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Edineia de Brito

EFEITOS DOS ANTIOXIDANTES ASSOCIADOS À CRIOTERAPIA NA RESPOSTA INFLAMATÓRIA APÓS EXERCÍCIOS RESISTIDOS EM VOLUNTÁRIOS SEDENTÁRIOS

Santa Maria, RS,
2018

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Dissertação apresentada ao Curso 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: Profº. Drº. Luis Ulisses Signori

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Aprovada em 27 de julho de 2018:

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Santa Maria, RS
2018

DEDICATÓRIA

Dedico esse trabalho aos meus pais, Nadir de Brito e Neide Conterato de Brito e a minha irmã Nelizete Conterato de Brito, a vocês com todo meu amor e gratidão.

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RESUMO

EFEITOS DOS ANTIOXIDANTES ASSOCIADOS À CRIOTERAPIA NA RESPOSTA INFLAMATÓRIA APÓS EXERCÍCIOS RESISTIDOS EM VOLUNTÁRIOS SEDENTÁRIOS

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Os exercícios resistidos (ER), ou de musculação promovem muitas adaptações no organismo, onde o treinamento e o tipo de programa influenciam nas adaptações fisiológicas, possibilitando a médio e longo prazo efeitos favoráveis na qualidade de vida. Entretanto, esta prática induz de forma geral, a uma resposta inflamatória aguda, que pode surgir em decorrência da lesão tissular advinda do estresse mecânico (que provoca lesões em tecidos contráteis e conjuntivos) e/ou do estresse metabólico (ativação hormonal e seus resultantes) causando lesões dos componentes celulares. Isso se deve em parte pelo estresse oxidativo, que induz a resposta inflamatória que se acompanha de leucocitose. Estes eventos são responsáveis pelo desconforto e a diminuição da função após os exercícios, os quais favorecem ao abandono da atividade física. Contudo, a utilização de agentes antioxidantes exógenos e a crioterapia podem reduzir o estresse oxidativo e consequentemente os danos tissulares musculoesqueléticos advindos dos ER de alta intensidade. Entretanto, a associação entre antioxidantes (vitaminas C e E, pré-exercício) e crioterapia (imersão na água a 15ºC, pós-exercício) ainda não foram avaliadas em ER de alta intensidade. O objetivo deste estudo é comparar os efeitos dos antioxidantes associados à crioterapia nos parâmetros de estresse oxidativo, marcadores inflamatórios e na leucocitose em resposta a sessão de ER em voluntários sedentários. A presente pesquisa se caracteriza como um Ensaio Clínico Randomizado, de braço único, cruzado uni-cego, realizada com quatorze voluntários sedentários saudáveis. O estudo compreendeu a avaliação da força muscular em quatro sessões de ER com intervalos de sete dias. A força muscular foi avaliada pelo teste de 10 repetições máximas (10RM). As intervenções compreenderam: a recuperação passiva, a recuperação com vitaminas C (1g) e E (800UI) 40 min pré-exercício, recuperação com crioterapia (imersão a 15ºC durante 10 min) imediatamente após os exercícios e a recuperação com a associação de vitaminas e crioterapia. Hemograma, fibrinogênio, proteína C reativa ultrassensível (PCR), creatina kinase (CK), lipoperoxidação (LPO), capacidade antioxidante contra o radical peroxil (ACAP) foram avaliados em basal, 0min, 30min e 120min. A dor muscular de início tardio (DOMS) foi avaliada 24 hrs após as sessões ER. A PCR (120min) e a CK (0min, 30min, 120min) aumentaram apenas na sessão controle. A recuperação com a associação das intervenções atenuou o aumento de neutrófilos jovens, dos marcadores inflamatórios, do estresse oxidativo e reduziu a DOMS após sessão de ER em voluntários sedentários, podendo desta forma auxiliar na recuperação de atletas em competição, bem como, indivíduos iniciantes nesta modalidade esportiva reduzindo o abandono a prática.

Palavras-chave: Exercício físico. Inflamação. Estresse oxidativo.

ABSTRACT

EFFECTS OF ANTIOXIDANTS ASSOCIATED WITH HYPOTHERMAL IN HEMATOLOGY DYNAMICS, INFLAMMATORY MARKERS AND OXIDATIVE STRESS PROVOKED BY STRENGTH TRAINING IN HEALTHY SEDENTARY

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Resistance exercises (RE) or bodybuilding promote many adaptations in the body, where the training and the type of program influence the physiological adaptations, allowing medium and long term favorable effects on the quality of life. However, this practice generally induces an acute inflammatory response, which can arise as a result of tissue damage resulting from mechanical stress (which causes lesions in contractile and connective tissues) and/or metabolic stress (hormonal activation and its consequences) causing lesions of the cellular components. This is due in part to oxidative stress, which induces the inflammatory response that accompanies leukocytosis. These events are responsible for the discomfort and the decrease of the function after the exercises, which favor the abandonment of the physical activity. However, the use of exogenous antioxidant agents and cryotherapy may reduce oxidative stress and consequently musculoskeletal tissue damage from high intensity ER. However, the association between antioxidants (vitamins C and E, pre-exercise) and cryotherapy (immersion in water at 15ºC, post-exercise) have not yet been evaluated in high intensity RE. The objective of this study is to compare the effects of antioxidants associated with cryotherapy on the parameters of oxidative stress, inflammatory markers and leukocytosis in response to an ER session in sedentary volunteers. The present study is characterized as a single-blind, randomized, single arm Randomized Clinical Trial performed with fourteen healthy sedentary volunteers. The study comprised the evaluation of muscle strength in four ER sessions with seven-day intervals. Muscle strength was assessed by 10 maximal repetitions (10RM). The interventions comprised: passive recovery, recovery with vitamins C (1g) and E (800UI) 40 min pre-exercise, recovery with cryotherapy (immersion at 15ºC for 10 min) immediately after exercise and recovery with the association of vitamins and cryotherapy. Hemoglobin, fibrinogen, C-reactive protein (CRP), creatine kinase (CK), lipoperoxidation (LPO), antioxidant capacity against peroxyl radical (ACAP) were evaluated at baseline, 0min, 30min and 120min. Delayed onset muscle soreness (DOMS) was assessed 24 h after ER sessions. CRP (120min) and CK (0min, 30min, 120min) increased only in the control session. Recovery with the combination of interventions attenuated the increase of young neutrophils, inflammatory markers, oxidative stress, and reduced DOMS after a RE session in sedentary volunteers, being able to help in the recovery of athletes in competition, as well as, beginners in this sport modality reducing the practice abandonment.

Keywords: Physical exercise. Inflammation. Oxidative stress.

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1. INTRODUÇÃO

A prática regular de atividade física possibilita a promoção e manutenção da saúde e reduz o risco de doenças crônicas e mortalidade (HASKELL et al., 2007). Os programas de exercícios resistidos (ER) podem aumentar a massa magra e reduzir a massa gorda e estão associados a redução de riscos para a saúde (DONNELLY et al., 2009). Entretanto, os ER de alto volume e curta recuperação provocam como resposta o estresse oxidativo (HUDSON et al., 2008), pois aumentam a produção das espécies reativas de oxigênio (ERO), podendo levar a lesão muscular e uma consequente resposta inflamatória (CRUZAT et al., 2007). Clinicamente, este mecanismo se manifesta pela dor muscular de inicio tardio (DMIT), a qual é mais prevalente em atletas no início da temporada esportiva ou em iniciantes da atividades físicas (CHEUNG; HUME; MAXWELF, 2003). Porém, a realização de programa de ER em alta intensidade estimula um processo adaptativo que aumenta a capacidade antioxidante enzimática do sangue e do músculo esquelético (RIETJENS et al., 2007).

No centro dessa resposta adaptativa está o estresse oxidativo. As ERO (ânion superóxido $O_2^{\circ-}$, peróxido de hidrogênio H_2O_2, radical hidroxil ^{-}OH) são os produtos intermediários do metabolismo celular oxidativo (BACHSCHMID; SCHILDKNECHT; ULLRICH, 2005). A formação do $O_2^{\circ-}$ reage com o óxido nítrico (NO) formando o peroxinitrito ($ONOO^-$), um potente e duradouro oxidante, que liga as ERO às de nitrogênio (ERON). Essas espécies reativas podem provocar a oxidação dos constituintes celulares (lipídios, proteínas e ácidos nucléicos) e a apoptose celular (BACHSCHMID; SCHILDKNECHT; ULLRICH, 2005; FREIN et al., 2005; FLEMING, 2004), que, nos ER, repercutem em resposta inflamatória aguda (HUDSON et al., 2008; RIETJENS et al., 2007). Entretanto, existem os mecanismos de defesa enzimáticos (superóxido dismutase - SOD, catalase, glutationa peroxidase – GPx e a Glutationa redutase) e não enzimáticos (extracelulares). As defesas não enzimáticas são, principalmente, o ácido úrico e as vitaminas A, C e E. Neste contexto, o dano oxidativo é um estado no qual o excesso da ERON se sobrepõe os sistemas antioxidantes (SCHNEIDER; OLIVEIRA, 2004; SINGH; JIALAL, 2006)

Essa resposta inflamatória é um processo fisiológico inerente a miorregeneração (SILVA; MACEDO, 2011), entretanto, iniciantes ou sedentários estão mais propensos ao abandono da prática esportiva devido a esta resposta inflamatória. Neste contexto, em que se deseja reduzir a resposta inflamatória e a DMIT após exercícios de alta intensidade, parece

racional atenuar a transdução do sinal inflamatório, através de intervenções que promovam a recuperação baseadas nos mecanismos envolvidos no estresse oxidativo, buscando atenuar o desconforto pós-exercício (CHEUNG; HUME; MAXWELF, 2003).

