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ORIGINAL ARTICLE

Ten weeks of branched-chain amino acid supplementation improves select performance and immunological variables in trained cyclists

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Abstract We examined if supplementing trained cyclists (32 ± 2 year, 77.8 ± 2.6 kg, and 7.4 ± 1.2 year training) with 12 g/day (6 g/day L-Leucine, 2 g/day L-Isoleucine and 4 g/day L-Valine) of either branched-chain amino acids (BCAAs, $n = 9$) or a maltodextrin placebo (PLA, $n = 9$) over a 10-week training season affected select body composition, performance, and/or immune variables. Before and after the 10-week study, the following was assessed: (1) 4-h fasting blood draws; (2) dual X-ray absorptiometry body composition; (3) Wingate peak power tests; and (4) 4 km time-trials. No group × time interactions existed for total lean mass ($P = 0.27$) or dual-leg lean mass ($P = 0.96$). A significant interaction existed for body mass-normalized relative peak power (19 % increase in the BCAA group pre- to post-study, $P = 0.01$), and relative mean power (4 % increase in the BCAA group pre- to post-study, $P = 0.01$). 4 km time-trial time to completion approached a significant interaction ($P = 0.08$), as the BCAA group improved in this measure by 11 % pre- to post-study, though this was not significant ($P = 0.15$). There was a tendency for the BCAA group to present a greater post-study serum BCAA: L-Tryptophan ratio compared to the PLA group

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($P = 0.08$). A significant interaction for neutrophil number existed ($P = 0.04$), as there was a significant 18 % increase within the PLA group from the pre- to post-study time point ($P = 0.01$). Chronic BCAA supplementation improves sprint performance variables in endurance cyclists. Additionally, given that BCAA supplementation blunted the neutrophil response to intense cycling training, BCAAs may benefit immune function during a prolonged cycling season.

Keywords Leucine · Isoleucine · Valine · Cycling · Peak power · Immunity

Introduction

There is substantial interest in nutritional supplementation in the endurance cycling world to enhance performance (Tokish et al. 2004) and/or immune function (Gleeson 2007) due to long seasons and high volume training. Concerning endurance activities, branched-chain amino acid (BCAA) supplementation has been of intense research interest given the ability of BCAAs to support muscle mass gains (Blomstrand et al. 2006), reduce catabolism (Greer et al. 2007), potentially mitigate central fatigue (Newsholme and Blomstrand 2006), and modulate immune function (Bassit et al. 2002). BCAAs are comprised of L-Leucine, L-Isoleucine and L-Valine, and are a triad of essential amino acids that, when ingested, have potent anabolic/anti-catabolic properties (Gleeson 2005). The current body of literature views BCAAs, especially L-Leucine, to be key mediators in activation of muscle protein synthesis (Anthony et al. 2001). Exercise, particularly moderate and intense endurance activities, increases energy expenditure as well as up-regulating catabolism of muscle proteins (Shimomura et al. 2004). Since BCAAs can be oxidized in muscle tissue, they are a primary nutrient of interest when endurance exercise is utilized (van Hall et al. 1996). Oxidation of BCAA breakdown transpires in the mitochondria, as transamination occurs to produce branched-chain α-keto acids then is broken down by branched-chain aminotransferase, subsequently decarboxylation to produce coenzyme A compounds, then catalyzed by the branched-chain α-keto acid dehydrogenase complex (Shimomura et al. 2004). Furthermore, given that BCAA oxidation is considerably up-regulated by strenuous aerobic efforts (Gibala 2007; Layman 2002; Rennie and Tipton 2000; Shimomura et al. 2004), BCAA supplementation either pre- or post-exercise may be able to circumvent some of the catabolic effects attained from strenuous endurance activities, due to increased circulating BCAAs thereby not necessitating proteolysis.

Research conducted in animal models has shown that acute BCAA supplementation increases endurance performance compared to a placebo and/or glucose blend, respectively (Calders et al. 1997, 1999). Previous human studies indicate that BCAAs supplemented (77 mg/kg body weight) prior to exercise resulted in greater muscle ammonia production, intracellular and arterial BCAA levels along with reducing endogenous muscle breakdown (MacLean et al. 1994). Other acute studies have shown that low doses (2.5 g) of BCAAs to elicit lower levels of perceived muscle soreness and a greater propensity for knee flexion torque in subsequent days (24 and 48 h) following a 3–90 min bouts of submaximal cycling (Greer et al. 2007). However, BCAAs ingested prior to performance of a 100 km time-trial have been shown to acutely have no effect in well trained cyclists, when added with glucose (Madsen et al. 1996). These outcomes have been similar for running performance (Newsholme et al. 1991).

Regarding longer-term supplementation paradigms, a previous investigation has found that BCAA supplementation at 12 g/day for 2 weeks along with an additional 20 g each prior to and following a single 120 min bout of endurance cycling has been associated with decreased serum creatine kinase and lactate dehydrogenase, suggesting that BCAA reduces muscle damage concomitant with endurance exercise as well as possibly having lingering effects on lower levels of intramuscular catabolism in the days following exertion (Coombes and McNaughton 2000). Furthermore, Crowe et al. (2006) reported that 6 weeks of L-Leucine supplementation (45 mg/kg bodyweight/day) led to greater power outputs delayed time to fatigue during a sprinting trial in outrigger canoeists. Null findings with acute BCAA supplementation prior to an exercise bout versus the positive findings after chronic supplementation may suggest that, like creatine monohydrate supplementation, there is a potential need for tissue BCAA 'saturation' to occur in order to experience ergogenic effects.

Notwithstanding, performance benefits of chronic BCAA supplementation, especially with well-trained cyclists, is sparse. Likewise, little current research speaks to outcomes of chronic BCAA supplementation regarding endurance cycling performance with continued supplementation over a training season. Thus, the purpose of this study is to investigate the chronic effects of BCAA supplementation on markers of endurance cycling performance throughout the duration of a 10-week training season.

Methods

Participants

Upon approval from the Auburn University Institutional Review Board, participants read and signed Informed Consent, prior to study participation. Inclusion criteria were

absences of precluding injuries that would inhibit cycling performance, males between ages of 18 and 55 years old, as well as a minimum of 1 year of cycling experience. Of the 18 participants who completed the study, 17 were road cyclists with the other remaining participant being predominantly focused on mountain bike riding, while of a subtly different modality, was not an outlier in dependent variables.

