MARTIM GOMES WEBER

O USO DE BCAA NA PREVENÇÃO DA DOR MUSCULAR DE INÍCIO TARDIO:

UMA REVISÃO SISTEMÁTICA E METANÁLISE

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Dissertação de Mestrado apresentado ao Programa de Pós-graduação Associado em Educação Física da Universidade Estadual de Londrina – UEL e Universidade Estadual de Maringá - UEM, como requisito parcial para a obtenção do título de Mestre em Educação Física.

Orientador: Profª. Drª Solange de Paula Ramos.
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WEBER, Martim Gomes. **O uso de BCAA na prevenção da dor muscular de início tardio:** uma revisão sistemática e metanálise. 2021. 59 f. Dissertação (Mestrado em Educação Física) - Universidade Estadual de Londrina, Londrina, 2021.

RESUMO

Os aminoácidos de cadeia ramificada (*Branched-chain Amino Acids* - BCAA) são suplementos utilizados como recurso ergogênico e na recuperação física em rotinas de treinamento físico. O BCAA tem sido sugerido como método de recuperação após dano muscular induzida por exercício (DMIE). A literatura sugere que o BCAA pode aliviar a dor muscular de início tardio (*delayed-onset muscle soreness* - DOMS) um dos sintomas do DMIE. No entanto, não há consenso sobre o protocolo de suplementação em relação a dose e tempo de administração. O objetivo do presente estudo foi realizar uma revisão sistemática da literatura com metanálise, sobre a eficácia da suplementação de BCAA para reduzir os sintomas de DOMS em indivíduos saudáveis, após uma única sessão de DMIE. Ensaios clínicos randomizados (RCT) foram pesquisados nas bases científicas Medline-Pubmed, Cochrane Library, Science Direct, SciELO, LILACS, SciVerse Scopus, periódicos Springer Link, Wiley Online Library e Scholar Google, até novembro de 2020. Dez RCT foram incluídos na revisão sistemática e nove na metanálise. Sete (70%) estudos demonstraram níveis reduzidos de DOMS após 24 a 72 horas. O BCAA em doses de até 255 mg / kg / dia, suplementado previamente ao DMIE em indivíduos treinados, pode atenuar os sintomas de DOMS. No entanto, a maior variabilidade entre os estudos, devido ao estado de treinamento, diferentes doses, tempo de tratamento e gravidade do EIMD não permite concluir se a suplementação de BCAA é eficiente em indivíduos não treinados, doses agudas e mais altas (> 255 mg / kg / dia). Os efeitos gerais do BCAA reduzem a DOMS em indivíduos treinados e não devem ser administrados após o protocolo EIMD.

Palavras-chave: dor; exercício físico; leucina; músculo esquelético.

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ABSTRACT

Branched-chain amino acids (BCAA) are used as a recovery method after exercise-induced muscle damage (EIMD). Although data suggest that BCAA may alleviate the delayed-onset muscle soreness (DOMS) evoked by EIMD, there is no consensus about the most effective supplementation protocol. To investigate the effects of BCAA on DOMS, a systematic review and meta-analysis was conducted on the effectiveness of BCAA supplementation to reduce DOMS symptoms in healthy subjects after a single session of EIMD. Randomized clinical trials (RCT) were searched in Medline, Cochrane Library, Science Direct, SciELO, LILACS, SciVerse Scopus, Springer Link journals, Wiley Online Library, and Scholar Google, until November 2020. Ten RCTs were included in the systematic review and nine in the meta-analysis. Seven studies demonstrated that BCAA reduced levels of DOMS after 24 to 72 hours. BCAA at doses up to 255 mg/kg/day, supplemented prior to EIMD, in trained subjects could blunt DOMS symptoms. However, high variability between studies due to training status, different doses, time of treatment, and severity of EIMD do not allow us to conclude whether BCAA supplementation is efficient in untrained subjects, applied acutely, and at higher doses (>255 mg/kg/day). The overall effects of BCAA reduced DOMS in trained subjects and should not be administered only after the EIMD protocol.

Key words: pain; physical exercise; leucine; skeletal muscle.

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LISTA DE ABREVIATURAS E SIGLAS

COX-2	Cicloxigenase-2
DMIE	Dano muscular induzido por exercício
eIF4E	Fator 4E de início da síntese proteica
4EBP	Proteína de ligação do eIF4E
CK	Creatina quinase
GRADE	Classificação de recomendações, avaliação, desenvolvimento e avaliação
GLUT-4	Proteína transportadora de glicose
LDH	Lactato Desidrogenase
LPS	Lipopolissacarídeo
DOMS	Delayed-Onset Muscle Soreness
NGF	Fator de crescimento neural
GDNF	Fator neurotrófico derivado de células da glia
BCAA	Aminoácidos de cadeia ramificada
mRNA	Ácido ribonucleico mensageiro
MTOR	Alvo da rapamicina em mamíferos
mTORC1	Complexo 1 do alvo da rapamicina em mamíferos
mTORC2	Complexo 2 do alvo da rapamicina em mamíferos

SUMÁRIO

1 INTRODUÇÃO

1.1 DANO MUSCULAR INDUZIDO POR EXERCÍCIO

A realização de contrações musculares excêntricas, com cargas de alta intensidade, e em movimentos não habituais promove dano muscular induzido por exercício (DMIE) (CLARKSON and HUBAL 2002; LEWIS *et al*., 2012; MUELLER-WOHLFAHRT *et al*., 2013; OWENS *et al*., 2019). O DMIE é caracterizado por dor muscular de início tardio (*delayed-onset muscle soreness* - DOMS), inflamação, sensibilidade e rigidez muscular e amplitude limitada de movimento (MUELLER-WOHLFAHRT *et al*., 2013; HARTY *et al*., 2019;), além do aumento dos níveis circulantes de mediadores inflamatórios e marcadores de dano muscular, como a creatina quinase (CK), mioglobina e lactato desidrogenase (LDH) (HUBAL; CLARKSON, 2009).

Os principais mecanismos da patogênese do DMIE estão relacionados ao dano mecânico ao tecido muscular, devido a contrações excêntricas ou concêntricas de alta intensidade, sendo observado em biópsias musculares a perda da integridade das linhas Z (HOTFIEL *et al.*, 2018) e desestruturação ou o rompimento de sarcômeros, devido ao alongamento não uniforme dos sarcômeros (MORGAN; PROSKE, 2004). Acredita-se que o alongamento não uniforme leve ao cisalhamento das miofibrilas ocasionando uma grande deformação nos túbulos T, desequilíbrio da homeostase dos íons cálcio, seja por meio de rompimento de membranas ou abertura de canais de cálcio ativados por estiramento (CLARKSON; HUBAL 2002; MORGAN; PROSKE, 2004; MIZUMURA; TAGUCHI, 2016; OWENS *et al*. 2019).

Ao longo das primeiras quatro horas após o dano muscular, há um aumento da migração de neutrófilos (leucócitos pró-inflamatórios) para o músculo danificado causando dano enzimático e oxidativo das fibras musculares e liberação plasmática de mioglobina (um marcador de dano muscular) (PAULSEN *et al*., 2012). A resposta inflamatória aguda dos neutrófilos é sucedida pela migração de macrófagos (após 4 horas), e posteriormente (12 a 24 horas) a produção de neurotrofinas responsáveis pelo aumento da dor muscular, num período de 24 a 72 horas após o exercício (MIZUMURA; TAGUCHI 2016). No período de sintomas de DOMS, o tecido é

invadido por macrófagos M1, pró-inflamatórios, que mantém a produção de mediadores inflamatórios e removem as células e matriz extracelular destruídas por ação dos neutrófilos (WALTON *et al*., 2019). Após a remoção da matriz e células danificadas, ocorrerá a recuperação funcional do tecido muscular (WALTON *et al*., 2019).

Os sinais clínicos do dano muscular induzido pelo exercício compreendem (PAULSEN *et al*., 2012):

- Perda de função: redução de força e potência muscular;
- DOMS: dor muscular com pico entre 24 a 48 horas, podendo manter sintomatologia até sete dias, dependendo do grupo muscular e extensão do dano;
- Limitação de amplitude de movimento: causada por edema, dor e dano ao tecido conjuntivo;
- Aumento da temperatura local;
- Aumento dos níveis séricos de marcadores de dano muscular, incluindo cretina quinase (CK), lactato desidrogenase (LDH), mioglobina, aldolase, aspartato aminotransferase, troponina, mioglobina de cadeia pesada, entre outros;

A perda de função é considerada o melhor padrão para definição do dano muscular induzido pelo exercício. De acordo com Paulsen et al. (2012), o dano muscular induzido pelo exercício pode ser classificado em:

- Leve: quando há perda de função de até 20%, e ocorrer recuperação funcional até 48 horas; variação dos níveis séricos de CK até 1000 U/L;
- Moderado: quando há perda de mais de 20% até 50% de função muscular após o exercício, com período de recuperação entre 2 a 7 dias. Ocorrendo variação dos níveis séricos de CK, mas com ampla variação entre indivíduos;
- Grande: perda de função muscular acima de 50%, com período de recuperação superior a 7 dias. Níveis de CK acima de 10000 U/L.

A DOMS é um dos principais e mais importantes sintomas do

DMIE, como descrito adiante. Diversas estratégias de recuperação pós-exercício têm sido investigadas para mitigar o desenvolvimento deste sintoma pós-exercício, incluindo o uso de suplementos nutricionais (CRUZAT *et al*., 2014; OWENS *et al*., 2019).

