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SUPLEMENTAÇÃO DE ÁCIDO ASCÓRBICO NA RESPOSTA INFLAMATÓRIA E FUNCIONALIDADE MUSCULOESQUELÉTICA APÓS EXERCÍCIOS FÍSICOS: UMA REVISÃO SISTEMÁTICA

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Dissertação apresentada ao Programa de Pós-Graduação em Reabilitação Funcional, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Reabilitação Funcional.**

Orientador: Prof. Dr. Luis Ulisses Signori
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RESUMO

SUPLEMENTAÇÃO DE ÁCIDO ASCÓRBICO NA RESPOSTA INFLAMATÓRIA E FUNCIONALIDADE MUSCULOESQUELÉTICA APÓS EXERCÍCIOS FÍSICOS: UMA REVISÃO SISTEMÁTICA

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Os exercícios físicos realizados regularmente promovem benefícios à saúde, porém, agudamente podem resultar em dano muscular e diminuição da funcionalidade. A suplementação de ácido ascórbico vem sendo utilizada para atenuar esses sintomas. Neste sentido, o objetivo do estudo foi revisar sistematicamente os efeitos da suplementação de ácido ascórbico sobre a resposta inflamatória, dor e funcionalidade musculoesquelética após exercícios físicos em voluntários saudáveis. A busca foi realizada nas bases de dados MEDLINE (PubMed), Cochrane CENTRAL, EMBASE, Sport Discus e Web of Science, com os descritores “Adult”, “Healthy volunteers”, “Athletes”, “Exercise” e "Ascorbic acid". Foram incluídos ensaios clínicos randomizados e controlados por placebo que avaliaram os efeitos da suplementação de ácido ascórbico sobre a resposta inflamatória, dor e funcionalidade musculoesquelética após exercícios físicos de voluntários saudáveis. Modelo de efeitos aleatórios foi usado para comparar as mudanças de pré e pós-suplementação de ácido ascórbico e placebo. Os dados foram relatados como diferença de média padrão (SMD) e intervalo de confiança de 95% (IC). Dos 1.161 estudos encontrados, 18 foram incluídos, com uma amostra de 313 participantes, com mediana de 24 anos. A suplementação de ácido ascórbico reduziu a lipoperoxidação imediatamente (SMD = -0,488; IC95% = -0,888 a -0,088; p = 0,017), 1h (SMD = SMD = -0,521; IC95% = -0,911 a -0,131; p = 0,009) e 1h e 2h (SMD = -0,449; IC95% = -0,772 a -0,126; p = 0,006) após os exercícios. Houve também redução nos níveis de IL-6 2h (SMD = -0,764; IC95% = -1,279 a -0,248; p = 0,004) e entre 1 e 2h (SMD = -0,447; IC95% = -0,828 para -0,065; p = 0,022). Os demais marcadores inflamatórios (creatina quinase e proteína C-reativa), nível de cortisol, dor e força muscular não mostraram diferença entre a suplementação de ácido ascórbico e placebo. Em conclusão, existe evidência de tamanho de efeito pequeno e moderado de que a suplementação de ácido ascórbico reduz o estresse oxidativo (lipoperoxidação) e a resposta inflamatória (IL-6) após exercício físico agudo em voluntários saudáveis.

Palavras-chave: Ácido ascórbico, Adulto, Atleta, Exercício, Inflamação, Voluntários saudáveis.

ABSTRACT

ASCORBIC ACID SUPPLEMENTATION IN INFLAMMATORY RESPONSE AND MUSCULOSKELETAL FUNCTIONALITY AFTER PHYSICAL EXERCISES: A SYSTEMATIC REVIEW

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Regular exercise promotes health benefits, but can acutely result in muscle damage and decreased functionality. Ascorbic acid supplementation has been used to alleviate these symptoms. In this sense, the aim of the study was to systematically review the effects of ascorbic acid supplementation on inflammatory response, pain and musculoskeletal functionality after physical exercise in healthy volunteers. The search was performed in the MEDLINE (PubMed), Cochrane CENTRAL, EMBASE, Sport Discus and Web of Science databases, with the descriptors "Adult", "Healthy volunteers", "Athletes", "Exercise" and "Ascorbic acid". We included randomized, placebo-controlled clinical trials evaluating the effects of ascorbic acid supplementation on inflammatory response, pain, and musculoskeletal functionality following physical exercise by healthy volunteers. Random effects model was used to compare pre- and post-supplementation changes in ascorbic acid and placebo. Data were reported as standard mean difference (SMD) and 95% confidence interval (CI). Of the 1,161 studies found, 18 were included, with a sample of 313 participants, with a median of 24 years. Ascorbic acid supplementation immediately reduced lipoperoxidation (SMD = -0.488; 95% CI = -0.888 to -0.088; p = 0.017), 1h (SMD = SMD = -0.521; 95% CI = -0.911 to -0.131; p = 0.009) and 1h and 2h (SMD = -0,449; 95% CI = -0,772 to -0,126; p = 0,006) after the exercises. There was also a reduction in IL-6 levels 2h (SMD = -0.764; 95% CI = -1.279 to -0.248; p = 0.004) and between 1 and 2h (SMD = -0.447; 95% CI = -0.828 to -0.065; p = 0.022). The other inflammatory markers (creatine kinase and C-reactive protein), cortisol level, pain and muscle strength showed no difference between ascorbic acid supplementation and placebo. In conclusion, there is evidence of small and moderate effect size that ascorbic acid supplementation reduces oxidative stress (lipoperoxidation) and inflammatory response (IL-6) after acute exercise in healthy volunteers.

Key words: Ascorbic acid, Adult, Athlete, Exercise, Inflammation, Healthy volunteers.

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LISTA DE ABREVIATURAS E SIGLAS

TNFα	Fator de necrose tumoral-alfa
IL-6	Interleucina-6
ERON	Espécies reativas de oxigênio e nitrogênio
DMIE	Dano muscular induzido pelo exercício
CK	Creatina Kinase
Ca^{2+}	Cálcio
ATP	Adenosina trifosfato
Mb	Mioglobina
LDH	Lactato desidrogenase
AST	Aspartato aminotransferase
DMIT	Dor muscular de início tardia
O_2^-	Superóxido
H_2O_2	Peróxido de hidrogênio
OH^-	Hidroxil
NO	Óxido nítrico
IL-1β	Interleucina-1β
DNA	Ácido desoxirribonucleico
SOD	Superóxido dismutase
GPx	Glutationa peroxidase
ADM	Amplitude de movimento articular
PCR	Proteína C ultrassensível
IL-1RA	Interleucina-1*receptor agonist*
IL-10	Interleucina-10
IL-8	Interleucina-8
MDA	Malondialdeído
SMD	*standard mean difference*
IC	Intervalo de confiança

SUMÁRIO

1 INTRODUÇÃO

A realização regular de exercícios físicos promove diversos benefícios à saúde, decorrentes da adaptação dos diversos sistemas ao estresse fisiológico a que são expostos (EGAN; ZIERATH, 2013; HASKELL et al., 2007). O processo inflamatório decorrente do exercício se trata de uma resposta de defesa do organismo frente ao estresse metabólico e mecânico (SMITH, 2000; TIDBALL, 2011) e envolve a liberação de citocinas (como o fator de necrose tumoral-alfa -TNFα e a interleucina-6 - IL-6) que exercem funções importantes no reparo do dano tecidual (CALLE; FERNANDEZ, 2010).

Agudamente, quando realizados em alta intensidade, especialmente por indivíduos destreinados, os exercícios induzem a um estado de estresse oxidativo, devido ao aumento das espécies reativas de oxigênio e nitrogênio (ERON), que é capaz de exceder a capacidade dos antioxidantes exógenos e endógenos podendo levar a lesão das macro estruturas musculares (POWERS; JACKSON, 2008; POWERS; NELSON; HUDSON, 2011). O resultado deste estado é uma maior concentração dos marcadores inflamatórios (TEIXEIRA et al., 2014) e dano muscular (CLARKSON; NOSAKA; BRAUN, 1992), que levam à diminuição da funcionalidade, em especial à redução da força (POWERS; NELSON; HUDSON, 2011).

Algumas estratégias não farmacológicas vêm sendo utilizadas para prevenir e tratar o dano muscular induzido pelo exercício (DMIE), tais como massagem, crioterapia, alongamentos, exercícios ativos de baixa intensidade e a suplementação de antioxidantes (HOWATSON; VAN SOMEREN, 2008; TORRES et al., 2012). Esta última vem sendo utilizada com o objetivo de aumentar a capacidade dos antioxidantes exógenos, otimizando a ação dos antioxidantes endógenos, atenuando a excessiva produção das ERON durante e logo após os exercícios, no entanto, sua eficácia ainda é controversa (PETERNELJ; COOMBES, 2011).

Dentre os antioxidantes exógenos, o ácido ascórbico, também conhecido como Vitamina C, vem sendo amplamente estudado nesta condição (BOHLOOLI et al., 2012; BUNPO; ANTHONY, 2016; NAKHOSTIN-ROOHI et al., 2008; PETERS; ANDERSON; THERON, 2001; THOMPSON et al., 2003, 2004), por se tratar de um antioxidante capaz de atuar diretamente nas ERON (CARR; FREI, 1999). Estudos prévios, como os de Nakhostin-Roohi et al. (2008) e Bohlooli et al. (2012), que investigaram os efeitos da suplementação de ácido ascórbico em voluntários saudáveis, demonstraram a redução dos marcadores inflamatórios e lipoperoxidação 24h após os exercícios. No entanto, Thompson et al. (2003) e Thompson et al. (2004) não observaram interferência desta suplementação nestes marcadores. Por outro lado,

Peters e colaboradores (2001) observaram efeito contrário no grupo suplementado, com aumento nos níveis séricos de Creatina Kinase (CK) 24h após o exercício. Em relação a função, a força do grupo suplementado com ácido ascórbico não foi diferente do grupo placebo nos estudos de Jakeman e Maxwell (1993) e Bryer e Goldfarb (2006), porém nos estudos de Close et al. (2006) e Thompson et al. (2001a), foram observados efeitos benéficos à suplementação.

Tendo em vista os resultados conflitantes entre os estudos, observa-se que os efeitos da suplementação de ácido ascórbico na resposta inflamatória e na funcionalidade ainda são controversos e sua prescrição é incerta, pois os resultados variam de acordo com a posologia, o tempo de suplementação e o tipo de exercício realizado. Neste sentido, a presente revisão sistemática ajudou a esclarecer os efeitos da suplementação de ácido ascórbico sobre a resposta inflamatória e a funcionalidade de voluntários saudáveis após exercícios físicos. O presente estudo está registrado no gabinete de projetos institucional (ANEXO A) e é apresentado em forma de artigo, a ser submetido à revista *Sports Medicine* (ANEXO B).

2 REVISÃO DE LITERATURA

A prática regular de exercícios físicos é benéfica à saúde, pois promove adaptações (EGAN; ZIERATH, 2013; HEINONEN et al., 2014) que resultam na redução da ocorrência de doenças crônicas (EGAN; ZIERATH, 2013) e diminuição da mortalidade (GARBER et al., 2011). Os programas de exercícios induzem diversas adaptações ao sistema musculoesquelético, as quais dependem especialmente da intensidade e divergem dentre as diversas modalidades dos exercícios (EGAN; ZIERATH, 2013).

As diferentes formas de contração muscular podem levar ao dano muscular, mas este fica mais evidente nas contrações musculares excêntricas (ASSUMPÇÃO et al., 2013; ENOKA, 1996), devido ao maior estresse mecânico (ENOKA, 1996; PROSKE; MORGAN, 2001). Acredita-se que os mecanismos responsáveis pelo dano muscular envolvam as vias mecânica e metabólica (EBBELING; CLARKSON, 1989; HUDSON et al., 2008; SIMÃO et al., 2012; TEE; BOSCH; LAMBERT, 2007; TORRES et al., 2012).