Familiarization

Participants arrived at the laboratory and filled out pre-exercise questionnaires regarding health, exercise readiness, and cycling history. After this, participants were fitted to a Velotron Dynafit pro cycle ergometer (Racermate, Inc. Seattle, WA, USA) and were allowed to pedal at a low intensity to determine the best fit. Participants then performed a 30 s Wingate maximal anaerobic test which consisted of 20 s of light pedaling followed by a 5 s acceleration phase, then a 30 s maximal effort where the flywheel resistance was set at 9 % of the participants' body mass. Data derived from the Wingate test were peak/mean power and relative peak/mean power. Of note, participants were allowed to use their personal biking shoes and pedals in order to clip into the crank arms of the cycle ergometer. Following a cool-down (unloading pedaling for 3–5 min depending on participant desire), participants had a 10 min break before completing a 4 km time-trial. The 4 km time-trial, utilized as a surrogate for endurance performance, was performed using the participant's road bike which was attached to a Computrainer (Racermate, Inc. Seattle, WA, USA) to adjust cycling resistance with a magnetic braking apparatus (Abbiss and Laursen 2005; Ansley et al. 2004). The participant's road bike was outfitted with a rear-wheel hub CycleOps power meter (Madison, WI, USA) which was synchronized to a handheld Garmin device (Garmin Edge 500, Olathe, KS, USA) in order to measure power output. The researcher then had the participant pedal at a self-selected cadence and magnetic brake resistance in which he would be comfortable completing the 4-km time-trial. This brake resistance was apparent to the tester but not the rider. The rider then performed the familiarization 4 km time-trial as quickly as possible. Data derived from this were 4 km time-trial time and average power over that time. If the resistance became too cumbersome then the participant was allowed to downshift in order to complete the time-trial; of note, if the participant down-shifted while maintaining a similar cadence then speed and power output decreased. Essentially, the participants were instructed to complete the trial as fast as possible and were permitted to change the brakeweight as necessary. In this manner, time to completion was obtained once the rider reached 4 km on the stationary trainer computer, and 4 km average power output was recorded from the Garmin bike computer. After completion of the 4 km time-trial, the participants then scheduled a time for pre-testing measures to begin which occurred approximately 1 week later.

Pre- and post-testing procedures as well as supplementation procedures

Participants came into the laboratory following a 4 h abstaining period from food and/or caffeine. Venous blood samples were drawn from an antecubital vein of participants, and placed into a 5 mL serum separator tube and 3 mL EDTA tube (BD Vacutainer, Franklin Lakes, NJ, USA) for subsequent analysis serum and whole blood analysis, respectively. Participants were then given a standardized cereal bar (2 g protein, 24 g carbohydrates, 3 g fat, 120 kcal) in order to prevent potential hypoglycemic events during cycling testing. Hydration status of participants was then measured by urine testing via a handheld refractometer (ATAGO 2393, Bellevue, WA, USA). The hydration cut off for testing was determined using a urine-specific gravity value of 1.020 g mL^{-1}. If participants produced a higher value than the aforementioned one then 0.5 L of water was required to be consumed before testing procedures could continue. Each participant then underwent a dual-energy X-ray absorptiometry (DEXA) scan on a Lunar Prodigy (GE Corporation, Fairfield, Connecticut, USA) in order to determine total body fat mass, total body lean mass, and dual leg lean mass. Doing in-house laboratory testing, the same-day reliability of the DEXA during a test-calibrate-retest on 10 participants produced intra-class correlation coefficients of 0.998 for total body fat mass [mean difference between tests (mean ± standard error) = 0.40 ± 0.05 kg], 0.998 for total body lean mass [mean difference between tests (mean ± standard error) = 0.29 ± 0.13 kg], and 0.998 for dual-leg lean mass [mean difference between tests (mean ± standard error) = 0.17 ± 0.09 kg].

Subsequent cycling testing mimicked the familiarization trial. Specifically, a Wingate test was performed as described above, and this was followed by the 4 km time-trial described above. Following cycling testing, participants were assigned into groups (based on study entry order), in a double blind manner. One group was instructed to consume supplement 'A' (12 g of BCAAs: BCAA 3.1.2; MusclePharm Corp., Denver, CO, USA) in capsule form (16 total capsules per day) for 10 weeks. Of the 12 g of BCAAs, 6 g = L-Leucine, 2 g = L-Isoleucine and 4 g = L-Valine. The second group was instructed to consume supplement 'B' (12 g of maltodextrin placebo, PLA; 16 total capsules per day) for 10 weeks. Furthermore, participants were instructed to consume 8 capsules on an empty (2 h post-prandial) stomach and eight capsules

following exercise sessions on training days. On non-training days, participants were instructed to consume eight capsules twice daily on an empty stomach.

Participants were instructed to maintain normal dietary habits over the duration of the investigation. Moreover, the participants were instructed to obtain at least 160 km (100 mi) of riding per week and were instructed to log their riding volume on a daily basis. E-mail contact was maintained with the participants throughout the duration of the investigation to ensure that participants did not report any adverse effects of either BCAAs or PLA and were adhering to the study. Following the 10-week supplementation and training period, post testing procedures were re-performed during the same time of day for each participant as described above.

Whole blood assessment for white blood cell differentials

On the days of blood collection during pre- and post-testing, all 3 mL EDTA tubes were refrigerated upon blood collection. In the evening, all tubes were transported to the CLIA certified Auburn University Medical Clinic, and complete blood count (CBC) panels were analyzed using Beckman-Coulter DxH 600 Hematology analyzer (Beckman Coulter, Fullerton, CA, USA). Specifically, the following parameters were determined: total white blood cells (WBCs), neutrophils (absolute counts and percentage of WBCs), lymphocytes (absolute counts and percentage of WBCs), and monocytes (absolute counts and percentage of WBCs) were determined.