1.2 Dor Muscular de Início Tardio

Aproximadamente 24 horas após o exercício, o músculo apresenta queda de desempenho (perda de função), redução da amplitude de movimento (edema), aumento de temperatura local e DOMS (OWENS *et al*., 2019; PAULSEN *et al*., 2012). A DOMS tem início entre 6 a 12 horas, com um pico de 24 a 48 horas, após o DMIE. A DOMS é um dos sintomas clássicos de DMIE após exercícios não habituais, de alta carga ou excêntricos (CLARKSON; HUBAL, 2002; LEWIS *et al*., 2012; MUELLER-WOHLFAHRT *et al*., 2013; OWENS *et al*., 2019).

Os sintomas de DOMS são caracterizados por hiperalgesia mecânica (maior sensibilidade aos estímulos dolorosos), com o envolvimento de um músculo ou um grupamento muscular (CLARKSON; NEWHAM, 1995; MUELLER-WOHLFAHRT *et al*., 2013). Os sintomas de DOMS incluem sensibilidade muscular, rigidez, amplitude limitada de movimento e desconforto durante a contração isométrica muscular, e pode prejudicar as funções musculares até 72 h ou mais após o exercício (CHEUNG *et al*., 2003; OWENS *et al*., 2019; SILVA *et al*., 2017). A DOMS pode prejudicar o desempenho físico e as atividades da vida diária devido a reduções na potência e força muscular, diminuição da coordenação do movimento, alteração da cinemática articular e aumento do risco de lesão articular (CHEUNG *et al*., 2003), prejudicando o desempenho físico e qualidade do treinamento nos dias subsequentes ao exercício, o que pode causar grandes prejuízos a atletas que têm pouco tempo para se recuperar entre sessões de treinamento ou competições (HARTY *et al*., 2019; HOTFIEL *et al*., 2019).

Os mecanismos indutores de DOMS foram associados ao dano miofibrilar das fibras musculares esqueléticas e perimísio, vacuolização dos túbulos t, alteração da homeostase do cálcio intracelular, reações inflamatórias, estresse oxidativo, liberação de fatores neurotróficos e lesão nervosa

(CLARKSON; HUBAL, 2002; MORGAN; PROSKE, 2004; PAULSEN *et al*., 2012; MIZUMURA; TAGUCHI, 2016; OWENS *et al*., 2019).

As teorias mais recentes sobre a hiperalgesia mecânica (sensibilidade aumentada de dor) sugerem que durante, e por até uma hora após o exercício, ocorre a liberação de bradicinina, por meio da ativação de receptores B2 de adenosina epitelial vascular. A bradicinina pode induzir não apenas excitação e sensibilização de nociceptores, mas também alterações na expressão de neuropeptídios em diversos tipos de células (MURASE *et al*., 2010), e é conhecida por sensibilizar fibras nervosas aferentes finas em diversos tecidos (MIZUMURA; TAGUCHI, 2016). Os nociceptores são divididos em duas classes com base em sua dependência do fator neurotrófico, um dos grupos expressa o receptor tirosina quinase A (Trk A) e sua sobrevivência depende do Fator de Crescimento Neural (NGF), o outro grupo expressa o receptor tirosina quinase (Ret), o componente de sinalização comum para o receptor é o Fator Neurotrófico Derivado da linha de Células Gliais (GDNF) (MURASE *et al*., 2013).

O receptor de bradicinina B2 pode estar ligado a hiperalgesia mecânica, contudo não foi observado aumento da secreção de bradicinina nos dias após o DMIE, quando é detectado sintomas de DOMS (BOIX *et al.,* 2002; MIZUMURA; TAGUCHI, 2016). Um estudo experimental com um inibidor do receptor de bradicinina demonstraram que a inibição do receptor B2 previamente a indução de DMIE inibe o desenvolvimento da DOMS (MUSASE *et al*., 2010). No entanto, a administração de inibidores após 24 h não apresenta efeitos sobre a inibição de DOMS (MUSASE *et al*., 2010). Desta forma, o receptor B2 e a bradicinina parecem estar envolvidos com o início do processo de hiperalgesia, sendo um mecanismo de sensibilização dos nociceptores para induzir a hiperalgesia mecânica, mas não o fator que dispara o potencial de ação das fibras nervosas aferentes finas (tipo C e Aδ) (MURASE *et al.,* 2010; MIZUMURA; TAGUCHI, 2016).

O agente que está relacionado a estimulação dos nociceptores das fibras aferentes tipo-C, é o NGF. O NGF é liberado no período de 12 a 48 horas após o DMIE, coincidindo com o período de pico da DOMS (MURASE *et al*., 2013; MIZUMURA; TAGUCHI, 2016). Em um estudo experimental, para confirmar o envolvimento do NGF na hiperalgesia, os autores utilizaram

anticorpos anti-NGF, via intramuscular, causando assim um bloqueio do NGF. Quando os anticorpos foram injetados até 6 horas após o DMIE foi possível inibir a hiperalgesia mecânica, mas não resultou efeito inibidor quando aplicado após 2 dias (MURASE *et al*., 2013; MIZUMURA; TAGUCHI, 2016). O NGF sensibiliza fibras nervosas de tipo C, ocorrendo assim a hiperalgesia mecânica retardada (MIZUMURA; TAGUCHI, 2016; MURASE *et al*., 2010).

Um estudo com camundongos demonstrou que a administração aguda ou crônica de BCAA diminuiu a expressão de NGF no hipocampo (SCAINI *et al*. 2013; WISNIEWSKI *et al*. 2015). Estudos com homens pré-diabéticos também revelaram que a suplementação de BCAA diminuiu os níveis circulantes de NGF (WOO *et al.* 2019). Desta forma, é possível que a administração de BCAA possa ter algum efeito sobre o desenvolvimento da DOMS.

A regulação positiva do GDNF é induzida pela enzima cicloxigenase-2 (COX-2), e também está relacionada com a manutenção da DOMS (MURASE *et al*., 2010). O aumento dos níveis circulantes de GDNF ocorre de 12 a 24 horas após o DMIE, e está associado ao desenvolvimento da hiperalgesia mecânica (MURASE *et al*., 2010). A COX-2 é produzida quando os mioblastos sofrem alongamento em contrações excêntricas, o que ocorre durante o DMIE (MURASE *et al*., 2010).

Drogas anti-inflamatórias não-esteroidais que inibem a COX-2, foram utilizadas em testes para reduzir a hiperalgesia mecânica após o exercício (MURASE *et al*., 2013). Dois inibidores mostraram supressão dos efeitos de hiperalgesia mecânica quando utilizados anteriormente ao DMIE, mas novamente quando utilizados 2 dias após o DMIE falharam em reverter a hiperalgesia mecânica (MURASE *et al*., 2013).

É possível observar que a bradicinina facilita a expressão da COX-2 nas células musculares o que induz a expressão do GDNF durante as 12 horas após o exercício (MIZUMURA; TAGUCHI, 2016; MURASE *et al*., 2013). Por sua vez o GDNF estimula a hiperalgesia muscular mecânica sensibilizando as fibras Aδ (MIZUMURA; TAGUCHI, 2016; MURASE *et al*., 2013).

A liberação de bradicinina durante a contração muscular parece regular a captação de glicose (de forma semelhante à ação da insulina) por meio do aumento da translocação do receptor GLUT-4 nas células musculares e a

liberação de BCAA (SCHIFMAN et al. 1980; TAGUCHI et al. 2000). Além disso, a isoleucina tem o mesmo efeito que o receptor GLUT-4 nas células musculares (TAGUCHI et al. 2000; ZHANG et al. 2016). É possível que a suplementação de BCAA possa diminuir a produção de bradicinina, uma vez que a isoleucina pode aumentar a translocação de GLUT-4. No entanto, estudos experimentais são necessários para confirmar esta hipótese.

Muitas estratégias de recuperação foram propostas para mitigar a DOMS, tais como, terapia de contraste de frio e calor, técnicas de massagem, ultrassom, corrente elétrica e homeopatia, a maioria delas visando atenuar a lesão muscular, mas com resultados controversos (CHEUNG *et al*., 2003; MALANGA; YAN; STARK, 2014; MIZUMURA; TAGUCHI, 2016; OWENS *et al*., 2019). Em vista dos recentes avanços na elucidação dos mecanismos de indução da DOMS, além dos efeitos positivos observados em alguns estudos, é possível que a suplementação com BCAA seja uma estratégia nutricional de prevenção da DOMS quando administrado em momento adequado.

1.3 Aminoácidos de Cadeia Ramificada

Métodos de recuperação baseados na suplementação nutricional tem o objetivo de fornecer elementos químicos e substâncias essenciais que podem prevenir DMIE e DOMS, induzindo efeitos anti-inflamatórios e antioxidantes, bem como acelerando a reparação de tecidos (CRUZAT *et al*., 2014; OWENS *et al*., 2019). Um dos suplementos estudados são os aminoácidos de cadeia ramificada (BCAA), uma mistura de três aminoácidos essenciais: L-leucina, L-valina, L-isoleucina. Ao contrário de outros aminoácidos que são metabolizados principalmente no fígado, o BCAA pode também ser oxidado e absorvido em outros tecidos, como o músculo estriado esquelético, rins e coração (BISWAS *et al.*, 2019).

A absorção celular de BCAA ocorre através de um complexo de proteínas heterodiméricas da membrana plasmática pela subunidade leve da permease de aminoácido (BISWAS *et al.*, 2019). Uma vez dentro das células, os aminoácidos são transportados a mitocôndria para oxidação, biossíntese de esteróis, corpos cetônicos e produção de glicose, participando assim do metabolismo energético durante e após o treinamento físico, como também

podem ser integrados em proteínas, participando da síntese proteica ou armazenados em grupos de aminoácidos (JACKMAN *et al*., 2017; BISWAS *et al.*, 2019; KAMEI *et al*., 2020).

