Journal of Human Kinetics volume 72/2020, 69-78 DOI: 10.2478/hukin-2019-0099
Section II - Exercise Physiology & Sports Medicine

sciendo

Effects of Oral Branched-Chain Amino Acids (BCAAs) Intake on Muscular and Central Fatigue During an Incremental Exercise

by
Mohammad Fayiz AbuMoh'd[1], Laila Matalqah[2], Zainalabidden Al-Abdulla[3]

The aim of this study was to investigate the effects of oral branched-chain amino acids (BCAAs) intake on muscular (creatine kinase and myoglobin) and central (serotonin) fatigue during an incremental exercise protocol and to determine the time to exhaustion. Sixteen male long-distance runners (25.7 ± 2.0 yrs) performed two trials, 14 days apart. Using a double-blind, placebo-controlled, randomised crossover design, participants ingested either 20 g of BCAAs (BCAA trial) or a placebo 1 hour prior to performing an incremental exercise session on a treadmill. The starting speed was 8 km/h and this was increased by 1 km/h every 5 minutes until volitional exhaustion. Blood analysis indicated that plasma levels of serotonin were lower in the BCAA trial (259.3 ± 13.5 ng/ml) than the placebo trial (289.1 ± 14.5 ng/ml) ($p < 0.05$). There was a similar pattern of results for free fatty acid ($p < 0.05$). The creatine kinase level was higher in the BCAA trial (346.1 ± 33.7 U/L) than the placebo trial (307.3 ± 30.2 U/L). No significant difference between trials was observed regarding the level of myoglobin ($p = 0.139$). Time to exhaustion was longer in the BCAA trial (50.4 ± 2.3 min) than the placebo trial (46.6 ± 3.2 min). In conclusion, oral intake of 20 g of BCAAs 1 hour prior to an incremental treadmill exercise session increased time to exhaustion, probably due to the reduction in serotonin concentration. As myoglobin levels were within the normal range in both trials, we conclude that the participants did not reach muscular fatigue.

Key words: *Serotonin, free fatty acid, endurance exercise, myoglobin.*

Introduction

Muscle fatigue is the main limiting factor for physical performance during prolonged exercise and is characterised by impaired excitation-contraction coupling (Marshall et al., 2014; Presland et al., 2005). Several studies have reported on metabolic and neuromuscular factors that cause fatigue during prolonged exercise in athletes (Presland et al., 2005; Skurvydas et al., 2011). However, fatigue can occur in the absence of underlying mechanisms. It has been suggested that depletion of muscle glycogen and hypoglycaemia reduce muscle function during long-distance running (Bailey et al., 1993). Fatigue mechanisms may also include muscle metabolite accumulation (Bingham et al., 2017; Skurvydas et al., 2011), production of reactive oxygen species (ROS) (Nielson et al., 2008), and altered motor unit recruitment patterns (Bingham et al., 2017). Additionally, it is well documented that leakage of intracellular enzymes into plasma that are associated with decreased metabolic stability, such as creatine kinase (CK) and myoglobin could affect performance (Koo et al., 2014; Skurvydas et al., 2011). However, cessation of prolonged exercise occurs not because of muscular fatigue, but due to central fatigue (Eichelberger and Bilodeau, 2007; Skurvydas et al., 2011).

Central fatigue has been described as a reduction in muscle force due to reduced central drive (Buhot et al., 2000; Millet et al., 2003) and is

[1] - *Department of Movement/Sports Sciences, Faculty of Physical Education, Yarmouk University, Irbid, Jordan. Shafiq Irshidat St.*
[2] - *Department of Basic Medical Sciences, Faculty of Medicine, Yarmouk University, Irbid, Jordan.*
[3] - *Department of Clinical Sciences, Faculty of Medicine, Yarmouk University, Irbid, Jordan.*

Authors submitted their contribution to the article to the editorial board.
Accepted for printing in the Journal of Human Kinetics vol. 72/2020 in April 2020.

associated with increased plasma levels of the neurotransmitter serotonin (Marshall et al., 2014; McLellan, 2013). Serotonin (5-hydroxytryptamine, 5-HT) is distributed throughout the brain (Buhot et al., 2000) and has been shown to modulate functions mediated by the cerebral cortex (Gendle and Golding, 2010). Synthesis of 5-HT is dependent on plasma levels of free tryptophan (TRP), which is the precursor of 5-HT (Gendle and Golding, 2010; McLellan, 2013). Increases in the level of 5-HT in the brain are dependent on the ratio of free TRP to large neutral amino acids (LNAAs), such as branched-chain amino acids (BCAAs) (Gendle and Golding, 2010; McLellan, 2013; Nemet and Eliakim, 2007).

