

ARYANE FLAUZINO MACHADO

**EFEITOS IMEDIATOS E TARDIOS DA IMERSÃO EM ÁGUA FRIA PÓS-
EXERCÍCIO: UMA REVISÃO SISTEMÁTICA E UM ENSAIO CLÍNICO
RANDOMIZADO**

Presidente Prudente

2015

ARYANE FLAUZINO MACHADO

**EFEITOS IMEDIATOS E TARDIOS DA IMERSÃO EM ÁGUA FRIA PÓS-
EXERCÍCIO: UMA REVISÃO SISTEMÁTICA E UM ENSAIO CLÍNICO
RANDOMIZADO**

Dissertação apresentada à Faculdade de Ciências e Tecnologia da Universidade Estadual Paulista "Júlio de Mesquita Filho" (FCT/UNESP) – Presidente Prudente, para obtenção do título de mestre no Programa de Pós-Graduação *Stricto Sensu* em Fisioterapia.

Orientador: Prof. Dr. Carlos Marcelo Pastre

Coorientador: Prof. Dr. Paulo Henrique Ferreira

Presidente Prudente


2015

FICHA CATALOGRÁFICA

	Machado, Aryane Flauzino
M129i	Efeitos imediatos e tardios da imersão em água pós-exercício: uma revisão sistemática e um ensaio clínico / Aryane Flauzino Machado - Presidente Prudente : [s.n.], 2015
	110 f.
	Orientador: Carlos Marcelo Pastre Coorientador: Paulo Henrique Ferreira
	Dissertação (mestrado) - Universidade Estadual Paulista, Faculdade de Ciências e Tecnologia
	Inclui bibliografia
	1. Recuperação da função fisiológica. 2. Crioterapia. 3. Imersão. I. Pastre, Carlos Marcelo. II. Ferreira, Paulo Henrique. III. Universidade Estadual Paulista. Faculdade de Ciências e Tecnologia. IV. Efeitos imediatos e tardios da imersão em água pós-exercício: uma revisão sistemática e um ensaio clínico

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação Serviço Técnico de Biblioteca e Documentação – UNESP, Campus de Presidente Prudente.

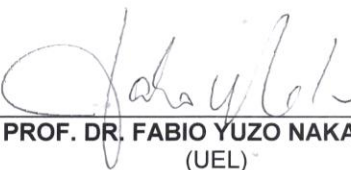
BANCA EXAMINADORA



PROF. DR. CARLOS MARCELO PASTRE
(ORIENTADOR)



PROF. DR. RAFAEL ZAMBELLI DE ALMEIDA PINTO
(FCT/UNESP)



PROF. DR. FABIO YUZO NAKAMURA
(UEL)



ARYANE FLAUZINO MACHADO

PRESIDENTE PRUDENTE/SP, 8 DE MAIO DE 2015.

RESULTADO: APROVADA

Dedicatória

*À minha amada família por todo
companheirismo, apoio e dedicação. Meus pais
Jurandir e Vânia e meu irmão Gabriel. Nossa
vitória, nossa conquista!*

Agradecimientos

Há muito o que e a quem agradecer.

À Deus minha infinita gratidão, pela paz interior e sabedoria. Obrigada por permitir a realização desse sonho. A Nossa Senhora Aparecida por me iluminar em todos os momentos da minha vida.

A minha família. Meus pais, amigos e parceiros, Vânia e Jurandir. Agradeço todo amparo e suporte em todo esse processo, e principalmente por tornarem esse sonho, um sonho nosso! Ao meu pequeno-grande homem, meu irmão Gabriel. À eles, que mesmo distantes, seja há 200 ou 15000 km, se fizeram presente, dedico e agradeço todo essa conquista. Serei eternamente grata a vocês. Minha inspiração de caráter, dedicação e amor. Amo vocês incondicionalmente!

Ao meu orientador, Prof. Carlos Marcelo Pastre. Agradeço pela oportunidade, pelos ensinamentos e pela amizade. Por me mostrar o caminho e me encorajar a ser e fazer sempre o melhor. Grandes desafios foram superados e oportunidades aproveitadas graças ao seu apoio. Obrigada por toda atenção e incentivo. Espero que essa jornada não acabe aqui!

Ao meu coorientador, Prof. Paulo H. Ferreira que me recebeu de portas e braços abertos em Sydney. Responsável por grande parte do crescimento profissional e pessoal que o estágio no exterior me proporcionou. Obrigada por toda atenção destinada a mim e a nossa pesquisa.

Ao querido Prof. Jayme Netto pelos abraços matinais e principalmente por todos os ensinamentos, sejam eles profissionais ou pessoais. Muito obrigada!

Ao amigo Prof. Fábio A. N. Martini que sempre me incentivou a seguir essa trajetória. Um grande amigo que não mediu esforços e que depositou uma grande confiança em mim. Serei eternamente grata!

Ao meu amado companheiro Rafael Rodrigues por todos os momentos. Pela paciência, atenção, dedicação e carinho em todo esse processo. Muito obrigada!

Aos meus companheiros de todos os dias Aline Castilho, Ítalo Lemes e Jéssica Micheletti. Obrigada por toda atenção, amizade e dedicação. Somos uma equipe incrível e assim, estamos aos poucos conquistando o que almejamos. Muito obrigada. Desejo a vocês sucesso. Muito sucesso!

Aos integrantes do Laboratório de Fisioterapia Desportiva (LAFIDE – FCT/UNESP), por toda dedicação destinada a essa pesquisa. Obrigada por terem me acolhido. Espero que eu tenha conseguido retribuir toda confiança e amizade. Orgulho-me sempre em fazer parte dessa equipe!

Aos integrantes do Arthritis and Musculoskeletal Research Group (AMRG – The University of Sydney) pela atenção, paciência, ensinamentos e recepção incrível! Espero reencontrá-los em breve. Agradeço especialmente ao Prof. Paulo, Prof. Evangelos Pappas, seus alunos de doutorado Marina Pinheiro, Amabile Dario e Matt Fernandez e os companheiros de viagem Ítalo R Lemes e Nathalie F Souto.

Aos meus amigos. A todos meus amigos que participaram dessa conquista. Aqueles que estiveram presente durante as conquistas e as dificuldades e que, de maneira singular, tornaram esse processo mais fácil. Em especial, as minhas amigas Aline, Jaqueline e Jéssica, que alegraram meu dia-a-dia, e que, compartilhando das mesmas alegrias e angústias, tornaram-se essenciais.

Aos membros da banca examinadora, Prof. Dr. Fabio Nakamura e Prof. Dr. Rafael Zambelli, pela disponibilidade em participar e pelas contribuições.

Aos funcionários da FCT/UNESP por toda atenção e dedicação, principalmente ao André T Meira que sempre esteve pronto a ajudar, com o máximo de educação, profissionalismo e simpatia.

Aos participantes dessa pesquisa que não hesitaram em colaborar momento algum. Que proporcionaram não só esse trabalho, mas também divertidas noites de coleta de dados.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (processos nº 2013/12474-7 e 2014/03778-5) pelo apoio financeiro destinado a essa pesquisa.

E por fim, agradeço a todos que contribuíram direta ou indiretamente para que mais essa etapa pudesse ser concluída. Muito obrigada!

Επίγραφε

*“Reconheça o que está ao alcance dos
seus olhos e o que está oculto tornar-se-á claro
para você.”*

(Autor desconhecido)

SUMÁRIO

Apresentação	14
Introdução	16
Artigo I: <i>Can the water temperature and immersion time influence the effects of cold water immersion on pain? A systematic review and meta-analysis</i>	18
Artigo II: <i>Immediate and delayed effects of cold water immersion after eccentric exercise-induced muscle damage: randomized controlled trial</i>	48
Conclusões	77
Referências	78
Anexos	80

Essa dissertação está apresentada em consonância com as normas do modelo alternativo de dissertação do Programa de Pós-Graduação *Stricto Sensu* em Fisioterapia da Faculdade de Ciências e Tecnologia da Universidade Estadual Paulista “Júlio de Mesquita Filho”. O conteúdo desse trabalho contempla o material originado a partir da pesquisa intitulada “*Efeitos imediatos e tardios da imersão em água fria pós-exercício: uma revisão sistemática e um ensaio clínico randomizado*” que foi realizada em duas etapas:

1-) Revisão Sistemática e Meta-análise, realizada na *The University of Sydney, Faculty of Health Sciences*, Sydney – NSW, Austrália, financiada pela Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Linha de Fomento: Bolsa de Pesquisa de Estágio no Exterior, processo: 2014/03778-5);

2-) Ensaio clínico randomizado, realizado na Univ. Estadual Paulista “Júlio de Mesquita Filho”, Faculdade de Ciências e Tecnologia, Presidente Prudente – SP, Brasil, financiada pela Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior – CAPES e posteriormente financiada pela Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Linha de Fomento: Bolsa de Mestrado no País, processo 2013/12474-7).

Assim, o presente material está dividido nas seguintes sessões:

- Introdução ao tema, para contextualização do tema pesquisado;
- Artigo I: Machado AF, Ferreira PH, Micheletti JK, Almeida AC, Lemes IR, Vanderlei FM, Netto Junior J, Pastre CM. *Can the water temperature and immersion time influence the effects of cold water immersion on pain? A systematic review and meta-analysis*; em revisão pelo periódico *Sports Medicine*;
- Artigo II: Machado AF, Almeida AC, Micheletti JK, Netto Junior J, Vanderlei FM, Netto Junior J, Pastre CM. *Immediate and delayed effects of cold water immersion after*

eccentric exercise-induced muscle damage: randomized controlled trial; em revisão pelo periódico *PLoS ONE*;

- Conclusões, a partir de ambas as pesquisas realizadas;
- Referências, em formato recomendado pelo *International Committee of Medical Journals Editors* (ICMJE), para as referências citadas na introdução.