Dentre os três aminoácidos que compõem o BCAA, a leucina participa da ativação do sistema chamado alvo da rapamicina em mamíferos (*mammalian target of rapamycin* - MTOR), que é uma via de sinalização central na regulação, crescimento e proliferação celular, que forma dois complexos, mTORC1 e mTORC2 (LYSENKO *et al*., 2018). Quando este sistema é ativado ocorre uma redução do sistema ubiquitina-proteassoma, que por sua vez atua na degradação das proteínas, inibindo o catabolismo proteico (LYSENKO *et al*., 2018). A estimulação da síntese de proteínas estimulada pela leucina, ativa o processo de tradução (de mRNA para proteínas) e um complexo molecular contento o fator de iniciação eucariótico 4E, importante para o processo de tradução, durante a depleção dos aminoácidos a proteína de ligação do eIF4E (4EBP) se liga ao fator 4E de início da síntese proteica (eIF4E) para diminuir a atividade do complexo da tradução. Quando a leucina ativa o complexo mTORC1, este fosforila a 4EBP, levando a sua dissociação de eIF4E, iniciando a tradução e um aumento da síntese de proteínas (KAMEI *et al*., 2020).

Além disso, a ativação de via de sinalização mTOR estimula a proliferação de miotubos, diminui a produção de citocinas inflamatórias e o estresse oxidativo (CRUZAT *et al*., 2014; DUAN *et al*., 2017; KAMEI *et al*., 2020; LYSENKO *et al*., 2018; NICASTRO *et al.,* 2012). Os efeitos biológicos da leucina na síntese e renovação de proteínas e redução de citocinas pró-inflamatórias pode ter efeito protetor contra o dano inflamatório e oxidativo muscular (LEE *et al*., 2017), contribuindo para a recuperação muscular mais rápida e atenuação dos sintomas de DOMS (BUONOCORE *et al*., 2015).

Os efeitos dos BCAA na prevenção de DOMS estão em debate na literatura, uma vez que alguns estudos demonstraram que a ingestão de BCAA diminuiu a dor muscular (HOWATSON *et al*., 2012; SHIMOMURA *et al*., 2010; ISHIKURA *et at*., 2014; DORREL; GEE, 2016; KEPHART *et al.,* 2016; REULE *et al.*, 2017; WALDRON *et al*., 2017; VANDUSSELDORP *et al*., 2018), enquanto outros não relataram efeitos (KOO *et al*., 2014; MOHAMEDI-PANAHI *et al.,* 2013; RA et al. 2013; RA *et al*., 2018), ou até mesmo aumentaram os sintomas de DOMS (KIRBY *et al*., 2012) após uma sessão de DMIE. No

entanto, diferentes conteúdos de BCAA e razões de aminoácidos, dose de BCAA, tempo de tratamento, tipo de protocolo DMIE e características do sujeito podem ser responsáveis por resultados controversos observados na literatura.

Considerando as evidências de que os BCAA podem ter propriedades biológicas que poderiam atenuar os sintomas de DOMS, mas resultados contraditórios ou com grande risco viés são encontrados em muitos estudos, é necessário determinar se existem evidências clínicas que apoiam o uso deste tipo de estratégia na prevenção de DMIE e qual o protocolo mais eficiente.

2 OBJETIVOS

O objetivo deste estudo foi realizar uma revisão sistemática da literatura sobre os efeitos da suplementação de BCAA na sintomatologia de DOMS após uma sessão de exercício físico, em indivíduos adultos saudáveis.

Objetivos secundários

Determinar se há evidência do efeito inibidor do BCAA sobre a DOMS.

Avaliar a qualidade da evidência para os efeitos da suplementação com BCAA em diferentes tempos (24 a 96 horas) após o DMIE;

Avaliar a qualidade da evidência para os efeitos da suplementação de BCAA em sujeitos treinados e destreinados;

Avaliar a qualidade da evidência para os efeitos da suplementação com BCAA em diferentes doses e tempos de tratamento;

Avaliar a qualidade da evidência para os efeitos da suplementação com BCAA em diferentes níveis de DMIE.

3 MATERIAL E METODOS

Para atender os objetivos, foi realizado uma revisão sistemática com metanálise intitulada: O uso do BCAA para prevenir a dor de acometimento tardio: uma revisão sistemática e metanálise.

4 RESULTADOS

4.1 ARTIGO 1

Artigo cientifico no formato para a revista Amino Acids.

The use of BCAA to prevent delayed-onset muscle soreness: a systematic review and meta-analysis.

Abstract

Branched-chain amino acids (BCAA) are used as a recovery method after exercise-induced muscle damage (EIMD). Although data suggest that BCAA may alleviate the delayed-onset muscle soreness (DOMS) evoked by EIMD, there is no consensus about the most effective supplementation protocol. To investigate the effects of BCAA on DOMS, a systematic review and meta-analysis was conducted on the effectiveness of BCAA supplementation to reduce DOMS symptoms in healthy subjects after a single session of EIMD. Randomized clinical trials (RCT) were searched in Medline, Cochrane Library, Science Direct, SciELO, LILACS, SciVerse Scopus, Springer Link journals, Wiley Online Library, and Scholar Google, until November 2020. Ten RCTs were included in the systematic review and nine in the meta-analysis. Seven studies demonstrated that BCAA reduced levels of DOMS after 24 to 72 hours. BCAA at doses up to 255 mg/kg/day, supplemented prior to EIMD, in trained subjects could blunt DOMS symptoms. However, high variability between studies due to training status, different doses, time of treatment, and severity of EIMD do not allow us to conclude whether BCAA supplementation is efficient in untrained subjects, applied acutely, and at higher doses (>255 mg/kg/day). The overall effects of BCAA on DOMS could be considered useful for improving muscle recovery by reducing DOMS in trained subjects and should not be administered only after the EIMD protocol.

Keywords: pain, physical exercise, leucine, skeletal muscle.

4.1.1 Introduction

Delayed-onset muscle soreness (DOMS) is one of the classical signs and symptoms of exercise-induced muscle damage (EIMD) after unaccustomed, high load, or eccentric exercises (Clarkson and Hubal 2002; Lewis et al. 2012; Mueller-Wohlfahrt et al. 2013; Owens et al. 2019). DOMS is characterized by mechanical hyperalgesia, peaking 24 to 72 hours after exercise, with the involvement of the entire muscle or a group of muscles (Clarkson and Newham 1995; Mueller-Wohlfahrt et al. 2013). DOMS symptoms include muscle tenderness, stiffness, limited range of motion, and discomfort during muscle isometric contraction, and could impair muscle function for up to 72h or more (Cheung et al. 2003; Owens et al. 2019; Silva et al. 2017). Due to reductions in muscle power and strength, decreased movement coordination, altered joint kinematic, and increased risk of joint injury, DOMS may impair physical performance and daily life activities (Cheung et al. 2003).

The DOMS pathogenesis had been attributed to myofibrillar damage of skeletal muscle fibers and perimysium, vacuolization of t-tubules, and altered calcium homeostasis, inflammatory reactions, oxidative stress, the release of neurotrophic factors, and nerve injury (Clarkson and Hubal 2002; Mizumura and Taguchi 2016; Owens et al. 2019; Paulsen et al. 2012). The most recent theories suggest that during EIMD, the activation of bradykinin B2 receptors induces upregulation of nerve growth factor (NGF) expression by muscle fibers after 12 to 24 h post-exercise (Mizumura and Taguchi 2016; Murase et al. 2010). NGF sensitizes type C nerve fibers to delayed mechanical hyperalgesia (Mizumura and Taguchi 2016; Murase et al. 2010). Bradykinin also increases cyclooxygenase-2 expression in muscle cells that induces the expression of glial cell line-derived neurotrophic factor (GDNF) after 12 h post-exercise (Mizumura and Taguchi 2016; Murase et al. 2013). GDNF also stimulates muscle mechanical hyperalgesia, sensitizing Aδ fibers (Mizumura and Taguchi 2016; Murase et al. 2013).

Although many recovery strategies have been proposed to mitigate DOMS, most of which target the pathogenesis of muscle injury, the results have been controversial (Cheung et al. 2003; Mizumura and Taguchi 2016; Owens et al. 2019). Recovery methods based on nutritional supplementation can provide

chemical elements and essential substances that may prevent EIMD and DOMS, inducing anti-inflammatory and antioxidant effects as well as accelerating tissue repair (Cruzat et al. 2014; Owens et al. 2019). One of the supplements that have been studied is branched-chain amino acids (BCAA), which is a mixture of three essential amino acids: L-leucine, L-valine, and L-isoleucine. These amino acids are abundant in skeletal muscle as the main amino acids of structural proteins and have a role in protein synthesis and energy metabolism during and after physical training (Jackman et al. 2017; Kamei et al. 2020). BCAA have many nutritional and functional roles, such as providing a source of energy during exercise, acting as a signaling factor for protein synthesis, inhibiting protein catabolism by the ubiquitin-proteasome pathway, stimulating myotube proliferation, and decreasing the production of inflammatory cytokines and oxidative stress (Cruzat et al. 2014; Duan et al. 2017; Kamei et al. 2020; Lysenko et al. 2018; Nicastro et al. 2012). These biological effects on protein synthesis and turnover, as well as inhibition of oxidative stress and inflammatory pathways may contribute to faster muscle recovery and, therefore, blunt the symptoms of DOMS.

The effects of BCAA on preventing DOMS is under debate in the literature since some studies demonstrated that BCAA intake decreased muscle soreness (Howatson et al. 2012; Shimomura et al. 2010; Waldron et al. 2017), while others reported no effects (Ra et al. 2013; Ra et al. 2018), or even increased DOMS symptoms (Kirby et al. 2012) after a bout of EIMD. However, different BCAA content and amino acid ratios, dose, time of treatment, type of EIMD protocol, and subject characteristics may account for the controversial results observed in the literature.

