The effect of branched-chain amino acid on muscle damage markers and performance following strenuous exercise: a systematic review and meta-analysis

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Abstract

This systematic review and meta-analysis determined whether the ergogenic effects of branched-chain amino acids (BCAA) ameliorated markers of muscle damage and performance following strenuous exercise. In total, 25 studies were included, consisting of 479 participants (age 24.3 ± 8.3 years, height 1.73 ± 0.06m, body mass 70.8 ± 9.5kg; females 26.3%). These studies were rated as fair to excellent following the PEDro scale. The outcome measures were compared between the BCAA and placebo conditions at 24 and 48 hours following muscle-damaging exercises, using standardised mean differences and associated p-values via forest plots. Our meta-analysis demonstrated significantly lower levels of indirect muscle damage markers (creatine kinase, lactate dehydrogenase and myoglobin) at 48 hours (SMD = -0.41; $p < 0.05$) post-exercise for the BCAA than placebo conditions, whilst muscle soreness was significant at 24 (SMD = $-0.28 \leq d \leq -0.61$; $p < 0.05$) and 48 (SMD = $-0.41 \leq d \leq -0.92$; $p < 0.01$) hours post-exercise. However, no significant differences were identified between the BCAA and placebo conditions for muscle performance neither at 24 nor 48 hours post-exercise (SMD = $0.08 \leq d \leq 0.21$; $p > 0.05$). Overall, BCAA reduced the level of muscle damage biomarkers and muscle soreness following muscle-damaging exercises. However, the potential benefits of BCAA for muscle performance recovery is questionable, and warrants further investigation to determine the practicality of BCAA for ameliorating muscle damage symptoms in diverse populations.

Novelty

- **BCAA reduces the level of creatine kinase and muscle soreness following strenuous exercise with a dose-response relationship**
- **BCAA does not accelerate recovery for muscle performance**

Introduction

Unfamiliar activities, particularly those that involve eccentric contractions, are known to cause exercise-induced muscle damage (EIMD) (Clarkson et al., 1992). The signs and symptoms of EIMD include prolonged impairment in muscle strength and joint range of motion (ROM), proprioception, delayed onset of muscle soreness (DOMS) and elevated muscle proteins in the blood (e.g., creatine kinase [CK]) (Ebbeling and Clarkson, 1989). The deterioration of muscular function and subsequent inflammatory response caused by EIMD could compromise athletic performance during the subsequent days. In fact, several studies have reported impairment in vertical jump (Byrne and Eston, 2002, Doma et al., 2017b), sprint and agility (Khan et al., 2016, Doma et al., 2018), running economy (Doma and Deakin, 2015, Doma and Deakin, 2013) and cycling power output (Hayter et al., 2016) during periods of EIMD following strenuous exercises. Therefore, to maintain performance levels, optimize the quality of subsequent training, and minimize the risks of injuries (Doma et al., 2017a), recovery strategies that minimize the level of EIMD should be considered (Doma et al., 2019a). One of various approaches could involve oral intake of legal substances, with branched-chain amino acids (BCAA) as a potentially effective supplement for this purpose.

The bioactive constituents of BCAA includes leucine, isoleucine and valine, three essential amino acids which are not synthesized in the body and must therefore be orally ingested (Mero, 1999). Of these amino acid compounds, leucine is known to offer the greatest anabolic potential for proteostasis (Mero, 1999). Whilst not fully understood, it has been speculated that supplemental amino acid after exercise may increase protein synthesis whilst mitigating the deleterious effects of EIMD, such as muscle proteolysis, inflammation and oxidative stress, by upregulating reactive oxygen species scavenging (Valerio et al., 2011). Given that increased inflammation and oxidative stress post-exercise exacerbates the level of

EIMD via the secondary muscle damage response (Smith et al., 2008), BCAA supplementation may enhance the recovery process associated with EIMD. In fact, evidence over the last two decades suggest that oral supplements of BCAA may exhibit prophylactic effects to ameliorate the signs and symptoms of EIMD. For example, several studies have reported that supplementation of BCAA reduced the level of muscle damage biomarkers (e.g., CK) (Coombes and McNaughton, 2000, Greer et al., 2007, Howatson et al., 2012, Matsumoto et al., 2009, Osmond et al., 2019, Ra et al., 2013a, Sharp and Pearson, 2010, Shimomura et al., 2010) and DOMS (Howatson et al., 2012, Jackman et al., 2010, Leahy and Pintauro, 2013, Matsumoto et al., 2009, Ra et al., 2013a, Reule et al., 2016, Shimomura et al., 2006), whilst improved muscular performance (e.g., isometric force) (Howatson et al., 2012, Osmond et al., 2019, Reule et al., 2016, VanDusseldorp et al., 2018, Greer et al., 2007, Waldron et al., 2017) for 24-48 hours post-exercise. However, BCAA supplementation has also been reported to have no impact on outcome measures associated with EIMD (Foure et al., 2016, Kephart et al., 2016, Lin et al., 2017, Panahi et al., 2013, Sheikholeslami-Vatani and Ahmadi, 2016). These equivocal findings may be attributed to the differences in study protocol between studies, including the supplementation methods of BCAA, sample characteristics, muscle-damaging protocols, and the type of EIMD outcome measures. Thus, a systematic exploration of the current literature may identify the overall effectiveness of BCAA supplement for post-exercise recovery by addressing the strengths and gaps in these previous studies.

Recently, systematic reviews with meta-analyses have examined the effects of BCAA supplementation on EIMD outcome measures. Previously, Rahimi et al. (2017) reported significant reduction in the overall outcome measures for CK and DOMS with the ingestion of BCAA. These findings were later confirmed by two other meta-analyses, with significantly greater reductions in CK (Hormoznejad et al., 2019) and DOMS (Fedewa et al., 2019) for the

BCAA condition compared to the placebo condition. Collectively, the meta-analyses published to date demonstrates the benefits of BCAA supplementation for muscle recovery following EIMD. Surprisingly, the recovery periods in these meta-analyses were either not reported, or combined from immediately post-exercise to several days post-exercise (Hormoznejad et al., 2019). This is despite that the time course of recovery period varies depending on the type of EIMD measured, which generally peaks at 24-48 hours post-exercise (Doma et al., 2019b, Doma et al., 2017b, Doma et al., 2015). When EIMD outcome markers were separated from 24 to 48 post-exercise in previous meta-analyses, the authors solely reported DOMS (Fedewa et al., 2019), or included no more than six studies (Rahimi et al., 2017), even though research in this area has been continuing for several decades. Furthermore, none of the existing meta-analyses have examined muscular performance measures, which is critical, given that such outcomes provide the best method of determining muscle injury (Warren et al., 1999). Considering that studies often report biomarkers of muscle damage (e.g., CK, lactate dehydrogenase [LDH] and myoglobin), DOMS and muscle performance as measures of indirect muscle damage, all these outcome measures should be examined during periods of EIMD. Thus, the current systematic review and meta-analysis examined whether commonly supplemented and commercially available BCAA ameliorates the signs and symptoms of EIMD and improve muscular function.