Baseado nestes pressupostos, diferentes estratégias de recuperação após exercícios e/ou atividades físicas de alta intensidade vem sendo estudadas, para moderar os efeitos do estresse oxidativo e consequentemente da resposta inflamatória (GULMEZ et al., 2007; BLOCK, et al., 2008) e da DMIT (CHEUNG; HUME; MAXWELF, 2003). Dentre essas estratégias, está a suplementação com o ácido ascórbico (vitamina C), os tocoferóis (vitamina E) (CRUZAT et al., 2007) e também a crioterapia, na forma de imersão em água fria (MISSAU et al., 2018).

A proposta deste estudo baseia-se em aumentar a capacidade antioxidante previamente aos exercícios através do ácido ascórbico (BRYER; GOLDFARB, 2006) e tocoferol (SILVA et al., 2010) e após o exercício atenuar a formação excessiva das ERON com a crioterapia (ALLAN; MAWHINNEY, 2017). Além disso, estudos avaliando a associação da suplementação das vitaminas (vitaminas C e E) e da crioterapia (imersão 15ºC) ainda não foram realizados. O objetivo da presente pesquisa é estudar os efeitos da suplementação das vitaminas C e E associadas à crioterapia, nas respostas fisiológicas e bioquímicas após exercícios resistidos em voluntários saudáveis destreinados.

2. REFERENCIAL TEÓRICO

A prática regular de atividades físicas é recomenda pelo *American College of Sports Medicine* e pelo *American Heart Association* (ACSM/AHA), sendo um importante fator na promoção e manutenção da saúde (HASKELL et al., 2007). Dentre os diferentes tipos de atividade física se destaca os benefícios dos ER (força ou musculação), uma modalidade de atividade física sistematizada, composta de variáveis (volume, intensidade, frequência, duração, recuperação, ordem dos exercícios, equipamentos e tipo de treinamento) que precisam ser controladas para otimizar os benefícios (RHEA et al., 2003).

Os exercícios físicos podem promover muitas adaptações ao organismo, que podem ser influenciadas pelo treinamento e tipo de programa. Os efeitos favoráveis dos exercícios a médio e longo prazo proporcionam melhora na qualidade de vida e também podem auxiliar na prevenção e/ou na reabilitação de doenças (GILLISON et al., 2009). Os ER estão associados à redução de fatores de risco para as doenças crônicas, aumento da massa magra e diminuição da massa gorda (DONNELLY et al., 2009).

A prática regular de exercícios, realizada de forma adequada, está associada à diminuição de episódios de infecção, possivelmente decorrentes da melhora de funções de neutrófilos, macrófagos e células natural Killeres (NK) (NIEMAN, 1997). Durante a prática dos exercícios, os leucócitos são alterados de acordo com a intensidade e duração da atividade realizada. Programas de exercícios de alta intensidade, em longo prazo, tendem a promover respostas anti-inflamatórias em resposta aos danos causados na musculatura esquelética (TERRA et al., 2012). Fatores neuroendócrinos e dano muscular parecem direcionar a resposta imune ao exercício (KOCH, 2010).

Entretanto, uma sessão de ER realizados em alta intensidade induz a uma resposta inflamatória aguda, pois provoca o aumento de marcadores inflamatórios, e leucocitose transitória (TEIXEIRA et al., 2012; TEIXEIRA et al., 2014). A inflamação induzida por esses exercícios pode surgir em decorrência da lesão tissular, devido ao estresse mecânico (que provoca lesões em tecidos contráteis e conjuntivos), ou ao estresse metabólico (ativação hormonal e seus resultantes) causando lesões dos componentes celulares (CRUZAT et al., 2007).

Os danos tissulares desencadeados pelos exercícios físicos intensos podem ser caracterizados por uma série de eventos (edema, calor e dor) que resultam em reações levando ao reparo do tecido lesado (BALBINO; PEREIRA; CURI, 2005). Esses sintomas estão

relacionados com a DMIT, que acometem atletas de alto nível e principiantes em atividades físicas, podendo levar a diminuição da função muscular e a dor, relacionadas com a descontinuidade dos programas de exercícios (CHEUNG; HUME; MAXWELF, 2003).

A formação de ERON é um fator determinante no curso da resposta inflamatória, pois seus efeitos estão relacionados com a sinalização celular advindos dos danos teciduais e com a intensidade da ação (BACHSCHMID; SCHILDKNECHT; ULLRICH, 2005). As ERO são os produtos intermediários do metabolismo celular oxidativo, que atuam como sinalizadores moleculares e incluem o ânion superóxido ($O_2^{\circ-}$), o peróxido de hidrogênio (H_2O_2) e o radical hidroxil ($^{\circ}OH$) (SCHIEBER; CHANDEL, 2014). O $O_2^{\circ-}$ reage com o óxido nítrico (NO) formando o peroxinitrito ($ONOO^-$), ligando as ERON. Em oposição as ERON, existem os mecanismos de defesa enzimáticos ou intracelulares (SOD, catalase, GPx e GSH) e não enzimáticos ou extracelulares (ácido úrico e as vitaminas A, C e E) (SCHNEIDER; OLIVEIRA, 2004).

O desequilíbrio entre os agentes oxidantes e antioxidantes é denominado estresse oxidativo, e que está presente nas respostas inflamatórias, mas do ponto de vista mecanicista, o estresse oxidativo é melhor definido como alterações na sinalização e no controle redox (JONES, 2006). O aumento transitório ou persistente nos níveis de ERON levam à modificação oxidativa de constituintes celulares (lipídios, proteínas e DNA) que, se não for contrabalançado, pode resultar em morte celular por necrose ou apoptose (BACHSCHMID; SCHILDKNECHT; ULLRICH, 2005; FREIN et al., 2005; FLEMING, 2004; LUSHCHAK, 2015). Neste contexto, o dano oxidativo é um estado no qual o excesso da ERON se sobrepõe os sistemas antioxidantes (SCHNEIDER; OLIVEIRA, 2004;SINGH; JIALAL, 2006). Entretanto, as ERON elevadas também atuam como sinalizadoras na manutenção das funções fisiológicas - processo denominado equilíbrio redox (SCHIEBER; CHANDEL, 2014). O efeito dos exercícios físicos sobre o equilíbrio redox é complexo e dependente da idade, do sexo e do nível de treinamento, bem como da intensidade e da duração do exercício.

Embora o treinamento moderado regular pareça ser benéfico para o estresse oxidativo e para a saúde, exercícios aeróbicos e anaeróbicos realizados de forma aguda e extenuantes (PINGITORE et al., 2015), dentre estes os ER realizados em alta intensidade, podem induzir a uma superprodução de ERON e aos danos oxidativos, que repercutem em resposta inflamatória aguda (RIETJENS et al., 2007; TEIXEIRA et al., 2012; TEIXEIRA et al., 2014). Entretanto, essas espécies reativas ainda podem induzir modificações na sinalização celular interferindo nas vias de transporte nuclear e citosólicas, a partir da

desestruturação das moléculas sinalizadoras atuantes na fase inflamatória ou reações de reparo, estando ligadas à expressão de mensageiros pró ou antiinflamatórios (KODIHA; STOCHAJ, 2012), que ativam a resposta imunitária e resulta na miorregeneração (BROWNING; CHATTERJEE; FISHER, 2012; JONES, 2006).

Agudamente, o exercício físico pode induzir ao dano oxidativo, em especial no sangue e no músculo esquelético, que pode persistir por vários dias após a realização dos exercícios (NIKOLAIDIS et al., 2008). Os exercícios de moderada intensidade (isométrico ou resistido) causam a formação de ERO, que podem causar o dano muscular e a disfunção contrátil (HE et al., 2016). Dentre os diferentes tipos de exercícios, os exercícios excêntricos são considerados os que mais provocam a resposta inflamatória local que pode aumentar gradualmente nos dias após o exercício (PAULSEN et al., 2010). Portanto, parece racional controlar a transdução do sinal inflamatório, através de estratégias de recuperação para atenuar o desconforto no pós-exercício (CHEUNG; HUME; MAXWELF, 2003).

Baseado nestes pressupostos, várias formas de recuperação que buscam melhorar a capacidade antioxidante ou reduzir o dano tecidual, têm sido estudado para atenuar os efeitos do estresse oxidativo e consequentemente da resposta inflamatória (BLOCK et al., 2008; LINDSAY et al., 2017). Dentre elas, se destacam o ácido ascórbico (vitamina C), os tocoferóis (vitamina E) e os agentes hipotérmicos (TRABER, 2006). O consumo de vitaminas (CANDIA-LUJÁN; DE PAZ FERNÁNDEZ; MOREIRA, 2015) , assim como a utilização dos agentes hipotérmicos (MISSAU et al., 2018) parecem colaborar para otimizar as adaptações do organismo, iniciadas pelo treinamento de força ou qualquer situação de prática esportiva.