Considering the evidence that BCAA may have biological properties that could attenuate DOMS symptoms, it is necessary to determine if clinical evidence supports its use in EIMD. The objective of this study was to conduct a systematic review and meta-analysis of the literature to elucidate the effects of BCAA supplementation on DOMS after EIMD. The study also describes any limitations and bias found in the BCAA clinical trials. The research question of this investigation is “Does BCAA supplementation decrease DOMS symptoms after a session of EIMD when compared to Placebo treatment in healthy subjects?”

4.1.2 Methodology

Search strategy

The preferred reporting items for systematic reviews and meta-analyses (PRISMA; https://www.equator-network.org/reporting-guidelines/prisma) guidelines were followed. No limitations of language, publication status, and year of publication were applied in the literature search. The literature search followed the PICOS (Patient, Intervention, Comparison/Control, Outcome, and Study) strategy and could include randomized clinical trials (RCTs), quasi-randomized clinical trials, case-control studies, and crossover trials. Two independent authors (MGW and SSD) searched the databases including the Cochrane Central Register of Controlled Trials (The Cochrane Library), MEDLINE (PubMed), Science Direct, The Scientific Electronic Library Online (SciELO), Latin-American and Caribbean Center on Health Sciences Information (LILACS), SciVerse Scopus, Springer Link journals, Wiley Online Library, and Scholar Google. Searches were performed until November 2020.

The search strategy used for MEDLINE is exemplified below and was adapted to search the other databases: injur*) OR lesion*) OR recover*) OR repair*) OR fatigu*) OR damage*) OR soreness) AND creatine kinase) AND Humans[Mesh])) AND muscle) AND Humans[Mesh])) AND ((((((((((exercis*) OR training) OR power) OR strength) OR aerobic) OR anaerobic) OR effort) OR athlet*) AND Humans[Mesh])) AND Humans[Mesh])) AND (((bcaa OR [branched chain amino acid] OR leucine OR valine OR isoleucine)) AND Humans[Mesh])) AND Humans[Mesh])) AND Humans[Mesh])) AND random* Filters: Humans.

Inclusion criteria

Only studies in which BCAA or its isolated components (L-leucine, Isoleucine, and Valine) were compared to placebo to prevent EIMD after a single session of exercise were eligible. The studies needed to be performed in adult healthy subjects, regardless of fitness status (sedentary, trained, athletes). The exercise protocol was required to be one session of physical exercise that caused EIMD and DOMS. The EIMD was required to be evaluated by at least two methods including decrement in muscle performance, increased levels of

circulating muscle damage markers (creatine kinase, lactate dehydrogenase, myoglobin), or decreased range of motion or edema, at least 48h or more after exercise. EIMD symptoms were classified as mild, moderate, or severe based on the criteria described by Paulsen et al. (2012).

Studies needed to describe BCAA composition, the individual dose (total amount or mg/kg), and duration of administration of BCAA. The control group should receive a placebo intervention with an inactive substance or a lower dose (<0.25mg/kg/day) of carbohydrates. The main outcome was the decrement in muscle pain, muscle soreness, and/or DOMS reported by participants evaluated by an algometer or perceived muscle pain reported on a visual analogue scale.

Data extraction and analysis

Information retrieved included: age, weight, physical fitness status, EIMD protocol, BCAA treatment protocol, and DOMS. DOMS values were converted to a standardized unit of measure (natural logarithm) to minimize methodological heterogeneity, according to the Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al. 2020). Quality of evidence was scored using the Grading of Recommendations, Assessment, Development, and Evaluation – GRADE (https://gradepro.org) (Schünemann et al. 2013).

The risk of bias for included studies was assessed as high, low, or unclear and the potential for bias was screened according to the Cochrane Collaboration's 'seven evidence-based domains' tables. In the meta-analysis, the overall and subgroup analyses for different follow-up time-points (24 to 96 h) were plotted with log-transformed mean and standard deviation. The authors were contacted up to three times to retrieve missing data. If no reply was obtained, values were extracted from the graphic data presentation using GetData Graph Digitizer (http://getdata-graph-digitizer.com/). The random-effect model was employed to compute a pooled estimate of mean difference (MD) and respective 95% confidence interval (95%CI). Heterogeneity between studies was detected by I^2 statistics. Risk of bias and meta-analysis was conducted on RevMan 5.1. (The Cochrane Collaboration; V.5.3).

4.1.3 Results

Studies

The database search yielded 2237 articles and after the removal of duplicates, 1136 records remained: 14 from Medline, six from SciELO, 10 from LILACS, 12 from Cochrane, 266 from Science Direct, 599 from Scopus, 151 from Springer, and 78 from Wiley (Figure1). After reading of the titles and abstracts, twenty-six studies remained, of which 16 papers were excluded after review (Armisasan et al. 2011; Asjodi et al. 2018; Atachak and Baturak 2012; Dorrell and Gee 2016; Gee and Deniel 2016; Ishikura et al. 2014; Jackman et al. 2010; Kephart et al. 2016; Koo et al. 2014; Leahy and Pintauro 2013; Lysenko et al. 2018; Matsumoto et al. 2009; Mohamad-Panahi et al. 2013; Nosaka et al. 2006; Sharp and Pearson 2010; Sheikholeslami-Vatani and Ahmadi 2016) because they did not fit the inclusion criteria. Screening the reference lists of the remaining articles identified one additional paper for inclusion (Kirby et al. 2012). The characteristics of the studies are presented in table 1.

Participants

A total of 160 participants were investigated (Table 1), 146 (91.3%) male and 14 (8.7%) female. Six (60%) studies investigated untrained or sedentary subjects (Fouré et al. 2016; Greer et al. 2007; Kirby et al. 2012; Ra et al. 2013; Ra et al. 2018; Shimomura et al. 2010). Recreationally resistance-trained participants were reported in three (30%) studies (VanDusseldorp et al. 2018; Waldron et al. 2018; Waldron et al. 2017) and one (10%) study included athletes (soccer, rugby) (Howatson et al. 2012).

Exercise-induced muscle damage protocol

Exercise protocols included resistance exercises (n=6, 60%) (Kirby et al. 2012; Ra et al. 2013; Ra et al. 2018; Shimomura et al. 2010; VanDusseldorp et al. 2018; Waldron et al. 2017), drop jumps (n=3, 30%) (Howatson et al. 2012; Kirby et al. 2012; Waldron et al. 2018), cycling (n=1, 10%) (Greer et al. 2007), and muscle electrical stimulation (n=1, 10%) (Foure et al. 2016) (Table 1).

EIMD symptoms were demonstrated in eight studies (80 %) with

increased serum levels of CK and other serum markers of muscle damage (Foure et al. 2016; Greer et al. 2007; Howatson et al. 2012; Ra et al. 2013; Ra et al. 2018; VanDusseldorp et al. 2018; Waldron et al. 2018; Waldron et al. 2017) and decreased muscle function (Foure et al. 2016; Greer et al. 2007; Howatson et al. 2012; Ra et al. 2013; Ra et al. 2018; VanDusseldorp et al. 2018; Waldron et al. 2018; Waldron et al. 2017) from 24 to 96 h after the EIMD session. Two (20%) studies demonstrated impaired muscle function after EIMD, but no significant change in CK levels over 72h of recovery (Kirby et al. 2012; Shimomura et al. 2010).

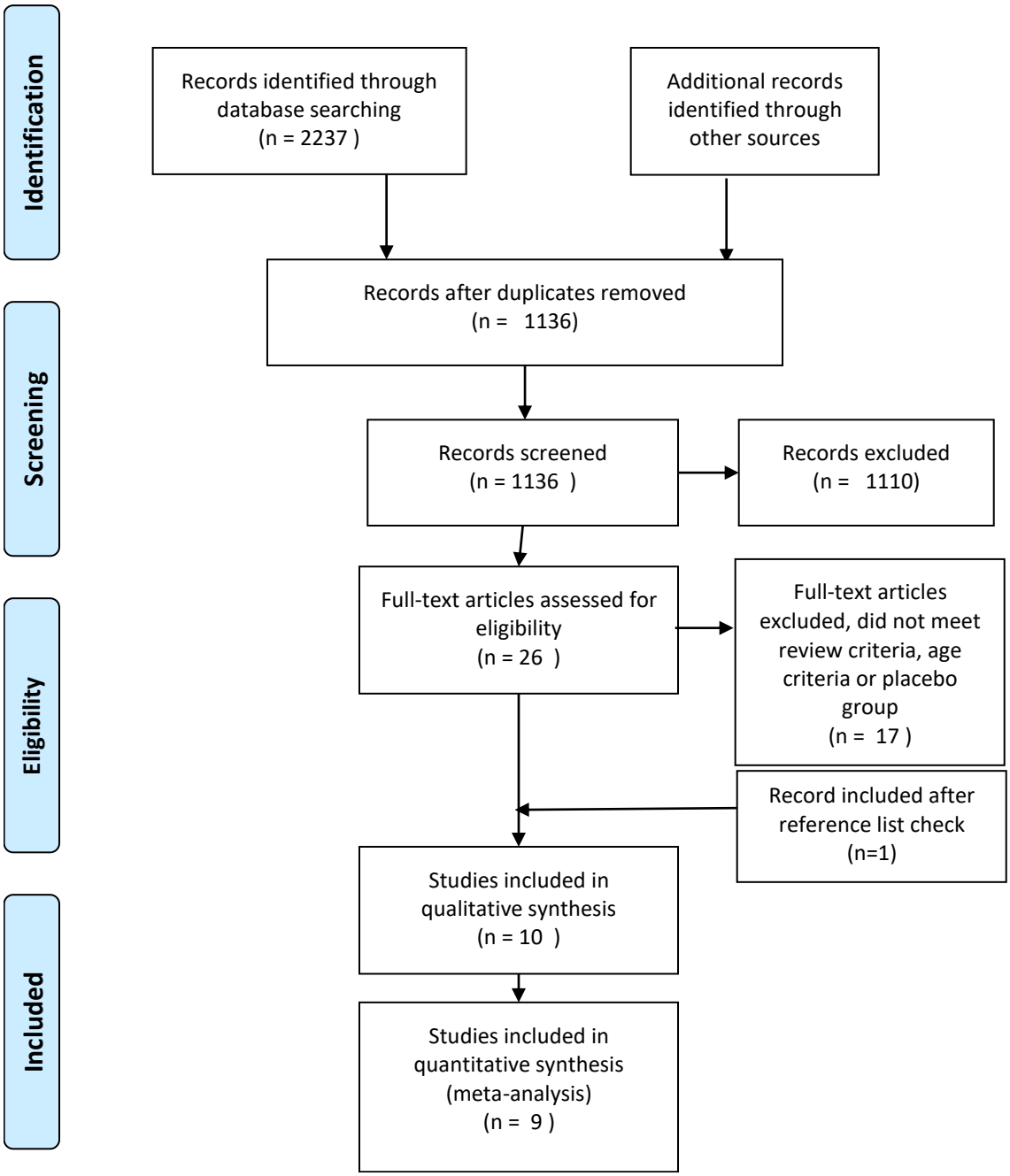

Figure 1. Study flow chart.