A via mecânica envolve a alta tensão exercida sobre a fibra muscular durante a contração muscular. Uma hipótese é que, inicialmente, ocorre a desorganização na estrutura das fibras musculares (PROSKE; MORGAN, 2001), com ruptura da linha Z e consequente extravasamento de cálcio (Ca^{2+}) intracelular, que leva a perda da homeostase celular (TEE; BOSCH; LAMBERT, 2007; TIDBALL, 2011). Dessa forma, vias dependentes de Ca^{2+} são ativadas e levam à degradação das fibras estruturais e contráteis do músculo (TEE; BOSCH; LAMBERT, 2007). A lesão pela via metabólica pode ser explicada a partir de duas hipóteses, a isquemia que ocorre durante o exercício prolongado (EBBELING; CLARKSON, 1989) e por consequência de deficiências energéticas no interior do músculo em atividade (TEE; BOSCH; LAMBERT, 2007). Ambas estão relacionadas à redução de adenosina trifosfato (ATP) e podem aumentar a vulnerabilidade das fibras musculares ao estresse mecânico, pois com a diminuição da atividade da membrana plasmática Ca^{2+}ATPase, elevam-se as concentrações de Ca^{2+} intracelular, o que resulta na degradação da fibra muscular (ARMSTRONG; WARREN; WARREN, 1991). Além disso, Tee et al (2007) relata um segundo estágio do DMIE, ocasionado pelo processo inflamatório e a consequente ativação dos neutrófilos que amplia área muscular lesada (POWERS; JACKSON, 2008).

As consequências estruturais do dano muscular compreendem sarcômeros enfraquecidos (PROSKE; MORGAN, 2001), que levam ao vazamento de proteínas intracelulares para a corrente sanguínea como a CK (EVANS et al., 1986; MAGAL et al., 2010), mioglobina (Mb) (KANDA et al., 2013), lactato desidrogenase (LDH) e aspartato

aminotransferase (AST). Essas alterações se manifestam pela dor muscular de início tardio (DMIT) (CHEUNG; HUME; MAXWELL, 2003), diminuição da força, da flexibilidade e a presença de edema (CLARKSON; NOSAKA; BRAUN, 1992; HYLDAHL; HUBAL, 2014). Esses sintomas começam a aparecer imediatamente após os exercícios, com picos entre 24 e 72h, desaparecendo de cinco a sete dias após o término dos exercícios (HYLDAHL; HUBAL, 2014). Podem variar de acordo com as características do protocolo de exercícios e do tipo de ação muscular (ASSUMPÇÃO et al., 2013; HOWATSON; VAN SOMEREN, 2008), sendo a intensidade dos exercícios o principal fator (EGAN; ZIERATH, 2013). Marcadores diretos de dano muscular compreendem as análises de amostras musculares (biópsias) e imagens por técnica de ressonância magnética (HOWATSON; VAN SOMEREN, 2008). Entretanto, essas técnicas provocam mais lesão e são de alto custo, não sendo usualmente utilizadas na prática clínica. Por outro lado, o dano muscular pode ser medido pelas alterações da dor (CHEUNG; HUME; MAXWELL, 2003), pela presença de marcadores inflamatórios na corrente sanguínea, ou por meio das variáveis relacionadas à funcionalidade (HYLDAHL; HUBAL, 2014).

A recuperação muscular envolve mecanismos inter-relacionados seguindo três estágios: degeneração/inflamação, fase de regeneração e a fibrose ou remodelamento (TIDBALL, 2005). O primeiro evento (degeneração/inflamação), que ocorre nos primeiros minutos e perdura até a primeira e segunda semanas após a lesão, é considerado o mais importante (FILIPPIN et al., 2009). Independente do tipo de lesão, a sinalização celular ocorre via estresse oxidativo (JONES, 2006).

Durante e logo após a realização dos exercícios físicos ocorre um aumento na produção das ERON (formadas pelos ânions superóxido (O_2^-) e peróxido de hidrogênio (H_2O_2) e radicais hidroxil (OH^-) e óxido nítrico (NO)), que estimulam a inflamação através da liberação de citocinas pró-inflamatórias (SMITH, 2000), as quais são potencializadas pela ação dos glóbulos brancos, em especial os neutrófilos, que levam a lesão das macro estruturas musculares (POWERS; JACKSON, 2008). Essas alterações são importantes para a fisiologia celular (POWERS; JACKSON, 2008) e fazem parte das adaptações relacionadas ao treinamento, independente da modalidade do exercício, no qual o período de recuperação deve ser respeitado e a resposta inflamatória é benéfica (SMITH, 2000; THOMPSON et al., 2001c). Além disso, são parte do estímulo que a longo prazo resultam na adaptação e no remodelamento muscular (COFFEY; HAWLEY, 2007). A inflamação se caracteriza pelo aumento da concentração de citocinas pró-inflamatórias, como o TNF- α, a interleucina-1β (IL-1β) e IL-6 (BERNECKER et al., 2013; PYNE, 1994). Esta última aumenta a síntese de citocinas anti-inflamatórias e a liberação do cortisol (PEDERSEN; STEENSBERG; SCHJERLING, 2001).

O estado de estresse oxidativo transitório que ocorre durante os exercícios físicos pode causar danos ao DNA, aos lipídios e às proteínas, sendo esses danos dependentes da intensidade e da duração dos exercícios (FINAUD; LAC; FILAIRE, 2006; HE et al., 2016; POWERS; JACKSON, 2008). O estresse oxidativo é definido pela alteração no equilíbrio redox (oxidação/redução) (JONES, 2006), e, durante e após os exercícios físicos, favorece o sistema pró oxidante, o que induz a uma resposta inflamatória (SMITH, 2000; THOMPSON et al., 2001c). Para manter o equilíbrio redox as células possuem um sistema antioxidante endógeno, que age para impedir ou retardar a oxidação de substratos e regular as ERON (POWERS; NELSON; HUDSON, 2011). Em resposta ao treinamento, o aumento da formação das espécies reativas auxilia as adaptações, pois a médio prazo aumentam a capacidade do sistema antioxidante endógeno (YAVARI et al., 2015), porém, de forma aguda este sistema é limitado.

Agudamente, em resposta aos exercícios realizados em alta intensidade, a produção das ERON supera a capacidade antioxidante enzimática (superóxido dismutase (SOD), catalase e glutationa peroxidase (GPx)) (JONES, 2006; POWERS; NELSON; HUDSON, 2011) e não enzimática (vitaminas A, C, E e ácido úrico), resultando na oxidação dos constituintes celulares (JONES, 2006; MILA-KIERZENKOWSKA et al., 2013; SUTKOWY et al., 2015) e, consequentemente, na resposta inflamatória exacerbada (BESSA et al., 2016). Clinicamente, essas alterações se manifestam pela DMIT (CHEUNG; HUME; MAXWELL, 2003), diminuição da funcionalidade (POWERS; NELSON; HUDSON, 2011) e dano muscular (CLARKSON; NOSAKA; BRAUN, 1992; POWERS; NELSON; HUDSON, 2011) que podem levar ao abandono da prática regular dos exercícios, especialmente de iniciantes (HOWATSON; VAN SOMEREN, 2008) e, principalmente, à redução da *performance* de atletas (PETERNELJ; COOMBES, 2011).

Estudos prévios demonstraram que a redução no pico de torque isométrico é o principal marcador funcional indireto do dano muscular, além de dor muscular, redução da amplitude de movimento (ADM) articular e presença de enzimas intramusculares (CK e Mb) no plasma sanguíneo (HICKS et al., 2016; STUPKA et al., 2001). A investigação do dano muscular decorrente de diferentes protocolos de exercícios demonstrou aumento da concentração de Mb 72h após exercícios excêntricos (KANDA et al., 2013) e da CK 12h após uma sessão de ciclismo (duração de 90min), que persistiu, tendo seu pico imediatamente após a terceira sessão (realizadas em três dias consecutivos), enquanto a Mb aumentou 1h e 3h após a primeira sessão e retornou aos valores basais após 12h (SUZUKI et al., 1999).

Para atenuar os danos musculares induzidos pelos exercícios físicos, várias estratégias vêm sendo estudas, como a crioterapia, a massagem e os alongamentos (HOWATSON; VAN

SOMEREN, 2008; TORRES et al., 2012), dentre estas, a suplementação de antioxidantes exógenos, que visa atenuar o aumento excessivo das ERON que ocorre durante e após os exercícios físicos (PETERNELJ; COOMBES, 2011; SMITH, 2000). Salienta-se que, neste momento, a capacidade do sistema antioxidante endógeno é limitada (YAVARI et al., 2015). A suplementação de antioxidantes exógenos vem sendo estudada em atletas (AGUILÓ et al., 2014; PETERNELJ; COOMBES, 2011), indivíduos ativos (DAVISON; GLEESON, 2006; POULAB et al., 2015; THOMPSON et al., 2001a, 2001b, 2004) e destreinados (CONNOLLY et al., 2006). Os suplementos mais estudados são as vitaminas C e E (HE et al., 2016), que associadas já demostraram atenuar a IL-6 em resposta a exercícios resistidos (FISCHER et al., 2004), porém apresentam pouca efetividade na redução da DMIT (CANDIA-LUJÁN; FERNÁNDEZ; MOREIRA, 2015).

O ácido ascórbico (vitamina C) é uma vitamina hidrossolúvel, essencial para o metabolismo normal do corpo humano (CARR; FREI, 1999), onde se encontra na forma de ascorbato, que neutraliza as ERON (CARR; FREI, 1999), em especial o radical O_2^-, e também o H_2O_2 e o hidroperóxido lipídico (POWERS et al., 2004), formando H_2O e O_2 (LEVINE; PADAYATTY; ESPEY, 2011). A oxidação do ascorbato leva a produção do radical monodehidroascorbato, que neutraliza as ERON, impedindo diretamente a oxidação das macromoléculas (BENDICH et al., 1986; SMIRNOFF, 2018). Indiretamente, atua na restauração do α-tocoferol (vitamina E), outro inibidor da lipoperoxidação (LI; HUANG; MAY, 2003; RIETJENS et al., 2002).

O ácido ascórbico se torna biodisponível 40 minutos após a sua ingestão oral (BATES; JONES; BLUCK, 2004), sendo a biodisponibilidade uma das formas de controlar as concentrações plasmáticas, além do transporte, reabsorção e excreção renal. Porém, 24h após a ingestão do ácido ascórbico, a sua concentração plasmática retorna aos seus níveis basais (LEVINE; PADAYATTY; ESPEY, 2011).

Estudos prévios que investigaram os efeitos da suplementação de 500mg de ácido ascórbico em indivíduos saudáveis observaram um efeito protetor ao dano muscular (JAKEMAN; MAXWELL, 1993), pelo aumento da capacidade antioxidante total (BOHLOOLI et al., 2012), com atenuação dos aumentos da CK e da lipoperoxidação 24h após o exercício aeróbico, mas sem modificar marcadores inflamatórios (BOHLOOLI et al., 2012). Da mesma maneira, em atletas de ultramaratona, a suplementação com 500mg não influenciou tais marcadores (AGUILÓ et al., 2014; NIEMAN et al., 2000), porém, quando suplementados com 1000mg (PETERS; ANDERSON; THERON, 2001) e 1500mg (NIEMAN et al., 2000; PETERS et al., 2001) foi observada redução do cortisol (PETERS et al., 2001; PETERS;

ANDERSON; THERON, 2001) e uma atenuação no aumento da proteína C ultrassensível (PCR) após 90km de ultramaratona (PETERS; ANDERSON; THERON, 2001), além de redução da concentração de interleucina-1RA (IL-1RA), interleucina-10 (IL-10) (PETERS et al., 2001),IL-6 e IL-8 (NIEMAN et al., 2000).

Doses maiores de ácido ascórbico também foram estudadas em indivíduos saudáveis e, após quatro semanas de suplementação de 1000mg, foi observado aumento da capacidade antioxidante total e atenuação do aumento da CK e da lipoperoxidação (malondialdeído - MDA) após exercício excêntrico (POULAB et al., 2015). A suplementação de 3000mg, ingerida por oito dias (três dias pré e cinco dias após exercícios excêntricos) se mostrou ineficaz para o dano muscular (CONNOLLY et al., 2006). Entretanto, a ingestão desta mesma dose por duas semanas antes e quatro dias após exercício excêntrico reduziu a dor muscular (24h após o exercício), preveniu a oxidação da glutationa e atenuou o aumento da CK (48h após o exercício), mas sem modificar a função muscular (BRYER; GOLDFARB, 2006).

