

Reduction of Muscle Injuries and Improved Post-exercise Recovery by Branched-Chain Amino Acid Supplementation: A Systematic Review and Meta-Analysis

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Review Article</p> <hr/> <p><i>Article History:</i> Received: 23 Feb 2019 Accepted: 29 Apr 2019 Published: 1 Jan 2020</p> <hr/> <p><i>Keywords:</i> BCAA Muscle Injury DOMS CK Meta-analysis</p>	<p>This meta-analysis and systematic review aimed to attain specific data on the effect of branched-chain amino acids (BCAAs) administration on muscle injuries and the indices of delayed-onset muscle soreness (DOMS) after exercise. Literature search was performed in databases such as Scopus, ISI, Web of Science, Scientific Information Database (SID), Cochrane Controlled Register of Trials (CENTRAL), and Cochrane library for the articles published until January 2017. The clinical trials examining the effects of BCAA administration on athletes were considered eligible. In total, 42 studies were evaluated in terms of eligibility, 26 of which were excluded from the meta-analysis. According to the meta-analysis, BCAA supplementation significantly reduced the levels of creatine kinase 24 hours post-exercise (mean difference: -129.55 [95% CI: -237.02--22.07] IU/l; P=0.018). However, BCAA administration could not decrease lactate dehydrogenase promptly (mean difference: -10.11 [95% CI: -21.76-1.53] IU/l; P=0.08) 24 hours post-exercise (mean difference: -14.66 [95% CI: -32.16-2.83] IU/l; P=0.10). Therefore, it could be concluded that BCAA consumption is inversely associated with DOMS at 24 hours (standardized mean difference [SMD] = -0.43 [95% CI: -0.71--0.16]; P=0.002), 48 hours (SMD=-0.55 [95% CI: -0.81--0.29]; P<0.0001), and 72 hours post-exercise (SMD=-0.44 [95% CI: -0.72--0.16]; P=0.002). Furthermore, the findings of the systematic review and meta-analysis indicated that BCAA supplementation could alleviate muscle damage within the first 24 hours after exercise, and it seems that the consumption of daily doses of BCAA is more effective in the recovery of athletes compared to the periodic doses.</p>

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Introduction

Muscle injury and delayed-onset muscle soreness (DOMS) after intensive and uncommon physical exercise expand gently and persist for several days [1]. DOMS is an indicator of muscle injury, affecting muscle performance in athletes. Abnormal muscle contractions extract the major magnitude of DOMS, which is perceived as pain in the skeletal muscles upon palpation or circulation after physical exercise, generally peaking within 24-48 hours after the exercise [2]. Due to muscle cell damage, several myocellular proteins (e.g., creatine kinase [3], myoglobin [4], and lactate dehydrogenase [5]) are released into the bloodstream, and their concentrations increase in the plasma. These proteins are often

used as the indirect markers of muscle fiber damage.

Various methods are applied for the assessment of DOMS, such as the visual analogue scale (VAS), which is used for pain measurement and determining the personal experience of DOMS on a scale of 0-10. Several interventions have been investigated for the prevention or reduction of DOMS and muscle injury and improvement of recovery from exercise-induced muscle damage, including pharmacological therapies and physical treatments [2, 6]. Moreover, numerous nutritional supplements have been assessed as the possible preventative/therapeutic agents, including branched-chain amino acids (BCAAs), leucine, isoleucine, and valine [7, 8]. BCAAs constitute 14-18% of the amino acids in muscle

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proteins. In contrast to the other essential amino acids that are mainly catabolized in the liver, BCAAs are primarily catabolized in muscles [9]. BCAAs are essential amino acids, which are involved in other mechanisms in addition to their function as essential amino acids, especially leucine. For instance, they regulate protein metabolism by suppressing protein degradation and promoting protein synthesis. As such, BCAA supplementation before or after physical exercise could accelerate the recovery of injured muscles [10].

Previous studies have described the beneficial effects of BCAA administration on the DOMS. Accordingly, BCAA supplementation before exercise could decrease DOMS and muscle injury within a short time post-exercise [9]. On the other hand, some studies have reported that BCAA supplementation could not reduce DOMS and muscle injury during recovery from eccentric exercise with higher intensity [5, 7, 11]. These findings suggest that BCAA

supplementation does not reduce DOMS and muscle injuries. Due to the inconsistencies in the findings regarding the effectiveness of BCAA supplementation in the alleviation of muscle injuries and DOMS, this meta-analysis aimed to evaluate the effects of BCAA supplementation on DOMS and muscle injury during exercise in athletes.

Materials and Methods

This review study was conducted in accordance with the guidelines of the PRISMA statement for the reporting of systematic reviews and meta-analyses. The population, intervention, comparator, outcome, and setting (PICOS) criteria used to perform this systematic review are presented in Table 1. Considering the study design (systematic review and meta-analysis), the local legislation ethical approval was not required. The study protocol has been registered on PROSPERO (code: CRD42017058985).

Table 1- PICOS (population, intervention, comparator, outcome, setting) criteria used to perform the systematic review

PICOS	Criteria
Population	Healthy active subjects
Intervention	BCAA supplementation
Comparator	Placebo group
Outcome	DOMS, LDH, and Creatine kinase
Setting	Clinical trials

Literature Search

Two authors (E. P. and M. M.) independently performed an extended literature search in databases such as Scopus, ISI, Web of Science, Scientific Information Database (SID), Cochrane Controlled Register of Trials (CENTRAL), and Cochrane library for the original, full-text articles published until January 2017. No restrictions were applied to the publication year, and all the studies published in English and Persian were selected. The literature search was conducted using various medical subject heading terms and keywords with all their possible combinations, including branched-chain amino acids or BCAA, leucine, isoleucine, valine, AND athletes, or exercise, sport, training, athletics AND muscle damage, muscle soreness, injury, creatine kinase, CK, creatine phosphokinase, phosphorus-creatine kinase, CPK, delayed-onset muscle soreness, lactate dehydrogenase, LDH, and recovery. The related article function was also used to expand the search, and the reference lists of the selected full-text articles were searched for other relevant articles.

Study Selection

BCAA supplementation was defined as any treatment containing valine, leucine, and isoleucine with or without combination with various forms of artificial nutrition as reported in the reviewed articles.

The inclusion criteria of the study were as follows: 1) studies conducted on patients aged more than 18 years; 2) studies conducted on athletes following regular exercise regimes; 3) controlled trials; 4) studies focused on BCAA supplementation; 5) trials reporting at least one of the outcomes considered in this systematic review and meta-analysis and 6) English or Persian articles. All the retrieved articles were assessed regardless of the form of the administered BCAAs (powder, pills or sports drinks).

The articles with the following criteria were excluded from further analysis: 1) observational studies (cohort studies, case-control studies, ecological studies, case reports, and case series); 2) studies using BCAA combined with other nutrients with potential metabolic activity (e.g., amino acids, nucleotides, creatine, and omega-3 fatty acids); 3) articles without full-text

availability, opinion pieces, review articles, and editorials.

Data Extraction

An electronic database was developed in Microsoft Excel to collect the data of all the relevant trials. The data were extracted independently by two researchers (E. P. and M. M.), and in case of disagreement, A. R. A. cross-examined the uncertain data after a consensus meeting. The extracted data from the trials included the name of the first author, country of origin, year of publication, study type (parallel/cross-over), gender of the patients, blinding, BCAA dosage, administration method, duration of supplementation, regimen of the control groups, type of sport/exercise, and measurement of various outcomes.

The primary objective of this systematic review and meta-analysis was to determine whether BCAA supplementation could affect recovery and muscle injury after exercise. The following sections represent the most frequent measurements in conducting the meta-analysis. In addition, data on the DOMS, serum creatine kinase (CK), and lactate dehydrogenase (LDH) levels before and after BCAA supplementation were extracted in order to assess the effects of BCAA supplementation. The quality of the retrieved studies was evaluated by two independent reviewers (E. P. and M. M.) based on the Jadad scale [12].

Quality Assessment

Two reviewers (M. R. and A. R.) performed the quality assessment of the retrieved studies independently. The Jadad scale was employed to assess the methodological quality of the selected studies based on the methods pertinent to randomization, double-blinding, and descriptions of the withdrawals within the score range of 0-5; the scores within the range of 0-2 were considered low, and the scores within the range of 3-5 were considered high.

