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Branched-chain amino acids supplementation enhances exercise capacity and lipid oxidation during endurance exercise after muscle glycogen depletion

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Aim. **It has been demonstrated that branched-chain amino acids (BCAA) transaminase activation occurs simultaneously with exercise-induced muscle glycogen reduction, suggesting that BCAA supplementation might play an energetic role in this condition. This study aimed to test whether BCAA supplementation enhances exercise capacity and lipid oxidation in glycogen-depleted subjects.**

Methods. **Using a double-blind cross-over design, volunteers (N.=7) were randomly assigned to either the BCAA (300 mg · kg · day $^{-1}$) or the placebo (maltodextrine) for 3 days. On the second day, subjects were submitted to an exercise-induced glycogen depletion protocol. They then performed an exhaustive exercise test on the third day, after which time to exhaustion, respiratory exchange ratio (RER), plasma glucose, free fatty acids (FFA), blood ketones and lactate were determined. BCAA supplementation promoted a greater resistance to fatigue when compared to the placebo (+17.2%). Moreover, subjects supplemented with BCAA showed reduced RER and higher plasma glucose levels during the exhaustive exercise test.**

Results. **No significant differences appeared in FFA, blood ketones and lactate concentrations.**

Conclusion. **In conclusion, BCAA supplementation increases resistance to fatigue and enhances lipid oxidation during exercise in glycogen-depleted subjects.**

KEY WORDS: Amino acids, branched-chain - Exercise - Glycogen - Citric acid cycle.

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The tricarboxylic acid cycle (TCA) is the major common pathway for the oxidation of carbohydrates, lipids and some amino acids. The TCA cycle is regulated directly by accessible pools of its two substrates oxaloacetate and acetyl-CoA, and its product citrate,[1, 2] suggesting that the continuous production of oxaloacetate can theoretically determine oxidative metabolic rate.[2] However, it is well established that only muscle and liver glycogen levels cannot support the great oxaloacetate demand imposed by either fasting or prolonged physical activity.[2] Hence, one can expect that lipid oxidation might be partially limited by carbohydrate availability.[3] Accordingly, muscle fatigue seems to coincide with depleted glycogen content during prolonged exercise. Thus it seems plausible to discuss that in this case, fatigue is related to provision mediated through a limited supply of substrate (*i.e.*, oxaloacetate) to the TCA cycle and/or limitations in the TCA flux due to reduced TCA intermediates concentration,[4] although the latter has been a matter of intense debate, for details, see the excellent review by Bowtell *et al*. 2007.[1]

The TCA cycle is characterized by a continuous generation of its intermediates, which releases CO_2

and other metabolites, such as citrate and glutamine. During catabolic conditions (*i.e.*, exhaustive exercise or fasting), a constant loss of carbon skeletons occurs, commonly referred as "cataplerosis", which need to be replenished by specific reactions aimed to promote TCA expansion, namely "anaplerosis". For example, our group has demonstrated that oxaloacetate can be generated through aspartate, asparagine and glutamate transamination.[5, 6]

Branched-chain amino acids (BCAA) which are primarily oxidated in skeletal muscle, may contribute to energy metabolism during exercise as energy sources and substrates to expand the pool of TCA intermediates through anaplerosis reactions.[7] Isoleucine and valine may increase succinyl-CoA availability, possibly leading to an increase in oxaloacetate concentration, which could hypothetically result in a higher FFA oxidation, especially in a glycogen-depleted condition (*i.e.*, during fasting or prolonged physical activity).

Also, it has been demonstrated that BCAA transaminase activation occurs simultaneously with exercise-induced muscle glycogen reduction.[3, 8, 9] In light of this, we hypothesized that glycogen depletion might enhance the BCAA contribution to energy provision, thus delaying the onset of fatigue. Therefore, the aim of this study was to test whether BCAA supplementation improves exercise capacity and FFA oxidation in glycogen-depleted subjects.