BCAAs (leucine, isoleucine and valine), which form one-third of total muscle protein (Mero, 1999), are the only amino acids metabolised in skeletal muscle (Koo et al., 2014). Oral BCAAs ingestion is commonly used by athletes (Choi et al., 2013; Wisnik et al., 2011) as a nutritional supplement (Burke, 2001; Nemet and Eliakim, 2007) to reduce central fatigue by lowering 5-HT activity (Choi et al., 2013) and increasing dopamine synthesis and release (Bailey et al., 1993), to trigger neural signals (Zheng et al., 2013), and to enhance physical performance (Choi et al., 2013). Several studies have indicated that taking BCAAs before and during prolonged exercise can alleviate central fatigue (Bailey et al., 1993; Burke, 2001; Nemet and Eliakim, 2007) and muscular fatigue (Areces et al., 2015; Bailey et al., 1993; Nemet and Eliakim, 2007), but another study found that BCAAs administration had no effect on performance (Nemet and Eliakim, 2007). Some of the studies into effects of BCAAs on serotonergic pathway response were not conducted on humans (Choi et al., 2013; Forrest et al., 2004; Jakeman et al., 1994). Choi and colleagues (2013) concluded that in rats oral BCAAs supplements reduced brain levels of serotonin and catecholamine during exercise. Despite this body of research, it remains unclear whether central or muscular fatigue is the main cause of exhaustion during prolonged exercise. Hence, the aim of this study was to examine whether, in long-distance runners, oral intake of BCAAs would reduce muscular (CK and myoglobin) or central (serotonin) fatigue during an incremental exercise protocol when compared with a placebo and to determine time to exhaustion under both conditions. We hypothesised that oral BCAAs intake would attenuate serotonin levels during exercise and thus enhance performance.

Methods

Participants

Sixteen male long-distance runners (age: 25.7 ± 2.0 years; body height: 172.6 ± 4.5 cm; body mass: 64.7 ± 4.5 kg; BMI: 21.0 ± 1.3 kg/m², resting heart rate: 61.2 ± 2.2 bpm, $VO_{2\ max}$ = 50.4 ± 7.2 ml/kg/min) who were members of the Jordan Military Sports Federation (population size = 21) participated in this study. They were healthy and free of any medical problems. Participants were asked to abstain from energy drinks and all ergogenic substances throughout the study. Volunteer athletes provided written consent prior to participation. The participants were instructed to maintain their regular training schedule, but to abstain from intense exercise for 72 hours prior to each trial. The study was approved by the internal Health Research Committee at the Yarmouk University.

Experimental design

This study was a randomised, double-blind trial with a crossover design. The protocol consisted of two trials: a BCAA (supplement) trial and a placebo trial, 14 days apart. Both trials started in the early morning (8:30 AM) to avoid effects of a circadian rhythm, as it is well established that athletic performance is influenced by the circadian rhythm (Russo et al., 2015). Similarly, physical performance is associated with a biological rhythm, which influences both the mental and physical energy that an athlete needs to perform at a given level (Chtourou et al., 2012). It is, therefore, difficult to compare results obtained at different times of the day. Three days prior to the commencement of the study, blood samples (10 ml) were collected from the antecubital median vein at 8:30 AM, after an overnight fast (approximately 9 hours) to determine baseline levels of the variables under investigation. The results are shown in Table 1. During this preliminary session, demographic information about participants was collected and participants were familiarised with the treadmill.

Procedures

One hour before starting the exercise trial, the athletes ingested an oral BCAAs supplement

or a placebo. After 45 minutes, the athletes were asked to warm up for 5 minutes on a treadmill at a speed of their choice (not exceeding the starting speed of the test protocol) and to stretch the lower limbs for 4-5 min. After 5 minutes of rest, the incremental exercise protocol started. At first, the athlete ran at 8 km/h on a treadmill (Techno-Gym, Lifefitness-6322; USA). Every 5 minutes the speed was increased by 1 km/h until volitional exhaustion was reached. Athletes were asked to raise their hand to indicate exhaustion so that distance and time to exhaustion could be recorded instantly. Blood samples were taken from all athletes immediately after the completion of each trial.