Materials and methods

This systematic review has been registered with PROSPERO (registration number: CRD42020191248), and the methodology was undertaken according to the PRISMA guidelines (Moher et al., 2009).

116 Inclusion and exclusion criteria

117 We included studies based on the following criteria: 1) studies that investigated the acute
118 responses to various muscle-damaging exercises, such as eccentric contractions, resistance
119 training, ballistic jumping and long-distance running in humans only; 2) the outcome
120 measures were compared between the BCAA and placebo (PLA) conditions; 3) the outcome
121 measures consisted of either biomarkers of indirect muscle damage (e.g., CK), inflammation
122 (e.g., interleukins), oxidative stress (e.g., malondialdehyde) and antioxidant status (e.g., total
123 antioxidant capacity), subjective measures of musculoskeletal pain and muscular performance
124 measures (e.g., isometric contractions, vertical jump); 4) the outcome measures (i.e.,
125 biomarkers of muscle damage, inflammation, oxidative stress, antioxidant status and
126 subjective measures of musculoskeletal pain) were collected for at least 24 hours following
127 the muscle-damaging exercise; and 5) the supplement at least consisted of a combination of
128 valine, leucine and isoleucine in any ratio, given that these are the most typical biochemical
129 constituents of BCAA. We excluded studies if: 1) conducted in animal models; 2) the
130 outcome measures were collected to determine chronic adaptations to training, rather than
131 acute responses; 3) a PLA condition was not included; 4) the paper was not written in
132 English; 5) findings were reported as a conference abstract, review or case report.

133

134 Search strategy

135 The search was conducted on the 6th of April, 2020, via five electronic databases, including
136 Cinhal, PubMed, Scopus, SPORTDiscus and Web of Science. Four strings of Mesh terms
137 were combined in PubMed, and two strings were combined for free text search for all
138 databases (refer to Supplementary 1 for full list of search terms). The reference lists of all
139 included studies and Google Scholar were also screened as a supplementary search.

140

141 Selection process

142 The abstract screening was independently completed by two authors (US and JC). Firstly, all
143 abstracts were highlighted as either 'yes' (definitely meeting the criteria), 'maybe' (possibly
144 meeting the criteria) or 'no' (not meeting the criteria) using the inclusion criteria. Any
145 discrepancies in screening was consulted by a third reviewer (KD) until a consensus was
146 reached. Following the abstract screening process, full text articles were further selected
147 based on the inclusion criteria by the two authors (US and JC).

148

149 Data extraction, assessment of quality, risk of bias and reporting of data

150 The descriptive information from each study was extracted for the following: 1) study aims;
151 2) research design (i.e., randomized, placebo control or cross-over); 3) participant
152 characteristics; 4) the amount of BCAA; 5) the type of outcome measures; and 6) the post-
153 exercise time points of when outcome measures were collected (i.e., 24- and 48-hours after
154 the muscle-damaging protocol). The quantitative results of the selected outcome measures
155 deemed appropriate for later meta-analyses was extracted as mean ± standard deviation to
156 construct forest plots. Based on previous recommendations (Johnston et al., 2018), the PEDro
157 scoring tool was modified by including additional criteria that assesses the methodological
158 quality of studies appropriate for oral supplements and EIMD responses. The PEDro scoring
159 tool originally consists of 11 items (Maher et al., 2003), with our additional items assessing
160 whether: 1) the supplements only consisted of leucine, isoleucine and valine; 2) the amount
161 of each amino acid was reported; 3) previous exposure to resistance training was confirmed
162 for each participant; and 4) the participants refrained from ingesting oral supplements with
163 anti-inflammatory and antioxidant agents. Each PEDro item was scored a 'one' or 'zero'

based on whether the item was met or not, respectively. The final additional item accounted for whether 1) participants avoided additional oral supplementation and/or medication two weeks prior to the study; 2) participants avoided additional oral supplementation and medication during the study; and 3) dietary habits were monitored during the study. The duration of refraining additional oral supplements prior to the study was set at 2-weeks, given that previous studies have used durations as minimal as 7 days for wash-out between BCAA and PLA conditions using a cross-over design (Leahy and Pintauro, 2013, Lin et al., 2017, Matsumoto et al., 2009). Collectively, a score of 'three' was given if all three factors were met, a score of 'two' if two factors were met, a score of 'one' if one factor was met, and 'zero' if none of the factors were met. Therefore, a maximum of 17 points was attainable with this modified PEDro scoring tool, with the quality classification as follows: excellent (15-17); good (12-14); fair (9-11); and poor (≤8) (Doma et al., 2020b). Studies rated as 'poor' were excluded from this systematic review. The participant selection bias was minimised by incorporating studies with all healthy adult participants, irrespective of sex and training background. Any outcome measure (i.e., muscle damage, inflammation, oxidative stress, anti-oxidant status, DOMS and muscle force) that consisted of less than six studies was not deemed appropriate for meta-analysis (Jackson and Turner, 2017). Thus, these excluded outcome measures were reported qualitatively using standard systematic review procedures (Moher et al., 2009).

Statistical methods

The Review Manager software (RevMan, version 5.3, Copenhagen: The Nordic Cochrane Centre, 2014) was used to conduct the meta-analysis for comparison in outcome measures between the BCAA and PLA conditions. All outcome measures that were deemed