Materials and methods

Study population

Seven healthy and physically active male volunteers (age: 24±2 years, body mass index: 22.3±2.5 kg m^{-2}, VO_{2peak}: 47.2±3.9 ml kg^{-1} min^{-1}) were selected to participate in this study.

The study protocol was approved by the University's Ethics Committee, and all eligible study subjects gave written, informed consent before their participation.

Overall design

A double-blind cross-over design was used, in which each subject completed two experimental conditions randomly. At baseline, subjects underwent maximal progressive treadmill exercise for VO_{2max} and anaerobic threshold determination (T1).

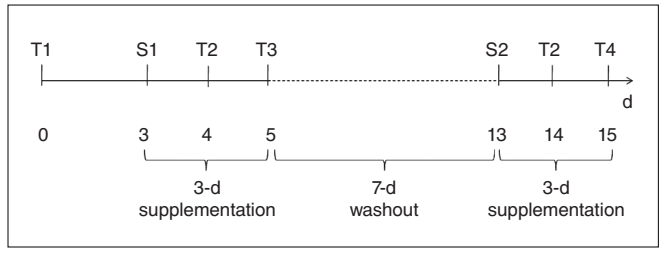

Figure 1.—Experimental design. Subjects (N.=7) were supplemented with either BCAA or placebo (S1) for 3 days. On the second day, they were submitted to a glycogen depletion protocol (T2). Then, they performed an exhaustive exercise test on the 3rd day (T3). After a 7-day washout period, subjects received BCAA or placebo in a cross-over fashion (S2) and then repeated T2. Thereafter, they performed other exhaustive exercise tests (T4). For further details, see *Overall Design.* T1 = $VO_{2\ max}$ test; T2 = glycogen depletion protocol; T3 and T4 = exhaustive exercise tests; S1 and S2 = BCAA or placebo supplementation.

One week after T1, subjects were supplemented with either BCAA (300 mg kg day^{-1}) or placebo (maltodextrine, at the same dose) for three days. On the second day of supplementation, subjects were submitted to an exercise-induced glycogen depletion protocol (T2). On the following day, after a 10-hour fast, subjects performed an exercise bout at 80% of their anaerobic threshold until exhaustion (T3 or T4). During this test, resistance to fatigue, respiratory exchange ratio (RER), plasma glucose, plasma free-fatty acids (FFA) and blood ketones were determined. After a seven-day washout period, subjects underwent the aforementioned protocol in a cross-over fashion. Participants were instructed to maintain the same food intake pattern during the trials. The experimental design is depicted in Figure 1.

VO_{2peak} and anaerobic threshold determination (T1)

Subjects completed a graded, continuous exercise test on a treadmill. The test commenced at 7 km/h with incremental increases in speed (1.2 km/h every 4 min with a 1 min interval for plasma determinations) until voluntary exhaustion. Gas exchange measurements (VO_2, VCO_2 , RER and $VO_{2\ peak}$ determination) were obtained continuously throughout the test by a portable espirometer (K4®). Blood lactate was analyzed every 4 minutes for anaerobic threshold determination. Attainment of VO_{2max} was accepted when two of three criteria were met: a plateau in VO_2, a respiratory exchange ratio (RER)> 1.1 and volitional exhaustion.

Glycogen depletion protocol (T2)

On the second day of supplementation in both experimental conditions, subjects underwent an exercise session aimed at glycogen depletion. This session consisted of treadmill running for 45 minutes at 70% $VO_{2\ peak}$, followed by two 10-minute sprints at 90% $VO_{2\ peak}$, with a two-minute interval. After this exercise the subjects remained fasted (~10 h) until the experiment the next morning. Similar protocols including continuous and interval exercise have been reported to give a reduced muscle glycogen level the following morning.[10, 11]

Exhaustive exercise tests (T3 and T4)

Subjects performed an exhaustive exercise test which consisted of treadmill running at 80% of their anaerobic threshold at a constant velocity (9.9±0.7 km/h) in glycogen depleted condition. Exhaustion was determined when the subjects were not able to maintain their initial speed or when test interruption was requested. Blood samples were collected before the test and every five minutes for plasma glucose and lactate determinations. Ketone bodies, FFA and ammonia were measured immediately before and after the test. RER was also determined at rest and every five minutes until exhaustion.