Serum BCAA and tryptophan analyses

Amino acids used for standards included: L-Leucine (99 % purity; EMD Millipore, Billerica, MA, USA), L-Isoleucine (99 % purity; Alfa Aesar, Ward Hill, MA), L-Valine (99 % purity; Alfa Aesar, Ward Hill, MA) and L-Tryptophan (99 % purity; Alfa Aesar, Ward Hill, MA), D-Leucine-d10 (99 % purity, CDN isotopes, Pointe-Claire, Quebec, CA), D-Valine-d8 (99 % purity, CDN isotopes, Pointe-Claire, Quebec, CA) and D-Tryptophan-d8 (99 % purity, CDN isotopes, Pointe-Claire, Quebec, CA). Hydrochloric Acid (HCl, 36–38 %) was purchased from Macron Fine Chemicals, Avantor Performance Materials (Center Valley, PA). Formic acid (LC–MS grade), acetonitrile (LC–MS grade) and water (LC–MS grade) were purchased form Sigma-Aldrich (St. Louis, MO).

Phosphate-buffered saline (PBS) was used for preparation of stock solutions and standard working solutions. Amino acid standards were dissolved in PBS to prepare a stock solution containing L-Leucine (240.0 μg/mL), L-Isoleucine (203.6 μg/mL), L-Valine (255.0 μg/mL) and L-Tryptophan (208.0 μg/mL), then the stock solution was diluted 200-fold in PBS to prepare a working solution. A serial dilution (1:5) of the standards solution containing all of the amino acids was prepared. Internal standards were mixed to prepare a working solution containing D-Leucine-d10 (1000 ng/mL), D-Valine-d8 (1220 ng/mL) and D-Tryptophan-d8 (1050 ng/mL).

Serum samples (5 μL) were diluted 200-fold to 1.0 ml in PBS. A 130 μL sample (diluted serum sample, standard or blank) was added to 100 μL of internal standard solution. Samples were deproteinated with the addition of 20 μL of HCl to a final volume of 230 μL, vortexed for 30 s and centrifuged for 20 min at 14,000*g*. A 100 μL aliquot of the resultant supernatant was transferred to glass vial and analyzed by liquid-chromatography tandem mass spectrometry (LC–MS/MS).

L-Leucine, L-Isoleucine, L-Valine and L-Tryptophan was quantified by LC–MS/MS using the internal standards, D-Leucine-d10 (for L-Leucine and L-Isoleucine), D-Valine-d8 and D-Tryptophan-d8. Analysis was performed on an Agilent 1290 UHPLC system coupled Agilent 6460 Triple Quad mass spectrometer (Agilent Technologies, Santa Clara, CA 95051, USA). The mobile phase consisted of 0.1 % (v/v) formic acid and acetonitrile. The samples were separated on ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) using a gradient from 2 to 5 % of acetonitrile for 1 min, then to 30 % for 1 min and kept at 30 % for 0.5 min. Samples (1 μL injection volume) were introduced into the mass spectrometer a flow rate of 0.5 mL/min using Agilent Jet Stream™ electrospray ionization (ESI) source. Nitrogen was used as the drying (10 L/min at 350 °C), nebulizer (45 psi), and collision gas. Capillary voltage was set at 4000 V. Mass spectra were acquired in positive-ion mode, and mass transitions were monitored using multiple-reaction monitoring; Transitions were: L-Leucine 132.2–86.2, L-Isoleucine 132.2–86.2, L-Valine 118.0-72.1, L-Tryptophan 205.1–188.1, D-Leucine-d10 142.2–96.2, D-Valine-d8 126.1–80.2, D-Tryptophan-d8 213.1–95.1. This method was linear from 1.00 to 1300 ng/mL for each amino acid with a lower limit of quantification (LLOQ) of 1.0 pg on column, accuracies ≥90 %, and coefficient of variation ≤15 %.

Statistics

Unless otherwise stated, all data are presented as mean ± standard error. All statistics were performed using SPSS v22.0 (Chicago, IL, USA), and an a priori alpha (α) level to detect significance was set at $P \leq 0.05$. Participant demographics between treatment groups (age, km ridden per week, average km ridden) were compared using independent *t* tests. For markers of performance, body composition, blood counts, and serum amino acids a 2 × 2 (group by time)

mixed factorial ANOVA was utilized to derive group × time interactions. In order to make the results more concise, if main group effects or main time effects were not significant or did not approach significance ($P > 0.10$), then these P values were not presented in the results section. If a significant group × time interactions or main effect for time α was obtained, subsequent paired samples t tests and independent t test were applied to locate specific differences, on within-subject and between-subject variables, respectively. Likewise, due to the small sample sizes, if a group × time interaction or main effect for time approached significance ($P \leq 0.10$), then 'forced' post hoc analyses were also explored.

Results

No between-group differences existed for riding volume over the study duration

No between-group differences existed for height ($P = 0.33$), pre-intervention body mass ($P = 0.88$), age ($P = 0.98$) and years cycling ($P = 0.53$) (Table 1). Furthermore, following the intervention, no between-group differences were found regarding total km ridden ($P = 0.29$) and/or average km/week ($P = 0.43$; Table 1).

BCAA supplementation increases cycling sprint power without altering body composition

No group × time interactions existed for body fat percentage ($P = 0.92$; Fig. 1a), total fat mass ($P = 0.78$; Fig. 1b), total lean mass ($P = 0.27$; Fig. 1c) or dual-leg lean mass ($P = 0.96$; Fig. 1c).

Interestingly, and in spite of total or dual-leg lean mass not being altered in the between groups, a group × time interaction was evident for peak power ($P = 0.02$; Fig. 2a), relative peak power (normalized to body mass, $P = 0.01$; Fig. 2b), and mean power ($P = 0.01$; Fig. 2c). Further analysis revealed that the BCAA group increased peak power by 20 % compared to the pre-study time point ($P = 0.01$). The BCAA group also experienced a 19 % increase in relative peak power ($P = 0.01$) compared to the pre-study time

Table 1 Participant demographics

Group	Number of participants	Pre mass (kg)	Height (cm)	Age (years)	Training age (years)	Total cycling (km)	Average cycling (km/wk)
BCAA	9	78.2 ± 3.9	172 ± 8	32.1 ± 2.9	8.2 ± 2.0	1861 ± 182	192 ± 20
PLA	9	77.4 ± 3.6	170 ± 8	32.2 ± 3.3	6.6 ± 1.7	2120 ± 150	212 ± 15

Total cycling (km) was the total distance logged by participants over the 10-week study. Average cycling was the average distance per week logged over the 10-week study