Statistical Analysis

The effect size estimated by the mean difference (MD) was used to perform the meta-analysis based on a fixed method. In addition, a random-

effects meta-analysis was conducted for each measurement where there was significant heterogeneity between the studies [13]. Heterogeneity was assessed using the I^2 index by assessing the null hypothesis that all the selected studies shared a common effect size. Heterogeneity was considered low at $I^2 < 30\%$, moderate at $I^2 = 30-75\%$, and high at $I^2 > 75\%$ [14]. In order to identify the potential source of heterogeneity, stratified analyses were performed based on indicators such as the BCAA dosage (≥ 100 mg/kg/day or < 100 mg/kg/day), duration of BCAA supplementation (acute/chronic), and study quality (low versus high). Acute supplementation referred to the interventions that were conducted in a single day.

Funnel plots were employed to visually inspect the presence of publication bias. For the further investigation of publication bias, Begg's rank correlation and Egger's linear regression were used. Data analysis was performed in Stata software version 12 SE (Stata Crop, College Station, TX, USA), and the P-value of less than 0.05 was considered statistically significant.

Results

Characteristics of the Selected Studies

As is depicted in Figure 1, the early electronic search resulted in 485 studies after the removal of the duplicates. After the screening of the titles and abstracts, 443 articles were excluded due to reporting unrelated data, using animal subjects, and conducting review studies. In total, 42 articles were evaluated in terms of eligibility, and 26 studies were excluded due to using a combination prepared with other ergogenic aids or amino acids [15-18], not reporting the measurements related to the research objectives [19-36], inaccurate reports [37], and publication in other languages than English or Persian [38-40]. Among 42 studies, 16 trials fitted the inclusion criteria of the meta-analysis. Table 2 shows the data on all the retrieved trials in this systematic review.

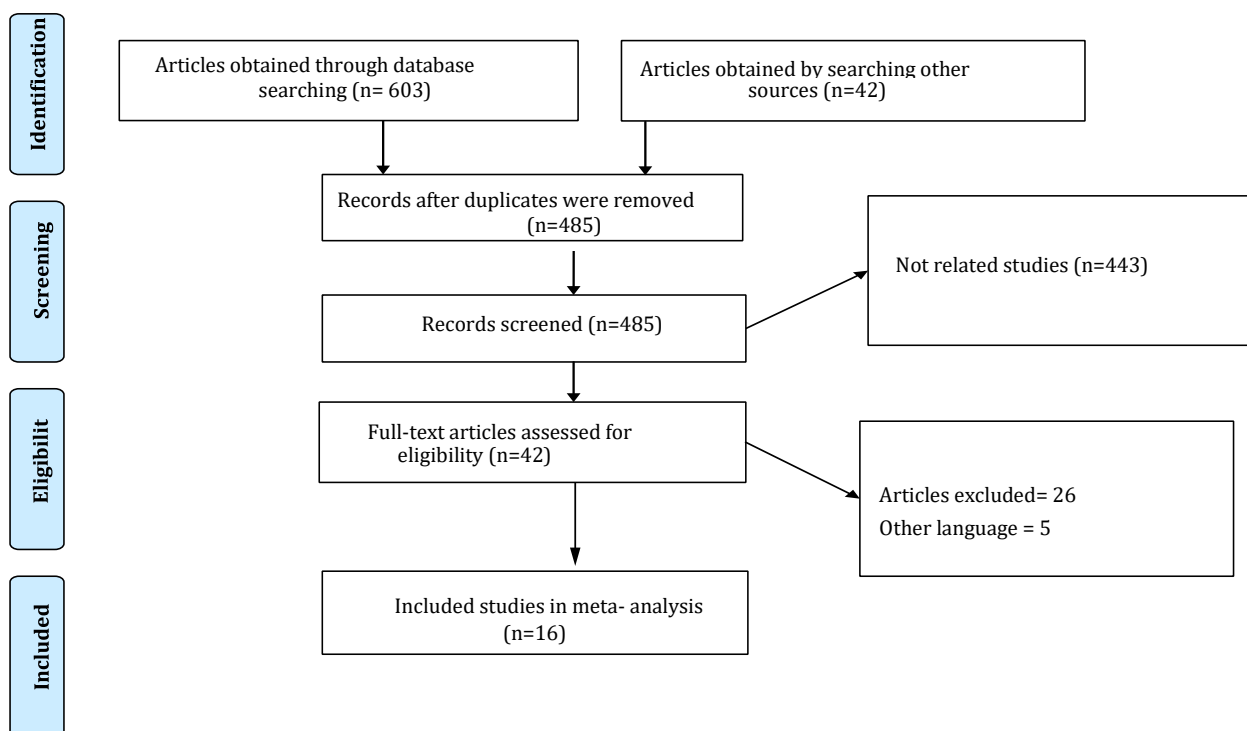


Figure 1- Flow diagram of literature search according to the PRISMA statement

Table 2- Characteristics of the Included Trials

Author (year)	Country	Study design	Gender	Blindness	Quality	N (int./plac.)	Duration	Supplement type	Placebo type	Supplement dose	Outcomes	Sport type	Results
Shimomura, Y (2010) ⁶⁵	Japan	Cross over	Female	Double	High	12 (12/12)	Acute	BCAA, green-tea, artificial sweetener,	Dextrin, green-tea, artificial sweetener,	4.5 g (1.2;2.3;1)	CK DOMS	Squat Exercise	Reduction in DOMS
Matsumoto, K (2009) ⁶⁶	Japan	Cross over	Both	Double	High	12 (12/12)	3 days	BCAA, Arg, CHO, artificial sweetener	Dextrin, Arg, CHO, artificial sweetener	20 g/day (5;10;5)	CK LDH DOMS	Long-distance running	Muscle soreness and fatigue sensation were lower in BCAA group
Howatson, G (2012) ⁶⁷	UK	Parallel	Male	Double	High	12 (6/6)	12 days	BCAA, aspartame, water	Aspartame, water	20 g/day (5;10;5)	CK DOMS	Resistance exercise	Significant reduction reported in CK and DOMS in BCAA group
Koba, T (2007) ⁶⁸	Japan	Cross over	Male	Double	High	8 (8/8)	4 days	BCAA, Arg, CHO	Dextrin, Arg, CHO	2 g/day (0.5;1;0.5)	LDH CK	Distance running	LDH level was reduced in the BCAA group
Shimomura, Y (2006) ⁶⁹	Japan	Cross over	Both	Single	Low	30 (30/30)	15 days	BCAA, green tea, non-nutritive sweetener	Dextrin, green tea, non-nutritive sweetener	4.5 g/day (1.2;2.3;1)	DOMS	Squat exercise	BCAA supplementation prior to squat exercise decreased DOMS and muscle fatigue
Koo, G. H (2014) ⁷⁰	Korea	Cross over	Male	Single	Low	5 (5/5)	7 days	BCAA	Not mentioned	3.15 g/day (0.8;1.55;0.8)	CK	Rowing	BCAA supplementation had no

Author (year)	Country	Study design	Gender	Blindness	Quality	N (int./plac.)	Duration	Supplement type	Placebo type	Supplement dose	Outcomes	Sport type	Results
Ra, S. G (2013) ⁷¹	Japan	Parallel	Male	Double	High	18 (9/9)	18 days	BCAA, artificial sweetener, flavor	Starch, artificial sweetener, flavor	9.6 g/day (2.4;4.8;2.4)	DOMS LDH	Eccentric exercise	positive effect on the fatigue. Blood biochemical markers for DOMS and muscle damage showed improvement in the BCAA supplementation on group rather than placebo.
Coombes, J. (1993) ⁷¹	Australia	Parallel	Male	Single	Low	16 (8/8)	7 days	BCAA	Not mentioned	12g/day (4;4;4)	CK-LDH	Healthy male	Branched Chain Amino Acids significantly reduce the levels of the intramuscular enzymes creatine kinase and lactate dehydrogenase
Greer, B. K (2007) ⁷²	USA	Cross-over	Male	Single	Low	6 (3/3)	Acute	BCAA, artificial sweetener, flavor	flavor, artificial sweetener	2.5 g (0.75;1.25;0.5)	CK LDH DOMS	Healthy male	BCAA supplementation attenuates muscle damage during prolonged endurance exercise
Jackman, S. R. (2010) ⁷³	UK	Parallel	Male	Single	Low	24 (12/12)	3 days	BCAA, artificially sweetener, flavor	artificially sweetener, flavored	29.2 g/day (6.8;14;8.4)	CK DOMS	Eccentric exercise protocol	BCAA supplementation may not attenuate muscle soreness
Barzegari, A (2011) ⁷⁴	Iran	Parallel	Male	Single	Low	19 (10/9)	Acute	BCAA	Dextrin	278 mg/kg (69.5;139;69.5) 518 mg/kg (129.5;259;129.5)	CK LDH	Wrestling	These results provide evidence that the use of two different dosage of BCAA could not decrease muscle damage.
Leahy, D. T. (2013) ⁵²	USA	Cross-over	Both	Double	High	20 (10/10)	4 days	BCAA, Chloride, sodium, potassium, Vitamin A, ascorbic acid, Vitamin E, thiamin, riboflavin, niacin, Vitamin B6	Lemonade powder, dextrose, citric acid, potassium, sodium artificial sweetener, magnesium oxide, flavor, lemon juice solids, soy lecithin,	1.22 g	DOMS	Squatting exercise	No significant effect of BCAA supplementation versus placebo was noted on muscle damage