BCAA or placebo supplementation

As mentioned earlier, subjects received either encapsulated BCAA (300 mg · kg · day $^{-1}$) or a placebo (maltodextrine, at same dose) supplementation in a randomized, cross-over, double-blind fashion. The supplementation was given three days prior to T3 and T4, including on the days of tests. The BCAA and placebo conditions were separated by a 7-day washout period and for the first trial (T3) 4 subjects were randomly submitted to BCAA supplementation and 3 subjects to placebo. For the second trial (T4), the opposite distribution was adopted. Adherence to the supplementation protocol was verified through personal communication on a daily basis.

Plasma analysis

Blood samples were drawn and immediately centrifuged at 4000 rpm for 15 minutes and stored at -20 °C until further analysis.

Plasma FFAs and ketones were analyzed using commercial kits (Sigma®, SP, Brazil). Plasma glucose and lactate were determined using automatic lactimeter/glucosimeter (Yellow Spring 2300®, OH, US). All analyses were performed in duplicate, and the mean value was calculated.

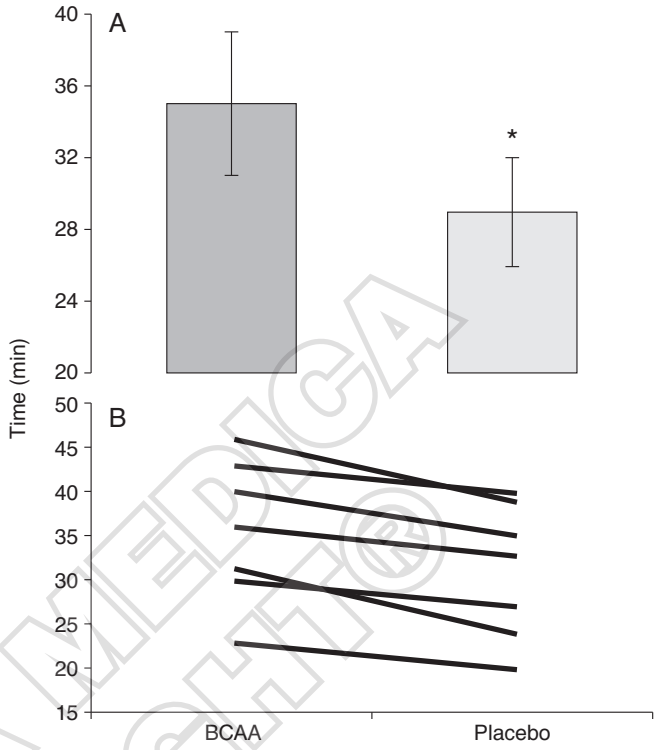

Figure 2.—Time to exhaustion (min) in maximal exercise (T3 and T4) after BCAA and placebo supplementation. The subjects' performance was significantly greater after BCAA supplementation when compared to placebo (*P=0.001). A) Mean ± SD; B) individual data.

Statistical analysis

SAS® proc Mixed Model was used to analyze repeated measures, and when applicable, Tukey Post hoc was used for multiple comparisons. All data is expressed as mean ± sd. The significance level adopted to reject the null hypothesis was P≤0.05.