BCAA branched-chain amino acid group, *PLA* placebo group

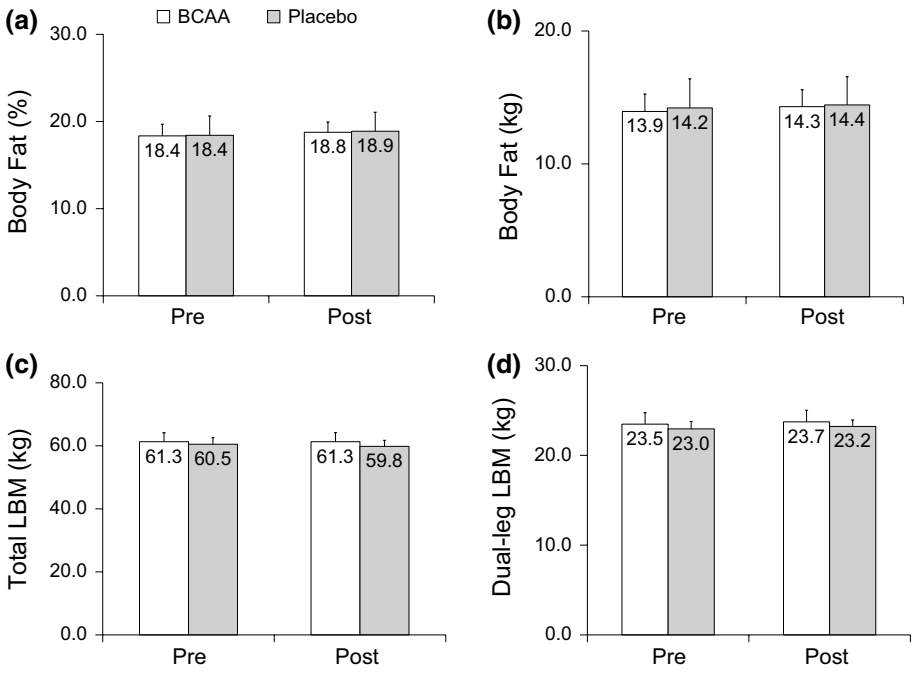

Fig. 1 Pre- and post-study body composition variables. Pre-study (*pre*) and 10-week post-study (*post*) body composition variables. No group *time interactions were observed for body fat percentage (panel **a**), body fat mass (panel **b**), total lean body mass (*LBM*; panel **c**), or dual leg *LBM* (panel **d**)

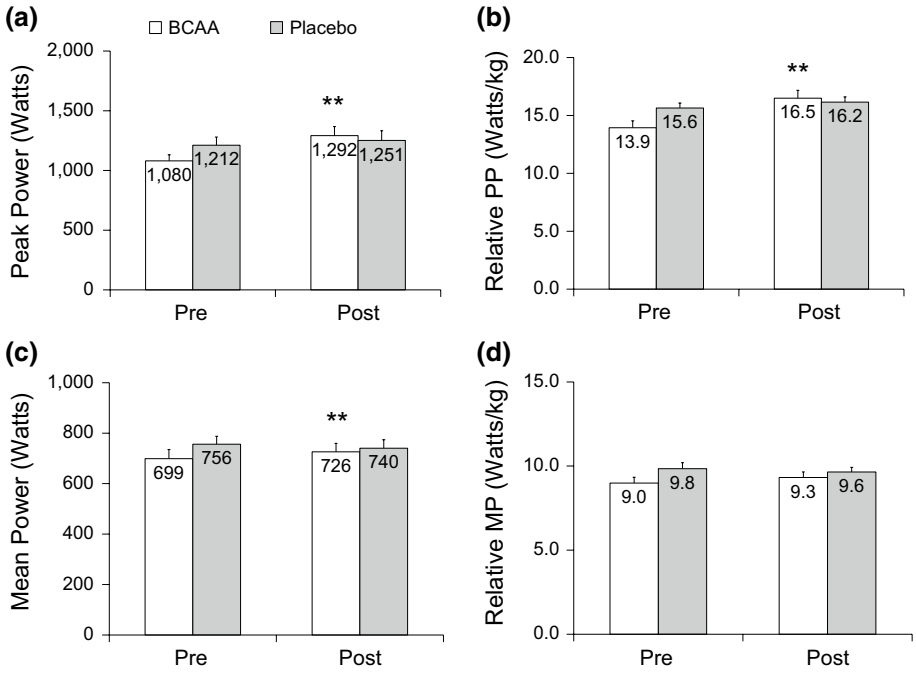

Fig. 2 Effects of chronic BCAA supplementation on Wingate variables in cyclists. Pre-study (*pre*) and 10-week post-study (*post*) Wingate variables. A group × time interaction was observed for peak power ($P = 0.02$), and the BCAA group increased peak power by 20 % compared to the pre-study time point (**$P = 0.01$) (panel **a**). A group × time interaction was also observed for relative peak power ($P = 0.01$), and the BCAA group increased relative peak power by 19 % compared to the pre-study time point (**$P = 0.01$) (panel **b**). A group × time interaction was observed for mean power ($P = 0.01$), and the BCAA group increased mean power by 4 % compared to the pre-study time point (**$P = 0.01$) (panel **c**). No group × time interactions was observed for relative mean power (*MP*; panel **d**)

point. In addition, the BCAA group experienced a 4 % increase in mean power ($P = 0.01$) compared to the pre-study time point. A group × time interaction failed to reach significance for relative mean power ($P = 0.35$; Fig. 2d).

Time to complete the 4 km time-trial approached a group × time interaction ($P = 0.08$; Fig. 3a). Further analysis revealed that the BCAA group improved on the 4 km time-trial to completion by 11 %, though this was not statistically significant ($P = 0.15$). Group × time interactions failed to reach significance for 4 km time-trial power and 4 k time-trial power/kg ($P = 0.26$, $P = 0.28$; Fig. 3b, c, respectively).