A maioria dos antioxidantes não enzimáticos conhecidos são advindos das dietas alimentares, dentre esses estão o ácido ascórbico (vitamina C), os tocoferóis (vitamina E), e os carotenóides (b-caroteno). Porém, também existem os antioxidantes não-enzimáticos, que se originam de fontes endógenas e podem ser produtos do metabolismo celular, sendo eles: glutationa, ácido úrico, ácido lipóico, bilirrubina e coenzima Q10 (PETERNELJ; COOMBES, 2011).

Durante os exercícios têm-se alta formação de ERO no músculo, que pode estar relacionado com o dano e função muscular. (PETERNELJ; COOMBES, 2011). A recuperação com suplementação de antioxidantes pode alterar as sinalizações durante o exercício podendo levar a alterações na absorção de glicose, na sensibilidade à insulina, na função de bomba de sódio e potássio, nos marcadores de biogênese mitocondrial, que

resultam na produção de força muscular (POWERS; JACKSON, 2008). Essa suplementação induz a uma proteção contra o dano celular induzido pelo estresse oxidativo (TRABER; STEVENS, 2011). No entanto, as ERON produzidas durante o exercício desempenham papéis importantes em vários processos celulares e suprimir sua formação com altas doses de antioxidantes pode ter um impacto prejudicial sobre a função celular (PETERNELJ; COOMBES, 2011).

O ácido ascórbico (vitamina C) que é um antioxidante hidrossolúvel que têm sido utilizado na tentativa de minimizar o dano tecidual causado pelo exercício (BRYER; GOLDFARB, 2006). Esta vitamina é usada na forma de suplementos e pode proporcionar a diminuição dos níveis de cortisol no pós-exercício (CARRILLO; MURPHY; CHEUNG, 2008), reduzindo os marcadores inflamatórios em pessoas destreinadas (THOMPSON et al., 2001), atenuando a peroxidação lipídica (POPOVIC et al., 2015) e resultando na diminuição da lesão muscular provocada pelo exercício (NAKHOSTIN-ROOHI et al., 2008).

A vitamina E refere-se a um grupo de compostos solúveis em gordura que incluem tocoferóis e tocotrienóis. O ∞-tocoferol é a forma mais biologicamente ativa e demonstrou proteger as células da peroxidação lipídica (JESSUP et al., 2003). Em exercícios excêntricos, a suplementação alimentar de vitaminas C e E por duas semanas atenuou a resposta de IL-6 frente aos ER (FISCHER et al., 2004). No entanto, quando utilizada somente a suplementação de vitamina E, essa não demonstrou ter efeito protetor contra a lipoperoxidação e os danos musculares induzidos pelo exercício (STEPANYAN et al., 2014).

Apesar dos efeitos antioxidantes previamente demonstrados, recente revisão sistemática demostrou que as vitaminas apresentam baixo efeito sobre a DMIT (CANDIA-LUJÁN; DE PAZ FERNÁNDEZ; MOREIRA, 2015), mas esses resultados contraditórios se devem a ampla variação da posologia empregada. Após 40 minutos da ingestão, a vitamina C encontra-se biodisponível (BATES; JONES; BLUCK, 2004; LEVINE; PADAYATTY; ESPEY, 2011), enquanto que a vitamina E torna-se biodisponível após 3h da sua ingestão (TRABER et al., 1998). Neste sentido, acredita-se que os melhores efeitos da suplementação das vitaminas C e E devem ocorrer quando estas estão biodisponíveis durante os exercícios, portanto a sua suplementação deve respeitar o tempo de absorção das mesmas.

A crioterapia (na forma de imersão na água fria) é frequentemente utilizada após o treinamento de força (BURKE et al., 2000; NISSAU et al., 2018). A aplicação desta terapêutica favorece a recuperação da DMIT e da função muscular após os exercícios (GLASGOW; FERRIS; BLEAKLEY, 2014; LINDSAY et al., 2017; MISSAU et al., 2018). A

melhora da fadiga mediada, reduções na tensão cardiovascular e remoção de subprodutos metabólicos musculares acumulados são alguns dos mecanismos relacionados a recuperação (IHSAN; WATSON; ABBISS, 2016). Após um exercício estressante, a crioterapia reduz a DMIT e acelera a recuperação da força (WHITE; WELLS, 2013). Estudo recente demonstrou que a aplicação da crioterapia (imersão na água fria a 15ºC, 10min) reduziu a resposta inflamatória, a leucocitose, a lipoperoxidação e a DMIT (MISSAU et al., 2018)..

O mecanismo induzido pela crioterapia é uma redução na temperatura do tecido, que exerce efeitos locais no fluxo sanguíneo, atenua a formação do edema, reduz o metabolismo celular e a velocidade de condução neural (WHITE; WELLS, 2013). Ainda, a redução da demanda de oxigênio atenua a formação das ERON (IHSAN; WATSON; ABBISS, 2016; ALLAN; MAWHINNEY, 2017). Recente meta-análise demonstrou os melhores parâmetros para a aplicação da crioterapia, sugerindo a temperatura da água entre 11 a 15ºC e o tempo de aplicação entre 11 a 15 minutos (MACHADO et al., 2016).

2.1 JUSTIFICATIVA

Os ER produzem estresse oxidativo, que alteram os marcadores inflamatórios e induzem as alterações hematológicas (leucocitose) durante e/ou logo após a sua prática (TEIXEIRA et al., 2012; TEIXEIRA et al., 2014). Entretanto, tais respostas estão ligadas ao volume e a intensidade desta prática. Estudos sugerem que os suplementos vitamínicos (vitaminas C e E) (POPOVIC et al., 2015; JESSUP et al., 2003) e a crioterapia (MISSAU et al., 2018) separadamente atenuam a o estresse oxidativo e o DMIT.

Estas formas de recuperação beneficiam indivíduos sedentários e iniciantes nesta modalidade esportiva, bem como atletas durante competições (natação, MMA), podendo auxiliar na resposta inflamatória durante e após a realização de exercícios e consequente regeneração muscular.

2.2 OBJETIVO GERAL

Verificar as alterações agudas na dinâmica hematológica, nos marcadores inflamatórios e no estresse oxidativo de voluntários sedentários saudáveis submetidos à prévia suplementação de antioxidantes (vitaminas C e E) associada à crioterapia (imersão 15ºC) após exercícios resistidos.

A seguir será apresentado os resultados do presente estudo que resultou no artigo que será submetido a revista ***The Journal of Strength and Conditioning Research***, Qualis A1 na área 21 (Educação Física).

3. ARTIGO

VITAMINS C AND E ASSOCIATED WITH CRYOTHERAPY IN THE RECOVERY OF THE INFLAMMATORY RESPONSE AFTER SESSION OF EXERCISES RESISTED IN DESTREINATED VOLUNTEERS: RANDOMIZED CLINICAL TRIAL

Short Title:

Vitamins and cryotherapy in recovery after resistance exercises

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VITAMINS C AND E ASSOCIATED WITH CRYOTHERAPY IN THE RECOVERY OF THE INFLAMMATORY RESPONSE AFTER SESSION OF EXERCISES RESISTED IN DESTREINATED VOLUNTEERS: RANDOMIZED CLINICAL TRIAL

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ABSTRACT

The objective of this research is to compare the effects of cryotherapy associated to vitamins (C and E) on the recovery of the inflammatory response from the resistance exercise session of untrained volunteers. Fourteen volunteers (26.2 ± 5 years-old, 25.8 ± 3 kg/m²) underwent 4 sessions of RE with different forms of recovery. The RE consisted of 4 sets of 10 maximal repetitions for each exercise (extensor bench, squat and leg press). The recoveries were randomized and comprised the passive (control), with previous supplementation (40min in 100mL of water) of vitamins C (1g) and E (800UI), with cryotherapy (immersion in water 15ºC for 10min) and the association (vitamins and cryotherapy). Hemogram, inflammatory markers (Protein C ultrasound - CRP and Creatine Kinase - CK) and parameters of oxidative stress (lipid peroxidation - LPO and antioxidant capacity - ACAP) were evaluated before (baseline) and after (0, 30 and 120 min) the RE sessions. Muscle pain, as primary outcome, was evaluated 24 hours after exercise. CRP (p = 0.010) and LPO (p <0.001) increased (120 min) only in passive recovery. Recovery with cryotherapy (30min), with vitamins and the association (0 and 30min) delayed increases in CK (p <0.001). ACAP increased (30 min) only in recovery with the association (p <0.011). The pain decreased in the recoveries with cryotherapy and association (p <0.001). The association of vitamins (C and E) with cryotherapy attenuated the inflammatory response and pain, favoring recovery after an acute RE session.

Key Words: Cold Therapy; Oxidative stress; Resistance training; Antioxidants

INTRODUCTION

Resistance exercise (RE) programs provide, in particular, hypertrophy and increased muscle strength (17), improving physical fitness (14), reducing the risk factors of chronic diseases, acting in the promotion and maintenance of health (16). However, depending on the training variables (intensity, duration, frequency), these exercises can acutely promote a mechanical and metabolic stress that lead to a greater depletion of energy substrates, accumulation of metabolites and muscle damage (28). This tissue damage leads to inflammation and leukocytosis, (32)(17)(24)(31) which is clinically manifested by delay onset muscle soreness (DOMS) (32)(24). This response is necessary for the resolution of any structural damage and is important for the adaptive response of skeletal muscle to exercise (34), but an excessive inflammatory response interferes with the functionality of athletes and can also lead to the abandonment of the practice of these exercises by beginners in this sport practice (13).

