.m.

LITERATURE CITED

1. Brouns, F., Saris, W.H.M., Stroecken, J., Beckers, E., Thijssen, R., Rehrer, N. J. & ten Hoor, F. (1989) Eating, drinking, and cycling. A controlled Tour de France simulation study, part I. Int. J. Sports Med. 10: S32–S40.

2. Saris, W.H.M., van Erp-Baart, M. A., Brouns, F., Westerterp, K. R. & ten Hoor, F. (1989) Study on food intake and energy expenditure during extreme sustained exercise: the Tour de France. Int. J. Sports Med. 10: S26–S31.

3. Jeukendrup, A. E. & Gleeson, M. (2004) Sports Nutrition: An Introduction to Energy Production and Performance. Human Kinetics, Champaign, IL.

4. Manninen, A. H. (2004) Protein hydrolysates in sports and exercise: a brief review. J. Sports Sci. Med. 3: 60–63.

5. Goldberg, A. L. & Chang, T. W. (1978) Regulation and significance of amino acid metabolism in skeletal muscle. Fed. Proc. 37: 2301–2307.

6. Wagenmakers, A.J.M., Brookes, J. H., Coakley, J. H., Reilly, T. & Edwards, R.H.T. (1989) Exercise-induced activation of branched-chain 2-oxo acid dehydrogenase in human muscle. Eur. J. Appl. Physiol. 59: 159–167.

7. Wagenmakers, A.J.M., Beckers, E. J., Brouns, F., Kuipers, H., Soeters, P. B., van der Vusse, G. J. & Saris, W.H.M. (1991) Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise. Am. J. Physiol. 260: E883–E890.

8. Wolfe, R. R., Goodenough, R. D., Wolfe, M. H., Royle, G. T. & Nadel, E. R. (1982) Isotopic analysis of leucine and urea metabolism in exercising humans. J. Appl. Physiol. 52: 458–466.

9. Knapik, J., Meredith, C., Jones, B., Fielding, R., Young, V. & Evans, W. (1991) Leucine metabolism during fasting and exercise. J. Appl. Physiol. 70: 43–47.

10. Wagenmakers, A. J. (1999) Amino acid supplements to improve athletic performance. Curr. Opin. Clin. Nutr. Metab. Care 2: 539–544.

11. Louard, R. J., Barrett, E. J. & Gelfand, R. A. (1990) Effect of infused branched-chain amino acids on muscle and whole-body amino acid metabolism in man. Clin. Sci. 79: 457–466.

12. Frexes-Steed, M., Lacy, D. B., Collins, J. & Abumrad. N. N. (1992) Role of leucine and other amino acids in regulating protein metabolism in vivo. Am. J. Physiol. 262: E925–935.

13. Nair, K. S., Matthews, D. E., Welle, S. L. & Braiman, T. (1992) Effect of leucine on amino acid and glucose metabolism in humans. Metabolism 41: 643–648.

14. Matthews, D. E. (2005) Observations of branched-chain amino acid administration in humans. J. Nutr. 135: 1580S–1584S.

15. Lynch, C. J., Halle, B., Fujii, H., Vary, T. C., Wallin, R., Damuni, Z. & Hutson, S. M. (2003) Potential role of leucine metabolism in the leucine-signaling pathway involving mTOR. Am. J. Physiol. 285: E854–E863.

16. Koopman, R., Wagenmakers, A. J., Manders, R. J., Zorenc, A. H., Senden, J. M., Gorseling, M., Keizer, H. A. & van Loon, L. J. (2005) The combined ingestion of protein and free leucine with carbohydrate increases post-exercise muscle protein synthesis in vivo in male subjects. Am. J. Physiol. 288: E645–E653.

17. Newsholme, E. A., Acworth, I. N. & Blomstrand, E. (1987) Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. In: Advances in Myochemistry (Benzi, G., ed.), pp. 127–138. John Libby Eurotext, London, UK.

18. Chaouloff, F., Kennett, G. A., Serrurrier, B., Merino, D. & Curzon, G. (1986) Amino acid analysis demonstrates that increased plasma free tryptophan causes the increase of brain tryptophan during exercise in the rat. J. Neurochem. 46: 1647–1650.