appropriate for meta-analyses were separately reported at 24 and 48 hours after the muscle-damaging protocol to gain an understanding of the time course recovery with the supplementation of BCAA. A weighted average was calculated for outcome measures with similar effect constructs within the same study to report a singular effect estimate (Moeyaert et al., 2017). For example, if a study reported CK, myoglobin and LDH as indirect muscle damage biomarkers, the measure of central tendency and dispersion were averaged, rather than being treated as separate effect estimates. This method correctly distributes the weight of studies with multiple outcomes and time points, and standardises the precision estimates of the resultant summary effect (Moeyaert et al., 2017). The forest plot was generated as a random effects model to control for inter-study heterogeneity, and the heterogeneity of each study was determined based on the I^2 statistic, with values of 25%, 50% and 75% classified as low, moderate and high, respectively. The effect estimate was calculated for each study and imputed in the meta-analysis to combine RCP and cross-over studies using the generic inverse-variance method in RevMan (Higgins et al., 2008). Forest plots were also generated with sub-group analysis to tease out the influence of study design (Higgins and Green, 2011), by calculating the effect estimates and the corresponding variance with separate equations (Uanhoro, 2017) for the randomized, placebo controlled (RCP) and cross-over (COR) designs (Doma et al., 2020b, Deeks et al., 2001). As it is uncommon for studies with cross-over designs to report the variance of the change in mean scores of outcome measures, the correlation coefficient required to calculate confidence intervals was set at 0.5, which is a relatively conservative level of association, and is recommended when dealing with missing values (Higgins and Green, 2011, Batson and Burton, 2016). Thus, the forest plots were generated by grouping the studies between the RCP and COR study designs, and SMD, Z-scores and associated p-values were calculated separately for each study design, along with the overall summary effect. The effect estimate was reported as standardised mean

differences (SMD), with values of 0.2, 0.5 and 0.8 were classified as small, moderate and large, respectively (Cohen, 1988). In addition, a meta-regression was performed to assess whether CK, DOMS and muscle performance measures were moderated by total dosage of BCAA, timing of dosage of BCAA (before, during and/or after the muscle-damaging protocol), the type of muscle-damaging protocol and PEDro scores at 24 hours and 48 hours after the muscle-damaging protocols. This meta-regression was completed using the Statistical Package of Social Sciences (SPSS, v25, IBM Corp., Armonk, NY, USA). The type of muscle-damaging protocol was separated by resistance exercises (e.g., traditional resistance exercise, isokinetic eccentric contractions, transcutaneous neuromuscular stimulation) and endurance exercises (e.g., running, cycling). A sensitivity analysis was conducted for all outcome measures by excluding each study in the meta-analysis to determine the influence that each study had on the overall effect estimate. Furthermore, studies that exhibited PEDro scores of a fair quality, or lower, were excluded in the meta-analysis to ascertain the influence that study quality had on the overall effect estimate.

Results

Systematic literature search

After removal of duplicate abstracts, a total of 2667 abstracts were screened according to the inclusion criteria (Figure 1). Upon completion of screening, 2603 abstracts were excluded, and the remaining 64 full text articles were screened, leaving 28 articles for inclusion. All studies were either conducted as a RCP design with a BCAA or PLA group, or a COR design, with BCAA and PLA conditions.

Figure 1 around here

237 Participants

238 Supplementary 2 presents the physical characteristics, sample size and outcome distribution
239 at baseline for the BCAA and PLA conditions for each study. There was a total of 365
240 participants from the RCP studies, with 181 participants in the BCAA condition and 184
241 participants in the PLA condition. The mean ± standard deviation for age, height and body
242 mass for the BCAA condition were 25.7 ± 11.8 years, 1.74 ± 0.05 m and 73.1 ± 7.5 kg,
243 respectively. For the PLA condition, the age, height and body mass were 25.4 ± 11.6 years,
244 1.74 ± 0.03 m and 73.9 ± 8.9 kg, respectively. Thus, the physical characteristics between the
245 BCAA and PLA conditions were similar for the RCP studies. For the COR studies, there was
246 a total of 114 participants, and the mean ± standard deviation for age, height and body mass
247 were 21.9 ± 1.5 years, 1.70 ± 0.09 m and 65.3 ± 12.1 kg, respectively. In addition, no
248 significant differences were identified in the outcome measures between the BCAA and PLA
249 conditions at baseline, except in one study for isokinetic torque (Kephart et al., 2016),
250 demonstrating that the outcome measures were relatively standardised between each
251 condition.

252

253 Methodological descriptions

254 The most frequently employed muscle-damaging exercise included multi-joint resistance
255 exercises (13 studies), followed by eccentric contractions (4 studies), cycling (3 studies) and
256 running (2 studies), with isometric neuromuscular stimulation (1 study), drop jumps (1 study)
257 and downhill walking (1 study) as the least common (Supplementary 3). The most common
258 biomarker for indirect muscle damage was CK (20 studies), followed by LDH (9 studies),
259 whilst myoglobin (3 studies) and creatine kinase myocardial band (2 studies) were the least
260 reported. Three studies reported on inflammatory markers, including interleukin-6 (IL-6),

monocyte and granulocyte elastase (GEL), and one study reported on oxidative stress, which was 8-hydroxydeoxyguanosine (OHdG). There were various visual analogue scales (VAS) scales used for DOMS measures, including the 10cm pain scale as the most common (6 studies), followed by 100mm pain scale (4 studies), 200mm pain scale (3 studies), and 0-10 points pain scale (3 studies), whilst the 6-point pain scale (1 study), 1-10 points pain scale (1 study) and 50mm pain scale (1 study) were the least reported. The most common type of muscle performance protocol included isometric contractions (7 studies), followed by vertical jump (3 studies), whilst isokinetic contractions (1 study) and leg press (1 study) were the least reported.

Methodological quality

The methodological quality according to the PEDro scores ranged from fair to excellent (Supplementary 4). The PEDro items that were identified by all studies included the following: eligibility criteria mentioned; confirmed the amount of BCAA content; all participants received either BCAA or placebo and appropriate statistical analyses were conducted to compare data between groups. Most studies addressed the following PEDro items: instructions for participants to refrain from pain medication/supplements and BCAA supplementation prior to and during the study; random allocation of participants into BCAA and PLA groups; baseline values were standardized between the BCAA and PLA groups; homogeneity of participants; use of a double-blind method; outcome measures were reported from more than 85% of the participants; and reporting of measure of dispersion (either standard deviation, standard error or confidence interval). The least reported PEDro items included the following: allocation of concealment; specificity of resistance training background; and reporting of the bioavailability of the BCAA supplement.

285

286 Quantitative analysis

287 According to the meta-analysis, the muscle damage biomarkers were significantly lower for
288 the BCAA condition than the PLA condition 48 hours post-exercise ($p = 0.007$; $Z = 2.68$;
289 Figure 2B) and approached significance at 24 hours post-exercise ($p = 0.06$; $Z = 1.89$), with
290 low inter-study heterogeneity (I^2 = 54% and 45%, respectively). However, the magnitude of
291 differences between conditions were small for both time points (SMD = -0.25 and -0.39,
292 respectively). For the DOMS measures, values were significantly lower for the BCAA
293 condition than the PLA condition at 24 hours ($p = 0.01$; $Z = 2.47$; Figure 3A) and 48 hours (p
294 $= 0.003$; $Z = 3.01$; Figure 3B), with high inter-study heterogeneity (I^2 = 73% and 72%,
295 respectively). There were no significant differences in muscle force measures between the
296 BCAA and PLA conditions at 24 hours ($p = 0.46$; $Z = 0.74$; Figure 4A) and 48 hours ($p =$
297 0.08; $Z = 1.76$; Figure 4B) post-exercise, with small inter-study heterogeneity (I^2 = 0% and
298 47%, respectively). In addition, the magnitude of differences between conditions were small
299 for both time points (SMD = 0.09 and 0.31, respectively).