Ressalta-se que cada artigo está apresentado de acordo com as normas dos seus respectivos periódicos, apresentadas em anexo ao final (com exceção das figuras que estão apresentadas no texto principal).

O processo de recuperação pós-exercício é fundamental para a preservação de estruturas e tecidos, bem como para a manutenção das funções motoras, visando à prevenção de agravos ou melhora da *performance* e rendimento do atleta. Seu objetivo principal é a restauração dos diferentes sistemas do corpo a condições basais, ou seja, a níveis pré-exercício^{1,2}.

Dentre as diversas técnicas que aceleram a recuperação pós-exercício, a crioterapia é um procedimento comumente utilizado na prática esportiva e é proposta para redução da resposta inflamatória em caso de lesão tecidual e dor muscular resultante do esforço físico, apresentando evidências particulares na aplicação após o dano muscular induzido por exercício (DMIE)³⁻⁵.

Uma das modalidades da crioterapia é a imersão em água fria (IAF), que consiste na imersão de parte do corpo em água com temperatura igual ou inferior à 15°C⁶. Essa estratégia de recuperação aparece no cenário atual como uma técnica eficaz e de baixo custo, facilmente reproduzida em diferentes situações. Os potenciais efeitos dessa estratégia de recuperação têm sido avaliados a partir de diferentes marcadores de dano muscular, tais como concentração sanguínea de creatina quinase (CK)^{5,7-9}, dor^{3,4,7-10}, alteração de sensibilidade¹⁰, percepção de recuperação¹¹ e força^{3-5,7-9,11}.

Sabe-se que a IAF é capaz de reduzir a temperatura do tecido muscular³, a permeabilidade celular de vasos sanguíneos, linfáticos e capilares devido à vasoconstrição, com consequente diminuição da difusão dos fluidos nos espaços intersticiais^{4,12,13} além de reduzir a velocidade da condução nervosa, a atividade do fuso muscular, a espasticidade e a dor³. Entretanto, autores afirmam que apesar dos efeitos relatados não há evidência fisiológica

clara para confirmar essas teorias e que os reais efeitos ainda não estão plenamente elucidados⁹.

Apesar dos resultados, os indivíduos ainda respondem de maneira diferente aos sinais e sintomas. Esse fato pode ser explicado pela utilização de diferentes metodologias adotadas e influenciado pela complexidade e especificidade de cada indivíduo¹⁴. Pastre *et al.*¹⁵ atribuíram parte do cenário aos diferentes métodos de controle utilizados pelos pesquisadores tanto na maneira de indução de estresse quanto à forma de aplicação da técnica. Glasgow *et al.*⁹ concordam ao afirmar que na prática clínica existe uma grande variação de protocolos de IAF, que se diferem principalmente quanto à temperatura da água e ao tempo de imersão. Essa variação corresponde à dose no qual o indivíduo é exposto, influenciando nos futuros desfechos.

Deve-se refletir à luz desta situação problema que requer uma melhor fundamentação científica a fim de eliminar possíveis vieses de rotina e de interpretação de resultados e objetivando identificar a melhor estratégia baseada na relação da dose-resposta na aplicação da IAF pós-exercício.

Assim, o objetivo da presente pesquisa foi determinar os efeitos da imersão em água fria pós-exercício, por meio de diferentes tipos de estudo, como a revisão sistemática e meta-análise e o ensaio clínico randomizado, além de identificar a melhor dose de aplicação dessa técnica, por meio da relação da dose-resposta e os possíveis efeitos deletérios.

Can water temperature and immersion time influence the effect of cold water immersion on pain? A systematic review and meta-analysis

Different protocols of CWI on pain: a meta-analysis

Aryane Flauzino Machado¹, Paulo Henrique Ferreira², Jéssica Kirsch Micheletti¹, Aline Castilho de Almeida³, Ítalo Ribeiro Lemes¹, Franciele Marques Vanderlei¹, Jayme Netto Junior¹, Carlos Marcelo Pastre¹

¹Univ. Estadual Paulista, Faculdade de Ciências e Tecnologia, Departamento de Fisioterapia, Presidente Prudente, Brazil

²The University of Sydney, Faculty of Health Science, Discipline of Physiotherapy, Sydney, Australia

³Univ. Federal de São Carlos, Centro de Ciências Biológicas e da Saúde, Departamento de Fisioterapia, São Carlos, Brazil

Corresponding author

Carlos Marcelo Pastre

305 Roberto Simonsen – Presidente Prudente / São Paulo, Brazil. Postcode: 19060-900

E-mail: pastre@fct.unesp.br

Telephone number: +55 18 3229 5528

ABSTRACT

Background: Cold water immersion (CWI) is a technique commonly used in post-exercise recovery. However, the procedures involved in the technique may vary, particularly in terms of water temperature and immersion time, and the most effective approach remains unclear.

Purpose: To determine the efficacy of CWI in pain management compared with passive recovery. We also aimed to identify which water temperature and immersion time provides the best results.

Methods: MEDLINE, EMBASE, SPORTDiscus, PEDro and The Cochrane Library databases were searched up to January 2015. Only randomized controlled trials that compared CWI to passive recovery were included in this review.

Results: Nine studies were included in the review and meta-analysis. The results of meta-analysis revealed that CWI has a more positive effect than passive recovery in terms of immediate (WMD=0.290, 95% CI [0.037, 0.543]; p=0.025) and delayed effects (WMD=0.315, 95% CI [0.048, 0.581], p=0.021). The pooled of studies that used water temperature of between 10-15°C demonstrated the best results for immediate (WMD=0.273, 95% CI [0.107, 0.440], p=0.001) and delayed effects (WMD=0.317, 95% CI [0.102, 0.532], p=0.004). In terms of immersion time, immersion of between 10-15 minutes had the best results for immediate (WMD=0.227, 95% [0.139, 0.314], p=0.000) and delayed effects (WMD=0.317, 95% [0.102, 0.532], p=0.004).

Conclusions: CWI is more effective than passive recovery in management of pain. The results also demonstrated the presence of a dose-response relationship, indicating that CWI with a water temperature of between 11 and 15°C and an immersion time of 11 to 15 minutes provided the best results.

Keywords: recovery of function; cryotherapy; immersion; muscle soreness.

1. BACKGROUND

Several post-exercise recovery techniques are currently employed in an attempt to return the body to its pre-exercise state [1,2]. Cold water immersion (CWI) has become popular in sports [3,4] as it is a low-cost technique that is easily performed in different situations [5] and has been found to minimize the immediate and delayed negative effects of exercise [6]. Compared to controlled interventions and other traditional recovery techniques, CWI achieves positive pain reduction results following a range of exercise types [7,8]. Yet the specific mechanisms associated with CWI response are unknown [9,10].

Despite its widespread use, significant procedural variations in CWI exist [11,12]. Investigations have suggested that water temperature contributes to the beneficial effect of CWI [6]. However, other factors may influence recovery. Pastre et al. [13] attribute response variation to differences in the application of CWI, such as water temperature, immersion time and type of CWI.

In recent years, the number of studies focusing on CWI has increased, and major systematic reviews have been performed to compare the effects of CWI and other pain recovery strategies [8,12]. However, the dose-response relationship of this technique has not yet been investigated. Bleakley et al. [12] found no clinical trials comparing different procedures while authors [10] showed that studies focusing on different CWI application strategies can contribute to determining the risks and benefits for athletes.

A systematic review involving the dose-response relationship will clarify the most effective method of application of CWI for post-exercise pain. Therefore, the purpose of this systematic review was to determine the efficacy of cold water immersion on management of pain compared with control intervention (passive recovery). An analysis of which dosage of application provides the best results, focusing on water temperature and immersion time, was also undertaken.

2. METHODS

This systematic review was registered in an international database of systematic reviews in health and social care. (Available: registration number CRD42015016573; <http://www.crd.york.ac.uk/PROSPERO/>).

2.1 Search strategies

Studies were selected after searching five databases (MEDLINE, EMBASE, SPORTDiscus, PEDro and The Cochrane Library) from the earliest record of each database to January 21, 2015. The terms and keywords used to search optimization were related to randomized controlled trials; cold water immersion and post-exercise recovery (see details in Appendix 1). The reference list of eligible clinical trials was searched by hand to complement the electronic searches.

2.2 Study selection

The studies selected involved CWI treatment of human participants and assessed the effect on immediate and/or delayed pain or muscle soreness. CWI was defined as immersion in water with a temperature less than or equal to 15°C [5,6,11]. To be eligible, studies had to 1) be randomized controlled trials, comparing cold water immersion and control conditions (passive recovery) post-exercise; 2) be studies that assessed muscle soreness; 3) be studies that used a single session of exercises; 4) apply CWI within 1 hour of the end of the exercise; 5) include only one immersion on the first day. Studies using intermittent immersions or more than one immersion on subsequent days were excluded. No restrictions were applied to the sample conditions (age, gender, exercise level) or language of the studies.

The study selection process was conducted by title, followed by abstract and full text (Figure 1). These steps were performed independently by two authors (ACA and JKM) and consensus was used to solve disagreements.

2.3 Data extraction

Outcome data, including mean scores, SDs (final values) and sample size was extracted by two reviewers (AFM, JKM). The data extraction process was performed using a standardized form that included details such as characteristics of participants, exercise procedures, cold water immersion procedures, outcome measures and methodological characteristics. Disagreements between authors regarding data extraction were resolved by consensus.