Supplementation protocol

For the BCCA trial, athletes ingested 20 g of BCAAs dissolved in 400 ml of water and 200 ml of strawberry juice one hour prior to the start of the incremental exercise protocol. Each gram of BCAAs supplement contained 300 mg of valine, 250 mg of leucine, and 100 mg of isoleucine. For the placebo trial, participants ingested a mixture of 400 ml of water and 200 ml of strawberry juice one hour prior to the test. Athletes ingested both drinks while sitting in the laboratory.

Blood sample analysis

The blood samples were centrifuged for biochemical analysis. Plasma was stored in a plain tube, centrifuged at 3500 rpm for 10 min and a serotonin level was analysed using (Cobas, 6000, Roche, Germany). Serum levels of free fatty acid (FFA) were analysed with RIA (Elecsys, 2010, Switzerland), serum CK using (Cobas, C 111, Roche, Germany) and serum myoglobin with (Immunlyte, 210, USA). The assays of FFA, CK, and myoglobin were stored in plain tubes and centrifuged at 5000 rpm for 5 min.

Statistical analysis

The Statistical Package for the Social Sciences Software (SPSS) version 18.0 was used for all analyses. A paired sample *t*-test was used to assess between-trial differences in biochemical responses and time to exhaustion. Data are presented as Mean ± SD. The significance level was set at $p < 0.05$.

Results

Time to exhaustion

Figure 1 illustrates that pre-exercise intake of BCAAs (50.4 ± 2.3 min) increased time to exhaustion by 3.818 min compared to the placebo (46.6 ± 3.2 min), with an effect size of 1.3 (t = -4.134, p = 0.001). The average maximal speed was 19 km/h in the BCAA trial and 18 km/h in the placebo trial. The normality of test was 0.9 for both the placebo and the BCAA trial. Furthermore, the 95% confidence interval of the difference was -5.7 at the lower bound and -1.8 at the upper bound.

Table1

Baseline values of biochemical variables

Variables	**Value** **Mean ± SD**
Plasma serotonin (ng/ml)	165.796 ± 13.840
Serum free fatty acid (mmol/l)	0.353 ± .0589
Serum creatine kinase (U/L)	95.31 ± 22.387
Serum myoglobin (ng/ml)	37.25 ± 6.952

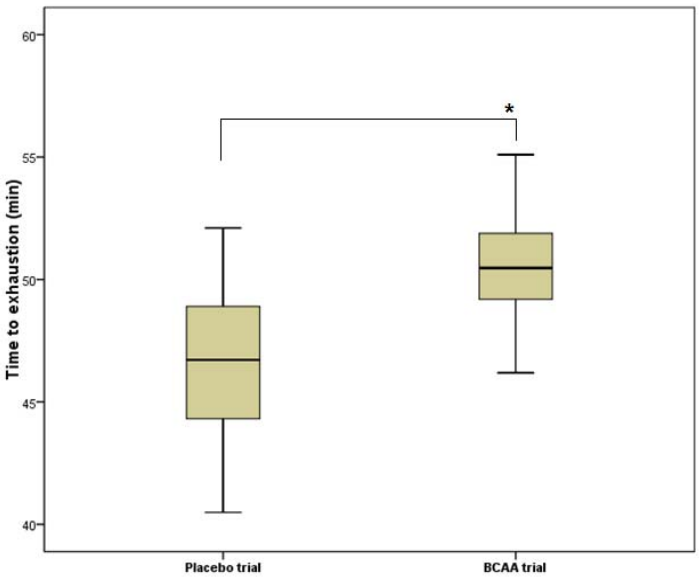

Figure 1

*Time to exhaustion in the BCAA and placebo trials. *Significantly different from the placebo trial (p = 0.001).*