Author (year)	Country	Study design	Gender	Blindness	Quality	N (int./plac.)	Duration	Supplement type	Placebo type	Supplement dose	Outcomes	Sport type	Results
Kim, D. H. (2013) ⁷⁵	Korea	Parallel	Male	Double	High	26 (13/13)	Acute	BCAA was dissolved in reverse osmosis water	artificial color, BHA. reverse osmosis water	80 mg/kg (19.2;36.8;16)	CK LDH	bicycle ergometer	BCAA supplementation may reduce the muscle damage associated with endurance exercise
Amirsasan, R (2011) ⁷⁶	Iran	Parallel	Male	Single	Low	19 (10/9)	Acute	BCAA	Dextrin	414 mg/day (103.5;207;103.5) 654 mg/day (163.5;327;163.5)	CK LDH	Wrestling	Different dosages of BCAA did not decrease the muscle damage
Fouré, A (2016) ⁷⁷	France	Parallel	Male	Double	High	26 (13/13)	5d	BCAA	Cellulose	100 mg/kg (25;50;25)	CK DOMS	Young healthy men	No significant effect of BCAA supplementation on versus placebo was noted on muscle damage
Mark Waldron (2017) ⁴⁸	UK	Parallel	Both	Double	Low	16 (8/8)	Acute	BCAA, dextrose	dextrose	87mg/kg (21.75;43.5;21.75)	CK DOMS	Resistance-trained athletes	Acute supplementation of BCAAs (0.087 g/kg) increased the rate of recovery in isometric strength and perceived muscle soreness compared with placebo

*Arg, Arginine; BCAA, Branched-chain amino acid; CK, Creatine kinase; DOMS, Delayed onset muscle soreness; LDH, Lactate dehydrogenase

In total, nine studies employed a parallel study design (41, 48, 67, 71, 73- 77), while seven studies used a cross-over design (52, 65, 66, 68-70, 72). The majority of the studies were conducted on male subjects. In addition, six trials investigated the effects of BCAA acute supplementation (48, 65, 72, 74-76). The duration of intervention in seven studies was less than or equal to one week (41, 52, 66, 68, 70, 73, 77), while in three trials, the duration was longer than one week (67, 69, 71). Based on the Jadad scale, the study quality of nine trials was high, while the quality of eight studies was considered to be low due to the lack of randomization or double-blinding.

BCAA Supplementation and CK

In total, nine (88 treatments and 88 placebos) [9, 41-48], six (64 treatments and 62 placebos) [41-43, 48-50], six (68 treatments and 66 placebos) [9, 42, 43, 48-50], and three studies (31 treatments and 31 placebos) [9, 42, 50] reported the effects of BCAA supplementation on serum CK levels to occur immediately, 24, 48, and 72 hours post-exercise, respectively. In one of the reviewed studies, two doses of BCAA were used, and two effect sizes were analyzed in the meta-analysis [49]. Accordingly, BCAA supplementation significantly reduced CK levels 24 hours post-exercise compared to the placebo group (mean difference=-129.55 [95% CI: -237.02--22.07] IU/l; P=0.018; Figure 2-b).

However, BCAA supplementation had no significant effect on the CK levels immediately (mean difference=-5.34 [95% CI: -22.18-11.48] IU/l; P=0.53), 48 hours (mean difference=-4.35

[95% CI: -22.35-13.64] IU/l; P=0.63), and 72 hours post-exercise (mean difference=-13.25 [95% CI: -28.79-2.27] IU/l; P=0.53) compared to the placebo group.

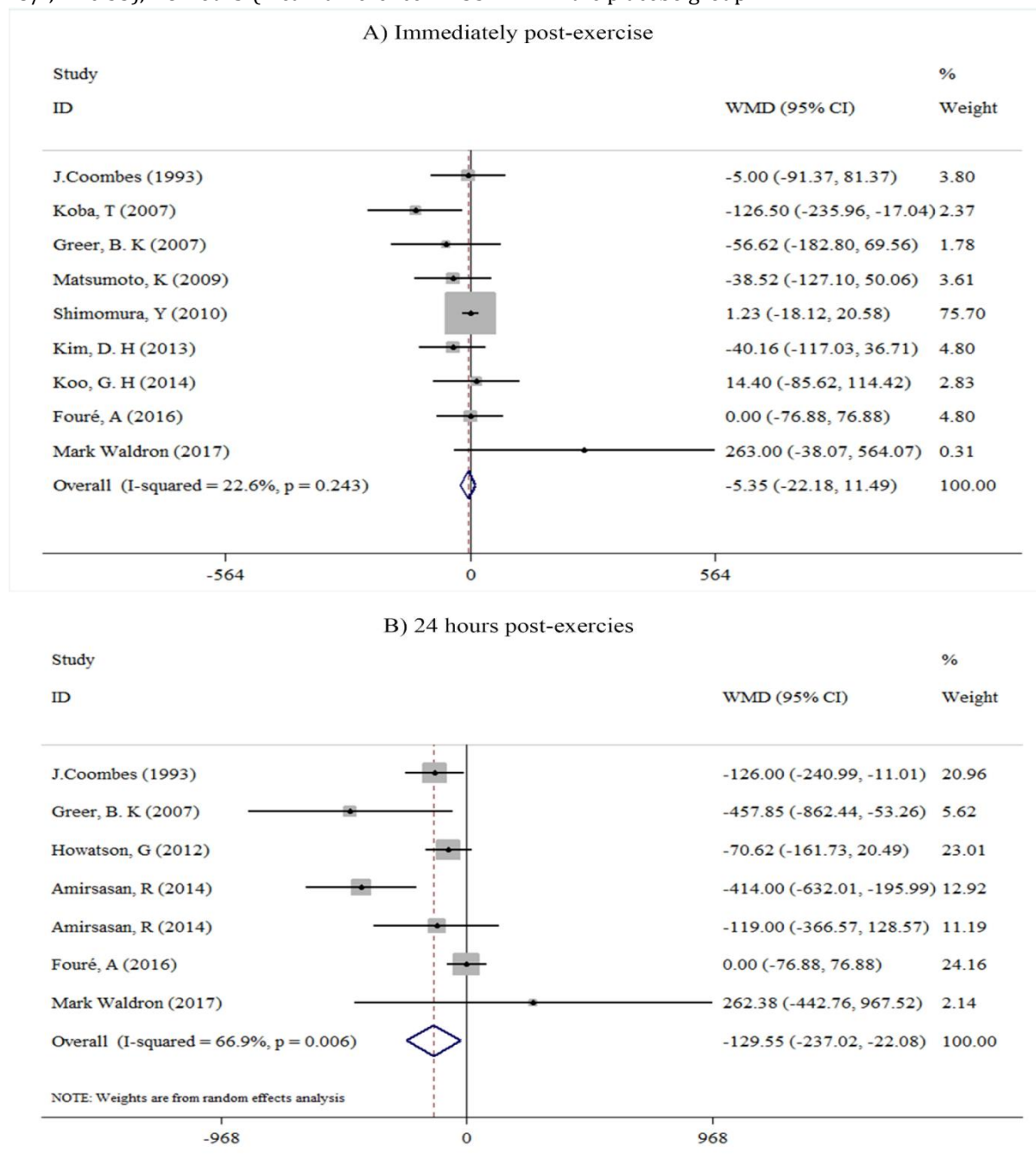


Figure 2. Forest Plot Details on Weighted Mean Difference at 95% Confidence Interval (CI) Regarding Impact of BCAA Supplementation on CK (Black squares show study-specific standardized differences [Std diff.] in means, and horizontal lines show 95% CI; area of black squares is proportional to specific-study weight in overall meta-analysis; center of black diamonds indicates pooled standardized difference in means, and their width represents pooled 95% CI.)