Delayed-onset muscle soreness

The outcomes for DOMS symptoms are presented in Table 1. Six (60%) studies reported reduced muscle soreness at different times ranging from 24 to 72 h after the exercise session (Greer et al. 2007; Howatson et al. 2012; Shimomura et al. 2010; VanDusseldorp et al. 2018; Waldron et al. 2018;

Waldron et al. 2017). Two studies reported no effects of BCAA up to 96h (Foure et al. 2016; Ra et al. 2013). However, one study demonstrated that muscle soreness was attenuated when administered three days before the EIMD protocol, but not when supplemented three days after the protocol (Ra et al. 2018). A single study demonstrated increased DOMS from 24 to 96 h post an EIMD protocol in subjects supplemented with a high dose of isolated leucine (Kirby et al. 2012).

BCAA supplementation

The BCAA was supplemented on the day of the EIMD protocol in all studies (Table 2). Acute (only on the day of exercise) administration was performed in two (20%) studies. However, the BCAA was also administered on the day before the EIMD protocol in two (20%) studies (Greer et al. 2007; Shimomura et al. 2010), 4 days before or 4 days after the EIMD protocol in one study (Ra et al. 2018), up 72 or 96 h after the protocol in four (40%) studies (Foure et al. 2016; Kirby et al. 2012; Waldron et al. 2018; Waldron et al. 2017), and on the days before and after the EIMD protocol in three studies (Howatson et al. 2012; Ra et al. 2013; VanDusseldorp et al. 2018) (Table 2).

The total BCAA per dose ranged from 5 to 10 g (Greer et al. 2007; Howatson et al. 2012), with a total amount ranging from 5.5 to 20 g on the day of the EIMD protocol (Howatson et al. 2012; Shimomura et al. 2010). The average amount per kg of body mass ranged from approximately 59.4 to 750 mg/kg/day (Greer et al. 2007; Howatson et al. 2012) (Table 2). To investigate the possible effects of different BCAA doses per day on EIMD, studies were analyzed in subgroups divided according to lower doses (<255 mg/kg/day) (Greer et al. 2007; Shimomura et al. 2010; VanDusseldorp et al. 2018; Waldron et al. 2018; Waldron et al. 2017) and higher doses (> 255 mg/kg/day).

Bias Risk

The absence of a sample size or statistic power calculation was observed in four (40%) studies (Ra et al. 2013; Ra et al. 2018; Waldron et al. 2018; Waldron et al. 2017). Dietary intake was monitored in only five (50%) studies (Foure et al. 2016; Greer et al. 2007; Kirby et al. 2012; Waldron et al. 2018; Waldron et al. 2017). Random sequence generation and allocation

concealment was not presented in three (30%) studies (Greer et al., 2007; Shimomura et al., 2010; Vandusseldorp et al., 2018). In one study, the blinding of researchers was not performed (Greer et al., 2007). (The risk of these studies is demonstrated in Figures 3 and 4 of our Annexes).

Table 1. Authors, study design, participant characteristics (age, sex, and fitness status), exercise-induced muscle damage (EIMD) protocol and outcomes for delayed-onset muscle damage (DOMS).

Author, year	Study Design	Participants	EIMD protocol	DOMS Outcome
Greer et al., 2007	RCT, crossover, blinded*	9 males (21.6 ± 3.2 years) Untrained	90 minutes of cycling at 55% VO_2max	↓ 24 h = 48 h
Shimomura et al., 2010	RCT, crossover, double-blinded	12 females (20 to 25 years) Untrained	7 sets of 20 squats (with body weight)	↓ 24 and 48 h = 72 and 96 h
Kirby et al., 2012	RCT, double-blinded	19 males (21.3 ± 1.6 years) Treated =10 Placebo =9 Untrained	100 drop jumps; 10 eccentric leg press contractions at 120% 1RM	↑ 24, 48, 72, and 96 h
Howatson et al., 2012	RCT, double-blinded	12 males (23 ± 2 years) Treated =6 Placebo = 6 Football and rugby athletes	100 drop jumps	↓ 24 and 48 h
Ra et al.,2013	RCT, double-blinded	18 males (22.5 ± 3.8 years) BCAA = 9 Placebo = 9 Sedentary	6 sets of 5 repetitions of eccentric elbow extension at 90% 1RM	= 24, 48, 72, and 96 h
Fouré et al.,2016	RCT, double-blinded	26 males BCAA: n=13, 22 ± 1 years; Placebo: n=13, 23 ± 2 years Untrained	Electrical stimulation of contractions in vastus lateralis and vastus medialis muscles	= 24, 48, 72, and 96 h

Cont. Table 1

Waldron et al., 2017	RCT, double-blinded		14 males (21.8 ± 1.6 years) 2 females (22 ± 1 years) BCAA: n=8 Placebo: n=8 Resistance-trained athletes	6 sets of 10 repetitions of back squats at 70% of 1 RM	↓ 24 and 48 h
Ra et al., 2018	RCT, double-blinded		15 males (21.5 ± 0.4 years) Pre BCAA: n=5 Post BCAA: n=5 Placebo: n=5 Untrained	Six sets of five repetitions of eccentric elbow extension at 90% of maximal voluntary isometric force	Pre group: = 24 h ↓ 48 and 72h No effects in the POST group
VanDusseldorp et al., 2018	RCT, double-blinded		20 males (22.3 - 1.5 year), BCAA: n=10 Placebo: n=10 Resistant-trained	10 sets of 8 repetitions at 70% 1RM squats using a Smith machine; five sets of 20 body-weight split-jumps	= 24 h ↓ 48 and 72 h
Waldron et al., 2018	RCT, double-blinded		15 males (21 ± 1 years) Leucine: n=8 Placebo: n= 7 Resistance-trained	100 drop jumps	↓ 24 and 48 h

* Authors did not report whether the researchers were blinded or not; = no differences in comparison to the control group; ↓ decreased DOMS compared to the control group; ↑ Increased DOMS compared to the control group.

Table 2. BCAA supplementation protocols.

Author, publication year	Composition Iso/Leu/Val	Total amount per dose (g)	Individual dose (mg/kg)	Supplementation on days before EIMD protocol	Supplementation in EIMD protocol	Supplementation on days after EIMD protocol	Placebo
Greer et al., 2007	1 : 2.5 : 1.5	5	~59.4 *	-	One dose 5 min before and another during (60min) EIMD protocol	-	Flavored water
Shimomura et al., 2010	1 : 2.3 : 1.2	5.5	~100 *	-	1 dose 15 minutes before EIMD protocol	-	5.5 g of dextrin
Kirby et al.,2012	0:1:0	Not informed	250	-	1 dose 30 minutes before + 1 dose immediately before, + 1 dose immediately after EIMD protocol	1 dose/day at 24, 48, 72, and 96 h	3 g artificial sweetener
Howatson et al., 2012	1:2:1	10	~280*	2 doses/day, 7 days	One dose 1h before + One dose immediately after EIMD protocol	2 doses/day, at 24, 48, 72, and 96 h	10 g artificial sweetener
Foure et al, 2016	1:2:1	Not informed	100	-	1 dose 30 minutes before + 1 dose immediately before, + 1 dose immediately	1 dose/day at 24, 48, 72, and 96 h	Microcrystalline cellulose (amount not described)

Cont. Table 2

Waldron et al., 2017	1:2:1	Not informed	87	-	1 dose 30 minutes before + 1 dose immediately after EIMD protocol	2 doses/day at 24, 48 h	0.25 g/kg of dextrose
Ra et al., 2018	1:2:1	3.2	~149*	Group PRE: one dose 3 days before EIMD	Group PRE: 3 doses 15 minutes before EIMD protocol	-	3.2 g of starch
				-	Group POST: 3 doses immediately after EIMD protocol	Group POST: 24, 48, and 72 h	
VanDusseldorp et al., 2018	1:3:2	Not informed	110	2 doses/day, 4 days before	2 doses before EIMD protocol	2 doses/day, 24, 48, and 72 h	110 mg/kg of maltodextrin
Waldron et al., 2018	0:1:0	Not informed	87	-	One dose 30 minutes before + One dose immediately after EIMD protocol	2 doses/day at 24, 48, and 72 h	0.3 g/kg of maltodextrin

EIMD: exercise-induced muscle damage; * individual doses were not informed in the article, but estimated by authors based on participants' mean body mass.

4.1.4 Meta-analysis

Visual analogue scales were employed to evaluate muscle soreness in all studies. All values were converted to natural logarithms, using mean and standard deviation, for entry in the meta-analysis. The meta-analysis was performed with subgroup analysis according to follow-up time from 24 to 96 h post-EIMD (peak symptoms of EIMD) (Figure 2). One article was not included in the meta-analysis because the DOMS score was presented as a percentage change from baseline (Waldron et al. 2017).