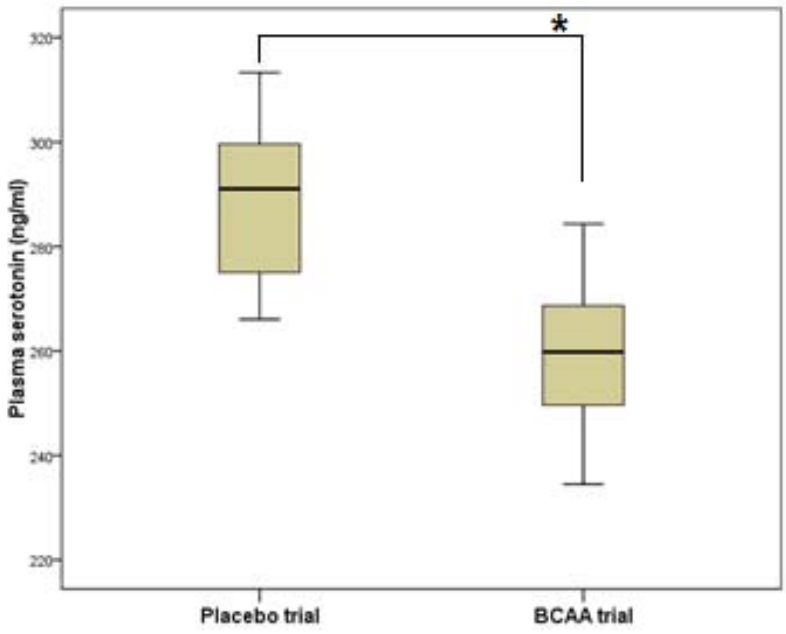

Figure 2

*Plasma serotonin in the BCAA and placebo trials. *Significantly different from the placebo trial (p = 0.001).*

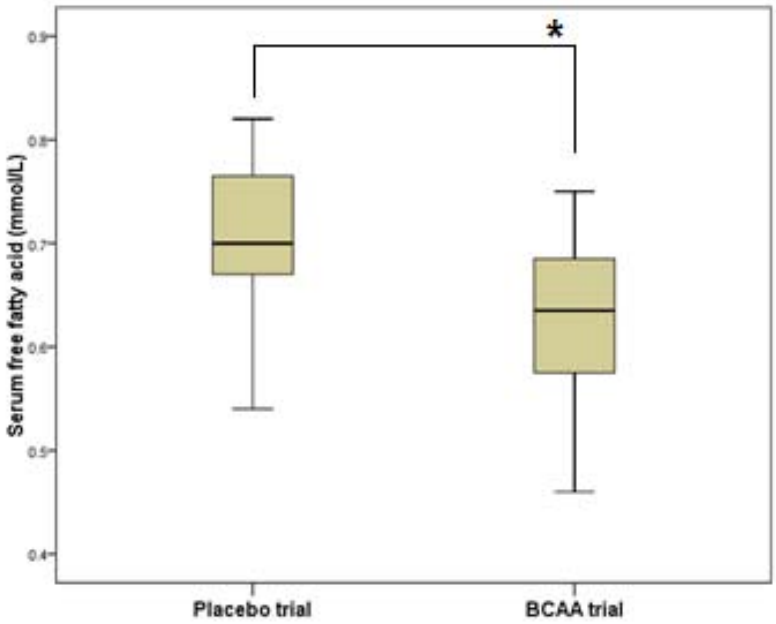

Figure 3

*Serum free fatty acid in the BCAA and placebo trials. *Significantly different from the placebo trial (p = 0.020).*

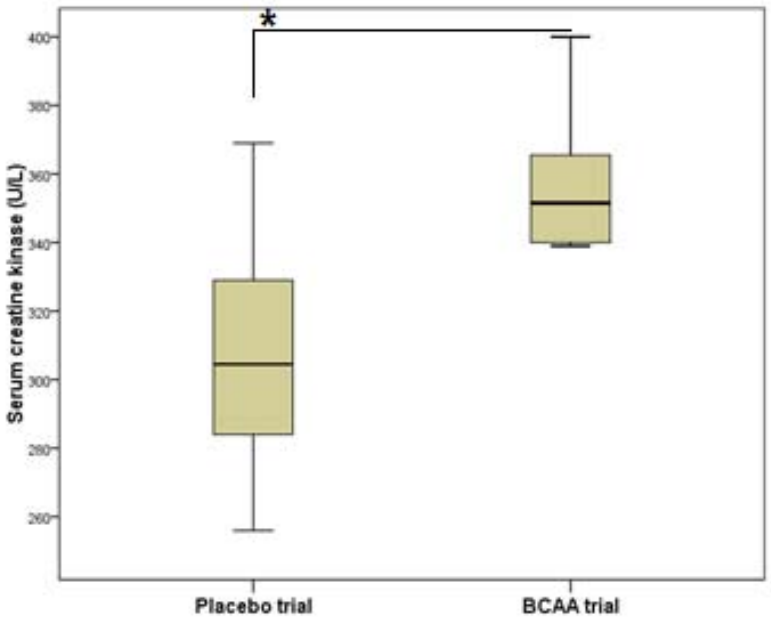

Figure 4

*Serum creatine kinase in the BCAA and placebo trials.*Significantly different from the BCAA trial (p = 0.001).*