Moderate heterogeneity was observed between the reviewed studies at 24 hours post-exercise ($I^2=66.9$; $P=0.006$), while heterogeneity was low immediately ($I^2=22.6$; $P=0.09$), 48 hours ($I^2=28.9$; $P=0.20$), and 72 hours post-exercise ($I^2=0.0$; $P=0.38$). Figure 3 shows the cumulative analysis of the effect of BCAA on CK levels post-exercise. However, sensitivity analysis provided no further data in this regard. With regard to 24

hours post-exercise, the subgroup analysis of the study duration (acute/more than one day of supplementation), quality (high versus low), and supplemented dose (less than 100 mg/kg or more than 100 mg/kg) indicated the study quality, intervention duration, and supplement dose to be the sources of heterogeneity. On the other hand, the funnel plot shows no publication bias between the reviewed trials.

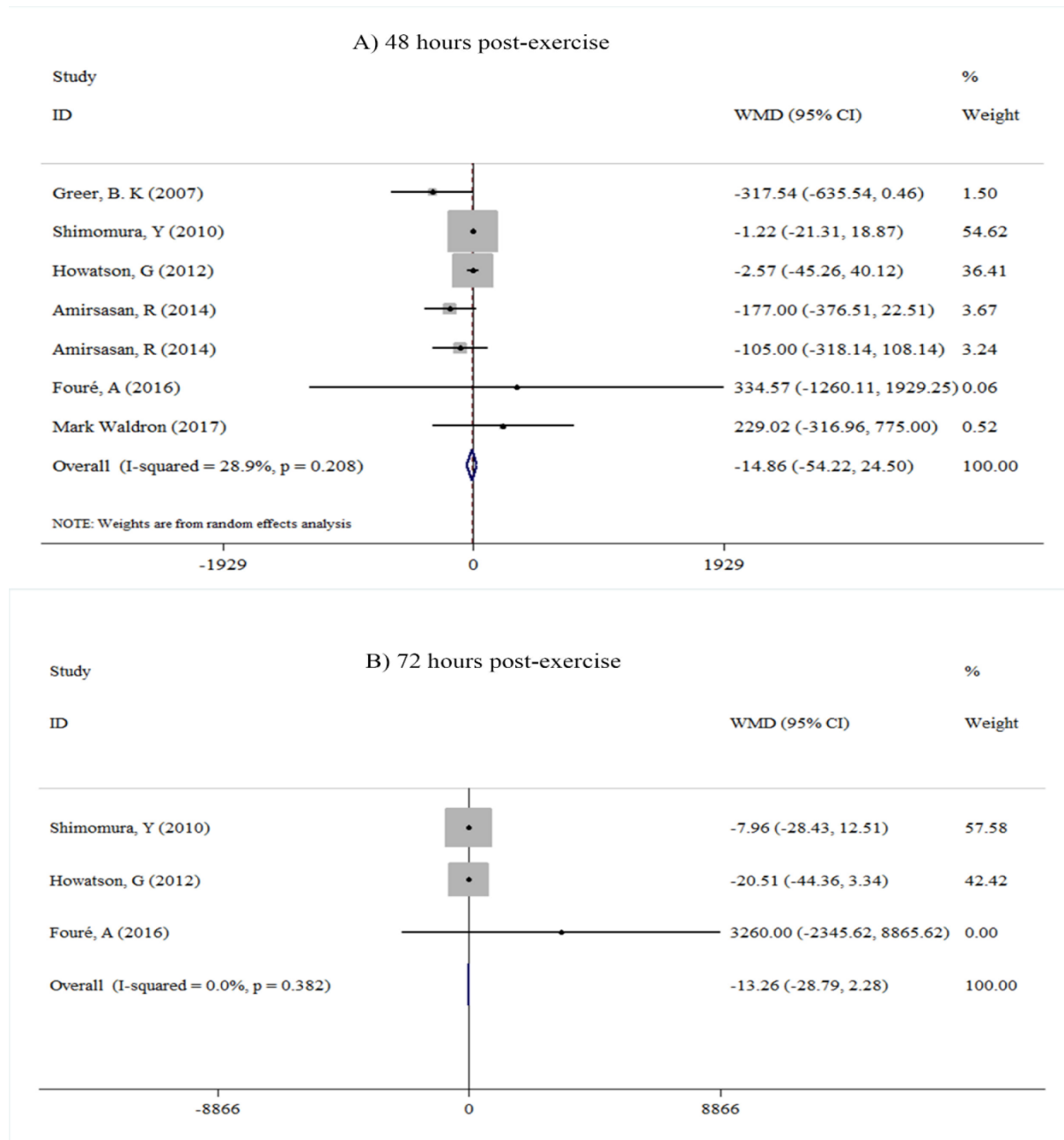


Figure 3. Cumulative Analysis Details on Weighted Mean Difference and 95% CI Regarding Impact of BCAA Supplementation on CK (Black squares show study-specific standardized differences [Std diff.] in means, and horizontal lines show 95% CI; area of black squares is proportional to specific-study weight in overall meta-analysis; center of black diamonds indicates pooled standardized difference in means, and their width represents pooled 95% CI.)

BCAA Supplementation and LDH

In total, seven studies provided adequate data to evaluate the effect of BCAA supplementation on LDH [41, 43-45, 47, 49, 51]. As is depicted in Figure 4, there was a trend in the reduction of LDH immediately (mean difference=-10.11 [95% CI: -21.76-1.53] IU/l; P=0.08) and 24 hours post-

exercise (mean difference=-14.66 [95% CI: -32.16-2.83] IU/l; P=0.10) in the BCAA group compared to the placebo group. Furthermore, the LDH levels were not significantly affected by BCAA supplementation 48 hours after exercise (mean difference=-7.50 [95% CI: -30.10-15.10] IU/l; P=0.51). Heterogeneity was not evident among the reviewed studies (I²=0.0).