300 ***Figure 2 around here***

301 ***Figure 3 around here***

302 ***Figure 4 around here***

303

304 Meta-regression analysis

305 According to the meta-regression, the amount of BCAA dosage significantly predicted the
306 muscle damage markers at 24 hours ($r^2 = 0.24$; unstandardised $\beta = -0.05$; $p = 0.02$) and 48
307 hours ($r^2 = 0.32$; unstandardised $\beta = -0.04$; $p = 0.02$) post-exercise. Furthermore, the PEDro

score at 24 hours post-exercise significantly predicted the muscle damage markers ($r^2 = 0.63$; unstandardised $\beta = 0.32$; $p > 0.01$), although not at 48 hours post-exercise ($r^2 = 0.45$; unstandardised $\beta = 0.20$; $p = 0.07$). However, the timing of BCAA ingestion and the type of muscle damage protocol were not significant moderators of the muscle damage markers ($p > 0.05$). For the DOMS measures, the PEDro scores were significant moderators at 24 hours ($r^2 = 0.0.57$; unstandardised $\beta = 0.41$; $p = 0.01$) and 48 hours ($r^2 = 0.71$; unstandardised $\beta = 0.58$; $p < 0.01$) post-exercise, although none of the other moderators (dosage, timing of BCAA ingestion and the type of muscle damage protocol) significantly predicted DOMS ($p > 0.05$). In addition, none of the selected moderators predicted muscle force measures ($p > 0.05$).

Sub-group analysis

For sub-group analyses, the variation in study design (i.e., RCP and COR) appeared to affect all included measures, with significantly greater CK values for PLA than BCAA in studies using the RCP design, although there were no differences in CK between conditions in the studies with COR at 24- and 48-hours post-exercise (Figures 2A and 2B). These trends were reversed for DOMS at 24- and 48-hours post-exercise (Figures 3A and 3B) and muscle force measures at 48 hours post-exercise (Figure 4B).

Sensitivity analysis

The muscle damage markers became significantly lower for the BCAA than the PLA conditions when several studies (Foure et al., 2016, Gervasi et al., 2020, Jackman et al., 2010, Kephart et al., 2016, Waldron et al., 2017, Lin et al., 2017, Sheikholeslami-Vatani and Ahmadi, 2016, Shimomura et al., 2010) were individually removed at 24-hours post-exercise. However, the meta-analysis for muscle damage markers remained similar when studies were

individually removed at 48-hours post-exercise. Similarly, the meta-analysis for DOMS remained similar when studies were individually removed at 24- and 48-hours post-exercise, and for muscle performance measures at 24-hours post-exercise. However, the meta-analysis for muscle performance measures was significantly greater for BCAA than PLA when studies by Foure et al. (2016) and Reule et al. (2016) were removed at 48-hours post-exercise. For study quality, the meta-analysis for muscle damage markers were altered when studies with 'fair' quality were removed, with no differences between the BCAA and PLA conditions at 24- ($p = 0.66$; $Z = 0.44$; SMD = -0.05) and 48-hours ($p = 0.08$; $Z = 1.75$; SMD = -0.26) post-exercise. However, results remained similar for DOMS at 24- ($p = 0.04$; $Z = 2.49$; SMD = -0.39) and 48-hours ($p = 0.008$; $Z = 2.05$; SMD = -0.65) post-exercise. Sensitivity analyses were not conducted for the muscle performance measures as all studies were rated above 'fair' quality.

Qualitative analysis

Given that the inflammatory markers were only reported by three studies and oxidative stress was reported by one study, these results were not included in the meta-analysis. Furthermore, none of the studies reported anti-oxidant status. Therefore, the findings for inflammatory and oxidative stress markers will be reported qualitatively hereon. With respect to inflammatory markers, Jackman et al. (2010) reported no differences in interleukin-6 between the BCAA and PLA conditions at 24 hours following eccentric knee extensor exercises. However, Matsumoto et al. (2009) showed significantly lower granulocyte elastase for the BCAA condition than the PLA condition at 24 hours after an intensified training period. Similarly, Kephart et al. (2016) showed significantly lower monocyte percentage for the BCAA condition than PLA condition at 24 hours following barbell back squats. For oxidative stress,

Ra et al. (2013a) reported no significant differences between the BCAA and PLA conditions at 48 hours following eccentric elbow flexor exercises.

Discussion

The results from the meta-analysis in the current systematic review revealed that BCAA significantly reduced biomarkers of muscle damage and DOMS for up to 48 hours after muscle-damaging exercises when compared to PLA. However, no significant differences were identified between BCAA and PLA conditions for muscle performance measures. Whilst the number of studies to conduct meta-analyses for inflammatory and oxidative stress markers was insufficient, two studies reported significantly lower levels of inflammation for the BCAA condition than the PLA condition for up to 24 hours post-exercise, although one study reported comparable measures of inflammation. Furthermore, no significant differences were found in oxidative stress between BCAA and PLA condition according to one study (Ra et al., 2013a). Overall, BCAA supplementation appears to be useful in ameliorating muscle damage and DOMS induced by strenuous exercise, although the inflammatory and oxidative stress responses are unclear. Thus, more studies are warranted to confirm the therapeutic effects of BCAA following strenuous exercises.

The results from our meta-analysis partly confirms previous meta-analyses that examined indirect measures of muscle damage with the ingestion of BCAA supplementation. For example, our meta-analysis is similar to Rahimi et al. (2017), who reported significantly lower levels of muscle damage for the BCAA condition than the PLA condition at 24 hours following muscle-damaging exercises based on six studies. However, our meta-analysis does not align with Rahimi et al. (2017) at 48 hours after muscle-damaging exercises, as they reported no differences in muscle damage between BCAA and PLA conditions. The

discrepancy in these findings may be attributed to Rahimi et al. (2017) only including two studies, whereas we included 15 studies, thus allowing for greater statistical power (Jackson and Turner, 2017). Hormoznejad et al. (2019) also showed that BCAA significantly lowered levels of muscle damage than PLA following muscle-damaging exercises based on eight studies. However, comparing our findings to that of Hormoznejad et al. (2019) is difficult, given that they combined post-exercise time points from less than 24 hours, to several days after muscle-damaging exercises, which limits the scope of time course recovery with BCAA. In addition, our meta-regression identified a novel dose-response relationship between BCAA and muscle-damage markers at 24- and 48-hours post-exercise. Thus, it appears that BCAA minimizes the level of muscle damage markers, and this level of attenuation may be augmented by ingesting a greater amount of BCAA.