Recentes revisões sistemáticas (CANDIA-LUJÁN; FERNÁNDEZ; MOREIRA, 2015; RANCHORDAS et al., 2018) analisaram os efeitos da suplementação de antioxidantes na DMIT. A metodologia dos estudos incluídos apresentou diversidades, como a dose de suplementação utilizada e os protocolos de indução da DMIT, o que tornou difícil emitir uma conclusão definitiva, sugerindo que a suplementação das vitaminas C e E apresentam melhores efeitos quando associadas, e apontando uma alta eficácia dos suplementos polifenólicos na redução da DMIT, em especial do suco de cereja, além de outros componentes antioxidantes, como a cafeína (CANDIA-LUJÁN; FERNÁNDEZ; MOREIRA, 2015). A suplementação com altas doses de antioxidantes apresentou pequena redução na DMIT até 6h, 24h, 48h, 72h e 96h após os exercícios, porém essa não foi considerada uma redução clinicamente relevante, pois não atingiu a diferença mínima importante, considerada de 1,4cm na escala subjetiva de dor (RANCHORDAS et al., 2018). Os efeitos da suplementação de antioxidantes no estresse oxidativo induzido pelo exercício também foram revisados sistematicamente (GUIMARÃES; VIANNA, 2013; SZUCK et al., 2011), não sendo apontada uma conclusão definitiva, por consequência das diferentes posologias, suplementos utilizados e período de utilização.

As revisões sistemáticas acima citadas incluíram estudos que realizaram a suplementação com diversos antioxidantes, de forma isolada ou associados, com distintas formas de administração (cápsulas, bebidas ou em forma de pó) e independente desta ter sido realizada somente após os exercícios. Além desses aspectos, o presente estudo difere dos já realizados por avaliar os efeitos da suplementação isolada de ácido ascórbico na resposta inflamatória após exercícios físicos em voluntários saudáveis. Salienta-se que esta pesquisa

respeitou o período de meia vida do antioxidante, não sendo incluídos estudos em que a suplementação tivesse sido interrompida em um período anterior à 24 horas antes da realização dos exercícios ou realizada somente após os exercícios, estando desta forma biodisponível para neutralizar as ERON durante e após os exercícios.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Revisar sistematicamente os efeitos da suplementação de ácido ascórbico sobre a resposta inflamatória e a funcionalidade musculoesquelética após exercícios físicos em voluntários saudáveis.

3.2 OBJETIVOS ESPECÍFICOS

Verificar se a suplementação de ácido ascórbico atenua a dor muscular, a inflamação, o estresse oxidativo e os marcadores plasmáticos de dano muscular após exercícios físicos em voluntários saudáveis.

Investigar se a suplementação de ácido ascórbico melhora a funcionalidade musculoesquelética após exercícios físicos em voluntários saudáveis.

4 ARTIGO

VITAMIN C SUPPLEMENTATION ON OXIDATIVE STRESS, INFLAMMATORY MARKERS, MUSCLE DAMAGE, SORENESS AND FUNCTIONALITY AFTER EXERCISE: A META-ANALYSIS OF RANDOMIZED CLINICAL TRIALS

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Abastract

Background Vitamin C (ascorbic acid) is able to attenuate the greater production of reactive species during exercise. The objective of this study was to systematically review the effects of Vitamin C supplementation on oxidative stress, inflammatory markers, muscle damage, muscle soreness and muscle functionality after a single bout of exercise.

Methods Major electronic databases (MEDLINE (PubMed), EMBASE, Cochrane CENTRAL, Web of Science and Sport Discus) were searched, from inception to May 2019 for placebo-controlled randomized clinical trials (RCTs) that evaluated the effects of Vitamin C supplementation on oxidative stress parameters, inflammation markers, muscle damage, muscle soreness, and muscle functionality after an single bout of exercise in healthy volunteers were selected. Random-effects modelling was used to compare mean changes from pre- to post-supplementation of Vitamin C versus Placebo in healthy volunteers. Data were reported as standard mean difference (SMD) and 95% confidence interval (CI).

Results A total of 18 RCTs, accounting for 313 participants (62% males, median age = 24 years) were included. Vitamin C supplementation reduced lipoperoxidation immediately (SMD = -0.488; 95% CI = -0.888 to -0.088), 1h (SMD = -0.521; 95% CI = -0.911 to -0.131) and between 1h and 2h (SMD = -0.449; 95% CI = -0.772 to -0.126) following exercise. Exercise induced interleukin-6 (IL-6) response was attenuated following 2h (SMD = -0.764; 95% CI = -1.279 to -0.248) and between 1 and 2h (SMD = -0.447; 95% CI = -0.828 to -0.065) after exercise. No effects of Vitamin C supplementation was found on Creatine Kinase (CK), C-reactive protein (CRP), cortisol levels, muscle soreness and muscle strength.

Conclusion There was evidence with small and moderate effects size that Vitamin C supplementation attenuates the exercise induced oxidative stress (lipoperoxidation) and inflammatory response (IL-6) after acute exercise.

Registration PROSPERO registration (2018: CDR42018094222).

Keywords: Adult, Ascorbic acid, Athlete, Exercise, Healthy volunteers, Inflammation, Oxidative stress.

List of abbreviations

SMD	standard mean difference
CI	confidence interval
IL-6	interleukin-6
CK	Creatine Kinase
CRP	C-reactive protein
RONS	reactive oxygen and nitrogen species
DOMS	delayed onset of muscle soreness
RCTs	randomized controlled trials
ROM	range of motion
GRADE	grading of recommendation, assessment, development and evaluation
MDA	Malondialdehyde
TBARS	Thiobarbituric Acid Reactive Substances
MVC	maximum voluntary contraction
TNFα	tumor necrosis factor-α

Key Points

- There is small and moderate evidence that Vitamin C supplementation reduces oxidative stress (lipoperoxidation) and the inflammatory response (IL-6) following acute exercise in healthy volunteers.
- Vitamin C supplementation does not attenuate muscle soreness reduction, plasmatic levels of CK, CRP, cortisol, nor improvement on muscle strength following exercise.

1 Background

Regular exercise promotes several benefits on physical and mental health [1,2], due to the adaptations of various systems, such as cardiovascular, musculoskeletal, endocrine and nervous, improving overall functioning and preventing diseases [3–5]. Such adaptations also affect longevity and enhance the quality of life [6]. However, acutely, when performed in high intensity, they lead to inflammation and muscular soreness [7,8], as a result of lesions of the macro structures of the musculoskeletal system [9,10].

Oxidative stress is shown, partially, by these inflammatory events, since the higher metabolic demand during exercise induces an increase in the production of reactive oxygen and nitrogen species (RONS) [7,10]. Since the antioxidant system (exogenous and endogenous) is acutely limited, RONS may exceed their capacity, resulting in oxidation of cellular constituents and injury [7,10]. Thus, immediately after an exercise session, there is a plasma increase of

inflammatory markers [8] and of intracellular proteins, such as creatine kinase (CK) [11], shown by muscle soreness [12] and decreased functionality [7]. Those events may lead to a reduction in athletes' performance [13] and to the abandonment of regular physical practice by beginners [14]. However, in medium to long term, the greater production of RONS leads to an increase in the endogenous antioxidant system capacity [15,16], which results in adaptations and muscle remodeling [17], and impacts on mortality reduction [6].

Vitamin C (Ascorbic Acid) supplementation has been used to increase antioxidant capacity and to attenuate the excessive production of RONS during and shortly after exercise, especially by athletes in sports competitions, although the results are still controversial [13]. Clinical trials have shown that vitamin C reduces lipoperoxidation [18,19] and interleukin-6 (IL-6) [20], an inflammatory marker, after exercises. However, these markers were not affected by vitamin C supplementation in other study [21]. Previous systematic reviews [22,23] evaluated the effects of antioxidant supplementation on delayed onset of muscle soreness (DOMS) and found a small reduction after high-dose supplementation [23]. Nonetheless, such reviews considered studies that carried out supplementation with several antioxidants, either alone or combined, with different dosages and different forms of administration (capsules, beverages or powder). Thus, the effects of isolated Vitamin C on inflammation and oxidative stress parameters following exercise are still unclear. The objective of this study was to systematically review the literature on the effects of vitamin C supplementation on oxidative stress, inflammatory markers, muscle soreness and the muscle functionality of healthy volunteers after exercise.

2 Methods

This systematic review was conducted according to the guidelines suggested by the Preference Report Items for Systematic Review and Meta-analyzes: the PRISMA Statement [24] and followed the recommendations of the Cochrane Handbook [25]. The protocol was registered in PROSPERO under the number CDR42018094222.

2.1 Literature Search Strategy

The search strategy considered the studies published from inception to May 2019 on MEDLINE (PubMed), EMBASE, Cochrane CENTRAL, Web of Science and Sport Discus databases, without restriction of year of publication or language.

The following descriptors were used: "Adult", "Healthy Volunteers", "Athletes", "Ascorbic Acid" and "Exercise", associated to a highly sensitive search strategy for clinical trials [26]. Also, there was a manual search in included articles' and previous reviews' reference

lists [22,23] and on the ClinicalTrials (website). The string used in PubMed it has been adapted for the other databases, and is available at Supplementary Table 1.

2.2 Eligibility Criteria

Two reviewers (NCR and ATD) independently assessed the identified studies and selected them, by title and abstract, according to the inclusion criteria: 1) Placebo-controlled randomized clinical trials (RCTs); 2) evaluated the effects of Vitamin C supplementation on oxidative stress parameters, inflammatory markers, muscle damage, muscle soreness and/or muscle functionality (functional capacity, strength, flexibility, power, endurance, range of motion (ROM), among others) immediately post and over different periods within the next five days following exercise, 3) included healthy adults (over 18 years), who could be athletes (individuals that exercise regularly to improve performance; that are registered in some sports federation and participate in official sports competitions, as their main activity), who are active individuals (physical exercise practitioners who do not meet the criteria for athlete definition) [27] or who are untrained, and 4) present at least one comparison between an intervention group with isolated Vitamin C supplementation against a placebo condition, that had been submitted to the same exercise protocol.

The abstracts that potentially meet the criteria or that did not provide sufficient information were selected for full-text evaluation. At this stage, the exclusion criteria were: (1) supplementation interrupted 24 hours prior to exercise; (2) supplementation performed only following exercise. Disagreements were resolved by consensus and, if necessary, by a third reviewer (LUS).

2.3 Data extraction

Through the use of standardized forms, two reviewers (NCR and CMP) independently conducted data extraction. Identification data (Authors, year of publication, country, study design, funding), sample characteristics (sample size, % of males, age, body mass index), from intervention and placebo groups (exercise type, intensity), vitamin C intake (dosage, length of use), and outcomes, including oxidative stress parameters outcome, including lipoperoxidation, malondialdehyde (MDA) and Thiobarbituric Acid Reactive Substances (TBARS), inflammation (IL-6 and CRP), muscle damage (CK), muscular soreness and muscle strength, measured trough maximum voluntary contraction (MVC) and peak torque were registered. Biomarkers from blood tissue, analyzed both on serum and plasma levels, collected from 24h, 48h and 72h after the exercise bout. Outcome data presented in graphs in the original papers were retrieved through PlotDigitizer software for Windows. Divergences between the evaluators were resolved by consensus or by the decision of a third reviewer (LUS). When

necessary, the main authors of the selected studies were contacted for additional information and data.

Two studies had more than one intervention arms and, for this meta-analysis, was extracted the intervention with the highest dose of administered Vitamin C and the group that underwent supplementation in only one day. In one study, some standard error values were 0 and were adopted for analysis as 0.001.

2.4 Risk of bias assessment and quality of evidence

The risk of bias was assessed by two independent reviewers (NCR and CMP), using the tool presented in the Cochrane Handbook for Systematic Reviews of Interventions, Version 5.1.0 [25]. The evaluated domains were random sequence generation, concealed allocation, blinding of participants, professionals and evaluators to outcomes, description of losses or exclusions, and selective reporting. For each of the domains, the risk of bias was characterized as "low", "high" or "uncertain". The certainty in the evidence and strength of the recommendations for each outcome was evaluated according to the grading of recommendation, assessment, development and evaluation (GRADE) [28,29].

2.5 Data analysis

The analyses were performed through the Comprehensive Meta-Analysis Software, version 3. Random-effects meta-analysis was performed and the data were presented by standard mean difference (SMD) together with 95% confidence intervals (95% CI) as effect size measurements, calculated by the difference of the mean values and standard deviation between baseline and post exercise, of the Vitamin C and Placebo groups, for each study, respectively. The considered effect sizes were small if SMD> 0.2, moderate if> 0.5 and great if> 0.8 [30] and a P value ≤ 0.05 was considered statistically significant.