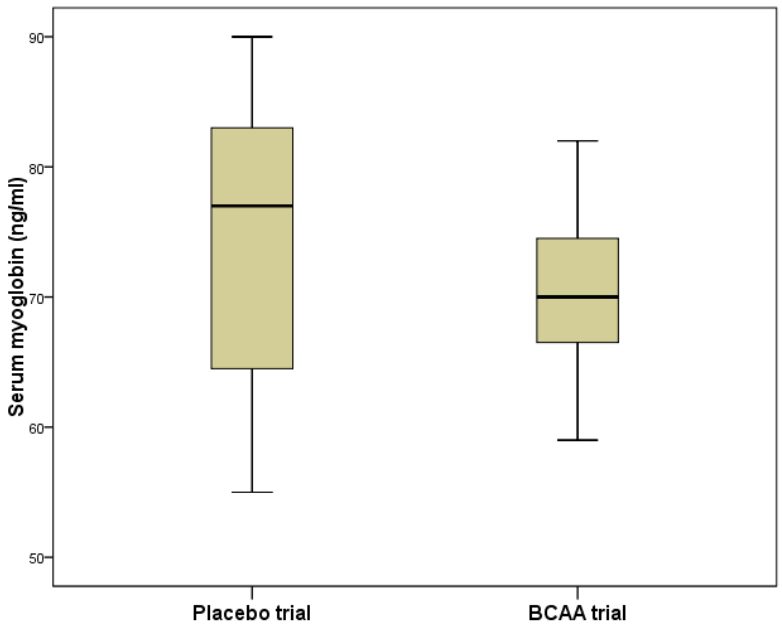

Figure 5

Serum myoglobin in the BCAA and placebo trials. Serum myoglobin is not significantly different between trials (p = 0.139).

Biochemical variables

Figures 2–5 illustrate biochemical variables in both trials. Analysis revealed that oral BCAAs intake decreased plasma serotonin levels compared to the placebo (t = -6.7, p = 0.001), with an effect size of 2.1 (BCCA: 259.3 ± 13.5 ng/ml; placebo: 289.1 ± 14.5 ng/ml) (Figure 2). The serum FFA level was lower in the BCAA trial (0.6 ± 0.08 mmol/l) than in the placebo trial (0.697 ± 0.080 mmol/L; t = -2.591, p = 0.020), with an effect size of 0.9 (Figure 3). The serum CK level was slightly higher after the BCCA trial (346.1 ± 33.7 U/L) than the placebo trial (307.3 ± 30.2 U/L; t = 3.8, p = 0.001), with an effect size of 1.2 (Figure 4). Myoglobin levels were similar after both trials (BCCA: 69.6 ± 7.9 ng/ml; placebo: 74.0 ± 11.1 ng/ml; t=-1.562, p = 0.139), with an effect size of 0.4 (Figure 5). The normality of the test in these variables was 1.0, 0.8, 0.07, and 0.8 for serotonin, FFA, CK, and myoglobin, respectively. Furthermore, the 95% confidence interval of the difference was -39.274 at the lower bound and -20.335 at the upper bound, -0.132 at the lower and -0.012 at the upper, 17.554 at the lower and 59.946 at the upper, and -10.343 at the lower and 1.593 at the upper bound for serotonin, FFA, CK, and myoglobin, respectively.

Discussion

The purpose of this study was to investigate the effect of oral intake of BCAAs on biomarkers of muscular and central fatigue and to determine time to exhaustion, in long-distance runners, during an incremental treadmill exercise test. We hypothesised that the intake of BCAAs supplement would alter serotonin concentration during exercise and that this would enhance performance.

Time to exhaustion

We found that the time to exhaustion was increased by pre-exercise administration of BCAAs, which could be due to the role of BCAAs

in sustaining prolonged physical effort (Burke, 2001; Nemet and Eliakim, 2007). This result is consistent with the study of Mittleman et al. (1999), which found that in a sample of 13 trained subjects, ingestion of 9.4 g or 15.8 g of BCCAs (women and men, respectively), 1 hour prior to performance of a cycling exercise protocol increased time to exhaustion (BCAA trial: 153 min; placebo: 137 min). The present study used a different mode of exercise, namely treadmill running. Another study (Stepto et al., 2011) showed that LNAAs enhanced agility performance by 5% and time to exhaustion by 3% in a sample of 15 sub-elite male soccer players. They examined the effects of 43 g of the LNAA supplement and a mixture containing 45.3 g of protein on fatigue, motor skills, and mental performance. They concluded that changes in TRP:LNAAs ratio contributed to enhanced performance.