With respect to DOMS, our meta-analyses were not in line with the meta-analysis by Rahimi et al. (2017), who showed no significant differences between BCAA and PLA conditions at 24 and 48 hours after muscle-damaging exercises. Similar to the muscle damage markers, Rahimi et al. (2017) only included three studies at 24 hours post-exercise and four studies at 48 hours post-exercise, which may have limited their statistical power. In fact, Fedewa et al. (2019) reported significantly lower levels of DOMS with BCAA than PLA for up to 48 hours post-exercise based on eight studies in their meta-analysis, and our meta-analysis supports these findings with 18 studies at 24 hours post-exercise and 14 studies at 48 hours post-exercise. Collectively, BCAA appears to be an ergogenic supplement to reduce the level of EIMD following muscle-damaging exercises from 24- to 48-hours post-exercise, with our meta-analysis confirming this effect with almost three fold the number of studies included than previous meta-analyses.

The anabolic and anti-catabolic properties of BCAA have been suggested as potential modulators that ameliorate the level of EIMD. For example, the ingestion of BCAA,

particularly from leucine, enhances protein synthesis, whilst concomitantly decreasing catabolic effects (Anthony et al., 2002). The ergogenic effects of BCAA supplementation for recovery has been supported at a sub-cellular level, by upregulating both mammalian target of rapamycin and ribosomal protein S6 kinase beta-1 following resistance exercises, which are key enzymes that orchestrates protein synthesis (Blomstrand et al., 2006). These processes have been speculated to minimize the level of cytoskeletal damage in the muscle, thereby limiting intramuscular leakage, such as CK, and sustaining the integrity of membrane-bound proteins. Furthermore, exogenous BCAA may attenuate the decrease of amino acid in the free muscle pool during exercise, which limits the initiation of muscle protein breakdown, and accelerate recovery (Greer et al., 2007). Interestingly, several studies included in our systematic review reported increases in leucine, isoleucine and valine post-exercise (Foure et al., 2016, Gervasi et al., 2020, Lin et al., 2017, Ra et al., 2018, Sheikholeslami-Vatani and Ahmadi, 2016, Shimomura et al., 2010), demonstrating the bioavailability of these amino acids with BCAA ingestion.

The EIMD response is considered biphasic, with the mechanical stress of the muscle-damaging exercise inducing a primary response, and a subsequent increase in inflammation and oxidative stress for several days post-exercise known as the secondary response (Hyldahl and Hubal, 2014). It has been speculated that the supplementation of BCAA may downregulate the secondary muscle damage response by increasing the bioavailability of amino acids (Howatson et al., 2012). Specifically, it has been suggested that BCAA can enhance glutamine synthesis via transamination of glutamate, which then influences the transcription of nuclear factor of activated beta cells, thereby attenuating local inflammation (for more information, see Nicastro et al. (2012)). Indeed, Matsumoto et al. (2009) reported significantly lower levels of GEL for the BCAA condition than PLA condition, indicating a suppression in elevated inflammatory responses with BCAA supplementation during periods

of EIMD. In addition, Kephart et al. (2016) reported significantly lower monocyte percentages for the BCAA condition than PLA condition, which are commonly known to differentiate into macrophages, and if increased, may further initiate an inflammatory response. Whilst there are findings supporting the anti-inflammatory role of BCAA following strenuous activity, much more research is needed to understand the pro- and anti-inflammatory effects of BCAA following strenuous activity.

The exercise-induced inflammatory response may also incur further skeletal muscle damage to previously damaged muscle fibers, and their neighboring non-damaged counterparts, by producing reactive oxygen species, which increases oxidative stress (Aoi et al., 2004). As far as we are aware, Ra et al. (2013a) are the only group of researchers who examined the effect of BCAA supplementation as a recovery intervention on oxidative stress following muscle-damaging exercises. Interestingly, comparable measures of 8-OHdG were found between BCAA and PLA conditions, indicating that the supplementation of BCAA had no influence on the level of oxidative stress. However, given the variety of oxidative biomarkers available, and the multiple ways in which BCAA supplementation and muscle-damaging protocols could be conducted, it is difficult to determine whether BCAA reduces the level of oxidative stress from one study. Furthermore, BCAA supplementation has been reported to reduce 8-OHdG levels for patients with liver cirrhosis, demonstrating that BCAA may reduce DNA damage in the muscle due to oxidative stress within a clinical context. Thus, similar to inflammatory responses, further research is warranted to examine the effects of BCAA on oxidative stress levels during periods of EIMD.

The current meta-analysis also demonstrated significantly lower levels of DOMS for the BCAA condition than the PLA condition for up to 48 hours following muscle-damaging exercises. It is hypothesized that the damage to the intermediate filaments of the myofibres activates groups III and IV afferent nociceptors, resulting in muscular pain (Ebbeling and

Clarkson, 1989). This process is supported by our meta-analysis with muscle damage biomarkers, suggesting that the lower levels of DOMS may have been attributed to suppressing muscular structural damage. In addition, inflammation of the perimysium or epimysium has been suggested to cause muscular pain (Proske and Morgan, 2001). Although the evidence of reduced inflammation with BCAA has only been reported by two studies (Matsumoto et al., 2009, Kephart et al., 2016), it is plausible that the anti-inflammatory properties of BCAA may contribute to a reduction in DOMS.

Whilst improvement in DOMS was apparent in our meta-analysis, BCAA supplementation was not favorable for any muscle performance measure. This is surprising, given that reduction in DOMS has been speculated to suppress neural inhibition during periods of EIMD, and accelerate restoration of muscle reflex sensitivity and performance (Nicol et al., 2003). Whilst the meta-regression suggested that dosage, timing of BCAA ingestion, the type of muscle-damaging protocol and the PEDro rating did not moderate muscle performance, the number of included studies were far less than the CK measure, possibly limiting statistical power. Thus, the lack of any effect of BCAA on muscle performance measures may still be attributed to distinct muscle-damaging exercises employed, particularly between endurance-oriented exercises (e.g., running, cycling), traditional resistance exercises and eccentrically-biased endurance exercises (e.g., downhill running). Furthermore, the type of muscle groups being assessed, the performance parameters selected (e.g., isometric contractions vs vertical jump height), and the fewer number of studies included when compared to muscle damage and DOMS measures may have also attributed to the limited effect of BCAA on muscle performance measure. However, this finding is plausible since previous reports have confirmed the different time course of muscle damage and performance measures in previous studies with muscle damaging exercises (Chen et al., 2020, Nosaka et al., 2006).