Heterogeneity was assessed by the Chi squared test and the I-squared test (I^2), considered low, moderate and high when I^2 values were < 25%, 25% - 50%, and > 50%, respectively [31]. In order to explore the heterogeneity, sensitivity analyses were carried out by withdrawing studies with athletes and anaerobic exercise, from subgroups (supplemented dose - up to 500mg and > 500mg, measurement method - MDA and TBARS, study design - cross and parallel, exercise intensity - moderate and high). In addition, meta-regressions tested the association between gender, age and time of supplementation with their effects on lipoperoxidation. Forest-plot graphs were used to present the main results. The publication bias was assessed by Funil visual analysis and the Egger test [32].

The analyses considered the outcomes measured between 0h and 24h, 24h, 48h and 72h immediately after exercise. In the period between 0h and 24h, a study [20] assessed outcomes

1h and 2h after exercises, with only the two-hour period being included in the 1h and 2h analysis, and another study [33] assessed outcomes 30min after exercise, being included in the analysis of 1h.

3 Results

3.1 Description of studies

Initially, of the 1.161 potentially relevant studies found, 18 RCTs [18–21,33–46] met the inclusion criteria. Fig. 1 shows the flowchart, detailing the number of studies excluded for each reason for exclusion. The included studies accounted for 313 participants, with a median age of 24 years. The supplementation dose ranged from 400mg to 3000mg and the supplementation period from one to 28 days. Supplementation was performed orally and the studies were published between 1993 and 2019. Aerobic exercise was used in 83% of the studies and anaerobic in 17%. Lipoperoxidation was evaluated in 67% of the included studies. There was the evaluation of IL-6 in 33% of the studies, CRP in 17%, CK in 50% and cortisol levels in 22%. Muscle soreness was evaluated in 39% of the studies, through visual scales with different metrics [20,34,35,37,41,42,44] and pressure algometry [34]. Musculoskeletal functionality was assessed in 33% of them, through muscle strength [20,34,36,37,41,44], ROM [37], flexibility and muscle tenderness [44]. Functionality was analyzed only by muscle strength, as it was the only variable evaluated in a sufficient number of RCTs to perform the meta-analysis. All selected studies were controlled by Placebo and eight of them presented crossover design [19,33,35,39–41,43,45]. Eight studies reported information on funding, of which six [21,33,35,38–40] refer to funding for research in universities or public bodies and two [20,41] were financed by companies. Two studies [34,36] reported only academic or technical support. In eight included studies [18,19,37,42–46], information on research funding was not reported. The characteristics of the studies are detailed in Table 1.

FIG 1

TABLE 1

3.2 Quantitative synthesis/Meta-analysis

3.2.1 Lipoperoxidation

Lipoperoxidation was evaluated in 12 studies using MDA [18,20,21,38,41,43,46] and TBARS [19,33,39,40,45] (n = Vitamin C: 140/Placebo: 140). Immediately after the exercises, there was a small reduction (SMD = -0.488; 95% CI = -0.888 to -0.088; p = 0.017; n = Vitamin C: 140/Placebo: 140; studies = 12; I^2 = 60.60; very low quality of evidence), moderate reduction 1h (SMD = -0.521; 95% CI = -0.911 to -0.131; p = 0.009; n = Vitamin C: 53/Placebo: 53;

studies = 5; I^2 = 0; moderate quality of evidence) and small reduction 1h and 2h (SMD = -0.449; 95% CI = -0.772 to -0.126; p = 0.006; n = Vitamin C: 76/Placebo: 77; studies = 7; I^2 = 0; moderate quality of evidence) after exercises (Fig. 2). Two hours, 24h, 48h, and 72h after exercise, there were no differences between groups and based on the GRADE approach, the quality of the evidence for this outcome was considered moderate (to 2h) and very low to the other moments (Supplementary Table 2). Potential publication bias was found by funnel chart (Supplementary Fig. 1) and Egger's test in lipoperoxidation analyses 0h (p = 0.022) and 24h (p = 0.038).

In sensitivity analyses (lipoperoxidation immediately after), the two studies with the athlete population [39,46] and one study that performed anaerobic exercise [18] were excluded, showing no difference. The subgroups were analyzed regarding the supplementation dose, with a group of studies that used doses up to 500mg [20,21,38,46] (SMD: -0.265; 95% CI = -0.956 to 0.425; p = 0.451; n = Vitamin C: 57/Placebo: 55; studies = 4; I^2 = 0) and greater than 500mg [18,19,33,39–41,43,45] (SMD: -0.624; 95% CI = -1.43 to -0.105; p = 0.018; n = Vitamin C: 83/Placebo: 85; studies = 8; I^2 = 0), with no evidence of differences between them, but with reduction of lipoperoxidation only in the subgroup of doses greater than 500mg. The same happened with the subgroups related to method of measurement of lipoperoxidation, MDA [18,20,21,38,41,43,46] (SMD: -0.348; 95% CI = -0.867 to 0.196; p = 0.205; n= Vitamin C: 85/Placebo: 83; studies = 7; I^2 = 33.94) and TBARS [19,33,39,40,45] (SMD: -0.713; 95% CI = -0.368 to -0.057; p = 0.033; n = Vitamin C: 55/Placebo: 57; studies = 5; I^2 = 77.74) and subgroups of exercise intensity, moderate [19,21,43] (SMD: -0.340; 95% CI = -1.024 to 0.343; p = 0.329; n = Vitamin C: 25/Placebo: 25; studies = 3; I^2 = 31.15) and high [18,20,40,41,45] (SMD: -1.039; 95% CI = -2.023 to -0.054; p = 0.039; n = Vitamin C: 46/Placebo: 44; studies = 5; I^2 = 78.86), with no evidence of differences between them, but with reduction of lipoperoxidation only in the subgroup of TBARS and high intensity. About the study design, with a study group that used crossover design [19,33,39–41,43,45] (SMD: -0.582; 95% CI = -1.168 to 0.113; p = 0.106; n = Vitamin C: 67/Placebo: 65; studies = 7; I^2 = 70.51) and another group that used parallel design [18,20,21,38,46] (SMD: -0.460; 95% CI = -0.965 to 0.044; p = 0.074; n = Vitamin C: 73/Placebo: 75; studies = 5; I^2 = 47.20), with no evidence of differences between them. Meta-regressions did not correlate gender, age and time of supplementation with the effects of vitamin C supplementation on lipoperoxidation immediately after exercise.

3.2.2 Inflammatory markers

The evaluation of IL-6 and CRP included six [19–21,42,43,46] (n = Vitamin C: 55/Placebo: 56) and three [20,21,33] (n = Vitamin C: 35/Placebo: 35) studies, respectively. The

IL-6 2h following exercise presented a moderate reduction (SMD = -0.764; 95% CI = -1.279 to -0.248; p = 0.004; n = Vitamin C: 31/Placebo: 32; studies = 3; I^2 = 0; moderate quality of evidence) and small in the interval between 1h and 2h after exercise (SMD = -0.447; 95% CI = -0.828 to -0.065; p = 0.022; n = Vitamin C: 55/Placebo: 56; studies = 6; I^2 = 0.08; moderate quality of evidence) (Fig. 2) when compared to controls. Immediately, 1h, 24h and 48h after exercise there was no difference between the groups supplemented with Vitamin C and Placebo and based on the GRADE approach, the quality of the evidence for this outcome was considered moderate in these moments. The CRP showed no difference between the groups at the evaluated moments, with moderate quality of the evidence (Supplementary Table 2).

FIG 2

3.2.3 Muscle damage

Nine studies [18,20,21,33,36,37,41,42,46] (n = Vitamin C: 93/Placebo: 92) assessed muscle damage from CK levels. Immediately, 1h, 2h, 24h, 48h and 72h after exercise, there was no difference between groups supplemented with Vitamin C and Placebo and based on the GRADE approach, the quality of the evidence for this outcome was considered very low (to 24h) and moderate to the other moments (Supplementary Table 2). Potential publication bias was found by funnel chart (Supplementary Fig. 1) and by the Egger test in CK analysis 24h after exercise (p = 0.011).

3.2.4 Cortisol

Four studies [19,20,41,43] analyzed cortisol levels (n = Vitamin C: 33/Placebo: 33) immediately, 1h, 1h and 2h, 24h, 48h and 72h after exercise. In none of the evaluated moments, differences between the supplements were observed and based on the GRADE approach, the quality of the evidence for this outcome was considered very low (to immediately, 1h and 2h, 24h), low (to 1h) and moderate (to 48h and 72h) (Supplementary Table 2). Potential publication bias was found by funnel chart (Supplementary Fig. 1) and by Egger test on cortisol analysis 0h (p = 0.013).

3.2.5 Muscle soreness

Seven studies [20,34,35,37,41,42,44] (n = Vitamin C: 70/Placebo: 68) evaluated muscle soreness. The meta-analysis results showed no difference between the groups supplemented with Vitamin C and Placebo immediately, 4h, 24h, 48h and 72h after exercise and based on the GRADE approach, the quality of the evidence for this outcome was considered very low (to 24h), low (to immediately and 48h) and moderate (to 4h and 72h) (Supplementary Table 2).

3.2.5 Muscle strength

Muscle strength was assessed through the MVC [36,37,44] (n = Vitamin C: 30/Placebo: 28) and isometric [41] and isokinetic [34] peak torque (n = Vitamin C: 19/Placebo: 19). Immediately, 24h, 48h and 72h after exercise, no difference was observed between groups supplemented with Vitamin C and Placebo (Supplementary Table 2). Based on the GRADE approach, the quality of the evidence for this outcome was considered very low (to MVC - 24h), low (to 72h) and moderate to the other moments (Supplementary Table 2). Potential publication bias was found by funnel chart (Supplementary Fig. 1) and the Egger test in the analysis of MVC 72h ($p = 0.010$).

3.3 Assessment of risk of bias and quality of evidence

Table 2 presents the final bias risk assessment of the included studies. Information on the randomization method and concealed allocation was unclear in the studies. Seventy-two percent reported blinding of participants and of involved staff and 61% of outcome assessors. Most studies (83.3%) were classified with low risk of bias for the domain: incomplete outcome data. In summary, the risk of bias for each study was considered unclear.

The quality of the evidence for each estimate of the effect of the result was evaluated through the GRADE system, indicating moderate quality in most analyses. The quality assessment is shown in Table 3.

TABLE 2

TABLE 3

4 Discussion

To our knowledge, this is the first systematic review to exclusively analyze the role of Vitamin C on muscle soreness, inflammatory markers, and musculoskeletal function of healthy volunteers after physical exercise. This study shows that Vitamin C supplementation decreases lipoperoxidation and the inflammatory response (IL-6) immediately (lipoperoxidation) and in the interval between 1h and 2h (lipoperoxidation and IL-6) following exercise, although it shows no effects on muscle damage, cortisol levels, CRP, or muscle soreness and strength.

The exercise-induced production of RONS has been explained in numerous ways [10], as by the increased mitochondrial activity and ischemia/reperfusion [47]. Acutely, they can exceed antioxidant capacity, leading to oxidative stress [7,10] and to harmful oxidation reactions with cellular components [48,49]. In this context, Vitamin C supplementation reduced lipoperoxidation immediately after and in the interval between 1h and 2h after exercise, due to its neutralizing effect on RONS, in addition to restoring α-tocopherol (Vitamin E), which is another inhibitor of lipoperoxidation [50]. Supplementation with antioxidants is performed with

the objective of assisting the endogenous antioxidant system in protecting the body against oxidative stress that occurs during and after high intensity physical exercises. However, the controversies regarding the Vitamin C supplementation are caused by the diversity of dosage and supplements, in particular [22].

According to the sensitivity analysis, no difference was observed in relation to exercise modality and population. Subgroup analyses also showed no difference between the evaluated groups, although supplementation with higher doses (> 500mg) of Vitamin C seems to be more effective in reducing oxidative stress immediately after exercise, since it reduces lipid peroxidation. These results are corroborated by previous RCTs [18,33]. Goldfarb and colleagues compared different doses of Vitamin C supplementation (500 vs. 1000mg) and demonstrated greater protection to protein damage at higher dose [45].