19. Hargreaves, K. M. & Pardridge, W. M. (1988) Neutral amino acid transport at the human blood-brain barrier. J. Biol. Chem. 263: 19392–19397.

20. Blomstrand, E., Hassmen, P., Ekblom, B. & Newsholme, E. A. (1991) Administration of branched-chain amino acids during sustained exercise—effects on performance and on plasma concentration of some amino acids. Eur. J. Appl. Physiol. 63: 83–88.

21. Mittleman, K. D., Ricci, M. R. & Bailey, S. P. (1998) Branched-chain amino acids prolong exercise during heat stress in men and women. Med. Sci. Sports Exerc. 30: 83–91.

22. Nielsen, B. & Nybo, L. (2003) Cerebral changes during exercise in the heat. Sports Med. 33: 1–11.

23. Watson, P., Strachan, A. T., Shirreffs, S. M. & Maughan, R. J. (2002) Branched-chain amino acids and prolonged exercise capacity in a warm environment. Proc. Nutr. Soc. 61: 109A (abs.).

24. Watson, P., Shirreffs, S. M. & Maughan, R. J. (2004) Effect of acute branched-chain amino acid supplementation on prolonged exercise capacity in a warm environment. Eur. J. Appl. Physiol. 93: 306–314.

25. Varnier, M., Sarto, P., Martines, D., Lora, L., Carmignoto, F., Leese, G. P. & Naccarato, R. (1994) Effect of infusing branched-chain amino acid during incremental exercise with reduced muscle glycogen content. Eur. J. Appl. Physiol. 69: 26–31.

26. Blomstrand, E., Andersson, S., Hassmen, P., Ekblom, B. & Newsholme, E. A. (1995) Effect of branched-chain amino acid and carbohydrate supplementation on the exercise-induced change in plasma and muscle concentration of amino acids in human subjects. Acta. Physiol. Scand. 153: 87–96.

27. Van Hall, G. J., Raaymakers, S. H., Saris, W.H.M. & Wagenmakers, A.J.M. (1995) Ingestion of branched-chain amino acids and tryptophan during sustained exercise in man: failure to affect performance. J. Physiol. 486: 789–794.

28. Madsen, K., MacLean, D. A., Kiens, B. & Christensen, D. (1996) Effects of glucose, glucose plus branched-chain amino acids, or placebo on bike performance over 100 km. J. Appl. Physiol. 81: 2644–2650.

29. Blomstrand, E., Hassmen, P., Ek, S., Ekblom, B. & Newsholme, E. A. (1997) Influence of ingesting a solution of branched-chain amino acids on perceived exertion during exercise. Acta. Physiol. Scand. 159: 41–49.

30. Stensrud, T., Ingjer, F., Holm, H. & Strømme, S. B. (1992) L-tryptophan supplementation does not improve running performance. Int. J. Sports Med. 13: 481–485.

31. Hefler, S. K., Wideman, L., Gaesser, G. A. & Weltman, A. (1995) Branched-chain amino acid (BCAA) supplementation improves endurance performance in competitive cyclists. Med. Sci. Sports Exerc. 27: S149 (abs.).

32. Shephard, R. J. (1997) Physical Activity, Training and the Immune Response. Cooper, Carmel, IN.

33. Mackinnon, L. T. (1999) Advances in Exercise and Immunology. Human Kinetics, Champaign IL.

34. Pedersen, B. K. & Bruunsgaard, H. (1995) How physical exercise influences the establishment of infections. Sports Med. 19: 393–400.

35. Bassit, R. A., Sawada, L. A., Bacurau, R. F., Navarro, F., Martins, E., Jr., Santos, R. V., Caperuto, E. C., Rogeri, P. & Costa Rosa, L. F. (2002) Branched-chain amino acid supplementation and the immune response of long-distance athletes. Nutrition 18: 376–379.

36. Rohde, T., MacLean, D. A. & Pedersen, B. K. (1998) Effect of glutamine supplementation on changes in the immune system induced by repeated exercise. Med. Sci. Sports Exerc. 30: 856–862.

37. Krzywkowski, K., Petersen, E. W., Ostrowski, K., Kristensen, J. H., Boza, J. & Pedersen, B.K. (2001) Effect of glutamine supplementation on exercise-induced changes in lymphocyte function. Am. J. Physiol. 281: C1259–C1265.