Oxidative stress (OS) is at the center of the cellular signaling of these events (12)(26)(17)(32)(24), including the imbalance between the production of reactive oxygen and nitrogen species (RONS) and the ability of cells to neutralize them through antioxidant defense (19)(25)(6). In this sense, therapeutic strategies are being studied in order to minimize OS, which can reduce muscle damage and benefit recovery and functionality (37)(10)(11).

Supplementation with exogenous antioxidants (vitamins C and E) is a non-invasive strategy to prevent or reduce OS (11). Ascorbic acid (vitamin C) is a water-soluble antioxidant, making it bioavailable in the cytosol (5)(20), since α-tocopherol (vitamin E) is stored and transported by lipids in the body, and presents its action on cell membranes (35). Vitamin C protects against OS-induced cell damage by eliminating RONS, but this action

depends on the neutralization of RONS performed by vitamin E (36). Vitamin C attenuates creatine kinase (CK) increase and reduces DOMS, (10). Vitamin E protects cells against OS and muscle damage (29). The combined supplementation of vitamins (C and E) plays an important role in inflammatory disorders and nitric oxide synthase (NO)(36) and presents better results in the reduction of DOMS (11).

Cryotherapy in the form of immersion in cold water has also been used to attenuate inflammation (21)(24), to reduce fatigue, to favor the removal of accumulated metabolites (18), to decrease DOMS and to improve muscle function after exercise (15,21,22)(37). Reduced temperature and decreased metabolism attenuate oxidative damage, (23)(21) (24) due to the reduction in RONS formation (8)(18)(1). Recent meta-analysis demonstrated the best parameters for the application of cryotherapy, suggesting the water temperature between 11 and 15ºC and the time application between 11 and 15 minutes (22).

The rationale of this research is based on evaluating a therapeutic strategy aimed at attenuating the OS imbalance that occurs during and after RE to reduce inflammatory response and muscle damage (17)(32). Hence, it is hypothesized that the effects on the increase of the antioxidant capacity through previous supplementation of vitamins C and E (33)(11) and the reduction in RONS formation through the application of cryotherapy (21)(8)(18)(1) have better effects on the inflammatory response following ER session. The aim of the present study is to evaluate the acute changes in hematological dynamics, inflammatory markers and parameters of OS and DOMS in untrained volunteers submitted to previous antioxidant supplementation (Vitamins C and E) associated with cryotherapy applied after RE.

METHODS

Experimental Approach to the Problem

This is a controlled, randomly assigned, crossover, uni-blind, four-arm therapeutic trial. The forms of recovery and the sequence of RE were randomized according to a computer-generated sequence. The study was divided into 5 days with 7 day intervals. The first day consisted of assessing muscle strength while the other 4 days comprised the forms of recovery (Passive Control, Vitamins C and E, Cryotherapy, Association [Vitamins + Cryotherapy]). The study allowed to compare the effects of 4 forms of recovery after RE on acute changes in DOMS (primary outcome), blood count, fibrinogen, C-reactive protein (CRP), creatine kinase (CK), lipoperoxidation (LPO), antioxidant capacity against radical peroxyl (ACAP) (secondary outcomes) of untrained volunteers.

Subjects

The sample consisted of 14 randomized volunteers in exercises of 4 sessions of RE followed by recoveries (Control passive, Vitamins C and E, Cryotherapy, Association), and based on previous studies (37)(24), in which the difference of 1.4 points (visual analogue scale) between the sessions and standard deviation of 1.6 points was estimated after the application of Cryotherapy. These values were maintained at a power of 80% and at $\alpha = 0.05$.

Inclusion criteria considered community volunteers who were clinically healthy, aged 20-35 years-old, with a body mass index (BMI) of less than 30 kg / m², who did not perform physical activity and / or exercise regularly (+ Once a week) and did not participate in diet programs, just as had no prior diagnosis of rheumatological, cardiovascular, metabolic, neurological, oncological, immunological and hematological diseases. Individuals who used

any type of vitamin and/or ergogenic food supplements, medication and / or were smokers were not included in the study. The subjects were oriented to maintain their daily routines and their eating habits.

Exclusion criteria considered the vonlunteers who presented, as the study was carried out, musculoskeletal injuries, used anti-inflammatory and/ or analgesic medication, just as the ones who presented, in one of the primary laboratorial assessment, inflammatory response (ultra-sensitive CRP >3 mg/ dL), glucose (>100 mg/ dL), leukocytosis (>11.000 x10³/mm³), hyperthermia (>38ºC), altered systemic blood pressure (>140/90 mmHg) and, yet, any symptom of soreness and / or discomfort prior to the primary assessment. Based on such criteria, 3 volunteers were excluded (elevated leukocytosis and CRP) and 1 volunteer did not conclude the study, which totalled the sample in 14 subjects. The flowchart of the study is shown in Figure 1. Fruit and alcohol consumption and physical activities were restrained 72h before data collection. The evaluation were made in the Sports Center of the Federal University of Rio Grande (FURG). The volunteers underwent clinical evaluation in order to determine their physiscal conditions and elegibility into the inclusion and exclusion criteria. Every volunteers who were eligible signed the consente, Research Ethics Committee in the Health Area at Federal University of Rio Grande (CEPAS/FURG protocolo nº 23116.002536/2010-48) e registrado no Clinical Trials (Identifier: NCT02902315).

Procedures

Muscle strength evaluation: The 10 maximal repetition test (10RM) was adopted for the performance of the training protocol with controlled overload (2). The selected exercises were the extensor bench, the squat and the leg press and performed in Physicus® equipment (Plus model, Brazil). During the collections, the recovery time of five minutes between exercises was adopted. The values of the maximum loads in the 10RM test were obtained

through 3 to 5 attempts, when the patient presented a concentric failure frame for the dynamic movement. At each new attempt, increments of 5 kg were added, being given a range of 3 to 4 minutes between each serie. In this way, the maximum load was validated according to what was obtained in the last execution (32)(31)(24).

Exercise session: Before the exercise session, volunteers were submitted to specific warm-up in each exercise (1 series of 15 repetitions with 40% of the maximum load obtained in the 10RM test). The exercise sessions were composed of 4 series of 10RM, with intervals of 1 minute between sets and 2 minutes between exercises. The sequence of RE (extensor bench, squat and leg press) was randomized by draw in closed brown envelope. Prior to 10RM testing and data collection, standardized instructions regarding the experimental procedure and technique for performing the exercises were provided. Verbal stimuli to the volunteer were performed during assessments and exercises (32)(31)(24). After the end of the exercise sessions the volunteers were released for their hydration with water.

Forms of recovery after exercise

Control: In the recovery control, the conducts of the exercise session were maintained and the passive recovery occurred with the volunteer sitting and performing no physical activity or other recovery forms.

Vitamin C and E: Supplementation with vitamins C (1g) (1 tablet, DEG, China) and vitamin E (800 IU) (1 tablet, Pharmanostra, Italy) (9) was performed by oral ingestion (100mL of water) 40 minutes before baseline blood collection. Vitamin C is bioavailable approximately 40 minutes after its oral intake (5)(20). On the other hand, vitamin E has its highest bioavailability in 3h after ingestion (35).

Cryotherapy: The session consisted of immersion in cold water (with water to the umbilical scar level) of the subjects' lower limbs in water at 15ºC, for a period of 10 minutes immediately after the exercise protocol (24).

Association: consisted in the previous supplementation of Vitamin C (1g) and E (800 IU) by oral intake (100mL of water) 40 minutes before baseline blood collection (9). After the RE session, blood was collected again and cryotherapy was recovered (immersion in cold water at the level of the umbilical scar at 15ºC for 10 minutes) (24).

Data collection: On the day of data collection volunteers had fasted for 12 hours. Blood samples were collected before RE (baseline), 0 minutes, 30 minutes and 120 minutes after the session. Twenty-four hours after the exercise session, the subjective perception of pain was assessed by the visual analogue scale (32)(24). Chart 1 shows the data collections and the analyzed variables

Biochemical Measures: Total cholesterol, triglycerides, high density lipoproteins (HDLc), glucose, uric acid, urea and fibrinogen were evaluated by commercial LAB TEST kits (Lagoa Santa, MG, Brazil). Low-density lipoproteins (LDLc) were calculated by the Friedewald formula. For the total plasma proteins the colorimetric method of Bioreto (Doles, GO, Brazil) was used. Lactate was evaluated by tapes (Roche Diagnostics GmbH, Mannheim, Germany). Glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) serum were measured by the IFCC method (HITACHI 917® apparatus, Roche Diagnostics, Florida, USA). Creatine kinase (CK) was assessed using reactive CK-NAC Liquiform (Mindray, model BS200, China). The C-reactive Ultrasensitive Protein was

evaluated by Nephelometry (Nephelometer Beckman Coulter, Immage model with reagents from CCRP IMMAGE laboratory, Fullerton, CA, USA). The erythrogram and leukogram tests were automatically processed (ABX kits, Horiba Diagnostica, Curitiba, Brazil) and microscopy. For quantification of the hematological variables the samples were counted twice and the values were expressed by the mean of the measurements (differences greater than 10% were repeated).