Overall, pooled data revealed a random effect for treatment ($z = 4.45$, $P<0.0001$) on DOMS decrements of -0.27 (95% CI: of -0.39 to -0.15) with low heterogeneity for log-transformed values in the overall analysis ($Chi^2 = 30.0$, $I^2 = 0\%$, $p = 0.52$). No significant differences ($Chi^2 = 3.51$, $I^2 = 14.4$, $p=0.32$) were detected between subgroups (follow-up 24 to 92h) (Figure 2). GRADE criteria indicated high certainty for 24, 48, and 72h analysis, and moderate for 96h. However, because a potential source of heterogeneity included subject training status (untrained or trained), treatment schedule (pre, during, and post EIMD protocol), the total amount of BCAA ingestion, and severity of EIMD, a second subgroup analysis by heterogeneity sources is presented in table 3.

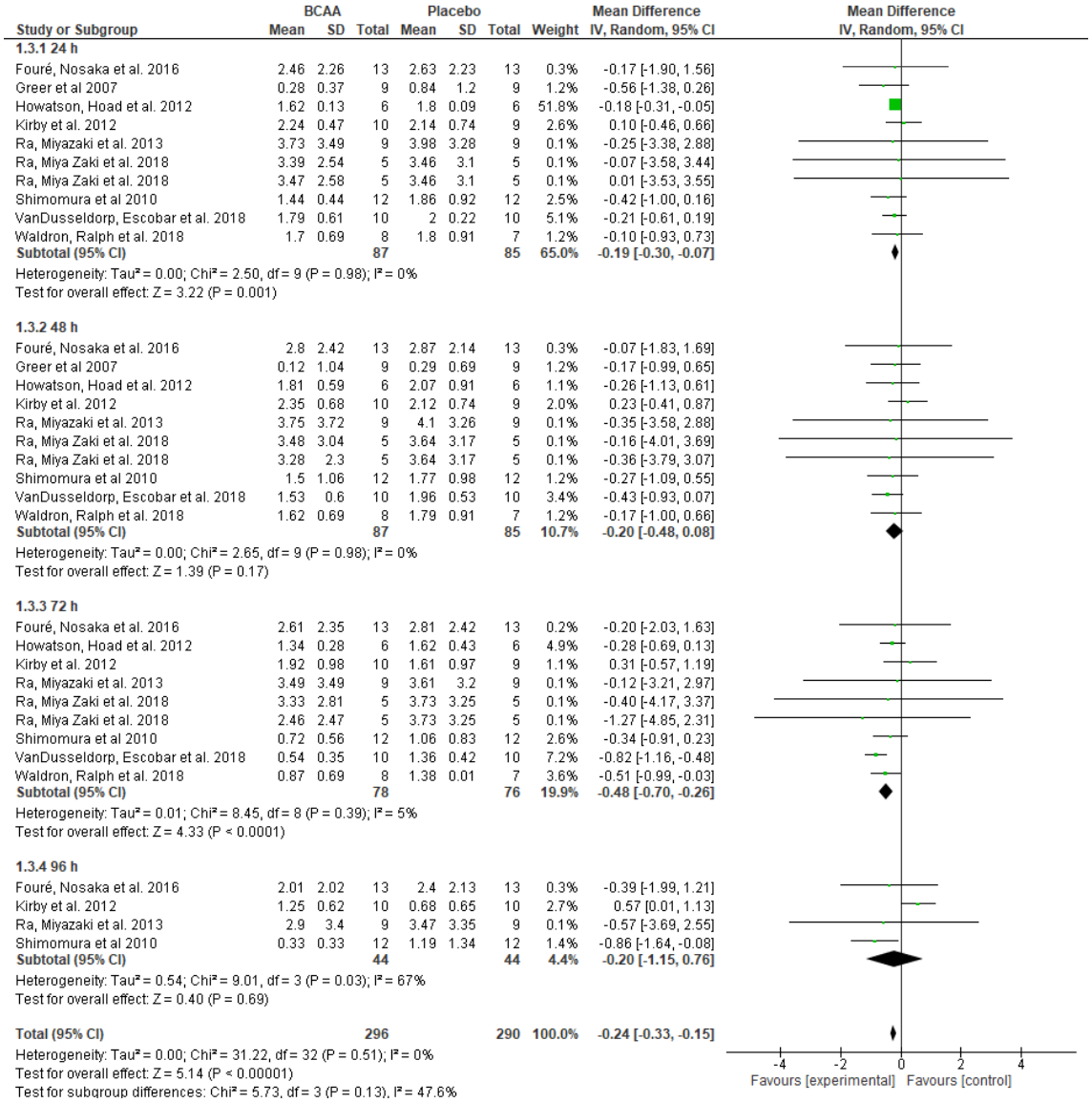

Figure 2. Meta-analysis of log-transformed values of selected studies.

Trained subjects presented a beneficial effect for BCAA at 24h and 72h, but with a moderate level of heterogeneity and quality of evidence at 72h (Table 3). The supplementation of BCAA only on the day of EIMD (acute supplementation) presented random effects favoring BCAA treatment, but with lower quality of evidence. Post-EIMD protocols (up to 72 hours) presented no effects favoring BCAA treatment. A large effect for DOMS decrements was observed in studies that supplemented BCAA chronically (pre to post) (Table 3).

Lower doses presented a random effect favoring BCAA treatment, but with a moderate degree of heterogeneity and quality of evidence (Table 3). Higher doses presented an effect only at 24 h (Table 3). Regarding EIMD severity, an effect favoring BCAA was observed for moderate levels of injury

(Table 3).

Table 3. Subgroup meta-analysis of participants stratified by fitness level, days of BCAA supplementation, mean daily intake.

Subgroup (n=number of studies; participants)	Z score	P	Random mean	95% CI	I^2	Certainty (GRADE)
Fitness levels						
Untrained (n=6; 113)						
24h (n=6; 113)	1.28	0.20	-0.23	-0.58 to 0.12	0%	+++
48h (n=6; 113)	0.16	0.87	-0.03	-0.44 to 0.37	0%	+++
72h (n=5; 107)	0.75	0.45	-0.17	-0.62 to 0.28	0%	+++
92h (n=4; 88)	0.40	0.69	-0.20	-1.15 to 0.6	67%	++
Trained						
24h (n=3; 45)	2.76	0.006	-0.21	-0.37 to -0.06	0%	++++
48h (n=3; 45)	0.42	0.68	-0.08	-0.47 to 0.31	0%	++++
72h (n=3; 45)	3.27	0.001	-0.56	-0.89 to -0.22	51%	+++
Period of intervention						
Acute						
24h (n=2; 42)	1.94	0.05	- 0.47	-0.73 to 0.00	0%	++
48h (n=2; 42)	0.75	0.46	- 0.22	-0.80 to 0.36	0%	++
Post-EIMD						
24h (n=4; 70)	0.10	0.92	0.02	-0.42 to 0.47	0%	++++
48h (n=4; 70)	0.24	0.81	0.06	-0.42 to 0.54	0%	++++
72h (n=4; 70)	1.58	0.11	-0.33	-0.74 to 0.08	0%	++++
96h (n=3; 46)	1.06	0.29	0.39	-0.34 to 1.12	19%	++++
Pre and Post EIMD						
24h (n=3; 50)	2.97	0.003	-0.18	-0.30 to -0.06	0%	++++
48h (n=3; 50)	1.78	0.08	-0.39	-0.81 to 0.04	0%	++++
72h (n=3; 50)	2.40	0.02	-0.56	-1.01 to 0.10	52%	+++
BCAA dose (mg/kg/day)						
Lower (<255)						
24h (n=4; 77)	1.99	0.05	- 0.29	- 0.56 to -0.01	0%	+++
48h (n=4; 77)	1.78	0.07	- 0.31	- 0.61 to 0.03	0%	+++
72h (n=3, 59)	4.36	<0.001	-0.63	-0.91 to -0.34	18%	+++
Higher (> 255)						
24h (n= 5;95)	2.65	0.008	-0.17	-0.29 to -0.04	0%	++++
48h (n= 5;95)	0.11	0.91	0.03	-0.45 to 0.51	0%	++++
72h (n= 5;95)	1.02	0.31	-0.19	-0.55 to 0.17	0%	++++
96h (n=3;64)	1.65	0.10	0.44	-0.08 to 0.96	0%	++++
EIMD severity						
Mild						
24h (n=2; 39)	1.31	0.19	-0.32	-0.79 to 0.16	0%	++++
48h (n=2; 39)	0.74	0.46	-0.22	-0.80 to 0.36	0%	++++
72h (n=2; 39)	2.36	0.02	-0.44	-0.80 to -0.07	0%	++++
Moderate						
24h (n=4; 74)	2.98	0.003	-0.19	-0.31 to -0.06	0%	++++
48h (n=4; 74)	0.71	0.48	-0.20	-0.76 to 0.35	0%	++++
72h (n=3;56)	1.35	0.18	-0.27	-0.67 to 0.12	0%	++++
96h (n=2;44)	0.59	0.56	-9.43	-1.81 to 0.99	0%	++++
Severe						
24h (n=3; 59)	0.63	0.53	-0.10	-0.43 to 0.22	0%	+++
48h (n=3; 59)	0.93	0.35	-0.18	-0.57 to 0.20	0%	+++
72h (n=3; 59)	1.02	0.31	-0.42	-1.23 to 0.39	47%	+++

BCAA: branched-chain amino acids; EIMD: exercise-induced muscle damage. GRADE score for evidence consistency and quality of evidence: ++++ high, +++ moderate, ++ low

4.1.5 Discussion

The results of the present systematic review and meta-analysis suggest that DOMS can be blunted by low doses of BCAA supplemented before exercise protocols that induce low to moderate EIMD in trained subjects. Although the majority of the studies demonstrated a beneficial effect (Greer et al. 2007; Howatson et al. 2012; Shimomura et al. 2010; VanDusseldorp et al. 2018; Waldron et al. 2018; Waldron et al. 2017), the different supplementation doses, time of intervention, subjects characteristics, physical fitness, EIMD protocols, and level of EIMD may have accounted for the high variability of outcomes between studies. Although the low degree of heterogeneity suggested no significant variability between standardized results of studies, we sought to consider these factors as a potential influence on DOMS outcomes. Considering the magnitude of DOMS symptoms and time to relapse of symptoms, as well as the overall meta-analysis, subgroup analysis for each variable of interest from 24 to 96 h was also performed. The analysis of quality of evidence suggested that most of the evidence for different variables and time presented high quality, based on GRADE criteria. However, the quality of evidence for treatments in untrained subjects, acute administration, lower doses, and severe EIMD was considered low to moderate, due to the low number of studies, risk of bias, and moderate heterogeneity at some time points of analysis.