Based on the critical appraisal of studies included in this systematic review, several recommendations can be provided for future research. Firstly, only eight studies reported on the bioavailability of leucine, isoleucine and valine, thus more studies should consider including these biomarkers to distinguish the ergogenic effects of BCAA from PLA. Reporting the ergogenic effects of leucine, isoleucine and valine separately will demonstrate the role that each amino acid has in assisting with the recovery process following strenuous exercises. This may particularly be evident for leucine, given that this amino acid has been considered to exhibit the greatest anabolic potential for proteostasis (Mero, 1999). Secondly, very few studies have examined the inflammatory and oxidative stress responses during periods of EIMD with the ingestion of BCAA. Monitoring these biomarkers will confirm whether BCAA constitutes of anti-inflammatory and anti-oxidant properties, and expand our knowledge on the potential mechanisms contributing to the amelioration of EIMD. Third, only three studies concealed allocation of participants into BCAA and PLA conditions, and future studies should account for this approach to limit selection bias. There are also studies that reported greater ergogenic effects of BCAA when combined with taurine (Ra et al., 2013b), although there is an insufficient number of studies to confirm this with a meta-analysis at present. Furthermore, although there are other commercially available supplements that are known to enhance recovery dynamics during periods of EIMD, such as plant-based extracts (Doma et al., 2020a, Doma et al., 2020b, Hill et al., 2021, Doma et al., 2021), the ergogenic effects of these combinations are not clear and warrant more research. Fourth, the majority of studies to date have examined the ergogenic effects of BCAA in adult, healthy populations. However, conditions with elevated levels of muscle damage, inflammation and oxidative stress are also apparent in elite athletes due to strenuous training, and older individuals with common clinical conditions, such as, but not limited to, osteoarthritis, cardiovascular diseases and diabetes. Thus, future research is encouraged to

504 determine whether BCAA may ameliorate the signs and symptoms of these population
505 groups in their respective, contextual applications. Finally, the meta-regression identified that
506 the PEDro score was a significant moderator for the magnitude of differences between
507 BCAA and PLA conditions for CK. Therefore, future studies examining effects of oral
508 supplementation on EIMD markers should consider addressing the modified PEDro items
509 employed in the current systematic review to optimize study quality.

510 With respect to the sub-group analysis, although study design appeared to exhibit a notable
511 influence on the overall effect estimate, the trends were dependent on the type of outcome
512 measure. For example, the RCP studies demonstrated greater benefits of BCAA than the
513 COR studies at 24- and 48-hours post exercise for muscle damage markers, suggesting that if
514 participants are separated into BCAA and PLA groups in future studies, the potential carry-
515 over effects may be nullified. However, this trend was reversed for DOMS and muscle
516 performance measures, with greater benefits of BCAA in the COR studies, exemplifying the
517 advantage of repeated measures design by minimizing inter-individual variability. Thus, our
518 findings do not allow for an appropriate consensus on the optimal study design for studies
519 examining the effectiveness of BCAA on recovery dynamics due to differences between
520 outcome measures. Nonetheless, the PEDro scores significantly predicted the overall effect
521 estimate according to our meta-regression, indicating that future studies should consider the
522 PEDro items, including the additional items included in this systematic review. For example,
523 studies employing an RCP design should make every effort to homogenize the sample
524 between the BCAA and PLA groups (e.g., resistance training background, supplement and
525 dietary history and sex). Studies with a COR design should ensure that the BCAA and PLA
526 conditions are separated by at least two weeks to minimize potential carry-over responses of
527 BCAA or EIMD. Furthermore, the participants should be instructed to refrain from ingesting

dietary supplements and medication that are known to ameliorate the signs and symptoms of EIMD during the course of the study, irrespective of the study design.

In conclusion, our systematic review and meta-analysis identified significant reductions in muscle damage markers at 48 hours after muscle-damaging exercises and DOMS for up to 48 hours after muscle-damaging exercises, but not for muscle performance measures. The lack of any differences in muscle performance for the meta-analysis may be attributed limited statistical power when compared to biomarkers, such as CK, and DOMS. However, given that muscle performance has been considered as the primary, indirect muscle damage measure (Warren et al., 1999), studies from hereon are encouraged to focus on ascertaining the ergogenic effects of BCAA as a recovery intervention for muscle function during periods of EIMD in diverse populations.

Figure captions

Figure 1. PRISMA flow chart

Figure 2. Forest plot for indirect muscle damage markers. A: at 24-hours after the muscle damaging exercise; B: at 48-hours after the muscle damaging exercise.

BCAA – branched chain amino acid; PLA – placebo; SUPP – supplementary; RCP – randomized, controlled placebo trial

Figure 3. Forest plot for delayed onset of muscle soreness. A: at 24-hours after the muscle damaging exercise; B: at 48-hours after the muscle damaging exercise.

BCAA – branched chain amino acid; PLA – placebo; RCP – randomized, controlled placebo trial

Figure 4. Forest plot for muscle force. A: at 24-hours after the muscle damaging exercise; B: at 48-hours after the muscle damaging exercise.

BCAA – branched chain amino acid; PLA – placebo; SUPP – supplementary; RCP – randomized, controlled placebo trial