Results

According to daily personal communication, subjects' compliance to supplementation protocol was

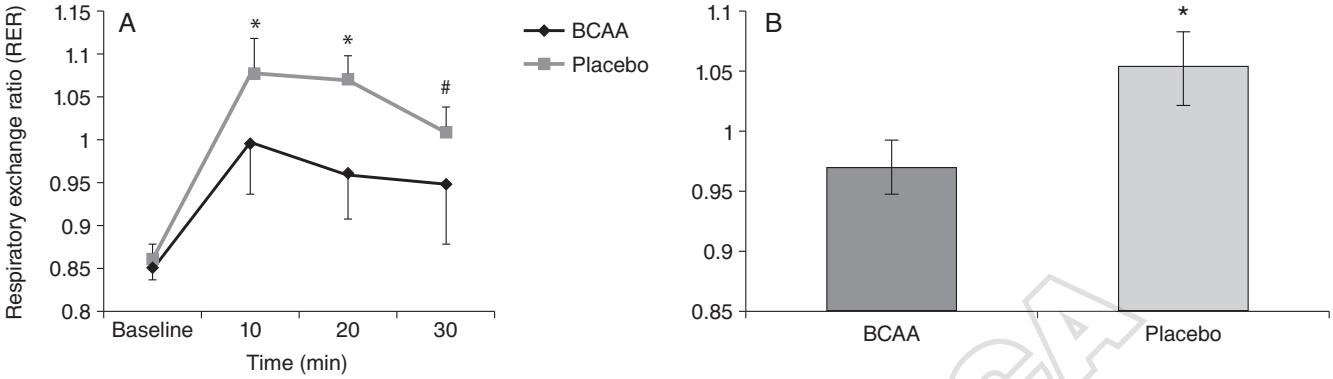

Figure 3.—Respiratory exchange ratio (RER) after BCAA and placebo supplementation. A) BCAA led to a significant reduced RER after 10 and 20 minutes (*P=0.01) during an exhaustive exercise test (T3 and T4), suggesting an increase in lipid oxidation. Also, a trend was noted toward an increase in RER after BCAA supplementation at 30 minutes (#P=0.08); B) pooled data only during exhaustive exercise test (10 + 20 + 30 minutes) also indicated lower RER after BCAA supplementation compared to placebo (*P=0.002).

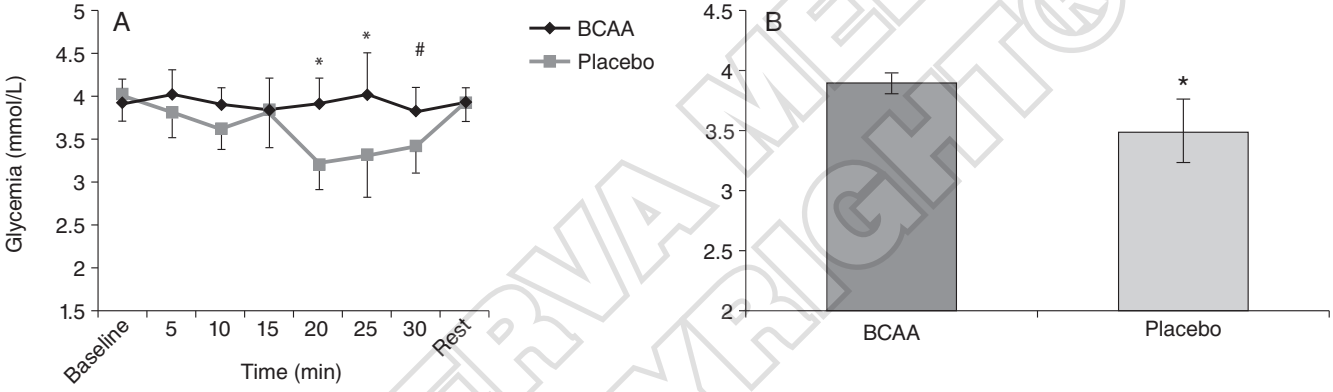

Figure 4.—Glycemic levels during exhaustive exercise test (T3 and T4) after BCAA and placebo supplementation. A) The subjects in BCAA condition showed greater glucose concentration than in placebo trial at 20, 25 (*P=0.04) and 30 minutes (# trend P=0.06); B) The pooled data throughout the exhaustive exercise test (5 + 10 + 15 + 20 + 25 + 30 minutes) also showed that BCAA supplementation prevents the glycemic fall observed in placebo (*P=0.001). To convert mmol/L to mg/dL, multiply by 18.