A positive effect of BCAA was observed in all studies with trained individuals or athletes (Howatson et al., 2012; Vandusseldorp et al., 2018; Waldron et al., 2018), with a high quality of evidence. However, a limitation of these studies is the unclear definition of “resistance-trained” subjects (VanDusseldorp et al. 2018; Waldron et al. 2018) since the authors only mention that the participants were experienced in resistance training. Despite this, we included resistance-trained individuals and athletes in the same subgroup analysis considering that participants presented adaptations in skeletal muscle due to training and can not be compared to untrained subjects. Some protective effects of BCAA in trained participants may be associated with better adaptation of skeletal muscle, including improved glucose metabolism and GLUT-4 expression, (Mujika and Padilla 2000), increased mobilization of

anti-inflammatory T regulatory cells (Tregs) (Dorneles et al. 2020), and anti-inflammatory/ tissue repairing M2 macrophages (Walton et al. 2019). GLUT-4 expression and Treg cells are upregulated by BCAA (Ikeda et al. 2017). Moreover, Treg activation suppresses muscle inflammation, decreasing the activation of the pro-inflammatory mediator cyclooxygenase-2 (Taguchi et al. 2000; Villalta et al. 2014). These factors may have accounted for a decrease in the inflammatory process and muscle damage in trained subjects.

Analysis of the time of administration demonstrated a high level of quality that post-EIMD supplementation had no clinically important effect on DOMS symptoms. However, acute and previous supplementation decreased DOMS at some time points. This evidence suggests that DOMS has no significant effects when administered after EIMD and DOMS. Recent theories about DOMS pathogenesis suggest that key mediators induced during exercise accounted for increased production of neurotrophins (NGF and GDNF) during the peak of muscle soreness (12 to 24 h) (Mizumura and Taguchi 2016; Murase et al. 2013; Murase et al. 2010). Thus, targeting the mediators released during exercise that induce the increase in late neurotrophins may be an efficient method of inhibiting DOMS. During exercise and one hour after an acute session of exercise, increased circulating and interstitial levels of bradykinin sensitize B2 bradykinin receptors in muscle and tendons (Langberg et al. 2002; Mizumura and Taguchi 2016; Murase et al. 2013; Ota et al. 2018; Taguchi et al. 2000). Activation of B2 bradykinin receptors stimulates the production of NGF in human tissues (Ochodnicky et al. 2013), which triggers mechanical hyperalgesia, stimulating afferent C-type fibers (Malik-Hall et al. 2005; Mizumura and Taguchi 2016). The bradykinin-induced up-regulation of NGF seemed to be essential for the development of DOMS (Murase et al. 2010). After muscle damage by lengthening contractions, muscles express increased levels of bradykinin followed by increased expression of NGF from 12h and peaking during mechanical hyperalgesia (Murase et al. 2010). Blocking bradykinin release 30 minutes before electrical stimulation of eccentric contraction blunted NGF production and mechanical hyperalgesia in rats (Murase et al. 2010). On the other hand, blocking bradykinin after exercise had no effects (Murase et al. 2010).

The release of bradykinin during muscle contraction seems to regulate

the glucose uptake (similarly to insulin) via increased translocation of the GLUT-4 receptor in muscle cells and the release of BCAA (Schifman et al. 1980; Taguchi et al. 2000). Curiously, isoleucine has the same effect as the GLUT-4 receptor in muscle cells (Taguchi et al. 2000; Zhang et al. 2016). It may be speculated that BCAA supplementation could blunt bradykinin production since isoleucine could increase GLUT-4 translocation. This may explain the absence of a protective effect in the study using only leucine BCAA (Kirby et al. 2012). Moreover, the effects of the previous administration of BCAA would be more efficient in abating DOMS, since treatment should be administered up to 30 minutes before exercise, as suggested by Murase and coworkers (2010). Corroborating this hypothesis, a previous study in mice demonstrated that acute or chronic administration of BCAA decreased expression of NGF in the hippocampus (Scaini et al. 2013; Wisniewski et al. 2015). Human studies in prediabetic men also revealed that BCAA supplementation decreased circulating levels of NGF (Woo et al. 2019). For instance, studies that began supplementation some days before the EIMD protocol and at least 15 minutes before the EIMD protocol (Greer et al. 2007; Howatson et al. 2012; Ra et al. 2018; Shimomura et al. 2010; VanDusseldorp et al. 2018) decreased DOMS symptoms. However, whether BCAA has any effect on bradykinin release is still unknown.

One study that reported increased muscle soreness supplemented participants exclusively with leucine (Kirby et al. 2012). Failure to blunt DOMS with leucine may demonstrate a possible relationship between the other two amino acids in this process. Indeed, another study also found that the use of isolated leucine in place of BCAA increased muscle soreness after EIMD (Osmond et al. 2019), so the combination of these amino acids (Leucine, Valine, and Isoleucine) seems to play an important role in reducing DOMS. On the other hand, Waldron et al (2018) observed no statistical difference but a large effect size for DOMS decrements after 24 and 48 h in subjects supplemented with isolated leucine. Caution should be taken when comparing studies since Kirby and coworkers (2012) supplemented with a high dose (750 mg/kg/day) and induced severe muscle damage, whereas Waldron and coworkers (2018) employed a lower dose (150 mg/kg/day) and mild EIMD. Moreover, the small number of participants (n=5) and missing information about

sample size calculations and statistical power may be a limitation of the study (Waldron et al. 2018). Further investigations on the effects of isolated amino acids on EIMD are necessary to clarify the effects of each amino acid on neurotrophin expression and DOMS.

GDNF is another neurotrophin expressed 12 to 24 h after EIMD and associated with DOMS symptoms (Mizumura and Taguchi 2016; Murase et al. 2013). GDNF expression is stimulated by activation of cyclooxygenase-2 during exercise (Murase et al. 2013). Interestingly, BCAA can inhibit mRNA expression of cyclooxygenase-2 on LPS-primed macrophages (Lee et al. 2017). Inhibition of cyclooxygenase-2 by BCAA administered before exercise may be another explanation as to why acute and previous chronic administration has better effects than post-EIMD treatment. Whether BCAA could reduce the cyclooxygenase-2 activation and GDNF expression in muscle cells needs further investigation.

BCAA also decreased the production of pro-inflammatory cytokines, suggesting an additional protective effect mediated by decreased inflammatory and oxidative damage (Lee et al. 2017). Thus, one of the beneficial characteristics of BCAA may be its antioxidant effects, since supplementation can assist in the antioxidant defense system, counteracting the negative effects of exercise-induced oxidative stress (Buonocore et al. 2015). In athletes it has been observed that the consumption of BCAA improves recovery after intense training, acting in the reduction of inflammation and oxidative stress (Buonocore et al. 2015). In this way, trained subjects may be more responsive to BCAA anti-inflammatory and inhibitory effects on bradykinin induced-production of NGF and cyclooxygenase induction of GDNF.

According to the World Health Organization, the daily requirement for BCAA in adults is 85 mg/kg/day (WHO 2007). We set a supplementation dose of up to 255 mg/kg/day (3X) as a lower dose. BCAA doses up to 255mg/kg/day seemed to have better results in relation to doses above 250mg/kg/day, however, this should be interpreted with caution since two of the studies (Greer et al. 2007; Waldron et al. 2018) induced mild EIDM. These studies also presented a risk of bias (Greer et al. 2007; Shimomura et al. 2010; VanDusseldorp et al. 2018) which decreased the quality of evidence to moderate. In this review, higher doses did not demonstrate a beneficial effect

on the BCAA group. Although lower doses of BCAA demonstrated anti-inflammatory properties (Lee et al. 2017), experimental studies showed that high doses of BCAA can induce oxidative stress, inflammation, and endothelial dysfunction (Wessler et al. 2019; Zhenyukh et al. 2018). Because of this, it is possible that a higher dose of BCAA has no effect or could impair muscle recovery, whereas lower doses demonstrated better effects in the present review. A protective effect was observed in one study with a lower dose and severe EIMD (VanDusseldorp et al. 2018). On the other hand, studies with high doses and moderate (Howatson et al. 2012) and severe EIMD (Ra et al. 2018) also found protective effects, but these studies had a low number of participants per group (5 to 6).

Another important fact was that BCAA appeared more efficient in mild and moderate muscle damage than in severe EIMD, with high quality of evidence. This fact highlights the hypothesis that BCAA may act on bradykinin and cyclooxygenase-2 inhibition so it could be very efficient when there is no extensive muscle necrosis and infiltration of inflammatory cells. However, only two studies evaluated mild EIMD (Shimomura et al. 2010; Waldron et al. 2018), and differences at 72h are not representative of a clinically significant effect since subjects are expected to be physically recovered 72h after the EIMD protocol (Paulsen et al. 2012). In moderate EIMD studies, a high quality of evidence was observed, but DOMS was significantly blunted at the 24h analysis. Otherwise, moderate EIMD studies included three studies with high BCAA doses (Foure et al. 2016; Howatson et al. 2012; Ra et al. 2013) and one study with a high risk of bias which analyzed only 24h and 48h (Greer et al. 2007). No significant protective effects could be attributed to BCAA supplementation in severe EIMD. However, a moderate quality level of evidence in severe EIMD analysis was attributed to the risk of bias and moderate heterogeneity in the included studies.