BCAA supplementation does not significantly alter fasting serum amino acids

Given that chronic BCAA supplementation increased peak and mean Wingate power in cyclists without increasing dual-leg lean tissue mass (i.e., an increase in power without an increase in hypertrophy), we were next interested in examining if there were between-group differences in fasting serum BCAAs, L-Tryptophan, and the BCAA: L-tryptophan ratio given that these variables are all related to the proposed central fatigue hypothesis (i.e., offsetting serum L-Tryptophan with BCAAs may allow more BCAAs to cross the blood–brain barrier which can enhance work output by reducing central fatigue) (Blomstrand 2001; Davis et al. 2000). No significant group × time interaction for serum BCAAs ($P = 0.13$; Fig. 4a) serum L-tryptophan ($P = 0.82$; Fig. 4b) or serum BCAAs: L-Tryptophan ratio ($P = 0.22$; Fig. 4c). Interestingly, there was a main effect for time regarding serum L-Tryptophan, whereby collapsing the mean of both groups over time revealed an increase in this circulating marker after the 10 week cycling intervention ($P = 0.05$); however, there were no within-group increases. A main effect for time also came near to statistical significance regarding serum BCAA: L-Tryptophan ratio, whereby collapsing the mean of both groups over time tended to decrease this measure after the 10-week cycling intervention ($P = 0.10$). Upon further post hoc analysis, there was a tendency for the BCAA group to present a greater post-study serum BCAA: L-Tryptophan ratio compared to the PLA group ($P = 0.08$).

BCAA supplementation blunts neutrophil increases in cyclists

Finally, we were interested in examining whether chronic BCAA supplementation affected whole blood immune markers given that: (1) rigorous endurance training can lead to increases in circulating neutrophils and decreases in circulating

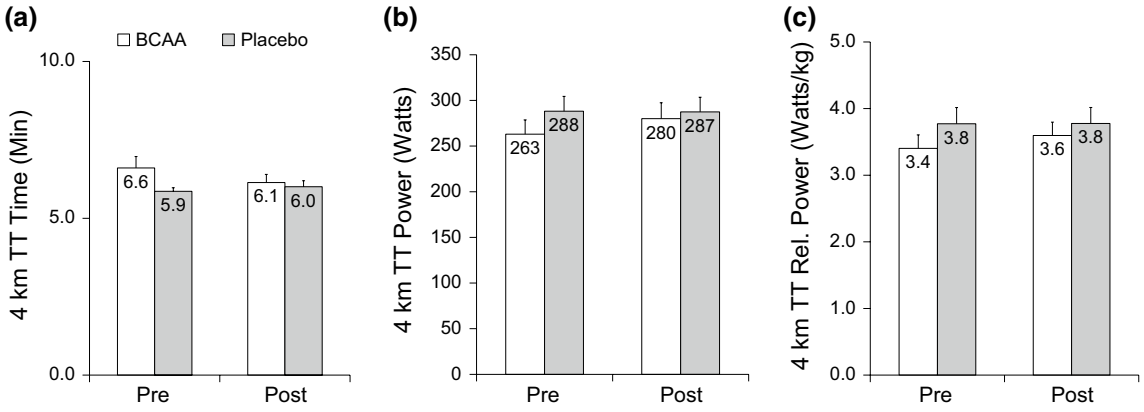

Fig. 3 Effects of chronic BCAA supplementation on 4 km time-trial measures in cyclists. Pre-study (*pre*) and 10-week post-study (*post*) 4 km time-trial measures. No significant group × time interactions were observed for 4 km time-trial (*TT*) time to completion (panel **a**), 4 km *TT* power (panel **b**) or 4 km *TT* relative power (panel **c**)

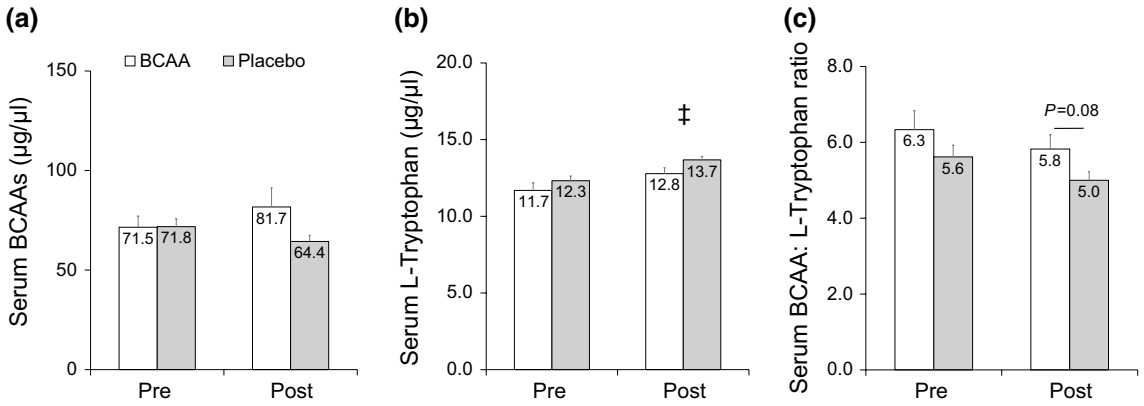

Fig. 4 Effects of chronic BCAA supplementation on fasting serum amino acids in cyclists. Pre-study (*pre*) and 10-week post-study (*post*) fasting serum amino acid analyses. No significant group × time interactions were observed for serum BCAAs (panel **a**), serum L-Tryptophan (panel **b**) or the serum BCAA: L-Tryptophan ratio (panel **c**). There was a main time effect for serum L-Tryptophan to increase from the pre- to post-study time point when both group means were collapsed over time ($^{‡}P < 0.05$). Likewise, there was a tendency for the serum BCAA: L-Tryptophan levels to decrease from the pre- to post-study time point when both group means were collapsed over time, and there was a tendency for this value to be greater in the BCAA group versus PLA group at the post-study time point ($P = 0.08$)

lymphocytes which, in turn, can lead to an immunocompromised state (Pedersen et al. 1997); and (2) BCAAs have been shown to be a viable energy for immune cells (Calder 2006). No group × time interaction existed for WBC counts ($P = 0.24$; Fig. 5a). Interestingly, a group × time interaction approached significance for percent neutrophils ($P = 0.06$; Fig. 5b) and a group × time interaction was significant for neutrophil number ($P = 0.04$; Fig. 5c). Regarding neutrophil percentages, post hoc analysis revealed a 4.6 % increase in the PLA group over time ($P = 0.01$), and a between group difference existed at the post-study time point ($P = 0.05$). Regarding neutrophil number, there was a significant 18 % increase within the PLA group from the pre- to post-study time points ($P = 0.01$), as well as a suggestive tendency between groups at the post-study time point ($P = 0.07$). There was also a tendency toward a group × time interactions for lymphocyte percentages ($P = 0.11$; Fig. 5d), but not lymphocyte numbers ($P = 0.69$; Fig. 5e). Monocyte percentages reached a significant group × time interaction ($P = 0.05$; Fig. 5f), but there were no significant differences when post hoc analysis was conducted. There was no significant group × time interaction for monocyte numbers ($P = 0.19$; Fig. 5g).