Oxidative stress: Evaluated through lipid damage measure (LPO) and a measure of antioxidant capacity against radicals (ACAP) peroxyl (RO_2•) (32)(24). Lipoperoxidation (LPO) was measured in the oxidation of Fe^{2+} by lipidic hydroperoxides in acid medium in the presence of Fe^{3+} Xylenol Orange complexing dye (SIGMA, SP, Brazil). Plasma samples were used in the analyzes. The determination was performed in a microplate reader, using a wavelength of 550 nm. Cumene hydroperoxide (SIGMA, SP, Brazil) was used as standard. The results are expressed as cumene hydroperoxide ηmol/g tissue.

For the evaluation of total antioxidant capacity against peroxyl radicals (ACAP) 10µl of diluted plasma were used to a concentration of 3.3 mg/ml protein and were pipetted into 8 wells of white bottom microplates. Then, 127.5 ul of the reaction buffer (30 mM HEPES (pH 7.2), 200 mM KCl and 1 mM $MgCl^2$) were added to the wells with samples. Four wells per sample received 7.5ul of 2,20-azobis-2-methylpropionamidine dihydrochloride (ABAP, 4 mM, Sigma-Aldrich, SP, Brazil) and four wells received 7.5 ul of ultrapure water. Basal plate fluorescence was measured using fluorimeter (Victor 2, Perkin-Elmer, Turku, Finland) at 35 ° C, where the peroxyl radicals are produced from the ABAP thermolysis. After reading the basal fluorescence, 10ul of 20,70-dichlorofluorescein-diacetate (H_2DCF-DA) were added to the wells, making a final concentration of 40uM. H_2DCF-DA was cleaved by esterases

present in the samples and the non-fluorescent compound H2DCF was oxidized by the peroxyl radicals, which was detected using the 488 and 525ηm wavelengths for excitation and emission, respectively. Fluorescence generation was monitored every 5 minutes for 1 hour. The fluorescence units were used for an area calculation by the time measurement, after adjustment to a second-order polynomial function. The data are expressed in the form (1 / (relative area with / without ABAP))(32)(24).

Statistical analyses

Data are expressed as mean ± standard deviation. The Kolmogorov-Smirnov test was used to verify the distribution of the data. Two-way ANOVA was used to compare the variables for repeated measures followed by Bonferroni post hoc. The significance level considered was 5% (p <0.05). The data were analyzed by the program GraphPad Prism 5 (GraphPad Software, San Diego, CA), Windows version, which was used for the analyzes.

RESULTS

The sample comprised 14 untrained volunteers with a mean age of 26.2 (± 5) years-old, with BMI 25.8 (±3) kg/m². Data from the sample characterization are shown in Table 1. Systolic blood pressure and diastolic blood pressure were similar between sessions. The intensity of exercise assessed by the lactate, after the RE sessions, increased approximately 5 times from baseline (recovery control pré: 1.8 ± 0.5 mmol/L pós: 10.2 ± 1.1 mmol/L; recovery vitamins pré: 1.7 ± 0.5 mmol/L pós: 10.3 ± 1.1 mmol/L; recovery cryotherapy pré: 1.7 ± 0.5 mmol/L pós: 10.4 ± 1.1 mmol/L; recovery association pré: 1.8 ± 0.5 mmol/L pós: 10.2 ± 1.0 mmol/L; Group: p = 0.966, Time: p < 0.001; Interaction: p = 0.860). The lipid profile (cholesterol, triglycerides, HDLc, LDLc), glucose, uric acid, urea, GOT and GPT were within recommended values for the age range and did not change among recovery forms.

The erythrogram results were within normal values, where, immediately after the RE, an increase of approximately 4% of the hematocrits, 3% of the red blood cells, 4% of the hemoglobins and 19% of the platelets was observed, and these values returned to basal levels in half an hour and 2 hours in all exercise sessions (data presented in Table 2).

Table 3 presents the Leukogram data. The total leukocytes and the segmented neutrophils increased 25% and 23%, respectively, immediately after the exercises, returning to baseline half an hour after the end the section, but 2h after the RE sessions these values again increased in relation to the other evaluated moments. The rods increased approximately 60%, 2h after the end of the RE in relation to the basal values in the control, vitamin and cryotherapy recoveries, but in the control and vitamins recoveries this increase also occurred in relation to the measurements immediately after the end of the exercises. Only in the recovery association no changes were observed during the period. Eosinophils did not change throughout the study (Group: $p = 0.220$, Time: $p = 0.104$; Interaction: $p = 0.767$). In all forms of recovery, the monocytes increased approximately 49% immediately after the RE, returning to the basal values in the other moments evaluated. Lymphocytes increased approximately 26% immediately after RE (0min) in vitamin, cryotherapy and combination recovery, and 30min after RE, they returned to baseline in all sessions.

Data on inflammatory markers, OS parameters and DOMS are presented in Figure 2. Fibrinogen did not change throughout the study (Figure 2A). CRP increased by 54% in 2h compared to baseline and 30min after ER only in the control recovery (Figure 2B). In the control recovery, the CK increased 19%, 21%, 30%, respectively, in 0min, 30min and 120min, in relation to the baseline values. In recovery cryotherapy increased 9% immediately after the ER, after returning to baseline and subsequently increased 2h after the exercise,

compared to baseline and 30 min. In recoveries vitamins and association, this increase occurred only 2 hours after exercise (Figure 2C). LPO increased by 67% in 2h after the end of the exercises only in the control recovery (Figure 2D). The ACAP increased 31% in 30min after the RE, in relation to baseline values, only in the association recovery (Figure 2E). The DOMS assessed 24 h after the exercise sessions presented a reduction of approximately 40% in cryotherapy recovery and 50% in association recovery (Figure 2F).

DISCUSSION

The main results of the present study demonstrate that the association of previous (40 min before) vitamin suplementation (C and E) and cryotherapy (immediately) after the RE session attenuates the increase of young neutrophils, of inflammatory markers (CK and CRP), reduces OS (increases antioxidant capacity and attenuates LPO), and decreases DOMS relative to other forms of recovery after ER session in untrained volunteers.

The results of the present study demonstrate that RE induce an expected leukocytosis and increased inflammatory markers, especially in passive recovery. The increase in blood reactivity occurs during and after the high intensity RE and presents several biomarkers (6), among such biomarkers, increases in leukocyte, platelet and inflammatory markers of acute phase protein concentrations and/or activities (32)(31). These results are due to hemoconcentration and the inflammatory response that occur during and immediately after the RE (31). The inflammatory response occurs due to the OS, where the excessive production of the RONS interferes in the oxidation of the cellular constituents (30)(23)(8), and is demonstrated in the present study by increases in CRP, CK, and LPO in passive recovery. These inflammatory cells and signaling molecules, including RONS and cytokines,

activate leukocytes (32), intervene in the process of myoregeneration that occurs during recovery of the damage induced by the high intensity RE (6). Thus, this research demonstrates that recovery with the association interfered in signaling, since it delayed the formation and/or recruitment of young neutrophils.

The present study demonstrates that supplementation of vitamins C and E attenuates muscle damage through the reduction of CRP, CK, and attenuates LPO. The reduction of muscle damage markers has already been demonstrated (10)(36). However, vitamin E supplementation (1200 IU/day for 3 weeks) showed no effect on CK after a RE session (3). The associated supplementation of vitamins (C and E) can optimize their antioxidant effects (36), which was demonstrated in the present study by attenuation of LPO in recovery with vitamins. Combined vitamin supplementation (C and E) decreases DOMS (11), but these results were not confirmed in the present study in agreement with another study (33).

Cryotherapy attenuated muscle damage (CRP and CK), LPO and decreased DOMS. The attenuation of CK (37)(24) and the LPO (24) and the reduction of DOMS (21) have been previously demonstrated after RE (24)(27). It is suggested that the reduction of DOMS by cryotherapy, occurs in part due to decreased nerve conduction velocity, ameliorating hyperthermia, reduction of muscle and central fatigue and reduction of metabolites (18). In the present study, cryotherapy reduced DOMS by approximately 40%, and the reduction in DOMS of 13-22% may be considered clinically relevant for athletes (7), emphasizing the effectiveness of the recovery form also for untrained volunteers.