In most of the included studies, the sample consisted only of males, which may indicate that the findings are mainly applicable to males, as it is highlighted in previous systematic review [23]. However, in the meta-regression, gender had no relation with the effect of supplementation on lipoperoxidation immediately after exercise. Likewise, the age and the supplementation period were not related to the effect found, demonstrating that Vitamin C supplementation over a long period of time does not show an additional effect (Supplementary Fig. 2). This is due to the fact that 40 minutes after its oral ingestion, it is already bioavailable [51] and after 24h its plasma concentration returns to basal levels [52].

The mechanical and metabolic stress due to the transient state of imbalance between the pro and antioxidant systems during acute exercise is responsible for muscle damage [53,54]. Such damage is shown, among other forms, by the presence of intramuscular enzymes, mainly CK, in the blood plasma [55,56], which shows the peak of its activity 24 hours after the exercises [57]. As observed, Vitamin C supplementation had no effect on CK at the evaluated moments, which may be justified by the half-life of the antioxidant [52].

There is an inflammatory response [56] associated to muscle damage, which involves the release of cytokines, such as tumor necrosis factor-α (TNFα) and IL-6, in order to repair damaged tissue [53]. Vitamin C supplementation reduced IL-6 levels shortly after exercise, a probable reflection of the reduction of oxidative stress parameters immediately after exercise, with a consequent delay in the signaling of the inflammatory response [7]. Inflammation and muscle damage are clinically manifested by muscle soreness and decreased muscle function, especially strength [7,10]. This review demonstrates that, until now, muscle soreness and strength are not altered by Vitamin C supplementation. Based on the results found, during sports competitions, Vitamin C supplementation may be an adopted by athletes as a strategy to aid in

the recovery after exercise and, in the case of beginners in sports, this supplementation may help in the adaptation and continuity of regular physical exercise practice. However, it is important not to extrapolate these results to the context of exercise / training programs, since the inflammatory response is necessary for muscle adaptation and remodeling [17].

No included study presented a high risk of bias in the evaluated domains, according to the Cochrane risk of bias tool [25], although the method used to generate the random sequence and allocation blinding was not clearly reported. Hence, it is suggested to perform further RCT with greater methodological transparency. The quality of the evidence was evaluated through observations on the risk of bias, indirectness, inconsistency (heterogeneity), imprecision and publication bias, combined by GRADE [28,29]. As found in previous systematic review [23], the quality of evidence ranged from very low to moderate, which indicates the need for further studies so that the true effect is estimated.

Among the limitations of this research, there are the unfeasibility of generalizing the findings to other populations, the small number of studies in each outcome and, therefore, the potential lack of statistical power and the difficulty in exploring heterogeneity and publication bias, the inclusion of studies with various forms of vitamin C supplementation (dosage, supplementation time), and different exercise modalities (aerobic and anaerobic) and populations (athletes, active individuals and untrained). However, it should be observed that sensitivity and subgroup analyses regarding exercise modality and population showed that such inclusions did not have a significant impact on the results of this research. In the present systematic review, by investigating the effects of vitamin C supplementation on variables related to musculoskeletal functionality, it was only possible to evaluate muscle strength, indicating the need of further RCTs that evaluate the effects of this supplementation on functional variables in this condition.

5 Conclusion

This systematic review and meta-analysis show that there is evidence with small and moderate effects size that Vitamin C supplementation reduces oxidative stress (lipoperoxidation) and the inflammatory response (IL-6) after acute physical exercise in healthy volunteers. However, such intervention does not show effects on the reduction of plasmatic levels of CK, CRP, cortisol or muscle soreness, nor improvement in muscle strength. In practice, Vitamin C supplementation may be considered as an option to favor the recovery after exercises and/or intense physical activities, especially during sports competitions and for beginners in physical exercise programs.

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Compliance with Ethical Standards

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Conflict of interest Natiele Camponogara Righi, Angélica Trevisan De Nardi, Caroline Montagner Pippi, Felipe Barreto Schuch and Luis Ulisses Signori declare that they have no conflicts of interest relevant to the content of this review.

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FIGURE LEGENDS

FIGURE 1. Flowchart of the study design.

FIGURE 2. Effect of Vitamin C supplementation on lipoperoxidation and IL-6.

FIGURE 1

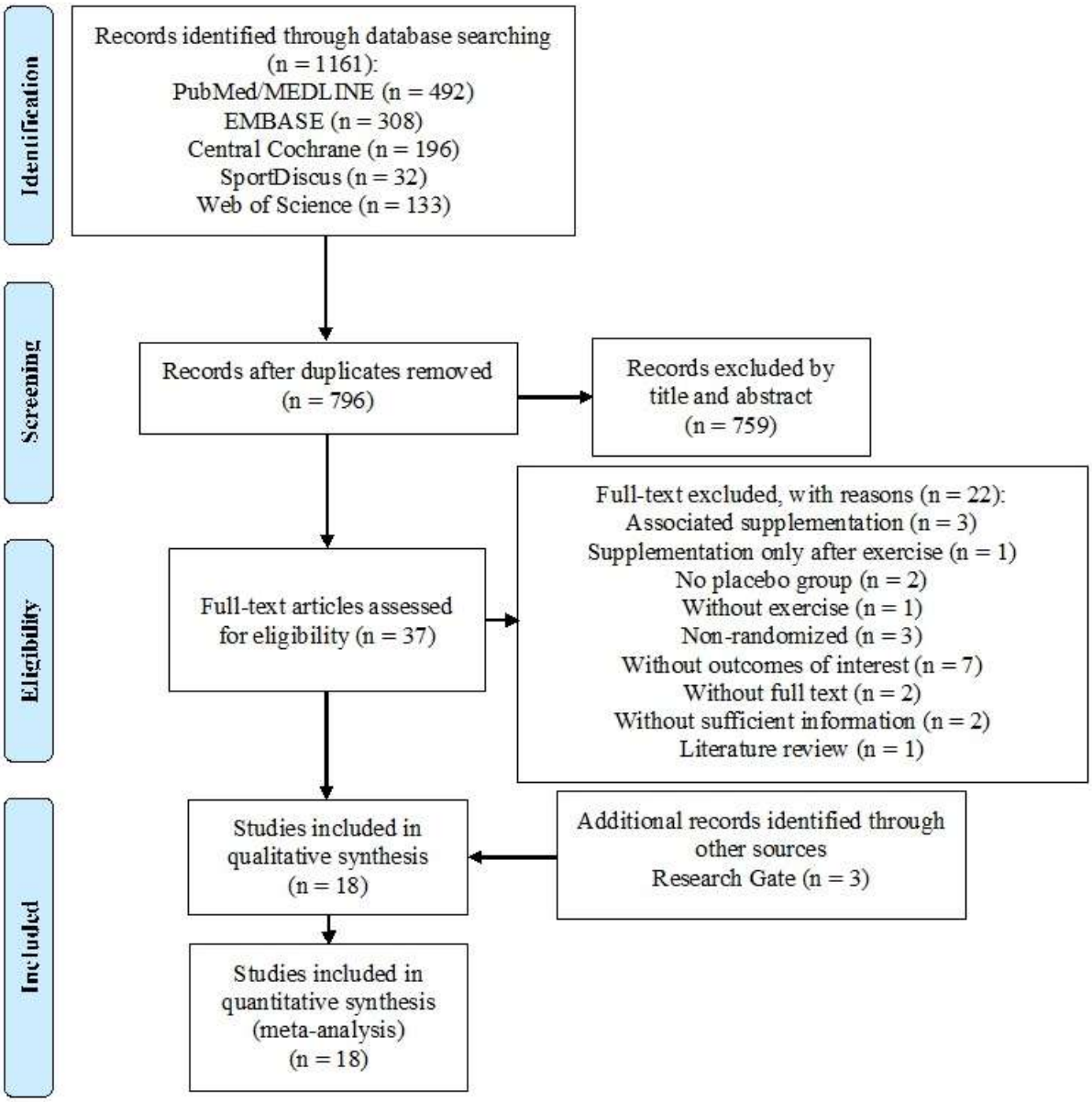

Identification
Records identified through database searching (n = 1161):
PubMed/MEDLINE (n = 492)
EMBASE (n = 308)
Central Cochrane (n = 196)
SportDiscus (n = 32)
Web of Science (n = 133)
Screening
Records after duplicates removed (n = 796)
Records excluded by title and abstract (n = 759)
Eligibility
Full-text articles assessed for eligibility (n = 37)
Full-text excluded, with reasons (n = 22):
Associated supplementation (n = 3)
Supplementation only after exercise (n = 1)
No placebo group (n = 2)
Without exercise (n = 1)
Non-randomized (n = 3)
Without outcomes of interest (n = 7)
Without full text (n = 2)
Without sufficient information (n = 2)
Literature review (n = 1)
Included
Studies included in qualitative synthesis (n = 18)
Additional records identified through other sources
Research Gate (n = 3)
Studies included in quantitative synthesis (meta-analysis) (n = 18)