Some studies included multiple observations. In such cases, data was extracted at a clinically relevant time point in order to analyze: immediate effects (up to 24 hours post-exercise) and delayed effects (after 24 hours post-exercise). For the delayed effects, the peak pain of the control group was considered, in order to minimize interference caused by the intervention. Pain scores were converted to a common 0-10 scale.

2.4 Quality Assessment

All studies included were assessed for methodological quality. This process was performed by two independent reviewers (AFM and JKM) using the PEDro Scale [14,15]. Each study was assessed for random allocation, concealed allocation, baseline comparability, blinding participants, therapists and assessors, adequate follow-up, intention-to-treat, between-group comparison, point estimates and variability. If trials had already been assessed and listed on the PEDro database, such scores were adopted. Methodological quality was not an inclusion criteria.

2.5 Data synthesis and analysis

Analysis of the temperature and immersion time of each study was performed. It was necessary to establish cutoff points for each of these covariates. For water temperature analysis two categories were created: severe cold, with water temperature between 5-10°C; and moderate cold, where temperature was between 11-15°C. Three categories were used for immersion time: short, immersions of 10 minutes or less; medium, immersions of 11-15 minutes; and longer, with immersions between 16-20 minutes.

Comprehensive Meta-Analysis software, version 2.2.04 (Biostat, USA) was used for all analysis and pooled estimates were calculated using a random-effect model, due to the heterogeneity of the studies (represented by I^2). Data was pooled in meta-analyses and described as weighted mean differences (WMD) with 95% confidence intervals (CI). The immediate and delayed effects were calculated in order to analyze the effect of cold water immersion, independent of water temperature and duration of immersion. In case of more than one intervention group per study, the group that represented the lowest effect size was used.

3. RESULTS

The database search identified 258 studies and 17 were chosen for full text review. Of these articles, eight were excluded: one was not a single exercise session, one used a cryotherapy technique other than cold water immersion and six did not feature an appropriate immersion, based on the inclusion criteria. Figure 1 shows the schematic process of the study selection based on a PRISMA flow diagram.

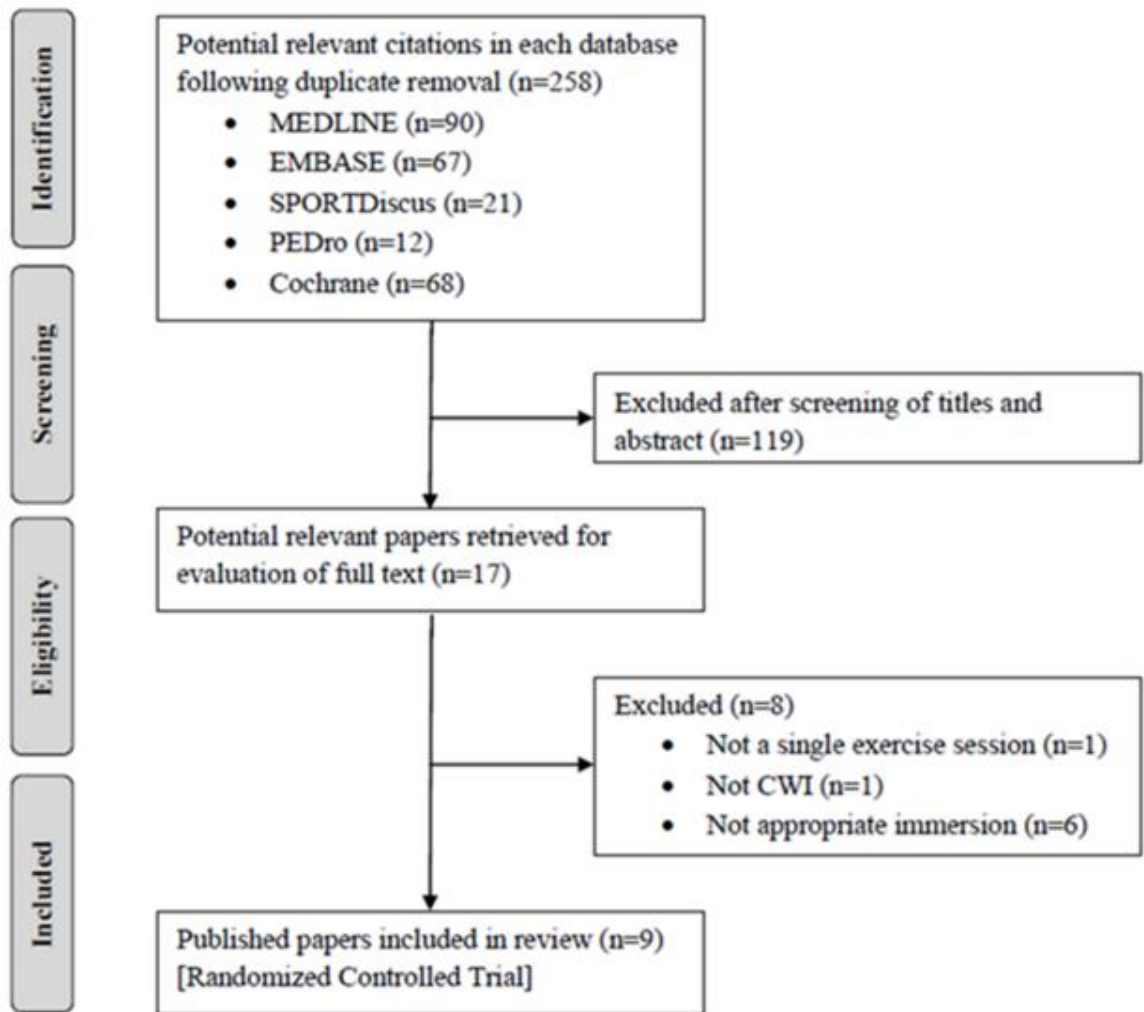


Fig.1 Flow chart for selection of studies

Assessment of the methodological quality of the studies included using the PEDro scale reported a mean of 4.2. Three studies [16–18] were considered as 'moderate quality' and another six studies as 'poor quality' [2,9,19–22]. Due to the type of intervention, blinding was often not possible, but 44.4% of the studies described adequate follow-up procedures (see details in Appendix 2). Figure 2 shows the number of clinical trials that fulfilled each criterion.

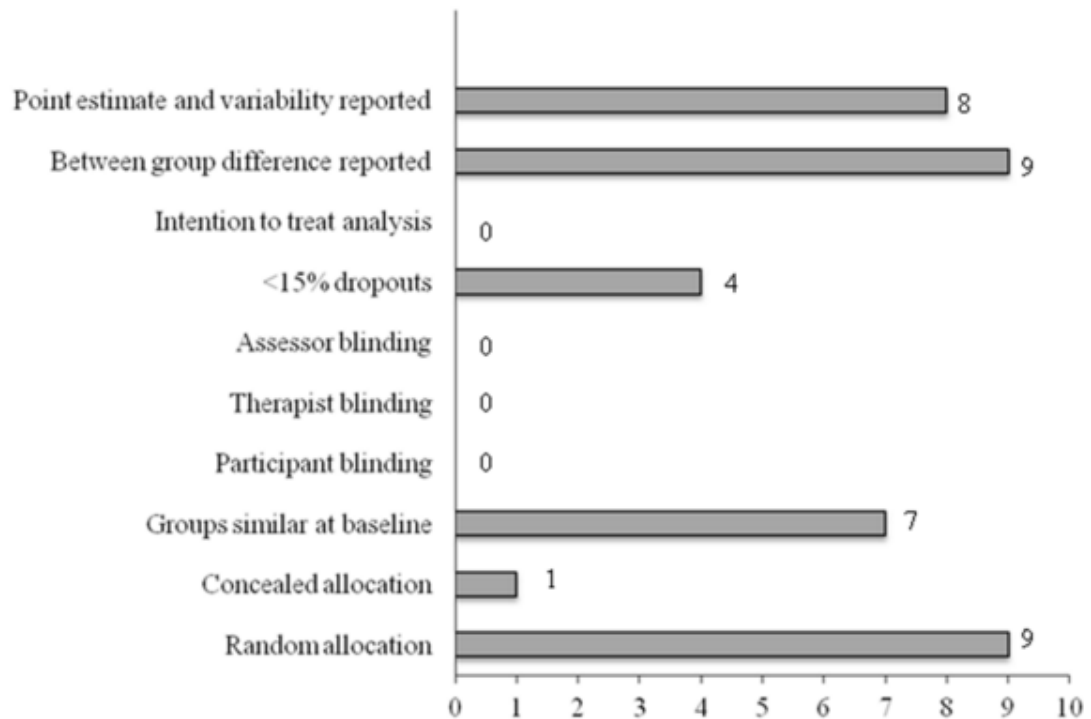


Fig.2 Number of trials for each PEDro criteria

The nine eligible studies were published between 2007 [9] and 2015 [16]. These studies comprised a total of 169 participants (male, n=141; female=28). The health conditions of the participants, the level of exercise, fluctuated between physically active and athletes.

The studies were from Australia [2,17,18,21], the United Kingdom [9,16,22] and USA[19,20]. All were randomized controlled trial type studies, while six were parallel group trials [9,16,19–22], and three used a cross-over design [2,17,18]. Exercise protocols consisted primarily of exercises that required high physical ability with possible subsequent onset of

pain, such as shuttle running [9,16], downhill running [19], Australian football match and training [17,21], high intensity intervals [2,18] and counter-movement jumps [22].