The present study demonstrates that the association of vitamins (C and E) and cryotherapy, attenuates the increase of young neutrophils, systemic inflammatory response

(delayed increase in CRP), muscle damage (delayed CK increase), stress oxidative (increased antioxidant capacity and decreased LPO) and reduced DOMS. The favorable isolated effects of recovery with vitamins (C and E) on muscle damage, oxidative stress (29), DOMS (10)(11) and recovery with cryotherapy on the reduction of CK (37)(24), LPO (24) and DOMS (27)(22)(24) have already been demonstrated. The mechanisms involved are related to OS, where vitamins increase the exogenous antioxidant capacity (4)(33)(11) and cryotherapy attenuates the formation of RONS (21)(8)(18)(1), which was demonstrated in the present study, because the association was the only form of recovery capable of increasing the antioxidant capacity 30 minutes after RE and the other forms of recovery (except the passive) reduced LPO. These results suggest that recovery with the association has more effective outcomes on OS after a RE session in untrained volunteers. However, the present study also demonstrated that recovery with cryotherapy had the same effect as recovery associated with DOMS. We believe that this is due to the fact that, in addition to the effects of cryotherapy on OE, this form of recovery decreases nerve conduction velocity, reduces muscle and central fatigue (18), which interferes with the perception of pain. Among the limitations of the present study, it is possible to emphasize the absence of evaluation of the enzymatic antioxidant capacity and uric acid, which could occur simultaneously to the biochemical collections, as well as the evaluation of these variables together with the DOMS.

PRACTICAL APPLICATIONS

To our knowledge, this is the first study to associate recovery forms with supplementation of exogenous antioxidants (vitamin C and E) and cryotherapy (water at 15ºC for 10 min) in recovery after a session of RE of untrained volunteers. The results of the present research demonstrate that the association of these recovery forms attenuates the

inflammatory response, muscle damage, oxidative stress and reduces DOMS. These results suggest that the association of exogenous antioxidants with cryotherapy may favor the adaptation of beginners to ER training and improve the functionality of athletes in competitive situations. However, this intervention may also interfere with the musculoskeletal adaptive processes derived from the RE, and its application with training programs should be further investigated.

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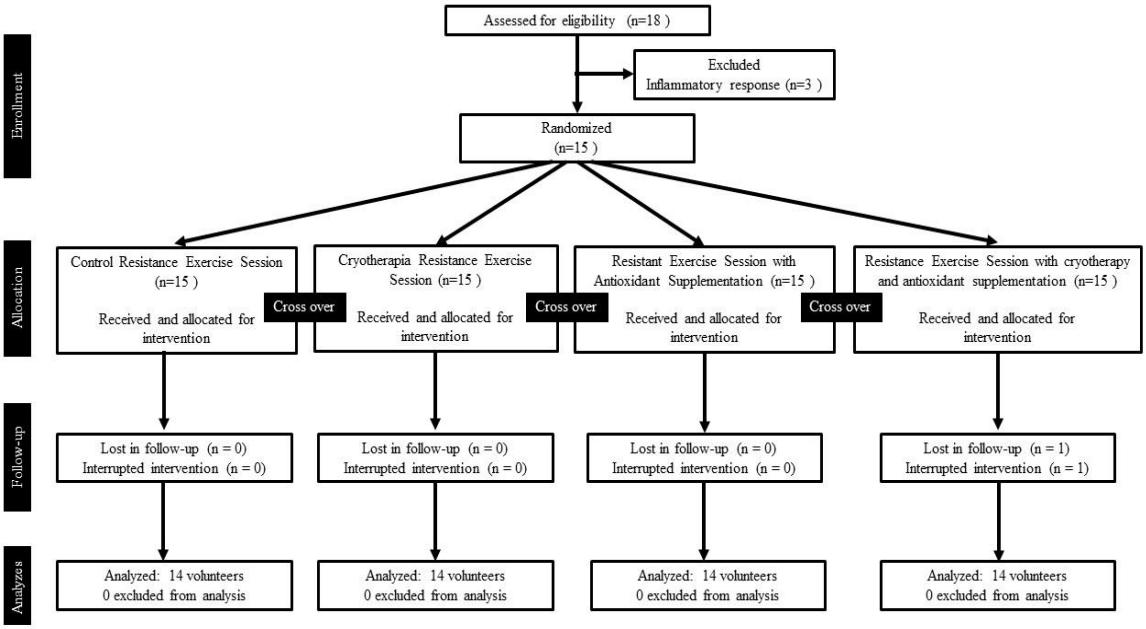
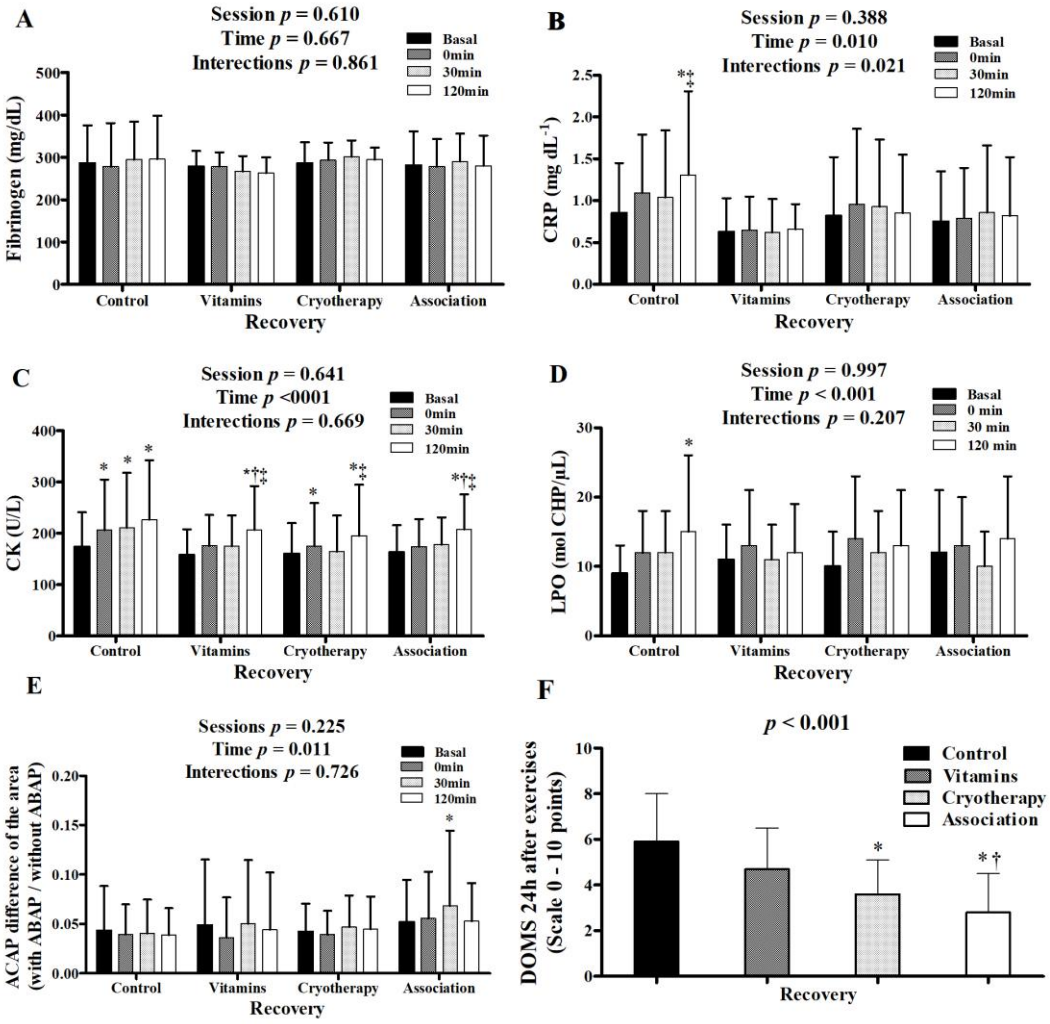
Figure subtitles

Figure 1. Flowchart of the study design.

Figure 2. Data on inflammatory markers, oxidative stress parameters and DOMS in different interventions after resistance exercise sessions.


Enrollment
Assessed for eligibility (n=18)
Excluded
Inflammatory response (n=3)
Randomized
(n=15)
Allocation
Control Resistance Exercise Session
(n=15)
Received and allocated for
intervention
Cross over
Cryotherapia Resistance Exercise
Session (n=15)
Received and allocated for
intervention
Cross over
Resistant Exercise Session with
Antioxidant Supplementation (n=15)
Received and allocated for
intervention
Cross over
Resistance Exercise Session with cryotherapy
and antioxidant supplementation (n=15)
Received and allocated for
intervention
Follow-up
Lost in follow-up (n = 0)
Interrupted intervention (n = 0)
Lost in follow-up (n = 0)
Interrupted intervention (n = 0)
Lost in follow-up (n = 0)
Interrupted intervention (n = 0)
Lost in follow-up (n = 1)
Interrupted intervention (n = 1)
Analyzes
Analyzed: 14 volunteers
0 excluded from analysis
Analyzed: 14 volunteers
0 excluded from analysis
Analyzed: 14 volunteers
0 excluded from analysis
Analyzed: 14 volunteers
0 excluded from analysis



Data are expressed as mean ± SD; *$p < 0.05$ vs. basal; †$p < 0.05$ *vs.* 0 min.; ‡$p < 0.05$ *vs.* 30 min. 2F: *$p < 0.05$ vs. control; †$p < 0.05$ *vs.* vitamins; CRP: C-reactive protein, CK: Creatine kinase, LPO: Lipid peroxidation; ACAP: Antioxidant Capacity Against Peroxyl radicals (RO_2•); DOMS: Delay onset muscle soroness.