FIGURE 2

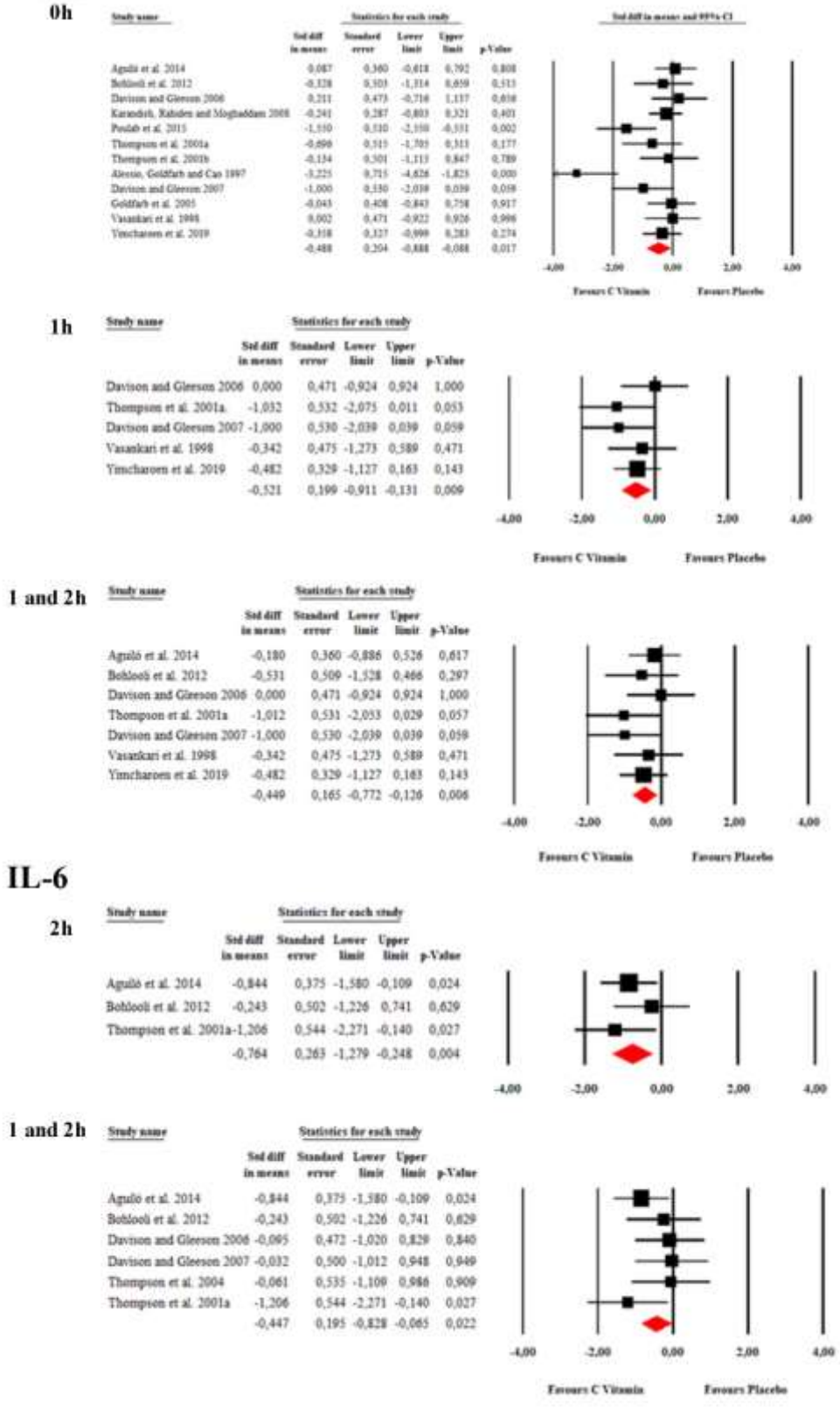

Lipoperoxidation
0h
Study name
Statistics for each study
Std diff in means
Standard error
Lower limit
Upper limit
p-Value
Std diff in means and 95% CI
Agulló et al. 2014 0,087 0,360 -0,618 0,792 0,808
Bohlooli et al. 2012 -0,328 0,303 -1,314 0,659 0,513
Davison and Gleeson 2006 0,211 0,473 -0,716 1,137 0,658
Karandish, Kabiden and Moghaddam 2008 -0,241 0,287 -0,803 0,321 0,401
Poulsen et al. 2015 -1,310 0,510 -2,310 -0,311 0,002
Thompson et al. 2001a -0,696 0,511 -1,701 0,311 0,177
Thompson et al. 2001b -0,134 0,501 -1,113 0,847 0,789
Alexiou, Goldfarb and Cao 1997 -3,225 0,715 -4,626 -1,823 0,000
Davison and Gleeson 2007 -1,000 0,530 -2,039 0,039 0,059
Goldfarb et al. 2005 -0,043 0,408 -0,843 0,758 0,917
Vasankari et al. 1998 0,002 0,471 -0,922 0,926 0,996
Yimcharoen et al. 2019 -0,318 0,327 -0,959 0,283 0,274
-0,488 0,204 -0,888 -0,088 0,017
-4,00 -2,00 0,00 2,00 4,00
Favours C Vitamin
Favours Placebo
1h
Study name
Statistics for each study
Std diff in means
Standard error
Lower limit
Upper limit
p-Value
Davison and Gleeson 2006 0,000 0,471 -0,924 0,924 1,000
Thompson et al. 2001a -1,032 0,532 -2,075 0,011 0,053
Davison and Gleeson 2007 -1,000 0,530 -2,039 0,039 0,059
Vasankari et al. 1998 -0,342 0,475 -1,273 0,589 0,471
Yimcharoen et al. 2019 -0,482 0,329 -1,127 0,163 0,143
-0,521 0,199 -0,911 -0,131 0,009
-4,00 -2,00 0,00 2,00 4,00
Favours C Vitamin
Favours Placebo
1 and 2h
Study name
Statistics for each study
Std diff in means
Standard error
Lower limit
Upper limit
p-Value
Agulló et al. 2014 -0,180 0,360 -0,886 0,526 0,617
Bohlooli et al. 2012 -0,531 0,509 -1,528 0,466 0,297
Davison and Gleeson 2006 0,000 0,471 -0,924 0,924 1,000
Thompson et al. 2001a -1,012 0,531 -2,053 0,029 0,057
Davison and Gleeson 2007 -1,000 0,530 -2,039 0,039 0,059
Vasankari et al. 1998 -0,342 0,475 -1,273 0,589 0,471
Yimcharoen et al. 2019 -0,482 0,329 -1,127 0,163 0,143
-0,449 0,165 -0,772 -0,126 0,006
-4,00 -2,00 0,00 2,00 4,00
Favours C Vitamin
Favours Placebo
IL-6
2h
Study name
Statistics for each study
Std diff in means
Standard error
Lower limit
Upper limit
p-Value
Agulló et al. 2014 -0,844 0,375 -1,580 -0,109 0,024
Bohlooli et al. 2012 -0,243 0,502 -1,226 0,741 0,629
Thompson et al. 2001a -1,206 0,544 -2,271 -0,140 0,027
-0,764 0,263 -1,279 -0,248 0,004
-4,00 -2,00 0,00 2,00 4,00
1 and 2h
Study name
Statistics for each study
Std diff in means
Standard error
Lower limit
Upper limit
p-Value
Agulló et al. 2014 -0,844 0,375 -1,580 -0,109 0,024
Bohlooli et al. 2012 -0,243 0,502 -1,226 0,741 0,629
Davison and Gleeson 2006 -0,095 0,472 -1,020 0,829 0,840
Davison and Gleeson 2007 -0,032 0,500 -1,012 0,948 0,949
Thompson et al. 2004 -0,061 0,535 -1,109 0,986 0,909
Thompson et al. 2001a -1,206 0,544 -2,271 -0,140 0,027
-0,447 0,195 -0,828 -0,065 0,022
-4,00 -2,00 0,00 2,00 4,00
Favours C Vitamin
Favours Placebo

TABLE 1 Characteristics of included studies

Study, Year	Study design	Sample characteristics		Intervention characteristics (Vitamin C supplementation)	Type of Exercise	Evaluated outcomes
Jakeman and Maxwell, 1993[36]	Parallel	Vitamin C: (n=8), 19.6(17.9-21.8) years, active men	Placebo: (n=8), 19.6(17.9-21.8) years, active men	400mg, 28 days	Aerobic – Box stepping (24 steps/min)	Muscle damage (CK), Muscle strength (maximal voluntary contraction)
Alessio, Goldfarb and Cao, 1997[40]	Crossover	Vitamin C: (n=9), 33±2.6 years, healthy men	Placebo: (n=9), 33±2.6 years, healthy men	1000mg, 1 day / 1000mg, 14 days	Aerobic – Running on a motorized ttreadmill at 80% V_{O2max}	Lipoperoxidation (TBARS)
Vasankari et al, 1998[39]	Crossover	Vitamin C: (n=9), 28.6(20-37) years, athlete men	Placebo: (n=9), 28.6(20-37) years, athlete men	2000mg, 1 day	Aerobic – 19km running exercise (4.5km warming up; 10.5km maximal, noncompetitive running and 4km cooling down)	Lipoperoxidation (diene conjugation)
Thompson et al, 2001a[20]	Parallel	Vitamin C: (n=8), 25±2 years, active men	Placebo: (n=8), 23±2 years, active men	400mg, 12 days	Aerobic – Loughborough Intermittent Shuttle Test[a] for 90min	Muscle soreness, Muscle damage (CK), Lipoperoxidation (MDA), Muscle strength (torque), Inflammation (IL-6, CRP), Cortisol
Thompson et al, 2001b[41]	Crossover	Vitamin C: (n=9), 28.4±1.3 years, active men	Placebo: (n=9), 28.4±1.3 years, active men	1000mg, 1 day	Aerobic – Loughborough Intermittent Shuttle Test [a] for 90min	Muscle soreness, Muscle damage (CK), Lipoperoxidation (MDA), Cortisol, Muscle strength (peak torque)
Thompson et al, 2004[42]	Parallel	Vitamin C: (n=7), 25.3±1.4 years, active men	Placebo: (n=7), 22.6±1.7 years, active men	400mg, 16 days	Aerobic – Running treadmill at 60% V_{O2max} (0% downhill for 15min + downhill 18% for 30min)	Muscle soreness, Muscle damage (CK), Inflammation (IL-6)
Goldfarb et al, 2005[45]	Crossover	Vitamin C: (n=12), 25±1.4 years, healthy men	Placebo: (n=12), 25±1.4 years, healthy men	500mg/1000mg, 14 days	Aerobic – Running for 30min at 75/80% V_{O2max}	Lipoperoxidation (TBARS)
Bryer and Goldfarb, 2006[37]	Parallel	Vitamin C: (n=10), 21.4±0.8 years, healthy men	Placebo: (n=8), 24.4± 1.7 years, healthy men	3000mg, 18 days	Anaerobic – Seventy eccentric actions using the elbow flexors	Muscle soreness, Muscle damage (CK), Muscle strength (maximal isomatric strength)

Close et al, 2006[34]	Parallel	Vitamin C: (n=10), 24±1.5 years, active men	Placebo: (n=10), 22.1± 0.4 years, active men	1000mg, 15 days	Aerobic – Downhill running for 30min at 60% V_{O2max}	Muscle soreness, Lipoperoxidation (MDA), Muscle strength (peak torque)
Connolly et al, 2006[44]	Parallel	Vitamin C: (n=12), 22.3±3.9 years, healthy men and women	Placebo: (n=12), 22.6±4.6 years, healthy men and women	3000mg, 8 days	Anaerobic – 40 (2×20) maximal eccentric contractions of the elbow flexors	Muscle soreness, Muscle strength (maximal isometric strength)
Davison and Gleeson, 2006[43]	Crossover	Vitamin C: (n=9), 26±2 years, active men	Placebo: (n=9), 26±2 years, active men	1000mg, 14 days	Aerobic – Ride a bicycle for 2.5h at 60% V_{O2max}	Lipoperoxidation (MDA), Inflammation (IL-6), Cortisol
Davison and Gleeson, 2007[19]	Crossover	Vitamin C: (n=8), 20±2.8 years, healthy men	Placebo: (n=8), 20±2.8 years, healthy men	1500mg, 2 days	Aerobic – Ride a bicycle for 2.5h at 60% V_{O2max}	Lipoperoxidation (TBARS), Inflammation (IL-6), Cortisol
Karandish, Rahiden and Moghaddam, 2008[38]	Parallel	Vitamin C: (n=25), 24±3 years, healthy women	Placebo: (n=24), 23±2 years, healthy women	500mg, 14 days	Aerobic – Running for 30min to 5-6 km h^{-1}	Lipoperoxidation (MDA)
Mizuma et al, 2009[35]	Crossover	Vitamin C: (n=14), 36.7±9.4 years, healthy men and women	Placebo: (n=14), 36.7±9.4 years, healthy men and women	3000mg, 8 days	Aerobic – Bicycle ergometer for 120min at fixed workloads to reach 80% of target heart rate	Muscle soreness
Bohlooli et al, 2012[21]	Parallel	Vitamin C: (n=8), 21.5±2.2 years, healthy men	Placebo: (n=8), 22.1±2 years, healthy men	500mg, 1 day	Aerobic – Running on treadmill for 30min at 75% V_{O2max}	Muscle damage (CK), Lipoperoxidation (MDA), Inflammation (IL-6 e CRP)
Aguiló et al, 2014[46]	Parallel	Vitamin C: (n=16), 37.2±5.4 years, athlete men	Placebo: (n=15), 39.5±5.6 years, athlete men	500mg, 15days	Aerobic – 15km run competition	Muscle damage (CK), Lipoperoxidation (MDA), Inflammation (IL-6)
Poulab et al, 2015[18]	Parallel	Vitamin C: (n=10), 24.15±1.75 years, active men	Placebo: (n=10), 24.15±1.75 years, active men	1000mg, 28 days	Anaerobic – Running on treadmill for 45min (9 sets of 5min/ 2min rest periods between sets) at 10° downhill and 80% V_{O2max}	Muscle damage (CK), Lipoperoxidation (MDA)
Yimcharoen et al, 2019[33]	Crossover	Vitamin C: (n=19), 22.4±2.2 years, healthy women	Placebo: (n=19), 22.4±2.2 years, healthy women	1000mg, 1 day	Aerobic – Cycling to 65–75% of maximum heart rate	Lipoperoxidation (TBARS), Muscle damage (CK), Inflammation (CRP)

n: sample size; min: minutes; h: hours; MDA: malondealdehyde; CK: creatine kinase; IL-6: interleukin-6; TBARS: plasma thiobarbituric acid reative substances; CRP: C-reactive protein.* walking, slow running and running

TABLE 2 Risk of bias of included studies

Study, Year	Bias domains					
	Randomization	**Allocation concealment**	**Blinding (participants and personnel)**	**Blinding (outcome assessment)**	**Incomplete outcome data**	**Selective reporting**
Jakeman and Maxwell, 1993[36]	Unclear	Unclear	Low	Low	Low	Unclear
Alessio, Goldfarb and Cao, 1997[40]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Vasankari et al, 1998[39]	Unclear	Unclear	Low	Unclear	Low	Unclear
Thompson et al, 2001a[20]	Unclear	Unclear	Low	Low	Low	Unclear
Thompson et al, 2001b[41]	Unclear	Unclear	Low	Low	Low	Unclear
Thompson et al, 2004[42]	Unclear	Unclear	Low	Low	Low	Unclear
Goldfarb et al, 2005[45]	Unclear	Unclear	Low	Low	Low	Unclear
Bryer and Goldfarb, 2006[37]	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Close et al, 2006[34]	Unclear	Unclear	Low	Low	Low	Unclear
Connolly et al, 2006[44]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Davison and Glesson, 2006[43]	Unclear	Unclear	Low	Unclear	Low	Unclear
Davison and Glesson, 2007[19]	Unclear	Unclear	Low	Unclear	Unclear	Unclear
Karandish, Rahiden and Moghaddam, 2008[38]	Unclear	Unclear	Unclear	Low	Low	Unclear
Mizuma et al, 2009[35]	Unclear	Unclear	Low	Low	Low	Unclear
Bohlooli et al, 2012[21]	Unclear	Unclear	Low	Low	Low	Unclear
Aguiló et al, 2014[46]	Unclear	Unclear	Low	Low	Low	Unclear
Poulab et al, 2015[18]	Unclear	Unclear	Low	Low	Low	Unclear
Yimcharoen et al, 2019[34]	Unclear	Unclear	Unclear	Unclear	Low	Low

‘‘Low” (low risk of bias), ‘‘High” (high risk of bias) or ‘‘Unclear” (no information or uncertainty over the potential for bias)

TABLE 3 A summary of GRADE's approach to rating quality of evidence

Analysis	Quality assessment						Quality	Importance
	N of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision		
Lipoperoxidation								
0h	12	RCT	Not serious	Very serious[b]	Not serious	Serious[a]	Very low	Important
1h	5	RCT	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
1 e 2h	6	RCT	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
IL-6								
2h	3	RCT	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
1 e 2h	6	RCT	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important

a = Few studies and small sample; b = High heterogeneity (over 50%); IL-6: interleukin-6; RCT: randomized clinical trial.