100%. Moreover, no reports were received of any deleterious effects during the study.

BCAA supplementation promoted a greater time to exhaustion (+17.2%) when compared to the placebo (Figure 2). It is important to highlight that all the subjects showed greater exercise capacity after BCAA supplementation (Figure 2B).

Moreover, a reduction in RER was observed after 10 (1 *vs*. 1.08) and 20 (0.96 *vs*. 1.07) minutes of the exhaustive exercise test (P<0.05), and a trend toward a lower RER at 30 minutes (0.95 *vs*. 1.1) (P=0.08) was observed following BCAA supplementation when compared to the placebo (Figure 3). The pooled RER data during the exhaustive exercise reveal that, in fact, BCAA supplementation diminished RER when compared to the placebo (0.97±0.02 *vs*. 1.05±0.03, P=0.002), suggesting a shift from carbohydrate to lipid oxidation.

Plasma glucose levels were higher at 20, 25 and 30 minutes (P=0.02, P=0.03 and P=0.06, respectively) after BCAA supplementation when compared to the placebo. We observed the same trend when considering pooled glycemic data during exhaustive exercise (3.5±0.08 *vs*. 3.1±0.2, P=0.001) (Figure 4).

There were no significant differences in plasmatic FFA (Figure 5), blood ketones (Figure 6) and lactate

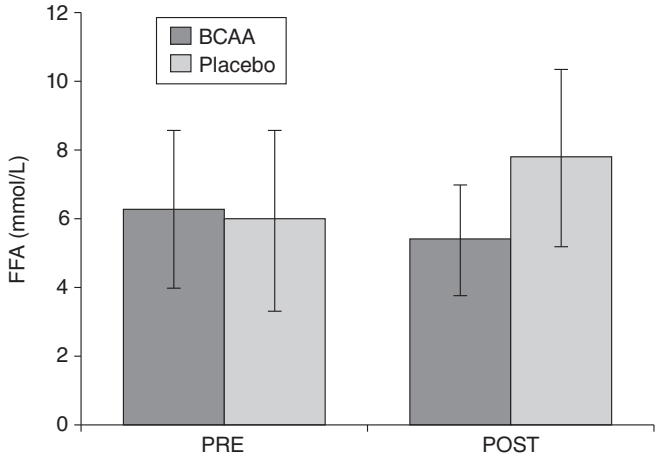

Figure 5.—Free fatty acids (FFA) concentration immediately before and after the exhaustive exercise test, following BCAA and placebo supplementation. No significant differences were noted.

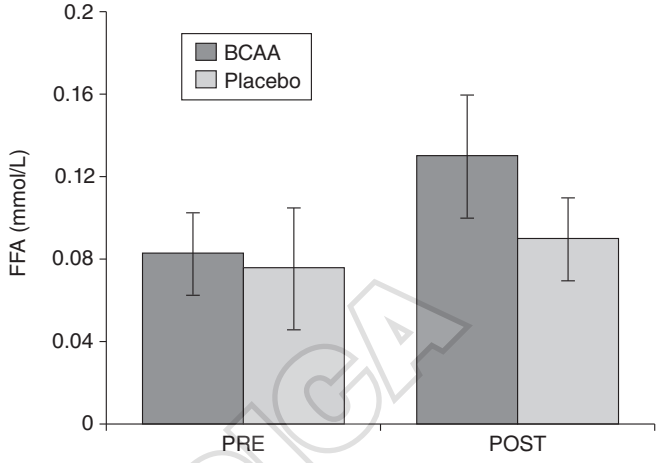

Figure 6.—Beta hydroxybutyrate concentration immediately before and after the exhaustive exercise test, following BCAA and placebo supplementation. There were no significant differences between the trials.