Chart 1: Data collection

Variables	**Pre-exercise**		**Resistance Exercise**	**Post exercise**			
	Vitamins 40 min	**Basal**		**0min**	**Cryotherapy**	**30min**	**120min**
Blood collections		X		X		X	X
Oxidative stress		X		X		X	X
Biomarkers		X		X		X	X
Biochemistry		X		X		X	X

Table 1. Physical and biochemical characteristics of volunteers.

Characteristic	Recovery				*p* Value
	Control	Vitamins	Cryotherapy	Association	
Systolic Blood Pressure (mmHg)	117 ± 5	119 ± 6	120 ± 6	119 ± 5	0.466
Diastolic Blood Pressure (mmHg)	79 ± 6	81 ± 7	81 ± 4	81 ± 5	0.432
Total cholesterol (mg/dL)	149 ± 30	140 ± 30	147 ± 35	147 ± 31	0.574
Triglycerides (mg/dL)	113 ± 69	86 ± 69	91 ± 58	83 ± 48	0.095
Hight-density lipoprotein cholesterol (mg/dL)	34 ± 6	36 ± 6	35 ± 7	37 ± 7	0.320
Low-density lipoprotein cholesterol (mg/dL)	92 ± 27	87 ± 27	94 ± 27	94 ± 26	0.658
Plasma glucose (mg/dL)	89 ± 8	82 ± 12	82 ± 12	84 ± 12	0.275
Uric acid (mg/dL)	5.0 ± 0.7	4.8 ± 0.7	5.2 ± 0.8	4.9 ± 0.6	0.125
Urea (mg/dL)	30 ± 7	30 ± 7	29 ± 9	30 ± 8	0.890
Glutamic-oxaloacetic transaminase (U/L)	29 ± 6	26 ± 6	27 ± 5	27 ± 9	0.510
Glutamic-pyruvic transaminase (U/L)	32 ± 10	32 ± 10	33 ± 8	30 ± 8	0.589

Data are expressed as mean ±SD;

Table 2. Erythrogram data in different interventions after resistance exercise sessions

Variables	Recovery	Blood Collections				*p* Value		
		Basal	0min	30min	120min	Recovery	Time	Interaction
Hematocrit (mL/%)	Control	47.2 ± 3	49.4 ± 3*	47.2 ± 3†	46.8 ± 3†	0.961	<0.001	0.661
	Vitamins	48.1 ± 3	49.9 ± 3*	47.1 ± 3†	47.5 ± 3†			
	Cryotherapy	47.1 ± 2	49.0 ± 3*	47.5 ± 2†	47.4 ± 2†			
	Association	47.2 ± 3	49.4 ± 3*	47.6 ± 2†	47.5 ± 3†			
Red blood cels ($x10^5/mm^3$)	Control	5.2 ± 0.3	5.4 ± 0.4*	5.2 ± 0.4†	5.2 ± 0.4†	0.948	<0.001	0.561
	Vitamins	5.2 ± 0.4	5.4 ± 0.4*	5.1 ± 0.3†	5.1 ± 0.4†			
	Cryoterapy	5.2 ± 0.3	5.4 ± 0.4*	5.2 ± 0.3†	5.2 ± 0.3†			
	Association	5.2 ± 0.3	5.3 ± 0.3*	5.1 ± 0.3†	5.2 ± 0.4 †			
Hemoglobin (g/dL)	Control	15.5 ± 0.9	16.2 ± 1.1*	15.5 ± 1.1†	15.4 ± 1.1†	0.878	<0.001	0.482
	Vitamins	15.8 ± 0.9	16.3 ± 0.1*	15.55 ± 0.9†	15.7 ± 1.0†			
	Cryotherapy	15.6 ± 0.6	16.2 ± 0.1*	15.74 ± 0.9†	15.7 ± 0.8†			
	Association	15.5 ± 0.7	16.1 ± 0.1*	15.58 ± 0.8†	15.4 ± 1.0†			
Plateles ($x10^3/mm^3$)	Control	230 ± 39	268 ± 55 *	239 ± 43†	241 ± 42†	0.178	<0.001	0.618
	Vitamins	204 ± 39	242 ± 41 *	213 ± 39†	214 ± 37†			
	Cryoterapy	222 ± 37	267 ± 39 *	237 ± 35†	237 ± 35†			
	Association	241 ± 40	291 ± 57 *	245 ± 29†	249 ± 31†			

Data are expressed as mean ±SD; *p<0,05 *vs* basal; †p<0,05 *vs* 0min; ‡p<0,05 *vs* 30min.

Table 3. Leucogram data in different interventions after resistance exercise sessions

Variables	Recovery	Blood Collections				*p* Value		
		Basal	0min	30min	120min	Recovery	Time	Interaction
Leukocytes (x10^3/mm^3)	**Control**	6879 ± 970	8429 ± 1426*	6593 ± 851†	8114 ± 1478*†‡	0.610	<0.001	0.973
	Vitamins	7402 ± 1230	9200 ± 1639*	7081 ± 1317†	9580 ± 2214*†‡			
	Cryotherapy	7095 ± 1400	8724 ± 1865*	6924 ± 1300†	8938 ± 2233*†‡			
	Association	7038 ± 1008	9129 ± 2202*	6860 ± 1179†	8919 ± 1750*†‡			
Segmented neutroplils (x10^3/mm^3)	**Control**	3719 ± 788	4699 ± 1021*	3908 ± 728†	5579 ± 1406*†‡	0.152	<0.001	0.149
	Vitamins	4539 ± 1246	5346 ± 1337*	4576 ± 1402	6875 ± 2332*†‡			
	Cryotherapy	4039 ± 1127	4913 ± 1258*	4187 ± 1208	5983 ± 2071*†‡			
	Association	4187 ± 913	5330 ± 1441*	4220 ± 985†	6177 ± 1647*†‡			
Young neutrophils (x10^3/mm^3)	**Control**	73 ± 15	84 ± 14	66 ± 9	124 ± 109*‡	0.756	<0.001	0.948
	Vitamins	74 ± 12	103 ± 41	76 ± 23	116 ± 54*‡			
	Cryotherapy	80 ± 25	97 ± 28	86 ± 53	124 ± 73*			
	Association	76 ± 23	98 ± 33	74 ± 22	107 ± 43			
Monocytes (x10^3/mm^3)	**Control**	302 ± 93	442 ± 110*	289 ± 69†	310 ± 104†	0.922	<0.001	0.790
	Vitamins	296 ± 82	470 ± 203*	259 ± 78†	280 ± 70†			
	Cryotherapy	317 ± 71	430 ± 106*	291 ± 76†	331 ± 85†			
	Association	288 ± 78	448 ± 194*	276 ± 64†	284 ± 112†			
Lymphocytes (x10^3/mm^3)	**Control**	2634 ± 512	3024 ± 735	2245 ± 516†	2484 ± 477†	0.559	<0.001	0.895
	Vitamins	2362 ± 332	3057 ± 984*	2053 ± 434†	2109 ± 429†			
	Cryotherapy	2435 ± 471	3035 ± 804*	2184 ± 344†	2238 ± 438†			
	Association	2325 ± 367	2868 ± 1249*	2149 ± 420†	2123 ± 345†			

Data are expressed as mean ±SD; *p<0,05 *vs* basal; $^{\dagger}p$<0,05 *vs* 0min; ‡p<0,05 *vs* 30min.

4. CONCLUSÃO

Após a realização deste estudo é possível afirmar que a suplementação prévia de vitamina C (1g) e E (800UI), associada à crioterapia (imersão na água fria, 10min, a 15ºC) pós exercício, atenua o aumento dos neutrófilos jovens, da inflamação e do estresse oxidativo e reduz a DMIT, quando comparado com o controle e as intervenções isoladas.

Esses resultados reforçam a hipótese, em relação à eficiência das intervenções na adaptação e/ou recuperação muscular, podendo auxiliar atletas no retorno ao treinamento, bem como os iniciantes na prática esportiva. É importante salientar que tais resultados não devem ser generalizados, e sim considerados quando se tratar de intervenção aguda, nos parâmetros descritos, para a recuperação após exercícios resistidos de alta intensidade.

Neste sentido, sugere-se a realização de mais estudos que abordem diferentes formas de recuperação pós ER, auxiliando o público praticante desta modalidade esportiva, bem como os iniciantes desta prática, com a finalidade de reduzir o abandono das atividades físicas, em especial os exercícios resistidos, vistos os seus benefícios para a saúde.

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ANEXOS

ANEXO 1 - Normas da Revista

Journal of Strength & Conditioning Research

Online Submission and Review System

The Journal of Strength and Conditioning Research (JSCR) is the official research journal of the National Strength and Conditioning Association (NSCA). The JSCR is published monthly. Membership in the NSCA is not a requirement for publication in the journal. JSCR publishes original investigations, reviews, symposia, research notes, and technical and methodological reports contributing to the knowledge about strength and conditioning in sport and exercise. All manuscripts must be original works and present practical applications to the strength and conditioning professional or provide the basis for further applied research in the area. Manuscripts are subjected to a ''double blind'' peer review by at least two reviewers who are experts in the field. All editorial decisions are final and will be based on the quality, clarity, style, and importance of the submission relative to the goals and objectives of the NSCA and the journal. Tips for writing a manuscript for the JSCR can be found at http://edmgr.ovid.com/jscr/accounts/Tips_for_Writing.pdf. Please read this document carefully prior to preparation of a manuscript. Manuscripts can be rejected on impact alone as it relates to how the findings impact evidence based practice for strength and conditioning professionals, end users, and clinicians. Thus, it is important authors realize this when submitting manuscripts to the journal.