The results suggest that supplementation of BCAA could blunt DOMS symptoms. However, practical recommendations include BCAA supplementation for trained subjects. Modest evidence suggests BCAA could decrease DOMS at low doses, but study limitations should be addressed in future studies to draw a definitive conclusion. Evidence still does not support BCAA supplementation for untrained subjects and severe EIMD. BCAA

supplementation does not improve DOMS symptoms when administered after EIMD.

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5 CONSIDERAÇÕES FINAIS

Os resultados demonstram que a suplementação de BCAA pode atenuar os sintomas de DOMS em sujeitos treinados e que o suplemento não tem efeito significante se administrado após o exercício. Os efeitos de doses menores de 255 mg/kg/dia demonstram efeito protetor até 72 horas, mas os estudos apresentaram moderada qualidade de evidência. Estudos futuros devem ser realizados para determinar o efeito do BCAA em sujeitos destreinados, em diferentes magnitudes de dano muscular e altas doses.

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APÊNDICES

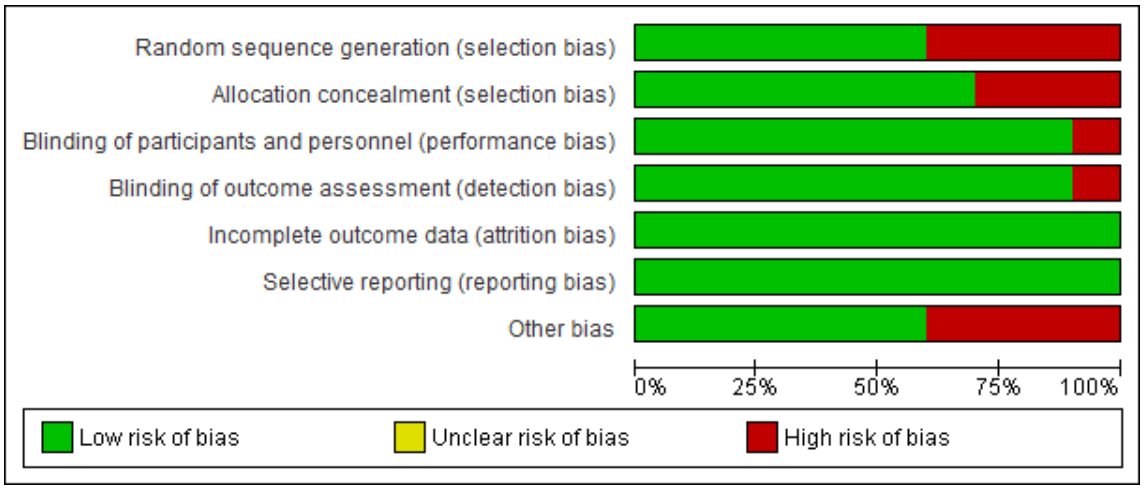

Figura 3. Porcentagem de risco de viés dos estudos incluídos na revisão sistemática.

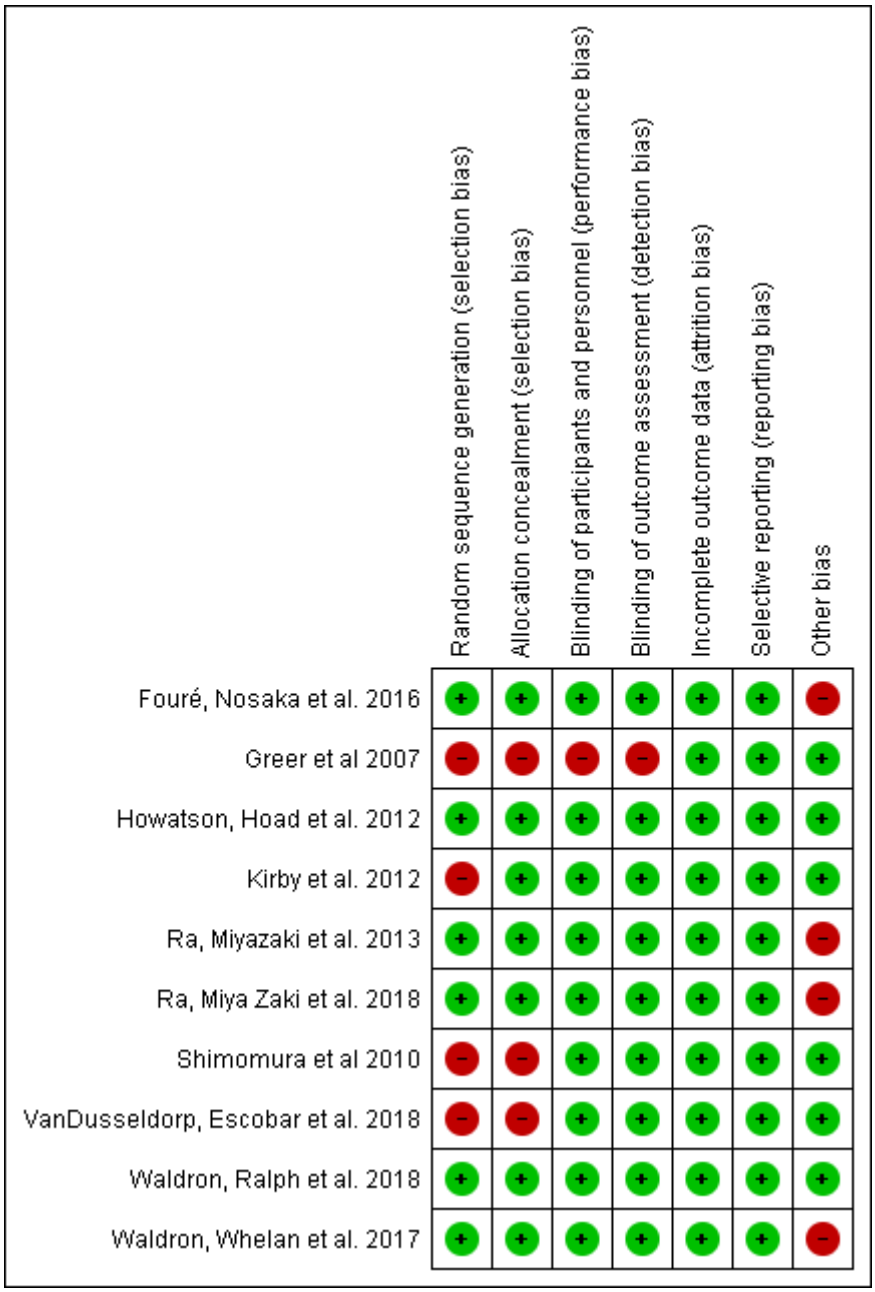

Figura 4. Tipos de risco de viés identificados nos artigos incluídos na revisão sistemática.

ANEXOS

ANEXO 1 - Normas para a revista Amino Acids

Amino Acids

The Forum for Amino Acid, Peptide and Protein Research

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Submission guidelines

Contents

Instructions for Authors

Authorship Policy

Authorship should incorporate and should be restricted to those who have contributed substantially to the work in one or more of the following categories:

Authorship Policy

Authorship should incorporate and should be restricted to those who have contributed substantially to the work in one or more of the following categories:

- Conceived of or designed study
- Performed research
- Analyzed data
- Contributed new methods or models
- Wrote the paper

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Types of papers

- Original Article manuscript length should not exceed 10 printed pages including tables and figures.
- Perspectives: Contributions under this format are usually invited, but may also be submitted unsolicited. They should address topics in amino acid and protein research of general interest. These include, but are not limited to research approaches, techniques and developments, data evaluation, ethics, health policies, meeting highlights etc. They are focused, personal reviews reflecting the perspective of the authors on a key subject in their area of expertise.The article should not exceed 2500 words (including a short, concise title, an abstract of maximal 50 words, main text and legends), 1 or 2 figures or tables (with legends), 12 references and 3 authors.
- Short Communications: The average length of short communications should not exceed 1500 words and a maximum of two figures or tables is accepted. The abstract should not exceed 80 words.
- Review Articles will be invited by the review editor, John D. Wade. Authors who would consider writing a review should please first contact John D. Wade at john.wade@florey.edu.au. Review articles should be about six to ten pages long.
- Letters to the Editor have to refer to an article published in Amino Acids and should be sent to Prof. Ellen I. Closs.
- For papers involving human subjects, adequate documentation should be provided to certify that appropriate ethical safeguards and protocols have been followed. Animal experiments should include a clear description of the method of anesthesia and killing.
- Amino Acids Protocols are peer reviewed manuscripts which should include the following sections: Abstract, introduction, extensive Material and Methods section including additional information on critical steps in the protocol, trouble shooting, equipment and timing, anticipated results and references. For further information please refer to the sample Amino Acids Protocols manuscript, which may be downloaded in pdf format at the link below:

Sample Amino Acid Protocol (Download pdf, 380 kB)

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

LaTeX macro package (Download zip, 188 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

References

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work. Order multi-author publications of the same first author alphabetically with respect to second, third, etc. author. Publications of exactly the same author(s) must be ordered chronologically.

- Journal article
  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI
  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book
  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter
  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document
  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Title page

Title Page

Please use this **template title page** for providing the following information.

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

For life science journals only (when applicable)

Trial registration number and date of registration

Trial registration number, date of registration followed by "retrospectively registered"

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations'.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

To be used for non-life science journals

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)