Interventions were varied. Water temperature ranged from 5 [19] to 15°C [2] and immersion time varied between 5 [18] and 20 [19] minutes. All studies used passive recovery in which participants had to remain seated with minimal movement. Immersion depth ranged from immersion of the lower limbs [9,19,22] to immersion of the whole body, excluding only the head and neck [18]. It was observed that six [2,9,16,17,19–21] of eight studies that evaluated delayed effect on pain found peak pain at 24 hours post-exercise, and only one [22] found peak pain at 48 hours post-exercise.

The characteristics of the included studies are summarized in Table 1.

Table 1. Characteristics of the included studies

Study, year	Study design	Characteristics of participants	Exercise protocol	CWI group	Control group	Pain assessment	Time of assessment	Time of analysis	PEDro Score
Leeder, 2015 [16]	Parallel groups	N=24 male; well trained 21±3 years	Intermittent shuttle running	14°C; 14 minutes; n=8 TI: Immediately post-exercise WL: DNR	Remained seated 14 minutes; n=8	VAS=0-200mm Squat at 90° knee flexion	24, 48, 72 hours post-exercise	24 hours post-exercise	6
Crystal, 2013 [19]	Parallel groups	N=20 male 21.2±2.3 years	Downhill run	5±2°C; 20 minutes; n=10 TI: DNR WL: Up to top of the thigh	Position: DNR; n=10	VAS= 0-100mm Leg soreness while walking down the stairs	Immediately, 1, 6, 24, 48 e 72 hours post-exercise	1 and 24 hours post-exercise	4
Getto, 2013 [20]	Parallel groups	N=23 13 male; 10 female Age: DNR	Exhaustive exercise session	10°C; 10 minutes; n=7 TI: Immediately post-exercise WL: Up to level of chest	Remained seated 10 minutes; n=8	VAS= 0-60 Calves, quadriceps, hamstrings, hip adductors, hip abductors and low back	Immediately post-exercise and immediately and 24 post-intervention	Immediately post-intervention and 24 hours post-exercise	3
Elias, 2013 [21]	Parallel groups	N=24 male; Australian football players 19.9±2.8 years	Australian football match	12°C; 14 minutes; n=7 TI: Within 12 minutes post-exercise WL: Up to xiphoid process	Remained seated 14 minutes; n=8	VAS= 0-100mm DNR	Immediately, 1, 24, 48 hours post-exercise	1 and 24 hours post-exercise	4
Elias, 2012 [17]	Cross-over	N=14 male; Australian football players 20.9±3.3 years	Australian football training	12°C; 14 minutes; n=14 TI: Within 12 minutes post-exercise WL: Up to xiphoid process	Remained seated; 14 minutes; n=14	VAS=0-100mm DNR	Immediately, 1, 24, 48 hours post-exercise	1 and 24 hours post-exercise	5
Stanley, 2012 [23]	Cross-over	N= 18 male; cyclist 27±7 years	High intensity interval session	14,2±0,6°C; 5 minutes; n=18 TI: 20 minutes post-exercise WL: Body excluding head and neck	Remained seated 10 minutes; n=18	VAS=1-10 Leg soreness	Immediately post-intervention	Immediately post-intervention	5

Brophy-Williams, 2011[2]	Cross-over	N= 8 male; well trained 20.9±1.2 years	High intensity interval session	15±1°C; 15 minutes; n=8 TI: Immediately post- exercise WL: Up to mid- sternum	Remained seated 15 minutes; n=8	VAS=0-7 DNR	24 hours post- exercise	24 hours post- exercise	4
Jakeman, 2009[22]	Parallel groups	N=18 female; athletes 19.9±0.97 years	Counter- movement jumps	10±1°C; 10 minutes; n=9 TI: Within 10 minutes post-exercise WL: Up to level of the superior iliac crest	Remained seated 10 minutes; n=9	VAS=0-10 Unweighted squat at 90° knee flexion (2 s)	1, 24, 48, 72, 96 hours post- exercise	1 and 48 hours post-exercise	3
Bailey, 2007[9]	Parallel groups	N=20 male; healthy 22.3±3.3 years	Intermittent shuttle running	10±0,5°C; 10 minutes; n=10 TI: Immediately post- exercise WL: Up to level of iliac crest	Remained seated 10 minutes; n=10	VAS=1-10 General whole- body soreness; palpation of major muscle group	Immediately, 1, 24, 48, 168 hours post- exercise	1 and 24 hours	4

TI: time of immersion; WL: water level; DNR: Data not reported; VAS: Visual Analog Scale; °C: degrees Celsius

3.1 Analysis of water temperature

Seven studies [9,17–22] provided data related to the immediate effects of cold water immersion. The subgroup analysis of the pooled results is shown in Figure 3. A general analysis of the immediate effects shows a significant pooled effect for cold water immersion (WMD=0.290, 95% CI [0.037, 0.543]; $p=0.025$). When subgroups were analyzed, it was observed that studies using a water temperature of between 11-15°C (moderate cold) produced better results than those using water between 5-10°C (severe cold). Therefore, temperatures higher than 10°C present the best results for immediate effect on pain (Severe cold: WMD=0.144, 95% CI [-1.299, 1.526], $p=0.875$]; Moderate cold: WMD=0.273, 95% CI [0.107, 0.440], $p=0.001$).

Eight studies [2,9,16,17,19–22] were included in the analysis of water temperature on delayed effects, with pooled results showing a tendency similar to immediate effect results (Figure 4). Overall pooled results, independent of water temperature, showed a statistically significant difference in favor of cold water immersion (WMD=0.315, 95% CI [0.048, 0.581], $p=0.021$). Analysis of subgroups revealed that water at temperatures of between 11-15°C showed the best results (Severe cold: WMD=0.057, 95 % CI [-1.483, 1.598], $p=0.942$]; Moderate cold: WMD=0.317, 95% CI [0.102, 0.532], $p=0.004$).

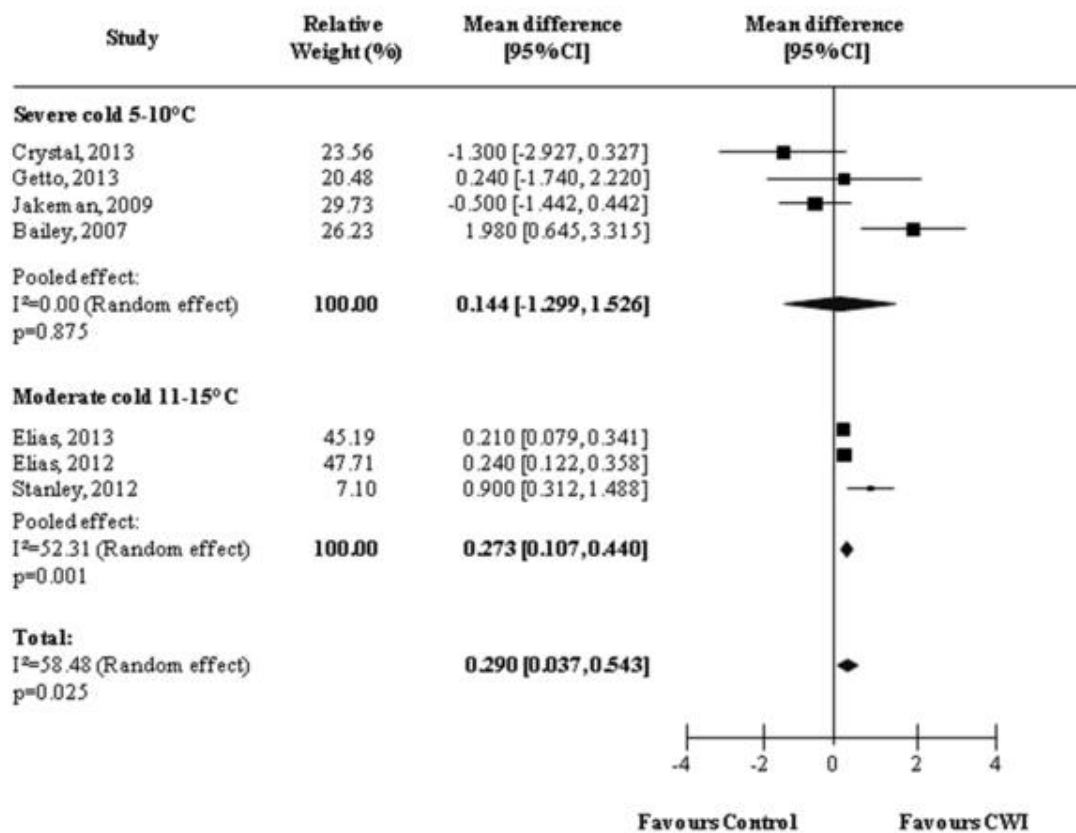


Fig.3 Forest plot of comparison: passive recovery and CWI. Subgroup: water temperature. Time point: immediate effect.