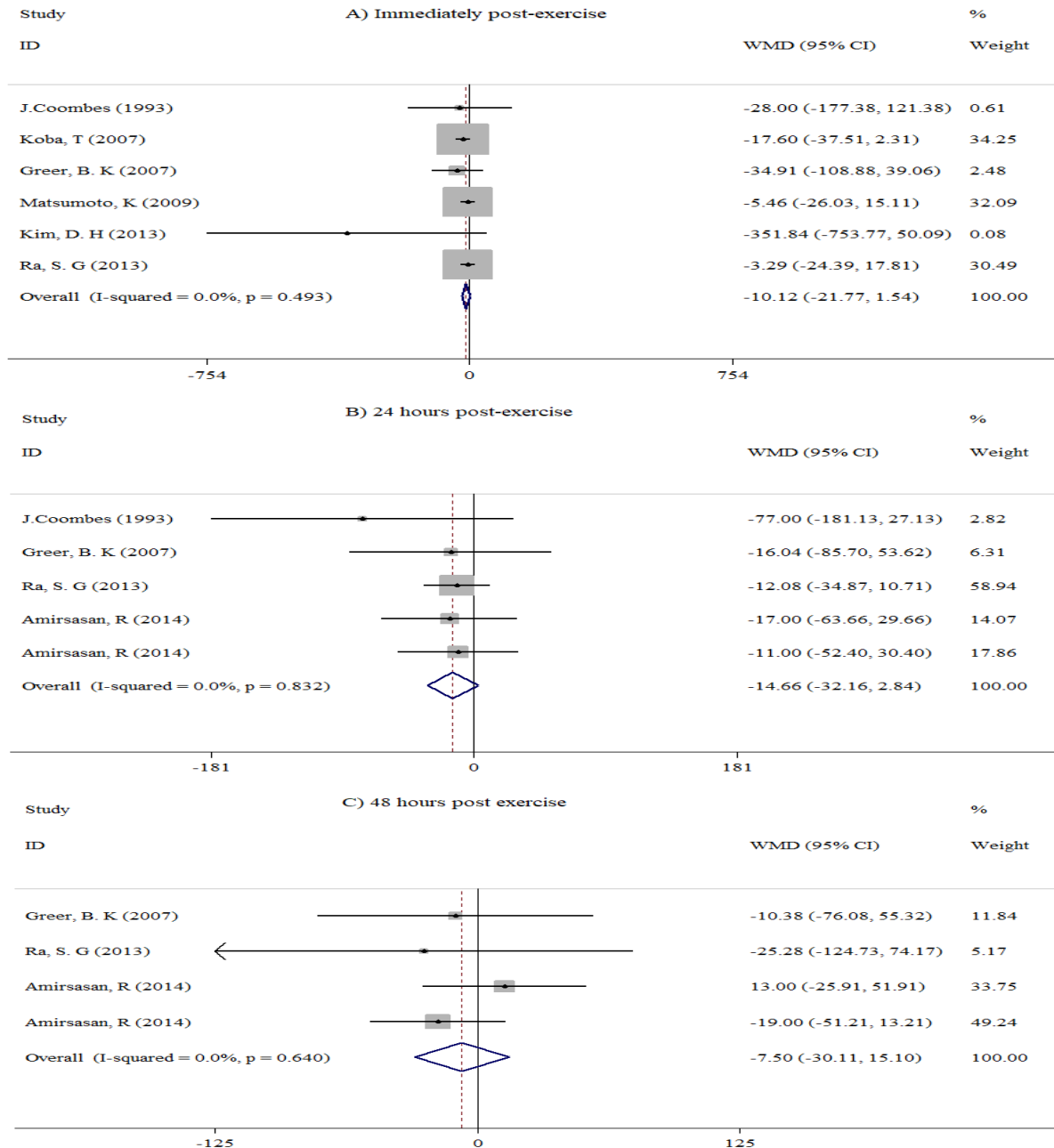


Figure 4. Forest Plot Details on Weighted Mean Difference and 95% CI Regarding Impact of BCAA Supplementation on LDH (Black squares show study-specific standardized differences [Std diff.] in means, and horizontal lines show 95% CI; area of black squares is proportional to specific-study weight in overall meta-analysis; center of black diamonds indicates pooled standardized difference in means, and their width represents pooled 95% CI.)

Figure 5 shows the cumulative analysis regarding the effect of BCAA on LDH levels post-exercise. However, sensitivity analysis provided no further data in this regard. With respect to the

trials considering LDH, neither Begg's and Egger's tests nor the visual inspection of the funnel plot indicated publication bias.

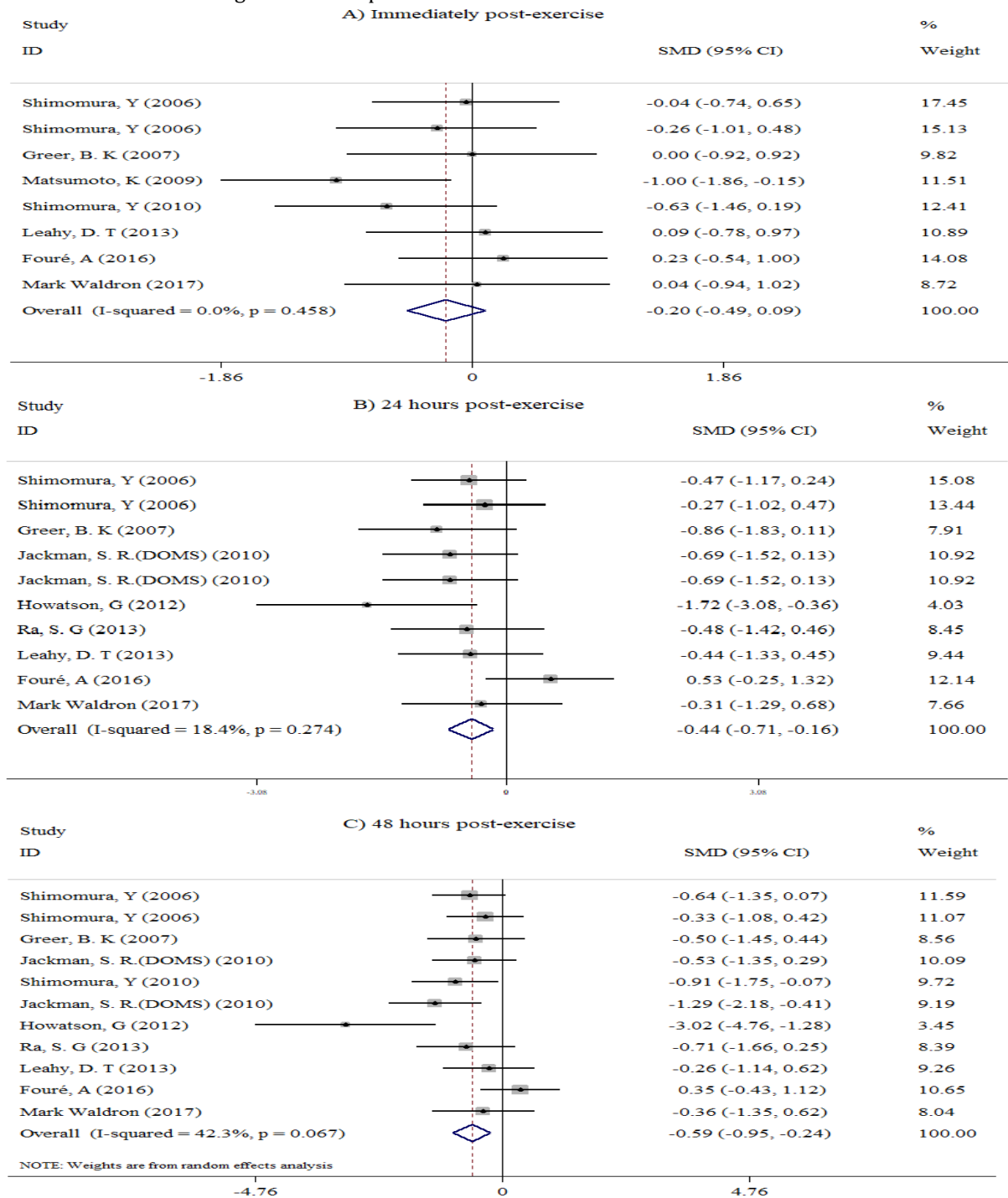


Figure 5. Cumulative Analysis Details on Weighted Mean Difference and 95% CI Regarding Impact of BCAA Supplementation on LDH (Black squares show study-specific standardized differences [Std diff.] in means, and horizontal lines show 95% CI; area of black squares is proportional to specific-study weight in overall meta-analysis; center of black diamonds indicates pooled standardized difference in means, and their width represents pooled 95% CI.)

BCAA Supplementation and DOMS

In total, 12 effect sizes were assessed in the meta-analysis in order to determine the effect of BCAA supplementation on DOMS [8, 9, 11, 42, 43, 47, 48, 50-52]. Two studies provided two different effect sizes [8, 11]. The results of the meta-analysis (figure 6) suggested that BCAA consumption was inversely associated with

DOMS at 24 hours (SMD=-0.43 [95% CI: -0.71--0.16]; P=0.002), 48 hours (SMD=-0.55 [95% CI: -0.81--0.29]; P<0.0001), and 72 hours post-exercise (SMD=-0.44 [95% CI: -0.72--0.16]; P=0.002). Moreover, no associations were observed between BCAA consumption and DOMS score immediately or 96 hours post-exercise (SMD=-0.19 [95% CI: -0.48-0.09]; P=0.18, SMD=-0.17 [95% CI: -0.48-0.14]; P=0.27, respectively).