Improvements in time to exhaustion have been attributed to the effect of BCAAs on psychomotor performance (Burke, 2001). Our findings are consistent with those of Wisnik et al. (2011), who examined the effects of ingestion of 7 g of BCAAs in soccer players. The BCAAs were administered 1 hour prior to a multiple-choice reaction time test during treadmill exercise (running-walking speed varied from 0 to 6.4 ms to simulate a soccer game). The results showed that reaction time was 10% lower for a BCAA trial compared to a placebo trial ($p < 0.05$), but the authors were unable to suggest a mechanism by which the effect of BCAAs on psychomotor performance could be linked to arousal. It has, however, been suggested that BCAAs supplementation reduces muscle soreness, which delays the perception of fatigue (Slater and Phillips, 2011), which could explain why BCAA administration increases time to volitional exhaustion.

Biochemical variables

The main finding of the study was that the plasma serotonin concentration following exercise to exhaustion was lower in the BCAA trial compared to the placebo trial. This neuropharmacological result could be due to attenuation of the elevation in a TRP:BCAAs ratio by administration of BCCAs (Burke, 2001; Nemet and Eliakim, 2007). It is well documented that prolonged physical exercise increases serotonin levels (Steinberg et al., 1998) rather than intermittent exercise (Eichelberger and Bilodeau, 2007). This is mainly due to slower oxidation of BCAAs during the recovery period (Nemet and Eliakim, 2007) and subsequently, it prevents an increase of plasma TRP concentration (Steinberg et al., 1998). However, in our study the plasma serotonin level was higher in both trials compared to baseline value (165.7 ± 13.8 ng/ml; Table 1) and normal range (68–232 ng/ml), indicating that during exercise the participants reached the point of 'central fatigue', due to the serotonergic system. The athletes in our study may have experienced an increase in serotonin concentration that minimised the CNS drive (Abbiss and Laursen, 2005) and had an adverse effect on excitation-contraction coupling in the later stages of the placebo trial. We suggest that central fatigue was lower in the BCCA trial, due to administration of BCAAs.

Not surprisingly, there was a decrease in plasma FFA in the BCAA trial compared to the placebo trial. This result may be explained by the role of BCAAs in facilitating delivery of energy to the working skeletal muscles (Burke, 2001; Nemet and Eliakim, 2007) and attenuating lipolysis during incremental treadmill exercise. In biochemical terms, muscular fatigue during prolonged physical exercise has been attributed to an increase in plasma FFA levels (Bailey et al., 1993). This could explain why time to exhaustion was lower in the placebo trial. This finding agrees with that of Wisnik et al. (2011), who reported that FFA concentration during the first half of a soccer game was higher in a placebo trial than in a BCAA trial. Many researchers have demonstrated that exercising on a bicycle ergometer produces much more plasma FFAs levels post-exercise in endurance athletes than untrained individuals (Bailey et al., 1993; Men'shikov, 2004; Nemet and Eliakim, 2007; Presland et al., 2005; Romijn et al., 1993). However, some of these studies did not include BCAA supplementation. Additionally, lipid oxidation is an important source of energy during prolonged physical exercise in athletes (Men'shikov, 2004; Tarnopolsky, 2004) and the body's ability to use fatty acids is increased during endurance exercise (Tarnopolsky, 2004). However, amino acid oxidation by skeletal muscles can provide up to 6% of total energy needed during prolonged endurance exercise

(Phillips et al., 1993), and more after BCAA supplementation. This may explain why FFA levels were higher in the placebo trial than the BCAA trial.

There was an increase in CK in the BCAA trial compared to the placebo trial. This result could have occurred because sustained skeletal muscle contractions (Areces et al., 2015) took place over a longer time and at a higher speed in the BCAA trial. Several studies have reported that prolonged exercise elevates levels of CK, myoglobin and lipoprotein (Nosaka and Clarkson, 1992; Smith et al., 2004) and that these increases are not necessarily linked to muscle damage (Smith et al., 2004). Importantly, if this elevation lasts for several days, its magnitude is linked to muscle damage-induced muscular fatigue (Nosaka and Clarkson, 1992). However, in this study, we measured CK levels only at the point of exhaustion. Additionally, serum CK values in both trials of this study may not have been related to rhabdomyolysis or muscle damage-induced muscular fatigue. This possibility is supported by the data on myoglobin levels, which were within the normal range in both trials (28–76 ng/ml). We suggest, therefore, that our participants did not reach the "values of CK-induced" muscular fatigue.