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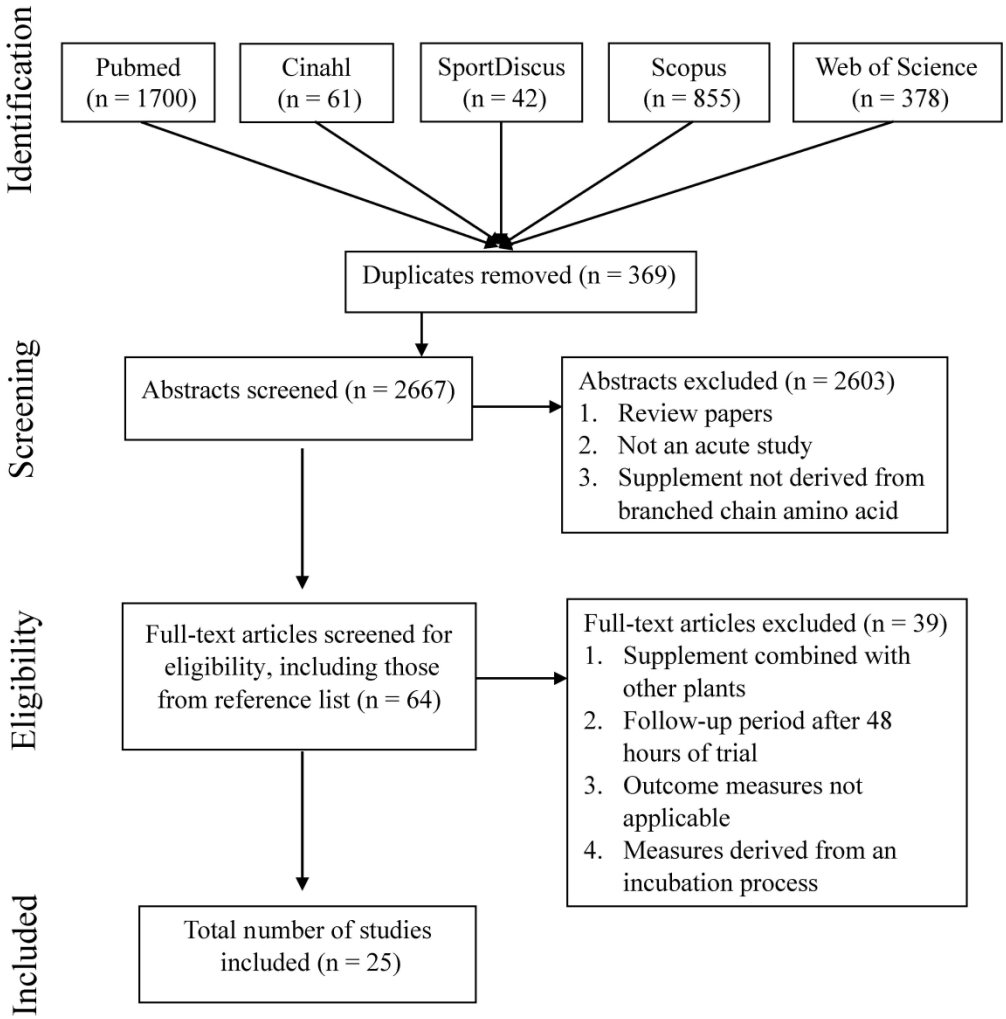

Figure 1

161x163mm (600 x 600 DPI)