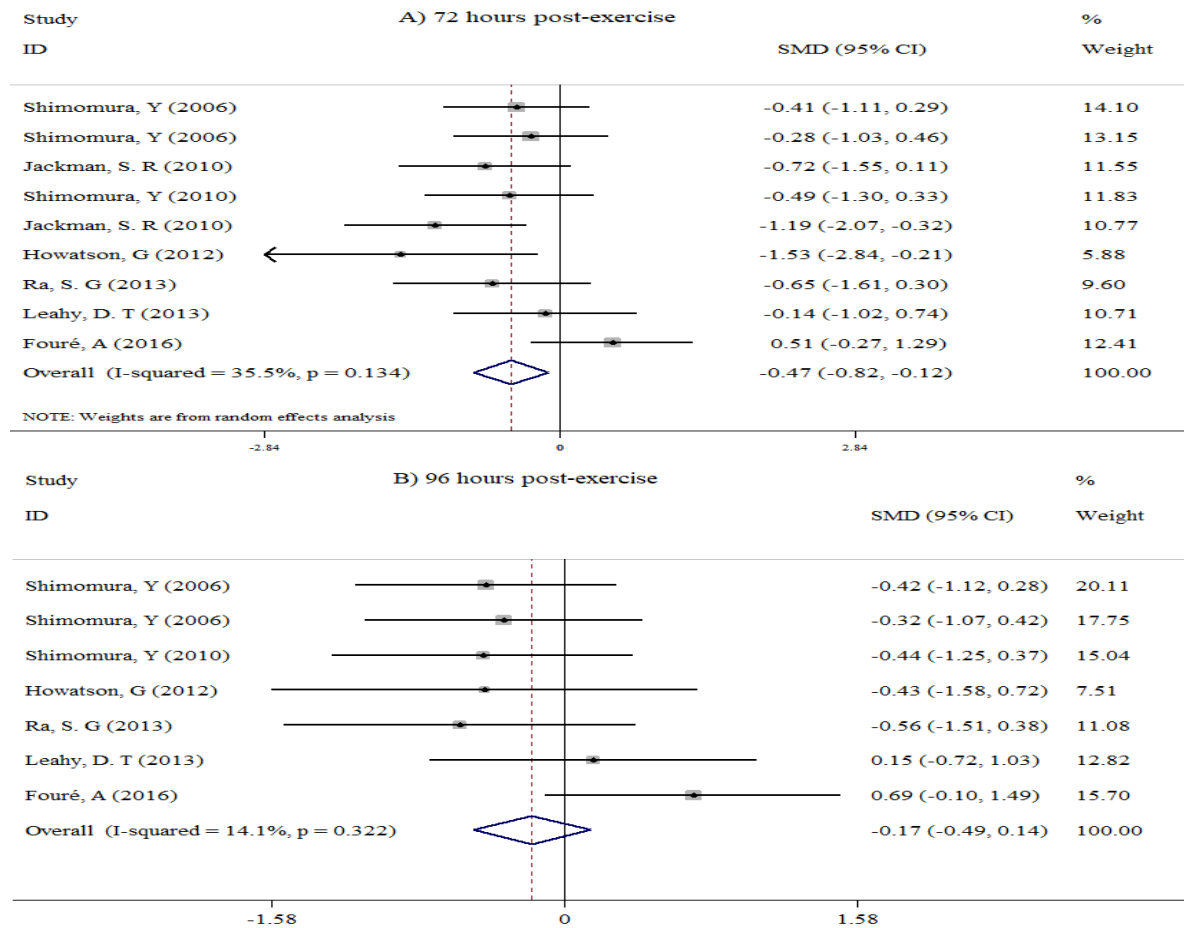


Figure 6. Forest Plot Details on Weighted Mean Difference and 95% CI Regarding Impact of BCAA Supplementation on DOMS (Black squares show study-specific standardized differences [Std diff.] in means, and horizontal lines show 95% CI; area of black squares is proportional to specific-study weight in overall meta-analysis; center of black diamonds indicates pooled standardized difference in means, and their width represents pooled 95% CI.)

Figure 7 shows the cumulative analysis regarding the effect of BCAA on DOMS post-exercise. There was low heterogeneity between the reviewed studies immediately (I²=0.0; P=0.45), 24 hours (I²=18.4; P=0.27), and 96 hours post-exercise (I²=14.1; P=0.32), while moderate heterogeneity was observed between the trials 48 hours (I²=42.3; P=0.06) and 72 hours post-exercise (I²=35.5; P=0.13). In

addition, the subgroup analysis indicated that the study duration (acute/more than one day of supplementation), study quality (high versus low), and supplemented dose (less than 100 mg/kg or more than 100 mg/kg) could be the sources of heterogeneity. However, sensitivity analysis indicated no changes in the results and publication bias.

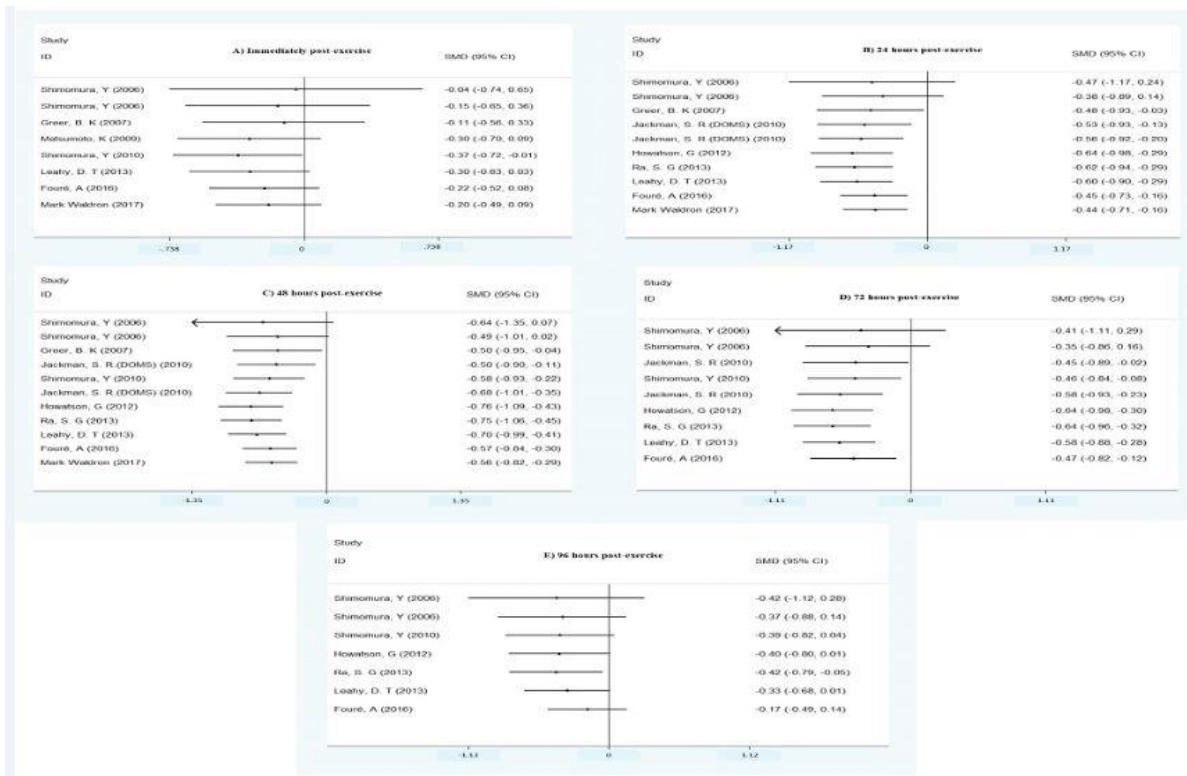


Figure 7. Cumulative Analysis Details on Weighted Mean Difference and 95% CI Regarding Impact of BCAA Supplementation on DOMS (Black squares show study-specific standardized differences [Std diff.] in means, and horizontal lines show 95% CI; area of black squares is proportional to specific-study weight in overall meta-analysis; center of black diamond's indicates pooled standardized difference in means, and their width represents pooled 95% CI.)

Discussion

To the best of our knowledge, the current meta-analysis is the first review study to provide new insight into the effects of BCAAs on post-exercise recovery at various time intervals, as well as some factors associated with muscle injury. This systematic review and meta-analysis demonstrated that BCAA supplementation significantly decreased CK levels at 24 hours post-exercise, while it did not decrease LDH immediately and 24 hours post-exercise. Additionally, BCAA consumption was reported to be reversely associated with DOMS at 24, 48, and 72 hours post-exercise.