Limitations

This study did not measure other hormones associated with central fatigue, such as prolactin and tryptophan, or regulators of the basal metabolic rate (BMR) that influence energy balance include thyroxine and triiodothyronine.

Conclusion

In conclusion, oral intake of BCAAs had a positive effect on long-distance runners' performance on an incremental treadmill test. This suggests that ingestion of 20 g of BCAAs dissolved in 400 ml of water with 200 ml of strawberry juice 1 hour prior to an incremental exercise session increases time to exhaustion, probably due to the reduction in plasma serotonin concentration, which delays onset of central fatigue. Our results showed that time to exhaustion was increased by oral intake of BCAAs, which have an important role in energy expenditure during exercise, as well as attenuating the exercise-induced increase in the TRP:BCAAs ratio and thus preventing high plasma serotonin levels.

Practical implications

Ingestion of an oral BCAAs supplement 1 hour prior to an incremental exercise session increases BCAAs oxidation and thus reduces plasma levels of FFAs and serotonin, which delays central fatigue in long-distance runners. Furthermore, ingestion of 20 g of a BCAAs supplement 1 hour prior to an incremental endurance exercise session improves performance by reducing serotonergic activity. Supplementation with 20 g of BCAAs increases time to exhaustion because it results in maintenance of the pre-exercise TRP: LNAAs ratio.

Acknowledgements

This study was partially supported by the Faculty of Scientific Research and Graduate Studies in Yarmouk University, Jordan. The authors thank Dr. Nabeel Al-Mushasha for advice on statistical analysis and the athletes for their participation and effort throughout the study.

References

Abbiss CR, Laursen PB. Models to explain fatigue during prolonged endurance cycling. *Sports Med*, 2005; 35(10): 865–898

Areces F, Gonzalez-Millan C, Salinero JJ, Abian-Vicen J, Lara B, Gallo-Salazar C, Ruiz-Vicente Coso DJ. Changes in serum free amino acids and muscle fatigue experienced during a half-ironman triathlon, *PLoS ONE*, 2015; 10(9): 1–11

Bailey SP, Davis JM, Ahlborn EN. Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. *Am Physiol Society*, 1993; 74(6): 3006–3012

Bingham A, Arjunan SP, Jelfs B, Kumar DK. Normalised mutual information of high-density surface electromyography during muscle fatigue. *Entrophy*, 2017; 19(697): 1–14

Buhot M, Martin S, Segu L. Role of serotonin in memory impairment. *Ann Med*, 2000; 32(3): 210–221

Burke LM. Branched-chain amino acids (BCAAs) and athletic performance. *Int Sport Med J*, 2001; 2(3): 1–7

Choi S, DiSilvio B, Fernstrom MH, Fernstrom JD. Oral branched-chain amino acid supplements that reduce brain serotonin during exercise in rats also lower brain catecholamines. *Amino Acids*, 2013; 45: 1133–1142

Chtourou H, Driss T, Souissi S, Gam A, Chaouachi A, Souissi N. The effect of strength training at the same time of the day on the diurnal fluctuations of muscular anaerobic performances. *J Strength Cond Res*, 2012; 26(1): 217–225

Eichelberger TD, Bilodeau M. Central fatigue of the first dorsal interosseous muscle during low-force and high-force sustained submaximal contractions. *Clin Physiol Funct Imaging*, 2007; 27: 298–304

Forrest CM, Mackay GM, Stoy N, Egerton M, Christofides J, Stone TW, Darlington LG. Tryptophan loading induces oxidative stress. *Free Radical Res*, 2004; 38(1): 1167–1171

Gendle MH, Golding AC. Oral administration of 5-hydroxytryptophan (5-HT) impairs decision making under ambiguity but not under risk: Evidence from the Iowa Gambling Task. *Hum Psychopharmacol Clin Exp*, 2010; 25: 491–499

Jakeman PM, Hawthorne JE, Maxwell SRJ, Kendall, MJ. Evidence for downregulation of hypothalamic 5-hydroxytryptamine receptor function in endurance-trained athletes. *Experimental Physiology*, 1994; 79: 461–464

Koo GH, Woo J, Kang S, Shin KO. Effects of supplementation with BCAA and L-glutamine on blood fatigue factors and cytokines in juvenile athletes submitted to maximal intensity rowing performance. *J Phys Ther Sci*, 2014; 26: 1241–1246

Marshall PWM, Lovell R, Jeppesen GK, Andersen K, Siegler JC. Hamstring muscle fatigue and central motor output during a simulated soccer match. *PLoS ONE*, 2014; 9(7): 1–11