Discussion

Presently there is a lack of literature concerning chronic amino acid supplementation and subsequent alterations in

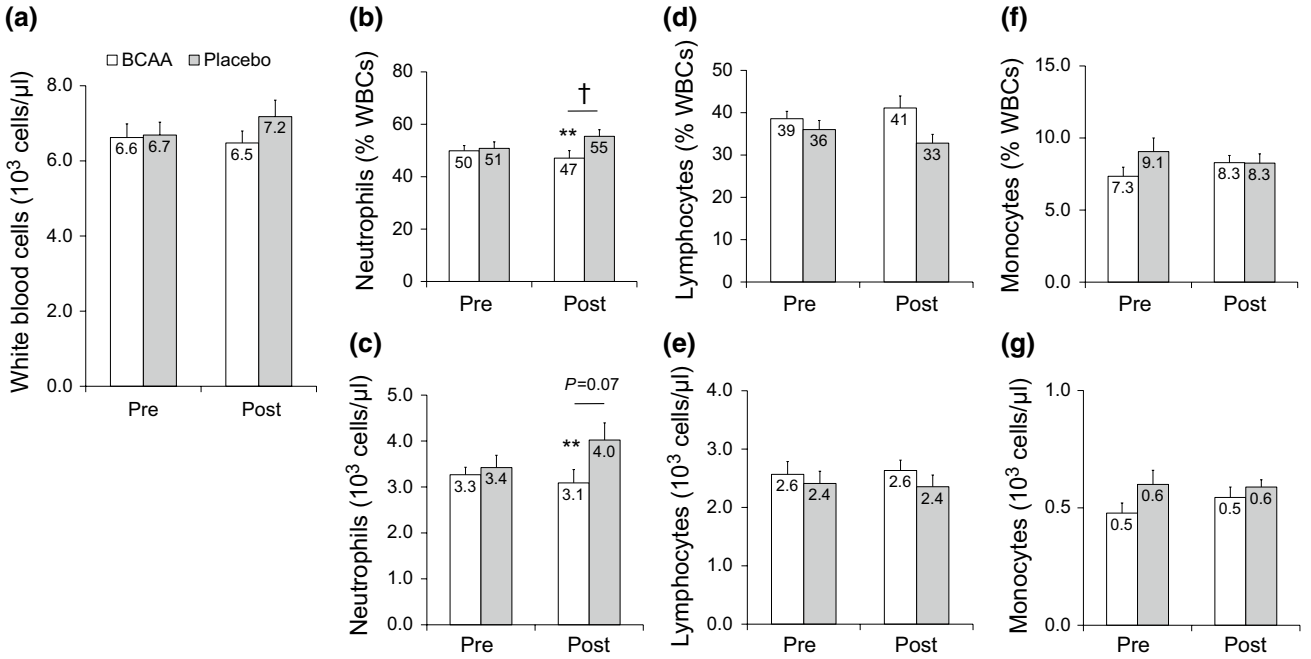

Fig. 5 Effects of chronic BCAA supplementation on immune variables in cyclists. Pre-study (*pre*) and 10-week post-study (*post*) immune variables. No significant group × time interactions was observed for while blood cell counts (panel **a**). A group × time interaction trend was observed for neutrophil percentages ($P = 0.06$; panel **b**). A group × time interaction existed for neutrophil number ($P = 0.04$), and there was an 18 % increase within the PLA group from the pre- to post-study time points (**$P = 0.01$) (panel **c**). No significant group*time interactions were observed for lymphocyte percentages and counts (panels **d**, **e**) or monocyte percentages and counts (panels **f**, **g**). Other symbols: †between-group difference at a given time point ($P < 0.05$)

performance variables in experienced endurance athletes. In this regard, this is the first study to our knowledge to investigate the how chronic BCAA supplementation affects anaerobic and aerobic variables in trained endurance cyclists. Overall, the major findings of this study are that chronic BCAA supplementation improves anaerobic measures associated with cycling sprint performance; specifically, Wingate performance measures which were significantly augmented only in the group ingesting 12 g BCAAs per day. Moreover, while fasting serum L-Tryptophan ratios increased with 10 weeks of cycling independent of treatment, chronic BCAA supplementation tended to prevent a further post-study decrease in fasting serum BCAA: L-Tryptophan ratio; a finding which may link BCAA supplementation to the aforementioned increase in performance variables. A secondary but noteworthy finding from this investigation was a blunting of elevated neutrophil values with chronic BCAA supplementation. Collectively, these findings are discussed in greater detail below.

BCAA supplementation enhances power output in cyclists

Echoing previous literature, our findings support that chronic BCAA supplementation increases anaerobic power capacity, although we did not observe an increase in total body and/or dual-leg lean mass. As mentioned previously, Crowe et al. (2006) have shown that L-Leucine supplementation over 6 weeks enhanced power performance in trained canoeists. Thus, our findings are in agreement with those reported by Crowe et al. in that chronic BCAA ingestion seems to enhance short-term power output in experienced athletes. Moreover, the current data as well as the data reported by Crowe et al. collectively suggest that BCAA supplementation may enhance power output across varying exercise modalities; that is to say that the benefits from BCAAs are not allocated to specific joints, muscles and/or types of movements. However, Crowe et al. did not assess changes in lean tissue mass in these athletes, so we cannot compare our null body composition findings to their findings in this regard.

Other investigations have illustrated that both acute and chronic BCAA supplementation protocols can enhance muscle functionality in the absence of hypertrophy. For instance, it has been reported that humans supplementing with BCAAs over 30 days experienced increases in forearm grip strength without concomitantly increasing skeletal muscle mass (De Lorenzo et al. 2003). Howatson et al. (2012) also reported that maximal voluntary contraction (MVC) was dampened after a muscle damaging exercise

bout in humans, though short-term BCAA supplementation was better able to preserve this post-bout decrease in MVC up to 96 h post-exercise, compared to placebo. Collectively, the results presented herein as well as the aforementioned literature suggests that BCAA supplementation enhances short-term powerful efforts without affecting muscle mass.