Within the past years, numerous studies have indicated that nutritional strategies could alleviate exercise-induced injuries and accelerate recovery. BCAA supplementation has been used in this regard more frequently compared to other supplements [53]. In the studies by Rahimi et al. [54] and Sorichter et al. [55], BCAA supplementation was evaluated in terms of the quantity of skeletal muscle damage, while in the present study, we assessed the effects of BCAA on the plasma levels of CK and

LDH, as well as DOMS at various time intervals in order to determine the effects of BCAA supplementation on post-exercise recovery. CK is considered to be a more accurate indicator for muscle damage and has been used in several studies for the evaluation of muscle injuries [56]. According to the results of this meta-analysis, BCAA supplementation could reduce CK levels 24 hours post-exercise, while the changes in the CK level immediately, 48, and 72 hours post-exercise were not considered significant. Furthermore, BCAA supplementation at 24 hours after exercise decreases CK levels. CK is an indicator of the gaps in sarcolemma or cell membrane damage [57]. Therefore, it could be concluded that athletes with exercise-induced muscle damage, BCAA supplementation could enhance cell membrane, while the presence of such beneficial effects may require athletes to take daily supplemental BCAA support. According to the results of this review, BCAA supplementation could reduce LDH levels immediately and 24 hours after exercise. LDH is an enzyme that converts pyruvate into lactate. Exercise causes a significant increase in LDH

depending on exercise intensity and duration [58, 59]. Some studies have denoted a significant increase in LDH levels between days two and five post-exercise [60, 61]. This is the reason for the fact that BCAA supplementation cannot reduce LDH levels at 48- and 72-hour intervals. According to the literature, the effects of BCAA on the reduction of CK and LDH may depend on exercise conditions, the primary site of muscle damage, and training conditions [54].

According to this review study, BCAA supplementation is inversely associated with DOMS at 24-, 48-, and 72-hour time intervals. The mechanism that causes muscle soreness following intense exercise remains unclear, while some studies have indicated that a possible cause for exercise-induced DOMS is oxidative stress and exercise-induced free radicals, as well as inflammation in the connective tissue elements [62, 63]. This may sensate nociceptors and increase pain perception [64]. According to the literature, BCAA supplementation could decrease oxidative stress and free radical levels in athletes [51]. In addition, BCAA uptake for protein synthesis may reduce CK flux, thereby diminishing secondary damage and limiting the extent of damage, which in turn leads to the reduction of soreness precipitation [50].

One of the strengths of this review study was that we examined the effects of BCAA supplementation at various time intervals so as to identify the optimal time for the use of this supplement in case of muscle damage. One of the limitations of the current review was that some factors might have influenced the inconclusive findings in the analysis, such as the difference in the duration of the studies (acute/more than one day of supplementation), study quality (high/low), and supplement dose (less than 100 mg/kg or more than 100 mg/kg). In the current review, we selected the studies with various doses of BCAA from 1.22 grams [52] to 29.2 grams [11]. In addition, the manufacturers of BCAA supplements were different, which might have influenced supplement bioavailability. Furthermore, it was not possible to examine the effects of other influential factors in the recovery of athletic, such as inflammation, neuromuscular function, and muscle function.

Conclusion

The current document-based data demonstrated that BCAA supplementation could reduce muscle damage within the first 24 hours after exercise.

In addition, use of daily BCAA doses could be more effective in the recovery of athletes compared to the periodic doses.

Conflicts of interest

None declared.

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References

1. Friden J, Kjörrell U, Thornell LE. Delayed muscle soreness and cytoskeletal alterations: an immunocytological study in man. *Int J Sports Med.* 1984; 5(1): 15-8.
2. Cheung K, Hume P, Maxwell L. Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med.* 2003; 33(2): 145-64.
3. Clarkson PM. Exertional rhabdomyolysis and acute renal failure in marathon runners. *Sports med.* 2007; 37(4-5): 361-3.
4. Smith JE, Garbutt G, Lopes P, Tunstall Pedoe D. 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(3): 292-4.
5. Del Coso J, Fernández de Velasco D, Abián-Vicen J, Salinero JJ, González-Millán C, Areces F, et al. Running pace decrease during a marathon is positively related to blood markers of muscle damage. *PLoS One.* 2013; 8(2): e57602.
6. Cleak MJ, Eston RG. Delayed onset muscle soreness: mechanisms and management. *J Sports Sci.* 1992; 10(4): 325-41.
7. Coombes JS, McNaughton LR. Effects of branched-chain amino acid supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise. *J Sports Med Phys Fitness.* 2000; 40(3): 240-6.
8. Shimomura Y, Yamamoto Y, Bajotto G, Sato J, Murakami T, Shimomura N, et al. Nutraceutical effects of branched-chain amino acids on skeletal muscle. *J Nutr.* 2006; 136(2): 529S-32S.
9. Shimomura Y, Inaguma A, Watanabe S, Yamamoto Y, Muramatsu Y, Bajotto G, et al. Branched-chain amino acid supplementation before squat exercise and delayed-onset muscle soreness. *Int J Sport Nutr Exerc Metab.* 2010; 20(3): 236-44.
10. Garlick PJ. The role of leucine in the regulation of protein metabolism. *J Nutr.* 2005; 135 (6 Suppl): 1553S-6S.
11. Jackman SR, Witard OC, Jeukendrup AE, Tipton KD. Branched-chain amino acid ingestion can ameliorate

- soreness from eccentric exercise. *Med Sci Sports Exerc.* 2010; 42(5): 962-70.
12. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials.* 1996; 17(1): 1-12.
13. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986; 7(3): 177-88.
14. Higgins JPT, Green S. *Cochrane handbook for systematic reviews of interventions*: John Wiley & Sons; 2011.
15. Hsu MC, Chien KY, Hsu CC, Chung CJ, Chan KH, Su B. Effects of BCAA, arginine and carbohydrate combined drink on post-exercise biochemical response and psychological condition. *Chin J Physiol.* 2011; 54(2): 71-8.
16. Knechtle B, Mrazek C, Wirth A, Knechtle P, Rüst CA, Senn O, et al. Branched-chain amino acid supplementation during a 100-km ultra-marathon-A randomized controlled trial. *J Nutr Sci Vitaminol (Tokyo).* 2012; 58(1): 36-44.
17. Nosaka K, Sacco P, Mawatari K. Effects of amino acid supplementation on muscle soreness and damage. *Int J Sport Nutr Exerc Metab.* 2006; 16(6): 620-35.
18. [18] Sharp CP, Pearson DR. Amino acid supplements and recovery from high-intensity resistance training. *J Strength Cond Res.* 2010; 24(4): 1125-30.
19. Apró W, Moberg M, Hamilton DL, Ekblom B, Rooyackers O, Holmberg HC, et al. Leucine does not affect mechanistic target of rapamycin complex 1 assembly but is required for maximal ribosomal protein s6 kinase 1 activity in human skeletal muscle following resistance exercise. *FASEB J.* 2015; 29(10): 4358-73.
20. Atherton PJ, Kumar V, Selby AL, Rankin D, Hildebrandt W, Phillips BE, et al. Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men. *Clin Nutr.* 2017; 36(3): 888-95.
21. Church D, Schwarz N, Spillane M, McKinley S, Andre T, Willoughby DS. A comparison of the effects of ursolic acid and l-leucine supplementation on IGF-1 receptor and AKT-mTOR signaling in response to resistance exercise in trained men. *J Int Soc Sports Nutr.* 2014; 11 (Suppl 1): P19.
22. Churchward-Venne TA, Breen L, Di Donato DM, Hector AJ, Mitchell CJ, Moore DR, et al. Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: A double-blind, randomized trial. *Am J Clin Nutr.* 2014; 99(2): 276-86.
23. Crowe MJ, Weatherson JN, Bowden BF. Effects of dietary leucine supplementation on exercise performance. *Eur J Appl Physiol.* 2006; 97(6): 664-72.
24. da Luz CR, Nicastró H, Zanchi NE, Chaves DF, Lancha AH Jr. Potential therapeutic effects of branched-chain amino acids supplementation on resistance exercise-based muscle damage in humans. *J Int Soc Sports Nutr.* 2011; 8: 23.
25. Dickinson JM, Gundermann DM, Walker DK, Reidy PT, Borack MS, Drummond MJ, et al. Leucine-enriched Amino acid ingestion after resistance exercise prolongs myofibrillar protein synthesis and amino acid transporter expression in older men. *J Nutr.* 2014; 144(11): 1694-702.
26. Dudgeon WD, Kelley EP, Scheett TP. In a single-blind, matched group design: Branched-chain amino acid supplementation and resistance training maintains lean body mass during a caloric restricted diet. *J Int Soc Sports Nutr.* 2016; 13: 1.
27. Freyssenet D, Berthon P, Denis C, Barthelemy JC, Guezennec CY, Chatard JC. Effect of a 6-week endurance training programme and branched-chain amino acid supplementation on histomorphometric characteristics of aged human muscle. *Arch Physiol Biochem.* 1996; 104(2): 157-62.
28. Kephart WC, Wachs TD, Thompson RM, Brooks Mobley C, Fox CD, McDonald JR, et al. Ten weeks of branched-chain amino acid supplementation improves select performance and immunological variables in trained cyclists. *Amino Acids.* 2016; 48(3): 779-89.
29. MacLean DA, Graham TE, Saltin B. Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. *Am J Physiol.* 1994; 267 (6 Pt 1): E1010-22.
30. Moberg M, Apró W, Ekblom B, Van Hall G, Holmberg HC, Blomstrand E. Activation of mTORC1 by leucine is potentiated by branched-chain amino acids and even more so by essential amino acids following resistance exercise. *Am J Physiol Cell Physiol.* 2016; 310(11): C874-84.
31. Moberg M, Apró W, Ohlsson I, Pontén M, Villanueva A, Ekblom B, et al. Absence of leucine in an essential amino acid supplement reduces activation of mTORC1 signalling following resistance exercise in young females. *Appl Physiol Nutr Metab.* 2014; 39(2): 183-94.
32. Rowlands DS, Nelson AR, Raymond F, Metairon S, Mansourian R, Clarke J, et al. Protein-leucine ingestion activates a regenerative inflammo-myogenic transcriptome in skeletal muscle following intense endurance exercise. *Physiol Genomics.* 2016; 48(1): 21-32.
33. Samuelsson H, Moberg M, Apró W, Ekblom B, Blomstrand E. Intake of branched-chain or essential amino acids attenuates the elevation in muscle levels of PGC-1 α mRNA caused by resistance exercise. *Am J Physiol Endocrinol Metab.* 2016; 311(1): E246-51.
34. Tang FC. Influence of branched-chain amino acid supplementation on urinary protein metabolite concentrations after swimming. *J Am Coll Nutr.* 2006; 25(3): 188-94.
35. Trabal J, Forga M, Leyes P, Torres F, Rubio J, Prieto E, et al. Effects of free leucine supplementation and resistance training on muscle strength and functional status in older adults: A randomized controlled trial. *Clin Interv Aging.* 2015; 10: 713-23.