McLellan TM. Protein supplementation for military personnel: A review of the mechanisms and performance outcomes. *J Nutr*, 2013; 143: 1820S–1833S

Men'shikov IV. Free fatty acids and Ca^{+2} in blood plasma of endurance-trained athletes after prolonged physical exercise. *Hum Physiol*, 2004; 30(4): 485–489

Mero A. Leucine supplementation and intensive training. *Sports Med*, 1999; 27(6): 347–358

Millet GY, Martin V, Lattier G, Ballay Y. Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *J Appl Physiol*, 2003; 94: 193-198

Mittleman KD, Ricci MR, Bailey SP. Branched-chain amino acids prolong exercise during heat stress in men and women. *Med Sci Sports Exerc*, 1999; 30: 83–91

Nemet D, Eliakim A. Protein and amino acid supplementation in sport. *Int Sport Med J*, 2007; 8(1): 11–23

Nielson HG, Skjonsberg OH, Lyberg T. Effect of antioxidant supplementation on leucocyte expression of reactive oxygen species in athletes. *Scand J Clin Lab Inv*, 2008; 86(7): 526–533

Nosaka TD, Clarkson PM. Relationship between post-exercise plasma CK elevation and muscle mass involved in the exercise. *Int J Sports Med,*, 1992; 13: 471–475

Phillips SM, Atkinson SA, Tarnopolsky MA, McDougall JD. Gender differences in leucine kinetics and nitrogen balance in endurance athletes. *J. Appl. Physiol*, 1993; 75: 2134–2141

Presland JD, Dowson MN, Cairns SP. Changes of motor drive, cortical arousal and perceived exertion following prolonged cycling to exhaustion. *Eur J Appl Physiol*, 2005; 95: 42–51

Romijn JA, Klein S, Coyle EF, Sidossis LS, Wolfe RR. Strenuous endurance training increase lipolysis and triglyceride-fatty acid cycling at rest. *J. Appl. Physiol*, 1993; 75(1): 108–113

Russo L, D'Eramo U, Padulo J, Foti C, Schiffer R, Scoppa F. Day-time effect on postural stability in young sportsmen. *Muscles, Ligaments Tendons J*, 2015; 5(1): 38–42

Skurvydas A, Brazaitis M, Venckunas T, Kamandulis S, Stanislovaitis A, Zuoza A. The effect of sports specialization on musculus quadriceps function after exercise-induced muscle damage. *Appl Physiol Nutr Metab*, 2011; 36: 873–880

Slater G, Phillips SM. Nutrition guidelines for strength sports: sprinting, weightlifting, throwing events, and bodybuilding. *J Sports Sci*, 2011; 29(S1): S67–S77

Smith JE, Carbutt G, Lopes P, Pedoe DT. Effects of prolonged strenuous exercise (marathon running) on biochemical and haematological markers used in the investigation of patients in the emergency department. *Br J Sports Med*, 2004; 38: 292–294

Steinberg LL, Sposito MM, Lauro FAA, Tufik S, Mello MT, Naffah-Mazzacoratti MG, Cavalherio EA, Silva AC. Serum level of serotonin during rest and during exercise in paraplegic patients. *Spinal Cord*, 1998; 36: 18–20

Stepto NK, Shipperd BB, Hyman G, McInerney B, Pyne DB. Effects of high-dose large neutral amino acid supplementation on exercise, motor skill, and mental performance in Australian rules football players. *Appl Physiol Nutr Metab*, 2011; 36: 671–681

Tarnopolsky M. Protein requirements for endurance athletes. *Eur J Sport Sci*, 2004; 4(1): 1–14

Wisnik P, Chmura J, Ziemba AW, Mikulski T, Nazar K. The effect of branched chain amino acids on psychomotor performance during treadmill exercise of changing intensity simulating a soccer game. *Appl Physiol Nutr Metab*, 2011; 36: 856–862

Zheng X, Qiu Y, Zhong W, Baxter S, Su M, Li Q, Xie G, Ore BM, Qiao S, Spencer MD, Zeisel SH, Zhou Z, Jia W. A targeted metabolomic protocol for short-chain fatty acids and branched-chain amino acids. *Metabolomics*, 2013; 9: 818–827

Corresponding author:

Mohammad Fayiz AbuMoh'd
Department of Movement/Sports Sciences, Faculty of Physical Education,
Yarmouk University, Irbid, Jordan. Shafiq Irshidat St.
Tel: +962799913157,
ORCID: 0000-0001-6969-4643
E-mail: famohammad@yu.edu.jo