Interestingly, chronic BCAA supplementation did not enhance 4 km time-trial time or 4 km time-trial power; both variables which are considered to be more endurance-associated compared to the Wingate test, given that the 4 km time-trial took ~6 min to complete and the Wingate took 30 s. Acute BCAA ingestion protocols have yielded similar results. For instance, Madsen et al. (1996) demonstrated that the acute ingestion of BCAAs + glucose did not improve 100 km time to exhaustion when compared to a glucose only and non-caloric placebo trials, despite increases in plasma BCAA levels during the cycling bout in the BCAA-supplemented group. Watson et al. (2004) replicated these findings, reporting that the acute ingestion of BCAAs did not improve cycling to exhaustion in glycogen-depleted subjects that exercised in a warm environment despite increases in plasma BCAAs. It should be noted that the 4 km cycling time trial employed herein was not nearly as 'aerobically oriented' compared to the 100 km time trials reported above and, thus, comparisons between data are limited. However, Toone and Betts (2010) have shown that including a BCAA-rich protein source with carbohydrates increased/worsened a 6 km time to completion by 6 % in competitive cyclists. Davis et al. (1999) also demonstrated that acute BCAA ingestion in conjunction with carbohydrates had no effect on endurance performance (shuttle-run) compared to carbohydrates alone. Finally, while animal models suggest that BCAAs may confer acute benefits to endurance performance (Calders et al. 1997, 1999), equivocal data also exist (Davis et al. 1999). Thus, it appears that acute and/or chronic BCAA supplementation, while enhancing power-associated variables, does not improve endurance performance variables. As it appears to be well established that acute BCAA ingest does not aid in endurance performance, the novel aspect of this investigation indicates that chronic BCAA supplementation does not seem to be efficacious in enhancing select endurance variables.

Finally, it is noteworthy to mention that, while chronic BCAA supplementation did not statistically increase fasting serum BCAA levels, it did tend to elevate the serum BCAA: L-Tryptophan ratio after the 10-week intervention compared to the PLA group. Central fatigue has been posited to arise from higher circulating levels of L-Tryptophan traversing the blood brain barrier and being converted into serotonin; this ultimately being linked to fatigue (Blomstrand 2001; Davis et al. 2000). Research focusing on performance outcomes has shown that ingestion of carbohydrates can favorably alter the BCAA: L-Tryptophan ratio and is linked to better time until exhaustion performance (Davis et al. 1992). Theoretically, given that BCAAs can also cross the blood–brain barrier, chronic BCAA supplementation with the intent of habitually disrupting the brain production of serotonin may also mitigate central fatigue (Blomstrand 2001). In light of the fact that we observed increases in anaerobic performance variables without increases in dual-leg lean mass, as well as a tendency for serum BCAA: L-Tryptophan ratios to be favorable altered, we posit that performance enhancement with chronic BCAA supplementation could be related to a central fatigue-mediated mechanism. However, this hypothesis is limited given that we did not use a mechanistic approach to decipher if preserved serum BCAA: L-Tryptophan ratios, and possible brain BCAA: L-Tryptophan ratios and/or serotonin levels were predictive of performance. To this end, more mechanistic animal models are needed in order to determine if chronic BCAA supplementation offsets brain serotonin production and whether this is associated with increases in anaerobic performance variables.

BCAA supplementation blunts neutrophil increases in cyclists

Intense exercise induces neutrophil proliferation (Peake 2002; Suzuki et al. 1996), and repetitive cycling bouts have been shown to increase neutrophilia and alter neutrophil function (Suzuki et al. 1999). Exercise-induced alterations in neutrophil number and/or function may be a maladaptive response, which can lead to a compromised immune function (Nieman 1997). Herein, we report an increase in neutrophil counts in PLA group from the pre- to post-study time point; an effect which may be due to altered neutrophil function. It has been posited that BCAAs are essential for lymphocyte and neutrophil function given that protein synthetic rates in these cells are driven by BCAAs (Calder 2006). BCAAs have also been shown to: (1) enhance neutrophil function by increasing phagocytotic capacity (Nakamura et al. 2007); and (2) enhance the ability of other immune cell types (lymphocytes, monocytes and macrophages) to proliferate in vitro in response to cytokines after a 30 km run (Bassit et al. 2002). Hence, with chronic BCAA supplementation, there may not be an impetus for up-regulating neutrophil counts due to an enhancement in neutrophil function. Currently we cannot support these findings outright as that was not a primary concern of this investigation, and specific cellular mechanics were not measured. However, our data supports chronic BCAA supplementation reduces the increase in neutrophil counts that occur with 10 weeks of high volume cycling, and this may favor an enhancement in immune function. In this regard, more mechanistic studies are warranted with regards to

how BCAAs affect immune cell function over chronic training interventions in endurance athletes.

Conclusions

This is the first study to our knowledge to explore the effects of chronic BCAA supplementation with regard to changes in performance variables relevant to endurance cyclists. Limitations to the current study include: (1) a relatively small sample size of cyclists, (2) the lack of sampling at intermittent time points (i.e., 2, 4 weeks, etc.), and (3) the lack of more mechanistic immune cell data to explain why neutrophil alterations occurred with BCAA supplementation. Furthermore, while participants were instructed to consume 8 capsules twice per day on an empty stomach, this paradigm is not 'consumer friendly', as many 'real-world' participants likely opt to consume BCAAs in powder form and/or with meals. Notwithstanding, this study illustrates that chronic BCAA supplementation improves sprint performance variables in well-trained road cyclists, particularly mean/peak power and relative mean/peak power but not time for a 4 km completion. Moreover, the alterations in circulating BCAA: L-Tryptophan ratios may be responsible for some of the performance benefits. BCAAs may also benefit immune function during a prolonged cycling season, although more research is needed to expand upon our findings.

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Compliance with ethical standards

Besides J.R.M., none of the authors have conflicts of interest. J.R.M. is a Ph.D. scientist employed by the MusclePharm Research Institute, but he substantially contributed to the study design and data write-up. Therefore, all co-authors agreed that his work into this project warranted co-authorship. It should also be noted that all participants gave their informed consent in writing prior to inclusion in the study. Identifying details (names, dates of birth, identity numbers and other information) of the participants are not published in the current work.