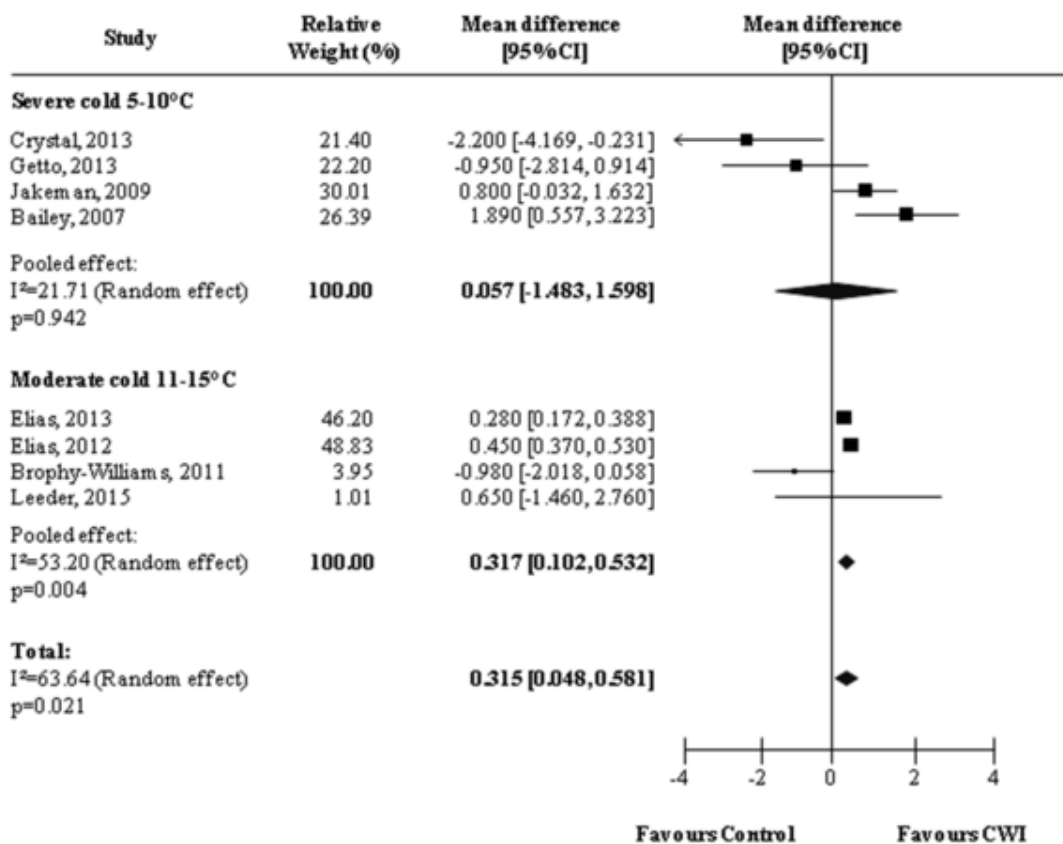


Fig.4 Forest plot of comparison: passive recovery and CWI. Subgroup: water temperature. Time point: delayed effect.

3.2 Analysis of immersion time

Figure 5 shows the results of analysis of immediate effect in relation to immersion time. Overall, CWI, was more effective than the control condition (WMD=0.290, 95% CI [0.037, 0.543]; p=0.025). Three categories were used for subgroup analysis: short, medium and longer immersion. The medium immersion category, which had duration of between 10-

15 minutes, was responsible for the best results in terms of immediate effects. Although there is only one study featuring 'longer immersion' [19] it was observed no effect for this category (Short immersion: WMD=0.646, 95% [-0.360, 1.652], p=0.208; Medium immersion: WMD=0.227, 95% [0.139, 0.314], p=0.000; Longer immersion: WMD=-1.300 [-2.927, 0.327], p=0.117).

In terms of delayed effects, the overall pooled effects of CWI described in the eight studies analyzed were positive (WMD = 0.315, 95% CI [0.048, 0.581], p = 0.021) (Figure 6). As with the immediate effects, an immersion time of between 11-15 minutes produced the best results (Short immersion: WMD=0.728, 95% [-0.561, 2.017], p=0.268; Medium immersion: WMD=0.317, 95% [0.102, 0.532], p=0.004; Longer immersion: WMD=-2.200 [-4.169, -0.231], p=0.029).

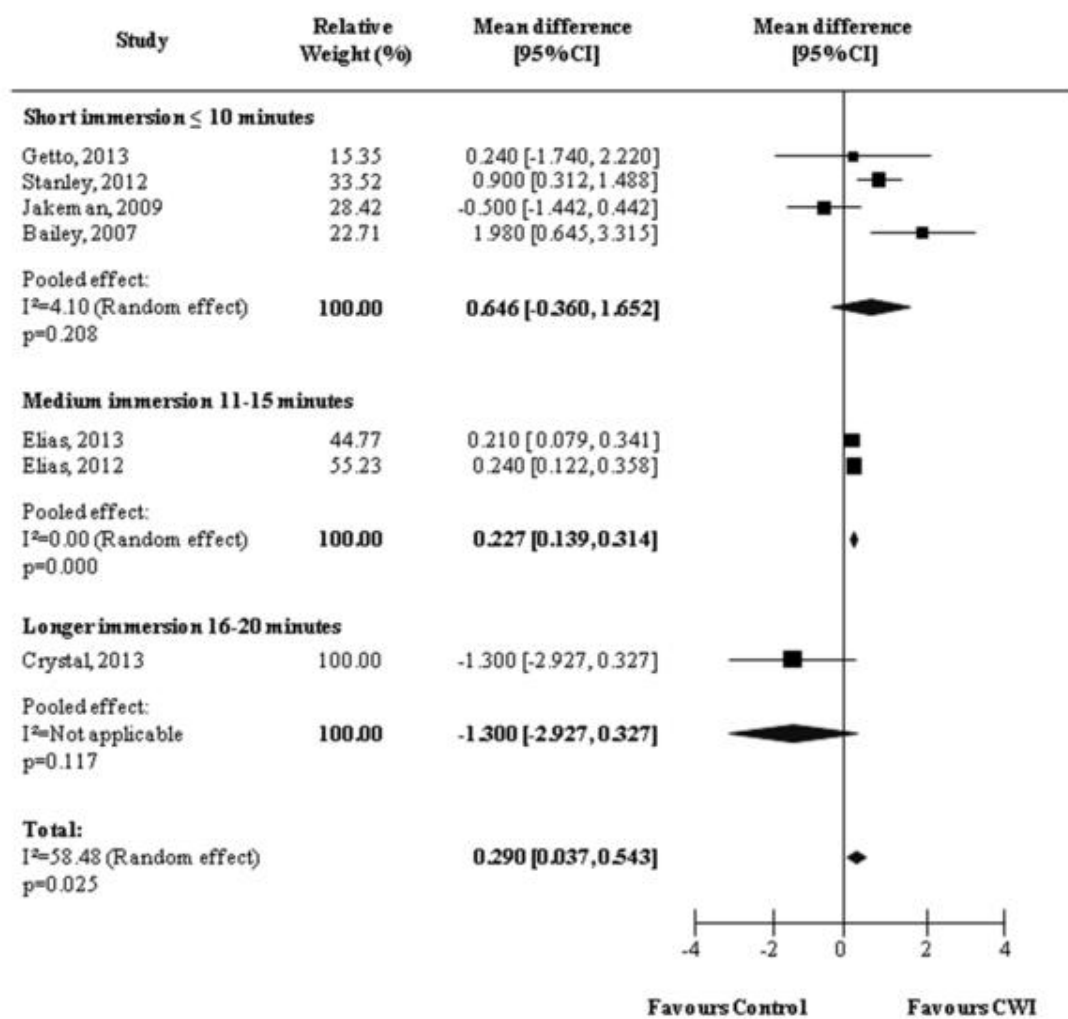


Fig.5 Forest plot of comparison: passive recovery and CWI. Subgroup: immersion time. Time point: immediate effect.