Supplementary Table 1 - Systematic review search strategy

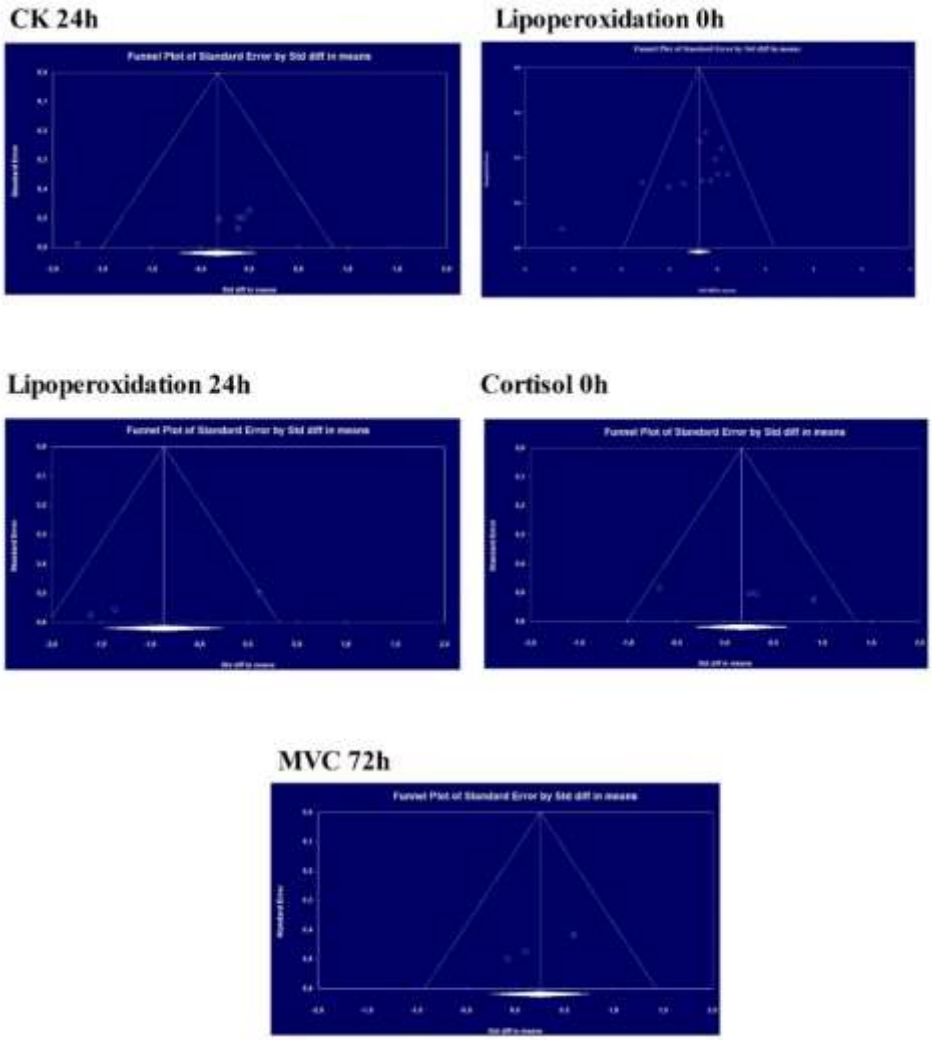
Numbers	Combiners	Terms
PubMed/MEDLINE		
#1	Population	((((("Adult"[Mesh] OR "Adults"))) OR (("Healthy Volunteers" [Mesh] OR "Healthy Volunteer" OR "Volunteer, Healthy" OR "Healthy Participants" OR "Healthy Participant" OR "Participant, Healthy" OR "Participants, Healthy" OR "Healthy Subjects" OR "Healthy Subject" OR "Subject, Healthy" OR "Subjects, Healthy" OR "Human Volunteers" OR "Human Volunteer" OR "Volunteer, Human" OR "Volunteers, Human" OR "Normal Volunteers" OR "Normal Volunteer" OR "Volunteer, Normal" OR "Volunteers, Normal"))) OR (("Athletes"[Mesh] OR "Athlete"))))
#2	Intervention	(("Ascorbic Acid"[Mesh] OR "Acid, Ascorbic" OR "L-Ascorbic Acid" OR "Acid, L-Ascorbic" OR "L Ascorbic Acid" OR "Vitamin C" OR "Hybrin" OR "Magnorbin" OR "Sodium Ascorbate" OR "Ascorbate, Sodium" OR "Ascorbic Acid, Monosodium Salt" OR "Ferrous Ascorbate" OR "Ascorbate, Ferrous" OR "Magnesium Ascorbate" OR "Ascorbate, Magnesium" OR "Magnesium di-L-Ascorbate" OR "Magnesium di L Ascorbate" OR "di-L-Ascorbate, Magnesium" OR "Magnesium Ascorbicum"))) AND (("Exercise"[Mesh] OR "Exercises" OR "Physical Activity" OR "Activities, Physical" OR "Activity, Physical" OR "Physical Activities" OR "Exercise, Physical" OR "Exercises, Physical" OR "Physical Exercise" OR "Physical Exercises" OR "Acute Exercise" OR "Acute Exercises" OR "Exercise, Acute" OR "Exercises, Acute" OR "Exercise, Isometric" OR "Exercises, Isometric" OR "Isometric Exercises" OR "Isometric Exercise" OR "Exercise, Aerobic" OR "Aerobic Exercise" OR "Aerobic Exercises" OR "Exercises, Aerobic" OR "Exercise Training" OR "Exercise Trainings" OR "Training, Exercise" OR "Trainings, Exercise")))
#3	Study desing	((clinical[Title/Abstract] AND trial[Title/Abstract]) OR clinical trials as topic[MeSH Terms] OR clinical trial[Publication Type] OR random*[Title/Abstract] OR random allocation[MeSH Terms] OR therapeutic use[MeSH Subheading]))
#4		**#1 AND #2 AND #3**
EMBASE		
#1	Population	Normal human OR Adult OR Athlete
#2	Intervention	Ascorbic Acid AND Exercise
#3	Study desing	Clinical study
#4		**#1 AND #2 AND #3**
Cochrane CENTRAL		
#1	Population	(MeSH descriptor: [Healthy volunteers] OR Healthy Volunteers) OR (MeSH descriptor: [Adult] OR Adult) OR (MeSH descriptor: [Athletes] OR Athletes)
#2	Intervention	(MeSH descriptor: [Ascorbic Acid] OR Ascorbic Acid) AND (MeSH descriptor: [Exercise] OR Exercise)
#3		**#1 AND #2**
SportDiscus Web of Science		
#1	Population	Healthy Volunteers OR Adult OR Athletes
#2	Intervention	Ascorbic Acid AND Exercise
#3		**#1 AND #2**

Supplementary Table 2 – Meta-analysis and summary of GRADE's approach to rating quality of evidence

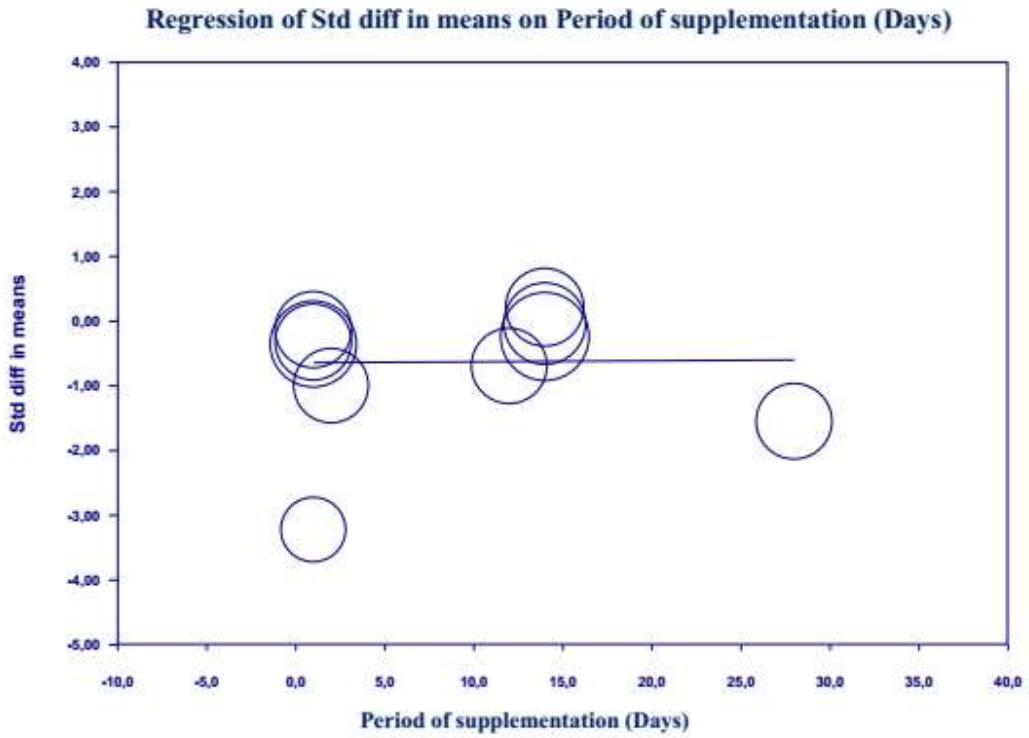
Analysis	Number of RCTs	N (Vitamin C/Placebo)	Meta-analysis			Heterogeneity			Quality assessment					
			SMD	95% CI		P-value	Q-value	I-squared	P-value	Risk of bias	Inconsistency	Indirectness	Imprecision	Quality	Importance
Lipoperoxidation															
2h	3	31/32	-0.465	-0.969	0.039	0.071	1.705	0	0.426	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
24h	3	24/24	-0.922	-2.004	0.161	0.095	6.220	67.85	0.045	Not serious	Very serious[b]	Not serious	Serious[a]	Very low	Important
48h	2	16/16	-0.122	-3.011	2.766	0.934	13.644	92.67	0.000	Not serious	Very serious[b]	Not serious	Serious[a]	Very low	Important
72h	2	16/16	0.352	-1.536	2.241	0.715	6.560	84.76	0.010	Not serious	Very serious[b]	Not serious	Serious[a]	Very low	Important
IL-6															
0h	6	55/56	-0.006	-0.379	0.367	0.974	1.263	0	0.939	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
1h	4	32/32	-0.235	-0.730	0.259	0.351	1.496	0	0.683	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
24h	3	23/23	-0.397	-1.123	0.329	0.284	0.576	0	0.448	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
48h	2	15/15	0.375	-0.682	1.432	0.487	0	0	1.000	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
CRP															
0h	3	35/35	0.024	-0.445	0.493	0.921	0.367	0	0.832	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
1h	2	27/27	0.014	-0.519	0.548	0.959	0.001	0	0.973	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
1 e 2h	3	35/35	-0.022	-0.491	0.447	0.927	0.408	0	0.816	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
2h	2	16/16	-0.072	-0.767	0.623	0.839	0.371	0	0.543	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
24h	2	16/16	0.243	-0.454	0.940	0.494	0.275	0	0.600	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
CK															
0h	8	85/84	-0.060	-0.362	0.243	0.700	2.499	0	0.927	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
1h	3	34/34	0.052	-0.425	0.529	0.830	0.871	0	0.647	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
2h	3	31/32	-0.186	-0.682	0.310	0.462	0.414	0	0.813	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
1 a 4h	6	67/66	-0.048	-0.390	0.293	0.782	1.718	0	0.887	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important