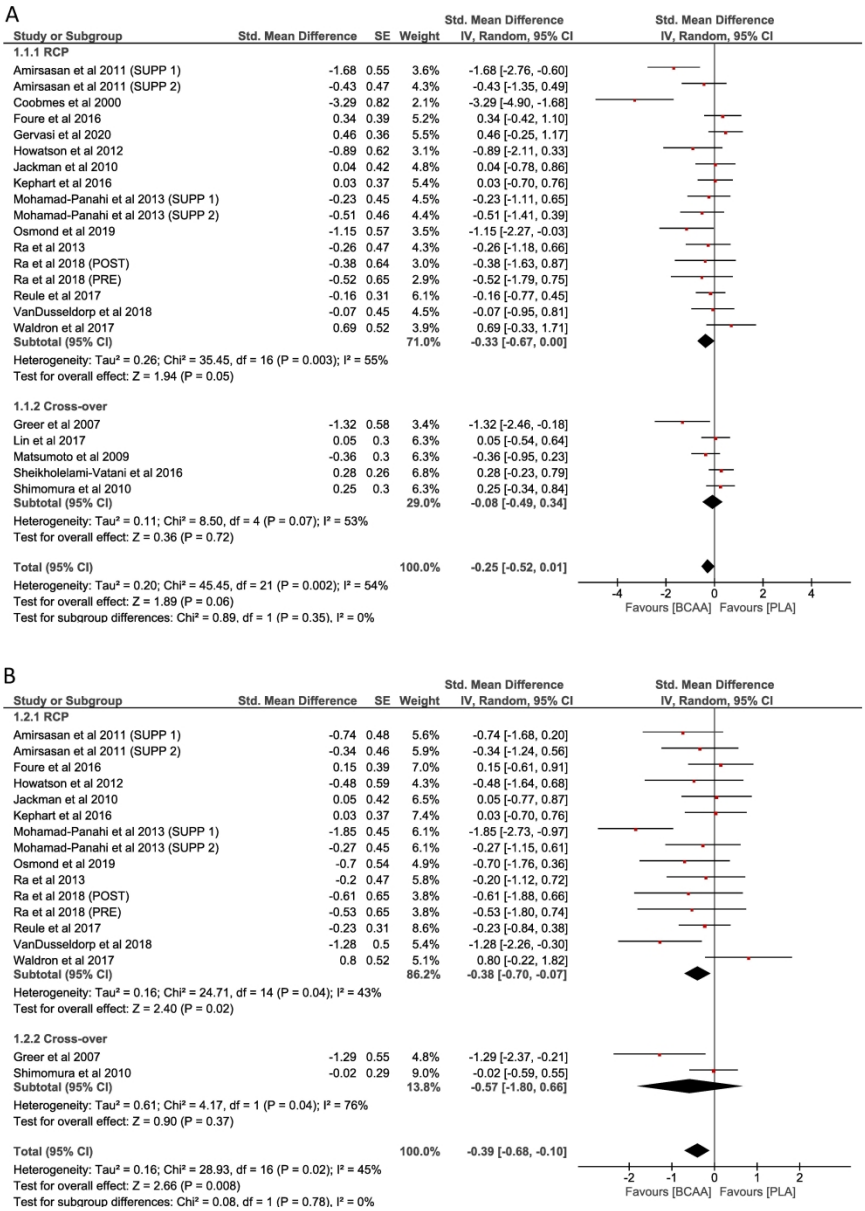

Figure 2

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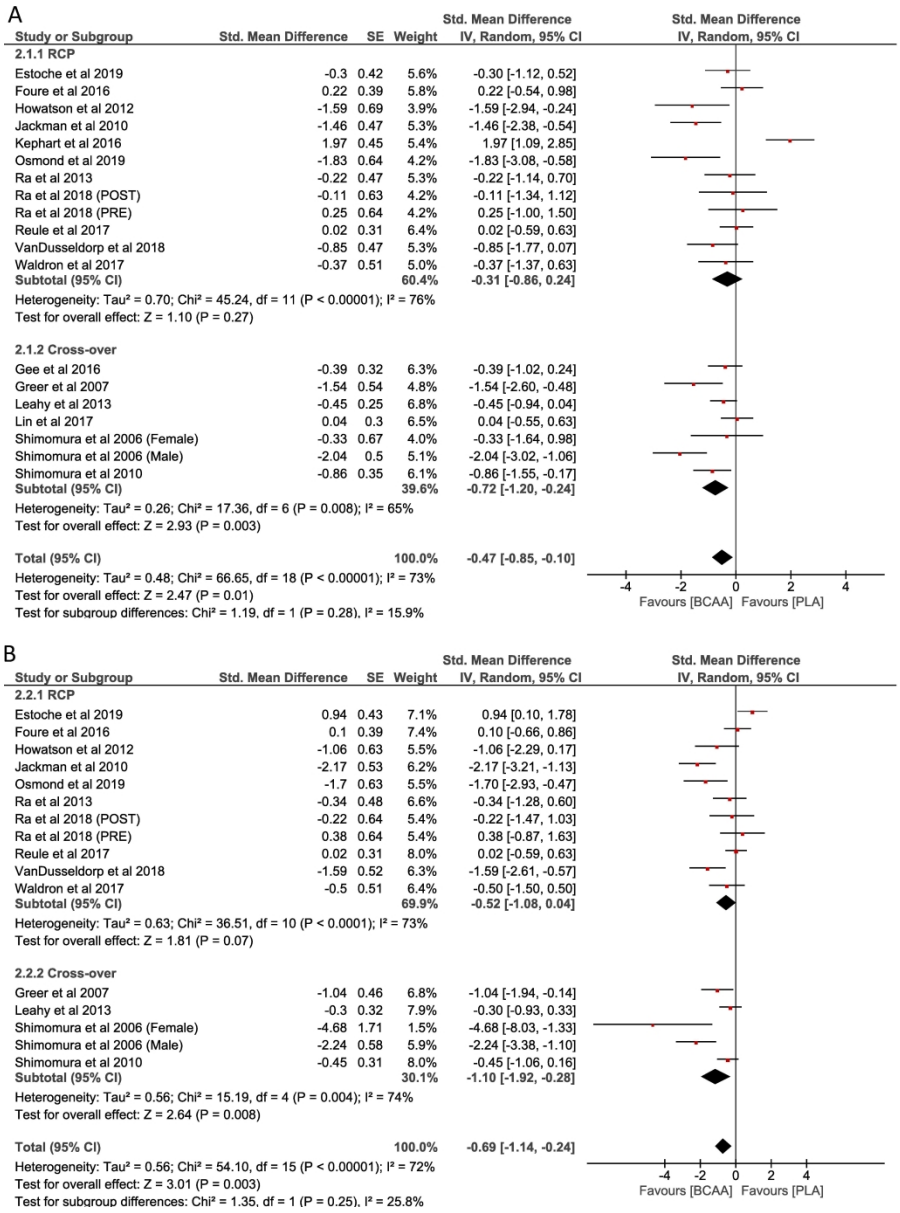

Figure 3

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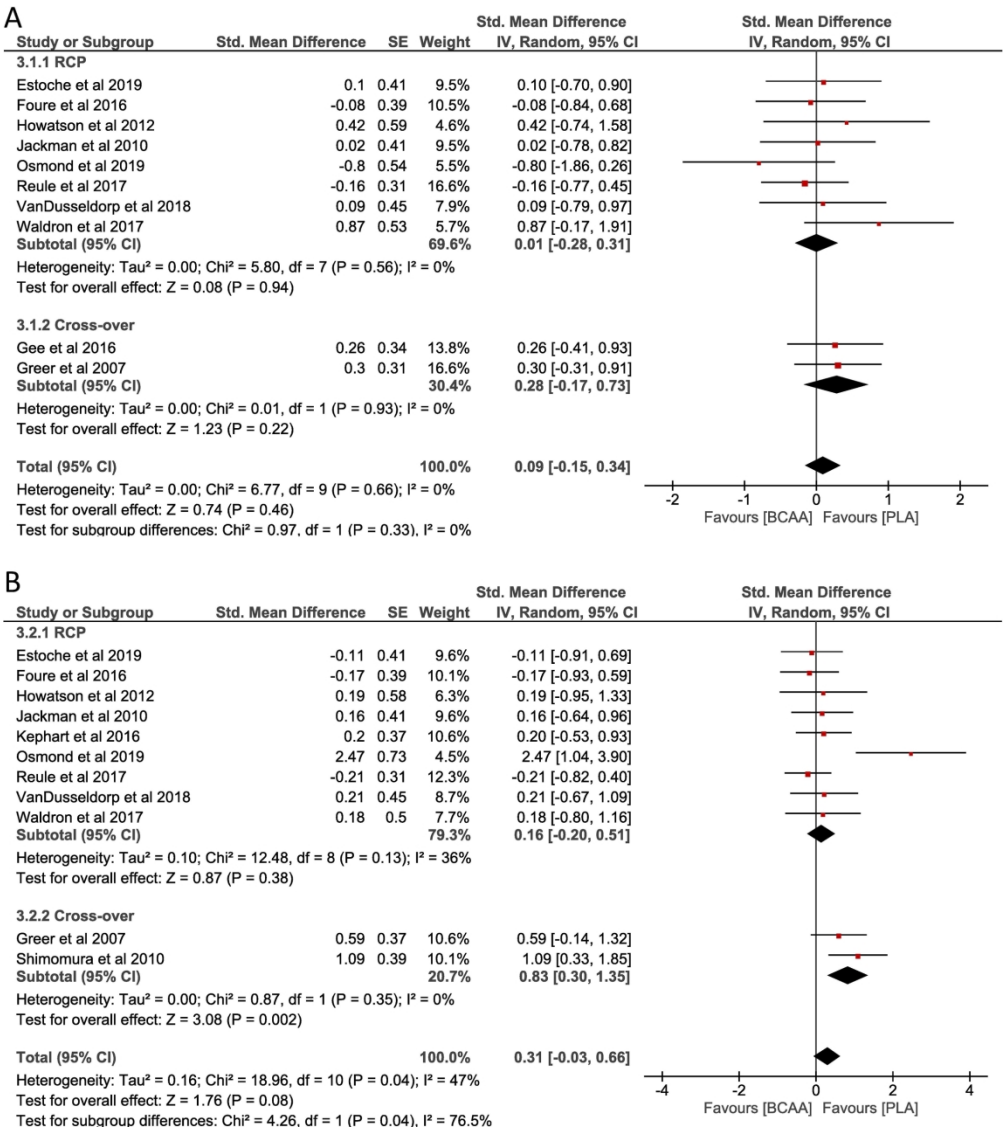

Figure 4

196x219mm (300 x 300 DPI)