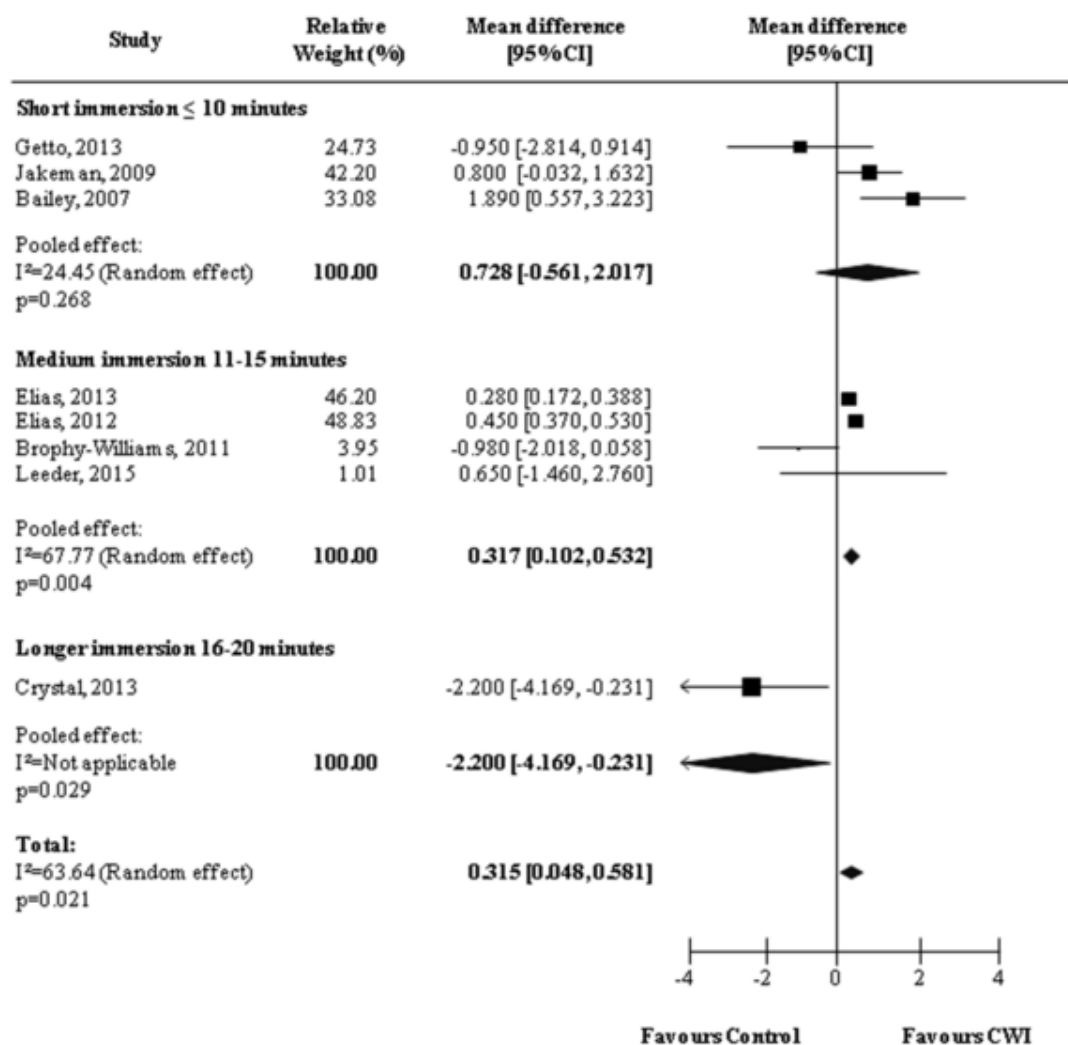


Fig.6 Forest plot of comparison: passive recovery and CWI. Subgroup: immersion time. Time point: immediate effect.

4. DISCUSSION

The results of meta-analysis of CWI as a post-exercise recovery technique and reliever of muscle soreness were consistent, and revealed the following findings: 1) independent of time and temperature, CWI produces generally positive results in terms of

both immediate and delayed effects; 2) immersion in water at temperatures between 11-15°C appeared to produce a greater reduction of muscle soreness after exercise; 3) 11-15 minutes appeared to be the optimal immersion time for the relief of muscle soreness caused by exercise.

The findings regarding CWI, independent of immersion temperature and time, are in accordance with previous reviews, such as the studies by Leeder et al. [8] and Bleakley et al. [12]. The authors claim that the technique is capable of altering blood flow, thereby causing vasoconstriction and redirection of the blood. The real effects of CWI have not been fully elucidated [4], but it has been speculated that this technique is able to reduce lymphatic and capillary cell permeability [24], resulting in vasoconstriction and consequent reduction of the inflammatory process caused by exercise [6]. The technique also can reduce nerve transmission and muscle spasm. The reduction of pain can therefore be explained by these factors, together with analgesia, which occurs in response to the reduction of the pain-spasm cycle [25].

While the effects of CWI have been widely investigated, opinions vary with regard to method of application, immersion time and water temperature [13]. One hypothesis is that outcomes may differ depending on the recovery strategy used. The variance in effects caused by temperature change observed in this review revealed that CWI was more effective in terms of both immediate and delayed effects when temperatures were in the 'moderate cold' category. The mean temperature of this category in studies was 12.7° C for immediate effect and 13.2° C for delayed effect.

The benefits of 'moderate cold' temperatures have not been discussed in clinical studies. However, it has been shown that immersion in very low temperatures can cause adverse effects, interpreted by the body as noxious stimuli, and that peak pain appears around

3°C [26]. This may explain the generally negative effects described in the study by Crystal et al. [19], which featured CWI at 5°C.

A study by Getto et al. [20] claims that short immersions are less efficient at lessening muscle pain caused by exercise, due to limited muscle temperature reduction [5]. Such statement confirms the findings of this study, which indicate that medium immersions of between 11 and 15 minutes produce better results than short immersions (≤ 10 minutes). Additionally, during the immediate effect, there is the presence of the category 'longer immersion', responsible for the worst results. Although pooled results were not available for this category due to an insufficient number of studies ($n=1$), it compared unfavorably with passive recovery. The study in question [19] considered the use of a very low temperature (5°C) for 20 minutes. Davis et al. [27] claimed that for CWI to produce harmful stimulation and pain, an application of ten seconds at low temperatures was required. Such effects can be exacerbated during long immersion conditions [28,29].

Accordingly, it is important to analyze studies by subdivisions of water temperature and immersion time. The limited number of studies, however, does not allow the implementation of closed protocol comparisons. The relationship between the two variables could allow objective inferences about the most effective recovery model to be used.

As Crystal et al. [19], other studies produced results that compared unfavorably with passive recovery. Getto et al. [20] used a scale that involved six different pain points, including the low back, to analyze pain. This type of evaluation considers a larger number of pain points than other studies, and can result in participant confusion in relation to the effects of CWI. Jakeman et al. [22] found that results of immediate effects of CWI compared unfavorably with control following countermovement jumps. Goodall et al. [30] and Howatson et al. [31] used similar exercise procedures and observed a reduction of pain in the

CWI group only after 24 hours post-exercise. These adverse results can be explained by the type of muscle stress and pain magnitude [32].

Overall, the studies selected for this review show similar models of inducing stress, represented by physical activities featuring high intensity of effort. This factor is relevant to data interpretation, as different types of stress provide different outcomes, as previously explained. For example, the characteristics of injuries induced in localized eccentric exercise can differ from those sustained during sporting activities, and respond differently to the application of CWI [7,32].

To the authors' knowledge this is the first systematic review and meta-analysis to investigate the effects of different CWI procedures, namely variations in water temperature and immersion time. The strengths of this systematic review relate mainly to the rationale of the study, which aims to analyze the dose-response relationship, which is still poorly investigated in studies of this nature. One of the limitations of the study is the poor methodological quality of the studies included, as assessed by PEDro Scale. Future trials should be attentive to the criteria for the development of a high quality study, which would result in surveys with greater scientific evidence. Another limitation is that the research focused only on pain. Although it is a key outcome in recovery of an athlete, further studies should consider the dose-response effect of CWI on other markers of muscle damage, in order to identify the best CWI recovery strategy based on different factors.

5. CONCLUSION

The findings of the present study suggest that CWI is more effective than passive recovery in management of pain. The results also demonstrate the presence of a dose-response relationship, indicating that CWI provides the most effective results at temperatures between

11 and 15°C, for 11 to 15 min. The low quality of the included studies should be considered. Higher quality studies are needed to investigate whether the dose-response relationship of the results can be reliably reproduced.

The findings of the study allow athletes using CWI to have a better understanding of the technique, resulting in a better dynamic in training and competition, leading to less aggressive and painful immersion. For those applying CWI, it will allow the use of improved logistics and therefore result in lower costs, due to the most effective use of immersion time and water temperature.

ACKNOWLEDGMENTS

The authors would like to thank the Univ. Estadual Paulista, The University of Sydney and the Sao Paulo Research Foundation (FAPESP).

6. REFERENCES

1. Bastos FN, Vanderlei LCM, Nakamura FY, et al. Effects of cold water immersion and active recovery on post-exercise heart rate variability. *Int J Sports Med.* 2012;33(11):873–9.
2. Brophy-Williams N, Landers G, Wallman K. Effect of immediate and delayed cold water immersion after a high intensity exercise session on subsequent run performance. *J Sports Sci Med.* 2011:665–670.
3. Pournot H, Bieuzen F, Duffield R, et al. Short term effects of various water immersions on recovery from exhaustive intermittent exercise. *Eur J Appl Physiol.* 2011;111(7):1287–95.
4. Broatch JR, Petersen A, Bishop DJ. Postexercise cold water immersion benefits are not greater than the placebo effect. *Med Sci Sports Exerc.* 2014;46(11):2139–2147.
5. Bleakley CM, Davison GW. What is the biochemical and physiological rationale for using cold-water immersion in sports recovery? A systematic review. *Br J Sports Med.* 2010;44(3):179–87.
6. Wilcock IM, Cronin JB, Hing WA. Physiological response to water immersion: a method for sport recovery? *Sports Med.* 2006;36(9):1–18.
7. Bieuzen F, Bleakley CM, Costello JT. Contrast water therapy and exercise induced muscle damage: a systematic review and meta-analysis. *PLoS One.* 2013;8(4):e62356.
8. Leeder J, Gissane C, Someren K Van, et al. Cold water immersion and recovery from strenuous exercise: a meta-analysis. *Br J Sports Med.* 2012 Mar;46(4):233-40.
9. Bailey DM, Erith SJ, Griffin PJ, et al. Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running. *J Sports Sci.* 2007;25(11):1163–70.
10. Glasgow PD, Ferris R, Bleakley CM. Cold water immersion in the management of delayed-onset muscle soreness: Is dose important? A randomised controlled trial. *Phys Ther Sport.* 2014.
11. Leal Junior EC, de Godoi V, Mancalossi JL, et al. Comparison between cold water immersion therapy (CWIT) and light emitting diode therapy (LEDT) in short-term skeletal muscle recovery after high-intensity exercise in athletes--preliminary results. *Lasers Med Sci.* 2011;26(4):493–501.
12. Bleakley C, Mcdonough S, Gardner E, et al. Cold-water immersion (cryotherapy) for preventing and treating muscle soreness after exercise. *Cochrane Database Syst Rev.* 2012 Feb 15;2:CD008262.