References

Abbiss CR, Laursen PB (2005) Models to explain fatigue during prolonged endurance cycling. Sports Med 30(10):865–898

Ansley L et al (2004) Regulation of pacing strategies during successive 4-km time trials. Med Sci Sports Exerc 36(10):1819–1825

Anthony JC, Anthony TG, Kimball SR, Jefferson LS (2001) Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. J Nutr 131:856S–860S

Bassit RA et al (2002) Branched-chain amino acid supplementation and the immune response of long-distance athletes. Nutrition 18:376–379

Blomstrand E (2001) Amino acids and central fatigue. Amino Acids 20:25–34

Blomstrand E, Eliasson J, Karlsson HK, Kohnke R (2006) Branched-chain amino acids activate key enzymes in protein synthesis after physical exercise. J Nutr 136:269S–273S

Calder PC (2006) Branched-chain amino acids and immunity. J Nutr 136:288S–293S

Calders P, Pannier JL, Matthys DM, Lacroix EM (1997) Pre-exercise branched-chain amino acid administration increases endurance performance in rats. Med Sci Sports Exerc 29:1182–1186

Calders P, Matthys D, Derave W, Pannier JL (1999) Effect of branched-chain amino acids (BCAA), glucose, and glucose plus BCAA on endurance performance in rats. Med Sci Sports Exerc 31:583–587

Coombes JS, McNaughton LR (2000) Effects of branched-chain amino acid supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise. J Sports Med Phys Fit 40:240–246

Crowe MJ, Weatherson JN, Bowden BF (2006) Effects of dietary leucine supplementation on exercise performance. Eur J Appl Physiol 97:664–672. doi:10.1007/s00421-005-0036-1

Davis JM, Bailey SP, Woods JA, Galiano FJ, Hamilton MT, Bartoli WP (1992) Effects of carbohydrate feedings on plasma free tryptophan and branched-chain amino acids during prolonged cycling. Eur J Appl Physiol 65:513–519

Davis JM, Welsh RS, De Volve KL, Alderson NA (1999) Effects of branched-chain amino acids and carbohydrate on fatigue during intermittent, high-intensity running. Int J Sports Med 20:309–314. doi:10.1055/s-2007-971136

Davis JM, Alderson NL, Welsh RS (2000) Serotonin and central nervous system fatigue: nutritional considerations. Am J Clin Nutr 72:573S–578S

De Lorenzo A, Petroni ML, Masala S, Melchiorri G, Pietrantuono M, Perriello G, Andreoli A (2003) Effect of acute and chronic branched-chain amino acids on energy metabolism and muscle performance. Diabetes Nutr Metab 16:291–297

Gibala MJ (2007) Protein metabolism and endurance exercise. Sports Med 37:337–340

Gleeson M (2005) Interrelationship between physical activity and branched-chain amino acids. J Nutr 135:1591S–1595S

Gleeson M (2007) Immune function in sport and exercise. J Appl Physiol 103:693–699. doi:10.1152/japplphysiol.00008.2007

Greer BK, Woodard JL, White JP, Arguello EM, Haymes EM (2007) Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise. Int J Sport Nutr Exerc Metab 17:595–607

Howatson G, Hoad M, Goodall S, Tallent J, Bell PG, French DN (2012) Exercise-induced muscle damage is reduced in resistance-trained males by branched chain amino acids: a randomized, double-blind, placebo controlled study. J Int Soc Sports Nutr 9:20. doi:10.1186/1550-2783-9-20

Layman DK (2002) Role of leucine in protein metabolism during exercise and recovery. Can J Appl Physiol Revue canadienne de physiologie appliquee 27:646–663

MacLean DA, Graham TE, Saltin B (1994) Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. Am J Physiol 267:E1010–E1022

Madsen K, MacLean DA, 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

Nakamura I, Ochiai K, Imai Y, Moriyasu F, Imawari M (2007) Restoration of innate host defense responses by oral supplementation of branched-chain amino acids in decompensated cirrhotic

patients. Hepatol Res Off J Jpn Soc Hepatol 37:1062–1067. doi:10.1111/j.1872-034X.2007.00166.x

Newsholme EA, Blomstrand E (2006) Branched-chain amino acids and central fatigue. J Nutr 136:274S–276S

Newsholme EA, Blomstrand E, Hassmen P, Ekblom B (1991) Physical and mental fatigue: do changes in plasma amino acids play a role? Biochem Soc Trans 19:358–362

Nieman DC (1997) Risk of upper respiratory tract infection in athletes: an epidemiologic and immunologic perspective. J Athl Train 32:344–349

Peake JM (2002) Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. Exerc Immunol Rev 8:49–100

Pedersen BK et al (1997) Exercise-induced immunomodulation—possible roles of neuroendocrine and metabolic factors International journal of sports medicine 18 Suppl 1:S2–7 doi:10.1055/s-2007-972695

Rennie MJ, Tipton KD (2000) Protein and amino acid metabolism during and after exercise and the effects of nutrition. Annu Rev Nutr 20:457–483. doi:10.1146/annurev.nutr.20.1.457

Shimomura Y, Murakami T, Nakai N, Nagasaki M, Harris RA (2004) Exercise promotes BCAA catabolism: effects of BCAA supplementation on skeletal muscle during exercise. J Nutr 134:1583S–1587S

Suzuki K et al (1996) Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J Appl Physiol 81:1213–1222

Suzuki K et al (1999) Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol 87:1360–1367

Tokish JM, Kocher MS, Hawkins RJ (2004) Ergogenic aids: a review of basic science, performance, side effects, and status in sports. Am J Sports Med 32:1543–1553. doi:10.1177/0363546504268041

Toone RJ, Betts JA (2010) Isocaloric carbohydrate versus carbohydrate-protein ingestion and cycling time-trial performance. Int J Sport Nutr Exerc Metab 20:34–43

van Hall G, MacLean DA, Saltin B, Wagenmakers AJ (1996) Mechanisms of activation of muscle branched-chain alpha-keto acid dehydrogenase during exercise in man. J Physiol 494(Pt 3):899–905

Watson P, Shirreffs SM, Maughan RJ (2004) The effect of acute branched-chain amino acid supplementation on prolonged exercise capacity in a warm environment. Eur J Appl Physiol 93:306–314. doi:10.1007/s00421-004-1206-2