24h	6	49/47	-0.343	-0.827	0.142	0.165	6.916	27.70	0.227	Not serious	Serious[c]	Not serious	Serious[a]	Very low	Important
48h	5	41/38	-0.297	-0.740	0.146	0.189	1.362	0	0.851	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
72h	5	41/39	-0.113	-0.553	0.327	0.615	0.593	0	0.964	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
Cortisol															
0h	4	33/33	0.182	-0.466	0.831	0.581	5.180	42.08	0.159	Not serious	Serious[c]	Not serious	Serious[a]	Very low	Important
1h	3	25/25	-0.167	-0.971	0.638	0.684	4.026	50.32	0.134	Not serious	Serious[c]	Not serious	Serious[a]	Low	Important
1 e 2h	3	25/25	0.020	-0.971	1.012	0.968	6.009	66.72	0.050	Not serious	Very serious[b]	Not serious	Serious[a]	Very low	Important
24h	2	16/16	-0.650	-2.228	0.928	0.420	4.563	78.09	0.033	Not serious	Very serious[b]	Not serious	Serious[a]	Very low	Important
48h	2	16/16	0.268	-0.432	0.968	0.453	0.733	0	0.392	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
72h	2	16/16	-0.384	-1.088	0.320	0.285	0.779	0	0.377	Not serious	Not serious	Not serious	Serious[a]	Moderate	Important
Muscle soreness															
0h	4	34/32	-0.192	-0.830	0.447	0.557	4.968	39.61	0.174	Not serious	Serious[c]	Not serious	Serious[a]	Low	Critical
4h	2	24/22	-0.379	-1.018	0.259	0.244	2.325	13.97	0.313	Not serious	Not serious	Not serious	Serious[a]	Moderate	Critical
24h	6	56/54	-0.242	-0.806	0.321	0.400	10.560	52.65	0.061	Not serious	Very serious[b]	Not serious	Serious[a]	Very low	Critical
48h	6	56/54	-0.259	-0.798	0.279	0.345	9.644	48.15	0.086	Not serious	Serious[c]	Not serious	Serious[a]	Low	Critical
72h	6	56/54	0.042	-0.333	0.417	0.828	1.103	0	0.954	Not serious	Not serious	Not serious	Serious[a]	Moderate	Critical
MVC															
0h	2	18/16	0.212	-0.464	0.889	0.538	0.102	0	0.749	Not serious	Not serious	Not serious	Serious[a]	Moderate	Critical
24h	3	30/28	0.563	-0.272	1.397	0.186	4.699	57.44	0.095	Not serious	Very serious[b]	Not serious	Serious[a]	Very low	Critical
48h	3	30/28	0.345	-0.175	0.866	0.194	0.524	0	0.770	Not serious	Not serious	Not serious	Serious[a]	Moderate	Critical
72h	3	30/28	0.253	-0.268	0.773	0.341	1.188	0	0.552	Not serious	Not serious	Not serious	Serious[a]	Low	Critical
Peak Torque															
24h	2	19/19	-0.256	-0.897	0.385	0.433	0.579	0	0.447	Not serious	Not serious	Not serious	Serious[a]	Moderate	Critical
48h	2	19/19	-0.041	-0.679	0.597	0.900	0.411	0	0.521	Not serious	Not serious	Not serious	Serious[a]	Moderate	Critical
72h	2	19/19	-0.150	-0.788	0.488	0.644	0.280	0	0.597	Not serious	Not serious	Not serious	Serious[a]	Moderate	Critical

[a] = Few studies and small sample; [b] = High heterogeneity (over 50%); [c] = Moderate heterogeneity (25-50%); RCT: randomized clinical trial; SMD: standard mean difference; CI: confidence interval; CK: creatine kinase; IL-6: interleukine-6; CRP: C-reactive protein; MVC: maximal voluntary contraction.



Supplementary Figure 1 - Funnel plot



Supplementary Figure 2 - Meta-regression for Period of supplementation (Days)

5 CONCLUSÃO

A suplementação com ácido ascórbico reduz o estresse oxidativo (lipoperoxidação) e a resposta inflamatória (IL-6), mas não apresenta efeitos sobre a dor muscular, força muscular, níveis de CK, PCR e cortisol. Baseando-se nesses resultados, a suplementação de ácido ascórbico pode ser considerada para atletas durante competições esportivas, bem como para iniciantes na prática do exercício. Na presente revisão sistemática, a análise dos efeitos da suplementação de ácido ascórbico sobre variáveis relacionadas à funcionalidade musculoesquelética foi possível apenas em relação à força muscular, por ser a única variável avaliada em um número de estudos suficiente para a realização da meta-análise. Nesse sentido, sugere-se a realização de mais ensaios clínicos que avaliem os efeitos desta suplementação sobre variáveis funcionais de voluntários saudáveis após a realização de exercícios físicos.

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ANEXOS

UNIVERSIDADE FEDERAL DE SANTA MARIA - UFSM

PROJETO NA ÍNTEGRA

Data/Hora: 17/06/2019 11:09
Autenticação: 8C3F.F671.D62C.951F.82B2.D734.FBC8.7247
Consulte em http://www.ufsm.br/autenticacao

Título: SUPLEMENTAÇÃO DE ÁCIDO ASCÓRBICO NO DANO E NA FUNCIONALIDADE MUSCULOESQUELÉTICA APÓS EXERCÍCIOS FÍSICOS: UMA REVISÃO SISTEMÁTICA

Número: 048129 **Classificação**: Pesquisa **Registrado em**: 31/01/2018

Situação: Em andamento **Início**: 31/01/2018 **Término**: 31/12/2019

Avaliação: Avaliado **Última avaliação**: 15/03/2019

Fundação: Não necessita contratar fundação **Número na fundação**: *Não se aplica*

Supervisor financeiro: *Não se aplica*

Proteção do conhecimento: Projeto não gera conhecimento passível de proteção

Tipo de evento: Não se aplica **Carga Horária**: *Não se aplica*

Alunos matriculados: *Não se aplica*
Alunos concluintes: *Não se aplica*

Palavras-chave: Voluntários saudáveis, Ácido ascórbico, Exercício, DOMS

Resumo: O exercício físico regular promove benefícios funcionais à saúde, porém, durante a realização de treinamento prolongado de alta intensidade, a produção de espécies reativas de oxigênio pode exceder a capacidade dos antioxidantes resultando em dano muscular e função muscular prejudicada. O dano muscular induzido pelo exercício (DMIE) se manifesta clinicamente pela dor muscular, diminuição da força, da flexibilidade e da funcionalidade. Algumas estratégias são utilizadas para prevenir e tratar o DMIE, como a suplementação de antioxidantes exógenos, como o ácido ascórbico (AA). No entanto, há controvérsias sobre os efeitos da suplementação de AA no DMIE. Neste sentido, esta revisão sistemática terá como objetivo verificar se a suplementação de ácido ascórbico atenua o dano muscular e a funcionalidade induzido pelo exercício em voluntários saudáveis.

Objetivos: OBJETIVO GERAL Estudar os efeitos da suplementação de ácido ascórbico sobre o dano e a funcionalidade musculoesquelética após exercícios físicos em voluntários saudáveis. OBJETIVOS ESPECÍFICOS Verificar se a suplementação de ácido ascórbico atenua o dano, a inflamação e a dor muscular esquelética em voluntários saudáveis. Verificar se a suplementação de ácido ascórbico melhora a funcionalidade em voluntários saudáveis. Verificar se a suplementação de ácido ascórbico atenua a estresse oxidativo em voluntários saudáveis.

Justificativa: Atualmente, é comum a utilização de suplementos antioxidantes no tratamento e prevenção da dor e do dano muscular induzidos pelo exercício físico, que afetam a funcionalidade musculoesquelética. No entanto, há controvérsias sobre os efeitos da suplementação de AA no DMIE. Neste sentido, uma revisão sistemática ajudará a determinar os reais efeitos da suplementação de AA no dano e na funcionalidade musculoesquelética após exercícios físicos em voluntários saudáveis.

Resultados esperados: Os reais efeitos da suplementação de AA no dano e na funcionalidade musculoesquelética após exercícios físicos em voluntários saudáveis.

PARTICIPANTES

MATRÍCULA	NOME	VÍNCULO	FUNÇÃO	C.H.*	INÍCIO	TÉRMINO
201510504	CAROLINE MONTAGNER PIPPI	Aluno de Graduação	Participante	2	01/08/2018	31/12/2019
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201770017	NATIELE CAMPONOGARA RIGHI	Aluno de Pós-graduação	Participante	10	31/01/2018	31/12/2019

* carga horária semanal

UNIDADES VINCULADAS

UNIDADE	FUNÇÃO	VALOR	INÍCIO	TÉRMINO
04.74.00.00.0.0 - PROGRAMA DE PÓS-GRADUAÇÃO EM REABILITAÇÃO FUNCIONAL	Responsável		31/01/2018	31/12/2019

CLASSIFICAÇÕES

TIPO DE CLASSIFICAÇÃO	CLASSIFICAÇÃO
Classificação CNPq	4.00.00.00-1 - CIÊNCIAS DA SAÚDE
Linha de pesquisa	00.00.00.00 - NOVAS LINHAS DE PESQUISA
Quanto ao tipo de projeto de pesquisa	2.03 - Projeto de Dissertação

ANEXO B – NORMAS DA REVISTA

Sports Medicine

Instructions for Authors

TYPES OF PAPERS

Please note:

The word counts given below do not include the abstract, references, figure legends or table captions.

Review Article. Word count up to 6000. Provides an authoritative, balanced, comprehensive, fully referenced and critical review of the literature.

Current Opinion. Word count 1500 to 3000. Places an area in perspective given that it is of current international interest and a consensus has not yet been reached; therefore, the arguments presented may be controversial, but at the same time must be balanced and rational.

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Systematic Review. Word count up to 10,000. Collates all empirical evidence that fits pre-specified eligibility criteria to answer a specific research question. It uses explicit, systematic methods that are selected with a view to minimizing bias, thus providing reliable findings from which conclusions can be drawn and decisions made. Please follow the reporting guidelines of PRISMA.

Original Research Article. Sports Medicine will consider high-quality original research with a strong link to clinical practice in the field of sport and exercise medicine.

Letter to the Editor. Word count up to 1000. Comment on an article published recently in the journal; a response to the comments would normally be sought from the authors of the original article and published in the same issue, where possible.

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The title page should include:

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A concise and informative title

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The e-mail address, and telephone number(s) of the corresponding author

If available, the 16-digit ORCID of the author(s)

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

Please note:

Please note that, for some articles (particularly, systematic reviews and original research articles), 250 words may not be sufficient to provide all necessary information in the abstract. Therefore, the abstract length can be increased from the 250-word limit (to up to 450 words) if the topic dictates, and to allow full compliance with the relevant reporting guidelines.

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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).

Headings

Please use the decimal system of headings with no more than three levels.

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

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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

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].

2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

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.

The entries in the list should be numbered consecutively.

Journal article

Smith JJ. The world of science. Am J Sci. 1999;36:234–5.

Article by DOI

Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. J Mol Med. 2000; https://doi.org/10.1007/s001090000086

Book

Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.

Book chapter

Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. pp. 251–306.

Online document

Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.

Always use the standard abbreviation of a journal’s name according to the ISSN List of Title Word Abbreviations, see

ISSN.org LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

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All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

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ARTWORK

For the best quality final product, it is highly recommended that you submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the quality of the artwork provided.

Electronic Figure Submission

Supply all figures electronically.

Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MS Office files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art

Definition: Black and white graphic with no shading.

Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

All lines should be at least 0.1 mm (0.3 pt) wide.

Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

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Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

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A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA Science Student Council 2006

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