13. Pastre CM, Bastos FN, Netto Jr J, et al. Métodos de recuperação pós-exercício: uma revisão sistemática. *Rev Bras Med Esporte*. 2009;15:138–144.
14. Maher CG, Sherrington C, Robert D, et al. Research report reliability of the PEDro Scale for rating quality of randomized. *Phys Ther*. 2003;713–721.
15. Macedo LG, Elkins MR, Maher CG, et al. There was evidence of convergent and construct validity of Physiotherapy Evidence Database quality scale for physiotherapy trials. *J Clin Epidemiol*. 2010;63(8):920–5.
16. Leeder JDC, Van Someren K a., Bell PG, et al. Effects of seated and standing cold water immersion on recovery from repeated sprinting. *J Sports Sci*. 2015;(January):1–9.
17. Elias GP, Varley MC, Wyckelsma VL, et al. Effects of water immersion on posttraining recovery in Australian footballers. *Int J Sports Physiol Perform*. 2012;7(4):357–66.
18. Stanley J, Buchheit M, Peake JM. The effect of post-exercise hydrotherapy on subsequent exercise performance and heart rate variability. *Eur J Appl Physiol*. 2012;112(3):951–61.
19. Crystal NJ, Townson DH, Cook SB, et al. Effect of cryotherapy on muscle recovery and inflammation following a bout of damaging exercise. *Eur J Appl Physiol*. 2013;113(10):2577–86.
20. Getto CN, Golden G. Comparison of active recovery in water and cold-water immersion after exhaustive exercise. *Athl Train Sport Heal Care*. 2013;5(4):169–176.
21. Elias GP, Wyckelsma VL, Varley MC, et al. Effectiveness of Water Immersion on Postmatch Recovery in Elite Professional Footballers. *Int J Sports Physiol Perform*. 2013:243–254.
22. Jakeman JR, Macrae R, Eston R. A single 10-min bout of cold-water immersion therapy after strenuous plyometric exercise has no beneficial effect on recovery from the symptoms of exercise-induced muscle damage. *Ergonomics*. 2009;52(4):456–60.
23. Stanley J, Buchheit M, Peake JM. The effect of post-exercise hydrotherapy on subsequent exercise performance and heart rate variability. *Eur J Appl Physiol*. 2012;112(3):951–61.
24. Ascensão A, Leite M, Rebelo AN, et al. Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. *J Sports Sci*. 2011;29(3):217–25.
25. Halson SL, Quod MJ, Martin DT, et al. Physiological responses to cold water immersion following cycling in the heat. *Int J Sport Physiol Perform*. 2008;3:331–346.
26. Sellwood KL, Brukner P, Williams D, et al. Ice-water immersion and delayed-onset muscle soreness: a randomised controlled trial. *Br J Sports Med*. 2007;41(6):392–7.

27. Davis KD, Pope GE. Noxious cold evokes multiple sensations with distinct time courses. *Pain*. 2002;98(1-2):179–185.
28. Yeargin SW, Casa DJ, McClung JM, et al. Body cooling between two bouts of exercise in the heat enhances subsequent performance. *J strength Cond Res / Natl Strength Cond Assoc*. 2006;20(2):383–389.
29. Versey NG, Halson SL, Dawson BT. Water immersion recovery for athletes: effect on exercise performance and practical recommendations. *Sports Med*. 2013;43(11):1101–30.
30. Goodall S, Howatson G. The effects of multiple cold water immersions on indices of muscle damage. *J Sports Sci Med*. 2008 Jun; 7(2): 235–241.
31. Howatson G, Goodall S, van Someren K a. The influence of cold water immersions on adaptation following a single bout of damaging exercise. *Eur J Appl Physiol*. 2009;105(4):615–21.
32. Pointon M, Duffield R, Cannon J, et al. Cold application for neuromuscular recovery following intense lower-body exercise. *Eur J Appl Physiol*. 2011;111(12):2977–86.

Appendix Table 1. Search strategy

MEDLINE (up to 21 January, 2015)

1. (randomized controlled trial) OR (controlled trial) OR (controlled clinical trial) OR (clinical trial)
2. (pain) OR (soreness) OR (muscle soreness) OR (delayed onset muscle soreness)
3. (cryotherapy) OR (cold water immersion) OR (cold-water immersion) OR (ice water immersion) OR (ice-water immersion) OR (ice bath) OR (ice-bath) OR (hydrotherapy)
4. (recovery) OR (post-exercise) OR (post exercise) OR (postexercise)
5. (animal) OR (animals)
6. 1 AND 2 AND 3 AND 4 NOT 5

EMBASE (up to 21 January, 2015)

1. (randomized controlled trial) OR (controlled trial) OR (controlled clinical trial) OR (clinical trial)
2. (pain) OR (soreness) OR (muscle soreness) OR (delayed onset muscle soreness)
3. (cryotherapy) OR (cold water immersion) OR (cold-water immersion) OR (ice water immersion) OR (ice-water immersion) OR (ice bath) OR (ice-bath) OR (hydrotherapy)
4. (recovery) OR (post-exercise) OR (post exercise) OR (postexercise)
5. 1 AND 2 AND 3 AND 4

SportDiscus (up to 21 January, 2015)

1. (randomized controlled trial) OR (controlled trial) OR (controlled clinical trial) OR (clinical trial)
2. (pain) OR (soreness) OR (muscle soreness) OR (delayed onset muscle soreness)
3. (cryotherapy) OR (cold water immersion) OR (cold-water immersion) OR (ice water immersion) OR (ice-water immersion) OR (ice bath) OR (ice-bath) OR (hydrotherapy)
4. (recovery) OR (post-exercise) OR (post exercise) OR (postexercise)
5. (animal) OR (animals)
6. 1 AND 2 AND 3 AND 4 NOT 5

The Cochrane Library (up to 21 January, 2015)

1. (randomized controlled trial) OR (controlled trial) OR (controlled clinical trial) OR (clinical trial)
2. (pain) OR (soreness) OR (muscle soreness) OR (delayed onset muscle soreness)
3. (cryotherapy) OR (cold water immersion) OR (cold-water immersion) OR (ice water immersion) OR (ice-water immersion) OR (ice bath) OR (ice-bath) OR (hydrotherapy)
4. (recovery) OR (post-exercise) OR (post exercise) OR (postexercise)
5. (animal) OR (animals)
6. 1 AND 2 AND 3 AND 4 NOT 5

Filter: trial

PEDro database (up to 21 January, 2015)

Abstract and title:

1. Pain
2. Soreness
3. Muscle soreness
4. Delayed onset muscle soreness

Therapy: hydrotherapy, balneotherapy

Subdiscipline: Sports

Method: clinical trial

*1, 2, 3, 4 were individually searched with the same therapy, subdiscipline and method.

Appendix Table 2. Physiotherapy Evidence Database Scores of Included Studies

Study	Random allocation	Concealed allocation	Groups similar at baseline	Participant blinding	Therapist blinding	Assessor blinding	<15% dropouts	Intention-to-treat analysis	Between-group difference reported	Point estimates / variability reported	Score (0-10)
Leeder, 2015	Yes	Yes	Yes	No	No	No	Yes	No	Yes	Yes	6/10†
Crystal, 2013	Yes	No	Yes	No	No	No	No	No	Yes	Yes	4/10*
Getto, 2013	Yes	No	No	No	No	No	No	No	Yes	Yes	3/10†
Elias, 2013	Yes	No	Yes	No	No	No	No	No	Yes	Yes	4/10*
Elias, 2012	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	5/10*
Stanley, 2012	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	5/10*
Brophy-Willians, 2011	Yes	No	No	No	No	No	Yes	No	Yes	Yes	4/10†
Jakeman, 2009	Yes	No	Yes	No	No	No	No	No	Yes	No	3/10*
Bailey, 2007	Yes	No	Yes	No	No	No	No	No	Yes	Yes	4/10*

* Trials assessed and listed on the PEDro Database


† Trial assessed by the 2 independent raters

Appendix: Registration PROSPERO. Available:

http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015016573#.VO99aP

[nF9bc](#)

UNIVERSITY *of* York
Centre for Reviews and Dissemination


National Institute for
Health Research

PROSPERO International prospective register of systematic reviews

The influence of different protocols of cold water immersion on pain: a systematic review and meta-analysis

Aryane Machado, Aline Almeida, Jéssica Micheletti, Paulo Ferreira, Carlos Marcelo Pastre

Citation

Aryane Machado, Aline Almeida, Jéssica Micheletti, Paulo Ferreira, Carlos Marcelo Pastre. The influence of different protocols of cold water immersion on pain: a systematic review and meta-analysis. PROSPERO 2015:CRD42015016573 Available from http://www.crd.york.ac.uk/PROSPERO_REBRANDING/display_record.asp?ID=CRD42015016573

Review question(s)

The purpose of this systematic review was to determine the efficacy of cold water immersion in management of pain compared with control interventions (passive recovery).

We also aimed to identify which dosage of application can provide the best results, focusing on the water temperature and the duration of immersion.

Searches

Studies were selected after searching five databases (MEDLINE, EMBASE, SPORTDiscus, PEDro and The Cochrane Library) from the earliest record to January 21, 2015. The terms and keywords used to search optimization were related with randomized controlled trials; cold water immersion and post-exercise recovery (see details in the accompanying PDF). Electronic searches were supplemented by hand searching the reference list of eligible clinical trials. No language restrictions were applied.

Link to search strategy

http://www.crd.york.ac.uk/PROSPEROFILES/16573_STRATEGY_20150106.pdf

Types of study to be included

Randomized controlled trials

Condition or domain being studied

Studies should involve treatment with cold water immersion (CWI) in human participants and should assess pain or muscle soreness at immediate and/or delayed effect. CWI was defined as immersion in water temperature $\leq 15^{\circ}\text{C}$.