(data not shown) concentrations between the BCAA and placebo conditions.

Discussion

The aim of the present study was to test whether BCAA supplementation was able to improve exercise capacity and FFA oxidation in glycogen-depleted subjects.

The main results of the present study demonstrate that 3-day BCAA supplementation improved exercise capacity, lipid oxidation and plasma glucose levels during an exhaustive exercise in glycogen-depleted subjects. On the other hand, BCAA supplementation did not affect plasma ketones, plasma FFA and lactate concentration.

TCA activity is regulated by the concentration of its intermediates and by the balance between anaplerotic and cataplerotic reactions.[1, 12] Theoretically, during a lower carbohydrate availability condition (*i.e.*, prolonged fasting and/or glycogen depletion), carbon skeletons provided by amino acids transamination are the main substrates that replenish TCA intermediates through anaplerotic reactions and hepatic gluconeogenesis. However, the studies regarding the contribution of supplementary amino acids as an energy source during glycogen depletion are contradictory. In fact, some authors indicate that BCAA transamination during lower carbohydrate availability might have zero effect on TCA intermediates concentration [13] or even stimulate cataplerosis, thus limiting oxidative activity.[14, 15] These latter authors hypothesized that, as a consequence of the initial BCAA aminotransferase reaction, the oxidation of BCAA places a carbon "drain" on the TCA cycle, which may lead to a reduction in the muscle concentration of 2-oxoglutarate or other TCAI. According to this theory, the BCAA-mediated drain of 2-oxoglutarate is normally counteracted by the regeneration of this intermediate through the alanine aminotransferase reaction, provided that sufficient glycogen is available to sustain the rate of pyruvate production. However, during conditions in which glycogen availability becomes limited, and particularly if the rate of BCAA oxidation is increased, it was suggested that the concentrations of 2-oxoglutarate and/or other TCA intermediates will decrease. These authors have further proposed that this scenario may lead to a "suboptimal concentration" of TCA intermediates and impair oxidative energy provision in skeletal muscle by reducing TCA flux. In contrast, our group did not previously observe any changes in the performance and TCA intermediates concentration of glycogen-depleted rats that were submitted to intensive exercise, supplemented with BCAA or isoleucine, leucine and valine alone (unpublished data). Additionally, Wagenmakers theory has also been refuted [13] demonstrating that BCAA ingestion,

during conditions of reduced glycogen availability, did not affect the concentration of 2-oxoglutarate or other TCA intermediates in human skeletal muscle during exercise. However, it is worthwhile to highlight that subjects were not completely glycogen-depleted (~200 mmol/kg dry wt).[13] In the present study, we used a severe protocol in order to induce glycogen depletion (see *Glycogen Depletion Protocol*) and despite the fact that we were not able to assess glycogen muscle content, we may presume the effectiveness of this maneuver based on previous studies, which used similar protocol.[10, 11] Therefore, whilst the extent of glycogen depletion could not be directly determined, it is far unlikely that a substantial glycogen diminution did not occur. Our findings also are in disagreement with hypothesis of Wagenmakers *et al*.,[15] since we observed a greater resistance to fatigue as a consequence of BCAA supplementation. Collectively, these studies suggest that any potential drain of TCA during exercise is likely to be small and to not significantly impact the total concentration of intermediates, even following rigorous glycogen depletion.