JSCR senior associate editors will administratively REJECT a paper before review if it is deemed to have very low impact on practice, poor experimental design, improperly formatted, and/or poorly written. Additionally, upon any revision the manuscript can be REJECTED if experimental issues and impact are not adequately addressed to reviewer satisfaction. The formatting of the manuscript is of great importance and manuscripts will be rejected if not PROPERLY formatted.

EDITORIAL MISSION STATEMENT

The editorial mission of the JSCR, formerly the Journal of Applied Sport Science Research (JASSR), is to advance the knowledge about strength and conditioning through research. Since 1978 the NSCA has attempted to ''bridge the gap'' from the scientific laboratory to the field practitioner. A unique aspect of this journal is the inclusion of recommendations for the practical use of research findings. While the journal name identifies strength and conditioning as separate entities, strength is considered a part of conditioning. This journal wishes to promote the publication of peer-reviewed manuscripts that add to our understanding of conditioning and sport through applied exercise and sport science. The conditioning process and proper exercise prescription impact a wide range of populations from children to older adults, from youth sport to professional athletes. Understanding the conditioning process and how other practices such as such as nutrition, technology, exercise techniques, and biomechanics support it is important for the practitioner to know.

Original Research

JSCR publishes research on the effects of training programs on physical performance and function to the underlying biological basis for exercise performance as well as research from a number of disciplines attempting to gain insights about sport, sport demands, sport profiles, conditioning, and exercise such as biomechanics, exercise physiology, motor learning, nutrition, and psychology. A primary goal of JSCR is to provide an improved scientific basis for conditioning practices.

Article Types

JSCR publishes symposia, brief reviews, technical reports and research notes that are related to the journal's mission. A symposium is a group of articles by different authors that address an issue from various perspectives. The brief reviews should provide a critical examination of the literature and integrate the results of previous research in an attempt to educate the reader as to the basic and applied aspects of the topic. We are especially interested in applied aspects of the reviewed literature. In addition, the author(s) should have experience and research background in the topic area they are writing about in order to claim expertise in this area of study and give credibility to their recommendations.

The JSCR strongly encourages the submission of manuscripts detailing methodologies that help to advance the study of strength and conditioning.

Manuscript Clarifications

Manuscript Clarifications will be considered and will only be published online if accepted. Not all requests for manuscript clarifications will be published due to costs or content importance. Each will be reviewed by a specific sub-committee of Associate Editors to determine if it merits publication. A written review with needed revisions will be provided if it merits consideration. Clarifications questions are limited to 400 words and should only pose professional questions to the authors and not editorial comments (as of 19.2). If accepted, a copy will be sent to the author of the original article with an invitation to submit answers to the questions in the same manner again with a 400 word limit.

Submissions should be sent to the JSCR Editorial Office via email:
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kraemer.45@osu.edu

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2. All authors should be aware of the publication and be able to defend the paper and its findings and should have signed off on the final version that is submitted. For additional details related to authorship, see "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" at http://www.icmje.org/.

3. The NSCA and the Editorial Board of the JSCR have endorsed the American College of Sports Medicine's policies with regards to animal and human experimentation. Their guidelines can be found online at http://www.editorialmanager.com/msse/. Please read these policies carefully. Each manuscript must show that they have had Institutional Board approval for their research and appropriate consent has been obtained pursuant to law. All manuscripts must have this clearly stated in the methods section of the paper or the manuscript will not be considered for publication.

4. All manuscripts must be double-spaced with an additional space between paragraphs. The paper should include a minimum of 1-inch margins and page numbers in the upper right corner

next to the running head. Authors must use terminology based upon the International System of Units (SI). A full list of SI units can be accessed online at http://physics.nist.gov/.

5. The JSCR endorses the same policies as the American College of Sports Medicine in that the language is English for the publication. ‘‘Authors who speak English as a second language are encouraged to seek the assistance of a colleague experienced in writing for English language journals. Authors are encouraged to use nonsexist language as defined in the American Psychologist 30:682- 684, 1975, and to be sensitive to the semantic description of persons with chronic diseases and disabilities, as outlined in an editorial in Medicine & Science in Sports & Exercise_, 23(11), 1991. As a general rule, only standardized abbreviations and symbols should be used. If unfamiliar abbreviations are employed, they should be defined when they first appear in the text. Authors should follow Webster’s Tenth Collegiate Dictionary for spelling, compounding, and division of words. Trademark names should be capitalized and the spelling verified. Chemical or generic names should precede the trade name or abbreviation of a drug the first time it is used in the text.’’

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Please Note

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2. Blind Title Page

A second title page should be included that contains only the manuscript title. This will be used to send to the reviewers in our double blind process of review. Do not place identifying information in the Acknowledgement portion of the paper or anywhere else in the manuscript.

3. Abstract and Key Words

On a separate sheet of paper, the manuscript must have an abstract with a limit of 250 words followed by 3 – 6 key words not used in the title. The abstract should have sentences (no

headings) related to the purpose of the study, brief methods, results, conclusions and practical applications.

4. Text

The text must contain the following sections with titles in ALL CAPS in this exact order:

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B. Methods. Within the METHODS section, the following subheadings are required in the following order: ''Experimental Approach to the Problem,'' where the author(s) show how their study design will be able to test the hypotheses developed in the introduction and give some basic rationales for the choices made for the independent and dependent variables used in the study; ''Subjects,'' where the authors include the Institutional Review Board or Ethics Committee approval of their project and appropriate informed consent has been gained. All subject characteristics that are not dependent variables of the study should be included in this section and not in the RESULTS; ''Procedures,'' in this section the methods used are presented with the concept of ''replication of the study'' kept in mind. ''Statistical Analyses,'' here is where you clearly state your statistical approach to the analysis of the data set(s). It is important that you include your alpha level for significance (e.g., P # 0.05). Please place your statistical power in the manuscript for the n size used and reliability of the dependent measures with intra-class correlations (ICC Rs). Additional subheadings can be used but should be limited.

C. Results. Present the results of your study in this section. Put the most important findings in Figure or Table format and less important findings in the text. Do not include data that is not part of the experimental design or that has been published before.

D. Discussion. Discuss the meaning of the results of your study in this section. Relate them to the literature that currently exists and make sure you bring the paper to completion with each of your hypotheses. Limit obvious statements like, ''more research is needed.''

E. Practical Applications. In this section, tell the ''coach'' or practitioner how your data can be applied and used. It is the distinctive characteristic of the JSCR and supports the mission of ''Bridging the Gap'' for the NSCA between the laboratory and the field practitioner.

5. References

All references must be alphabetized by surname of first author and numbered. References are cited in the text by numbers [e.g., (4,9)]. All references listed must be cited in the manuscript and referred to by number therein. For original investigations, please limit the number of references to fewer than 45 or explain why more are necessary. The Editorial Office reserves the right to ask authors to reduce the number of references in the manuscript. Please check references carefully for accuracy. Changes to references at the proof stage, especially changes affecting the numerical order in which they appear, will result in author revision fees. End Note Users: The Journal of Strength & Conditioning Research reference style, http://endnote.com/downloads/style/journal-strength-conditioning-research may be downloaded for use in the End Note application: http://endnote.com/downloads/style/journal-strength-conditioning-research.

Below are several examples of references:

Journal Article

Hartung, GH, Blancq, RJ, Lally, DA, and Krock, LP. Estimation of aerobic capacity from submaximal cycle ergometry in women. Med Sci Sports Exerc 27: 452–457, 1995.

Book

Lohman, TG. Advances in Body Composition Assessment. Champaign, IL: Human Kinetics, 1992.

Chapter in an edited book

Yahara, ML. The shoulder. In: Clinical Orthopedic Physical Therapy. J.K. Richardson and Z.A. Iglarsh, eds. Philadelphia: Saunders, 1994. pp. 159–199.

Software

Howard, A. Moments ½software_. University of Queensland, 1992.

Proceedings

Viru, A, Viru, M, Harris, R, Oopik, V, Nurmekivi, A, Medijainen, L, and Timpmann, S. Performance capacity in middle-distance runners after enrichment of diet by creatine and creatine action on protein synthesis rate. In: Proceedings of the 2nd Maccabiah-Wingate International Congress of Sport and Coaching Sciences. G. Tenenbaum and T. Raz-Liebermann, eds. Netanya, Israel, Wingate Institute, 1993. pp. 22–30.

Dissertation/Thesis

Bartholmew, SA. Plyometric and vertical jump training.Master's thesis, University of North Carolina, Chapel Hill, 1985.

6. Acknowledgments

In this section you can place the information related to Identification of funding sources; Current contact information of corresponding author; and gratitude to other people involved with the conduct of the experiment. In this part of the paper the conflict of interest information must be included. In particular, authors should: 1) Disclose professional relationships with companies or manufacturers who will benefit from the results of the present study, 2) Cite the specific grant support for the study and 3) State that the results of the present study do not constitute endorsement of the product by the authors or the NSCA. Failure to disclose such information could result in the rejection of the submitted manuscript.

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