36. Areces F, Salinero JJ, Abian-Vicen J, González-Millán C, Gallo-Salazar C, Ruiz-Vicente D, et al. A 7-day oral supplementation with branched-chain amino acids was ineffective to prevent muscle damage during a marathon. *Amino Acids*. 2014; 46(5): 1169-76.
37. Hasan M, Maghsoud P, Fatemeh M. The effect of one period HIIT training and BCAA supplementation on indicators muscle damage in non-athlete men. *Eur J Exp Biol*. 2012; 2(6): 2001-3.
38. Liu JH, Zhou ZH, Ou MH, Wang K, Shi YQ. Effects of branched-chain amino acid supplementation on training-induced muscle damage in rowing athletes. *Chinese Journal of Clinical Rehabilitation*. 2003; 7: 3402-3.
39. Bregani ER, Aliberti S, Guariglia A. Creatine combined with branched-chain amino acids supplement in speleological practice. A scientific controlled trial. *Med Sport (Roma)*. 2005; 58(3): 233-9.
40. Myojin C, Ueshima S, Kawanishi M, Tokimoto M, Matsunami T, Sagawa K, et al. Reducing Effects of branched-chain amino acids and the citric acid on fatigue caused by exercise. *Japanese Pharmacology and Therapeutics*. 2016; 44(2): 227-34.
41. Coombes JS. The effects of branched chain amino acid supplementation on indicators of muscle damage after prolonged strenuous exercise [dissertation]. University of Tasmania - Launceston. 1993.
42. Fouré A, Nosaka K, Gastaldi M, Mattei JP, Boudinet H, Guye M, et al. Effects of branched-chain amino acids supplementation on both plasma amino acids concentration and muscle energetics changes resulting from muscle damage: A randomized placebo controlled trial. *Clin Nutr*. 2016; 35(1): 83-94.
43. Greer BK, Woodard JL, White JP, Arguello EM, Haymes EM. Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise. *Int J Sport Nutr Exerc Metab*. 2007; 17(6): 595-607.
44. Kim DH, Kim SH, Jeong WS, Lee HY. Effect of BCAA intake during endurance exercises on fatigue substances, muscle damage substances, and energy metabolism substances. *J Exerc Nutrition Biochem*. 2013; 17(4): 169-80.
45. Koba T, Hamada K, Sakurai M, Matsumoto K, Hayase H, Imaizumi K, et al. Branched-chain amino acids supplementation attenuates the accumulation of blood lactate dehydrogenase during distance running. *J Sports Med Phys Fitness*. 2007; 47(3): 316-22.
46. 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(8): 1241-6.
47. Matsumoto K, Koba T, Hamada K, Sakurai M, Higuchi T, Miyata H. Branched-chain amino acid supplementation attenuates muscle soreness, muscle damage and inflammation during an intensive training program. *J Sports Med Phys Fitness*. 2009; 49(4): 424-31.
48. Waldron M, Whelan K, Jeffries O, Burt D, Howe L, Patterson SD. The effects of acute branched-chain amino acid supplementation on recovery from a single bout of hypertrophy exercise in resistance-trained athletes. *Appl Physiol Nutr Metab*. 2017; 42(6): 630-6.
49. Amirsasan R, Nikookheslat S, Sari-Sarraf V, Kaveh B, Letafatkar A. The Effects of Two Different Dosages of BCAA Supplementation on A Serum Indicators of Muscle Damage in Wrestlers. *International Journal of Wrestling Science*. 2011; 1(2): 32-6.
50. Howatson G, Hoad M, Goodall S, Tallent J, Bell PG, French DN. 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*. 2012; 9: 20.
51. Ra SG, Miyazaki T, Ishikura K, Nagayama H, Komine S, Nakata Y, et al. Combined effect of branched-chain amino acids and taurine supplementation on delayed onset muscle soreness and muscle damage in high-intensity eccentric exercise. *J Int Soc Sports Nutr*. 2013; 10(1): 51.
52. Leahy DT, Pintauro SJ. Branched-chain amino acid plus glucose supplement reduces exercise-induced delayed onset muscle soreness in college-age females. *ISRN Nutr*. 2013; 2013: 921972.
53. Sousa M, Teixeira VH, Soares J. Dietary strategies to recover from exercise-induced muscle damage. *Int J Food Sci Nutr*. 2014; 65(2): 151-63.
54. Rahimi MH, Shab-Bidar S, Mollahosseini M, Djafarian K. Branched chain amino acid supplementation and exercise induced muscle damage in exercise recovery: a meta-analysis of randomized clinical trials. *Nutrition*. 2017; 42: 30-6.
55. Soricichter S, Puschendorf B, Mair J. Skeletal muscle injury induced by eccentric muscle action: muscle proteins as markers of muscle fiber injury. *Exerc Immunol Rev*. 1999; 5: 5-21.
56. Clarkson PM, Kearns AK, Rouzier P, Rubin R, Thompson PD. Serum creatine kinase levels and renal function measures in exertional muscle damage. *Med Sci Sports Exerc*. 2006; 38(4): 623-7.
57. Howatson G, van Someren KA. The prevention and treatment of exercise-induced muscle damage. *Sports Med*. 2008; 38(6): 483-503.
58. Mena P, Maynar M, Campillo JE. Changes in plasma enzyme activities in professional racing cyclists. *Br J Sports Med*. 1996; 30(2): 122-4.
59. Priest JB, Oei TO, Moorehead WR. Exercise-induced changes in common laboratory tests. *Am J Clin Pathol*. 1982; 77(3): 285-9.
60. Nosaka K, Clarkson PM, Apple FS. Time course of serum protein changes after strenuous exercise of the forearm flexors. *J Lab Clin Med*. 1992; 119(2): 183-8.
61. Friden J, Sfikianos PN, Hargens AR. Blood indices of muscle injury associated with eccentric muscle contractions. *Journal of Orthopaedic Research*. 1989; 7(1): 142-5.
62. Radák Z, Pucsek J, Mecseki S, Csont T, Ferdinandy P. Muscle soreness-induced reduction in force generation is accompanied by increased nitric oxide