It has been suggested that TCA flux is regulated by the condensation of proper amounts of oxaloacetate and citrate.[2] Thus, the continuous production of oxaloacetate can be considered a key step in oxidative metabolism. As glycogen concentration itself cannot support the oxaloacetate demand imposed by severe fasting and exhaustive exercise, FFA oxidation becomes limited by carbohydrate availability.[2, 3] Assuming the veracity of this hypothesis, it was expected that a possible BCAA supplementation-mediated augmentation in oxaloacetate concentration would lead to increased lipid oxidation. In spite of no changes in serum FFA concentration, we observed with interest that BCAA supplementation promoted a decreased RER when compared to the placebo, suggesting increased lipid oxidation. Considering again that TCA may be a limiting factor for lipid oxidation by the skeletal muscle during exhaustive exercise,[2] our findings suggest that BCAA supplementation may contribute to this process, possibly expanding TCA flux. Furthermore, the reduced RER can also reveal a BCAA supplementation-induced glycogen-sparing effect, which might explain the greater exercise capacity. In fact, we and others [16] verified that BCAA supplementation promotes a higher hepatic and muscle glycogen concentration in fasting and after exercise. However, caution should be exercised because we were not able to perform muscle biopsies; therefore, we cannot conclude if TCA intermediates (*i.e*., oxaloacetate) and muscle glycogen concentration are indeed affected by BCAA supplementation as we previously hypothesized.

Alternatively, we cannot rule out the hypothesis that the improved glycemia maintenance during exercise as a result of BCAA supplementation might also have contributed to a better exercise capacity, since even a slight reduction of glycemia seems to be associated with fatigue. Importantly, our findings suggest that BCAA supplementation appears to prevent the exercise-induced hypoglycemia, particularly in glycogen depleted subjects. Opposing our results, Tang [17] observed no plasma glucose changes in swimmers supplemented with BCAA for 15 days after a 25-minute crawl stroke session. Nonetheless, it is important to highlight that these athletes were not in a glycogen-depleted condition. Calders *et al*.[18] verified that rats receiving BCAA significantly improved their resistance to fatigue compared with those receiving saline, but not with those supplemented with glucose. Furthermore, these authors observed that when glucose is administered before exercise, the supplementary administration of BCAA had no additional effect on performance. Overall, these findings corroborate the hypothesis that the effect of BCAA administration on performance could be related to carbohydrate availability during exercise.

In addition to the hypothesis that BCAA supplementation could enhance lipid oxidation, we speculated that, in glycogen-depleted subjects, this supplement would inhibit or attenuate plasma ketones production as a result of a greater TCA expansion. However, no significant differences were noted in beta hydroxybutyrate concentration. Moreover, we cannot prove the hypothesis that BCAA supplementation-induced RER reduction would occur in parallel to lactate concentration decreasing. In contrast to our findings, De Palo *et al*.[19] found a decreased lactate concentration in athletes supplemented with BCAA for one month. The authors conclude that the lower lactate level at the end of an intense muscular exercise may reflect an improvement of BCAA use, due to the chronic supplementation with BCAA. Apparently, the short-term supplementation used in our study may partially explain this discrepancy. Thus, blood ketones and lactate concentration did not pro-

vide evidence for explaining the higher exercise capacity after BCAA supplementation in the present study.

Of note, the reader should be aware that practical application of the present findings has some limitations, since it seems unreasonable to give supplementary amino acids instead carbohydrate to an individual in glycogen depleted state. In fact, one can say that of more practical relevance are the previous findings that BCAA conferred no further benefit to exercise performance when subjects were also give glucose prior to exercise. Perhaps most importantly, however, is the fact that our experimental design allowed us to obtain direct evidence that BCAA ingestion do not induce fatigue in glycogen depletion state, contrasting the previous hypothesis by Wagenmakers *et al*.[14, 15] Additionally, even though our results are consistent in all of the subjects, our sample is rather small, which warrants further investigation.

In summary, BCAA supplementation increases resistance to fatigue and enhances lipid oxidation during exercise in glycogen-depleted subjects. These actions do not seem to be related to changes in plasma FFA, lactate and blood ketones concentration. Further studies should consider the use of a muscle biopsy aimed to investigate the mechanisms underlying these findings.

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