Participants/ population

To be eligible, studies had to be:

- 1) randomized controlled trial, comparing cold water immersion and control conditions (passive recovery) post-exercise;
- 2) studies that used a single session of exercises;
- 3) CWI applied within one hour post-exercise;
- 4) only one immersion on the first day.

Studies using intermittent immersions or more than one immersion in subsequent days were excluded.

Intervention(s), exposure(s)

Studies should involve treatment with cold water immersion in human participants vs control conditions (passive

recovery)

Comparator(s)/ control

Non-exposed control group / passive recovery

Outcome(s)

Primary outcomes

Muscle soreness

Secondary outcomes

None

Data extraction, (selection and coding)

Outcome data including mean scores, SDs (final values) and sample size were extracted by two review authors (AFM, JKM). The data extraction process was performed using a standardized form that also included details as characteristics of participants, exercise protocol, cold water immersion protocols, outcome measures and methodological characteristics. Disagreements between authors regarding data extraction were resolved by consensus.

Some studies included multiple observations, therefore we extracted data at clinically relevant time point: immediate effect (up to 24 hours post-exercise) and delayed effect (after 24 hours post-exercise). For delayed effect, we considered the peak pain of control group, in order to minimize interference caused by the intervention. Pain scores were converted to a common 0-10 scale.

Risk of bias (quality) assessment

All studies included will be assessed for methodological quality. This process will be performed by two independent reviewers (AFM and JKM) using the PEDro Scale^{14,15}. Each study will be assessed for random allocation, concealed allocation, baseline comparability, blinding participants, therapists and assessors, adequate follow-up, intention-to-treat, between-group comparison and point estimates and variability. A score greater or equal to 7 will be considered 'high quality', a score of 5 or 6 will be considered 'moderate quality' and less than or equal to 4, 'poor quality'. If trials are already assessed and listed on the PEDro database, we will adopt these scores. Methodological quality was not an inclusion criteria.

Strategy for data synthesis

The analysis will be based on temperature and duration of immersion of each study. For this, it will be necessary to establish cutoff points for each of these covariates. In water temperature analysis two categories will be used: severe cold, with water temperature between 5-10°C; and moderate cold, 11-15°C. In duration of immersion, three categories: short immersion, immersions of 10 minutes or less; medium immersion, 11-15 minutes; and longer immersion, with immersion between 16-20 minutes.

Analysis of subgroups or subsets

Comprehensive Meta-Analysis software, version 2.2.04 (Biostat, USA) will be used to conduct all analyses and the pooled estimates will be calculated using random-effects model. Data will be pooled in meta-analyses and described as weighted mean differences (MD) with 95% confidence intervals (CI). The immediate and delayed effects will be calculated in order to analyze the cold water immersion effect, independent of water temperature and duration of immersion. In cases of more than one intervention group for study, the group used will be that which represented the lowest effect size.

Contact details for further information

Miss Machado

305, Roberto Simonsen

aryfmachado@yahoo.com.br

Organisational affiliation of the review

Faculdade de Ciências e Tecnologia - Universidade Estadual Paulista "Júlio de Mesquita Filho"

www.fct.unesp.br

Review team

Miss Aryane Machado, UNESP
Miss Aline Almeida, UFSCAR
Miss Jéssica Micheletti, UNESP
Dr Paulo Ferreira, University of Sydney
Dr Carlos Marcelo Pastre, UNESP

Anticipated or actual start date

01 July 2014

Anticipated completion date

31 March 2015

Funding sources/sponsors

This systematic review was funded by FAPESP (Sao Paulo Research Foundation); protocol number: 2014/03778-5

Conflicts of interest

None known

Language

English

Country

Australia, Brazil

Subject index terms status

Subject indexing assigned by CRD

Subject index terms

Humans; Pain; Water

Stage of review

Ongoing

Date of registration in PROSPERO

09 February 2015

Date of publication of this revision

09 February 2015

Stage of review at time of this submission

	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

PROSPERO

International prospective register of systematic reviews

The information in this record has been provided by the named contact for this review. CRD has accepted this information in good faith and registered the review in PROSPERO. CRD bears no responsibility or liability for the content of this registration record, any associated files or external websites.

Immediate and delayed effects of cold water immersion with different dosages after eccentric exercise-induced muscle damage: a randomized controlled trial

Cold water immersion with different dosages after eccentric exercise

**Aryane Flauzino Machado^{1†}; Aline Castilho de Almeida^{2†}; Jéssica Kirsch Micheletti^{1†};
Franciele Marques Vanderlei^{3†}; Marcelo Fernandes Tribst³; Jayme Netto Junior^{3†};
Carlos Marcelo Pastre^{3†*}**

¹Programa de Pós-Graduação em Fisioterapia. Univ Estadual Paulista, Presidente Prudente/SP, Brazil

²Programa de Pós-Graduação em Fisioterapia. Univ Federal de São Carlos, São Carlos/SP, Brazil

³Departamento de Fisioterapia. Univ Estadual Paulista, Presidente Prudente/SP, Brazil

[†]Laboratório de Fisioterapia Desportiva. Univ Estadual Paulista, Presidente Prudente/SP, Brazil

* Corresponding author:

E-mail: pastre@fct.unesp.br (CMP)

ABSTRACT

Purpose: To compare the effects of two strategies of cold water immersion (CWI), using different water temperatures, with passive recovery post-exercise, in the management of markers of muscle damage and to observe whether any of the techniques used caused deleterious effects on performance.

Materials and Methods: 60 healthy male participants performed an eccentric exercise protocol to induce muscle damage and were then randomized to one of three groups (CWI1: 15 min at 9°C; CWI2: 15 min at 14°C; CG: control group). Levels of creatine kinase, muscle soreness, pain threshold, perception of recovery and maximal voluntary isometric contraction were monitored up to 96 hours post-exercise.

Results: Regarding the immediate effect, cold water immersion, independent of water temperature, presented an earlier recovery for soreness and the CWI2 group presented an early recovery for performance ($P<0.05$). It was observed that there were no deleterious effects and no evidence was found to suggest a dose-response relationship.

Conclusions: The use of CWI is recommended as a post-exercise recovery technique and the application for 15 min at 14°C is considered the best dosage when the aim is early performance recovery; however the application should be appropriate to the specific intended outcome.

Keywords: recovery of function; cryotherapy; immersion; muscle soreness; muscle strength.

1. BACKGROUND

Intense, eccentric or unaccustomed exercise has been commonly documented as exercise able to produce delayed onset muscle soreness (DOMS) and alter various markers of muscle damage [1–5]. Responses such as a decrease in muscle function can also be related to muscle damage [6], demonstrating that these types of exercises can influence the performance and recovery of athletes [2]. Different scenarios of recovery strategies are currently being investigated in order to minimize performance decrements, such as massage, active recovery, contrast water therapy and cryotherapy [6, 7, 8].

Cold water immersion (CWI) is one recovery technique which is commonly used by athletes post-exercise to promote the restoration of body systems to baseline conditions and establish the physiological system to a pre-exercise state [9, 10]. The effects of CWI such as cooling the body tissues, reducing lymphatic, capillary and cellular permeability and decreasing nerve conduction velocity, muscular spindle activity and spasticity, have been discussed in several studies [2, 11, 12]. Despite these responses, the specific mechanism of CWI is unknown [12] and can change according to the type of exercise performed prior to the CWI and the methodology adopted for immersion [13], presenting specific evidence after exercise-induced muscle damage (EIMD) [2,11,12].

Several clinical trials and systematic reviews have compared the effects of CWI with other post-exercise recovery strategies [1-3; 5-10; 14-17]. Recent reviews found that, compared to control interventions and other traditional techniques of recovery, CWI is a more effective technique to reduce pain after a range of exercises [8, 18]. Nevertheless, studies that use different methodologies for the application of CWI, such as water temperature, duration of immersion and type of CWI, for example continuous or intermittent immersion [14] and the dose-response relationship of this technique have not yet been fully investigated.

Bleakley *et al.*, 2012 [4] claimed that there are still insufficient studies available to determine the best method of CWI. From this statement, it can be understood that conclusions about the effects of CWI are not well elucidated and require further research, including studies which approach the dose-response relationship. The purpose of this study was to analyze the immediate and delayed effects of CWI as a mode of post-exercise recovery on the management of some markers of muscle damage. The primary objective was to compare the effects of two strategies of CWI, using different water temperatures, with passive recovery post-exercise. Furthermore, from these findings, to observe whether any of the techniques used caused deleterious effects on performance on subsequent days post-exercise. Considering the positive results of previously cited studies, the hypothesis of this study was that CWI would present better results when compared with passive recovery. Moreover, considering that CWI is more efficient than other modalities for reducing neural conduction velocity [19], together with the knowledge that reduction in performance is proportional to muscle cooling [20, 21], another hypothesis was that CWI may have a negative influence on performance, related to the deleterious effects, especially when applied at 9°C.

2. METHODS

2.1 Participants

Sixty young, healthy male participants (aged 18-25 years, height 1.74 ± 0.06 m, body mass 74.4 ± 11.1 kg and body mass index 24.4 ± 4.1 kg . m⁻²) participated in this study, as show in the flowchart (Fig. 1). To be included, the participants were required to report the absence of anemia, inflammation, diabetes, cardiovascular diseases and muscle injuries in the lower limbs and/or spine in the previous six months. Participants were required to abstain from anti-inflammatory and analgesic drugs and not perform any exercise during the study.

Prior to data collection, a medical evaluation was performed to ensure that the participants were fully able to participate in the study.

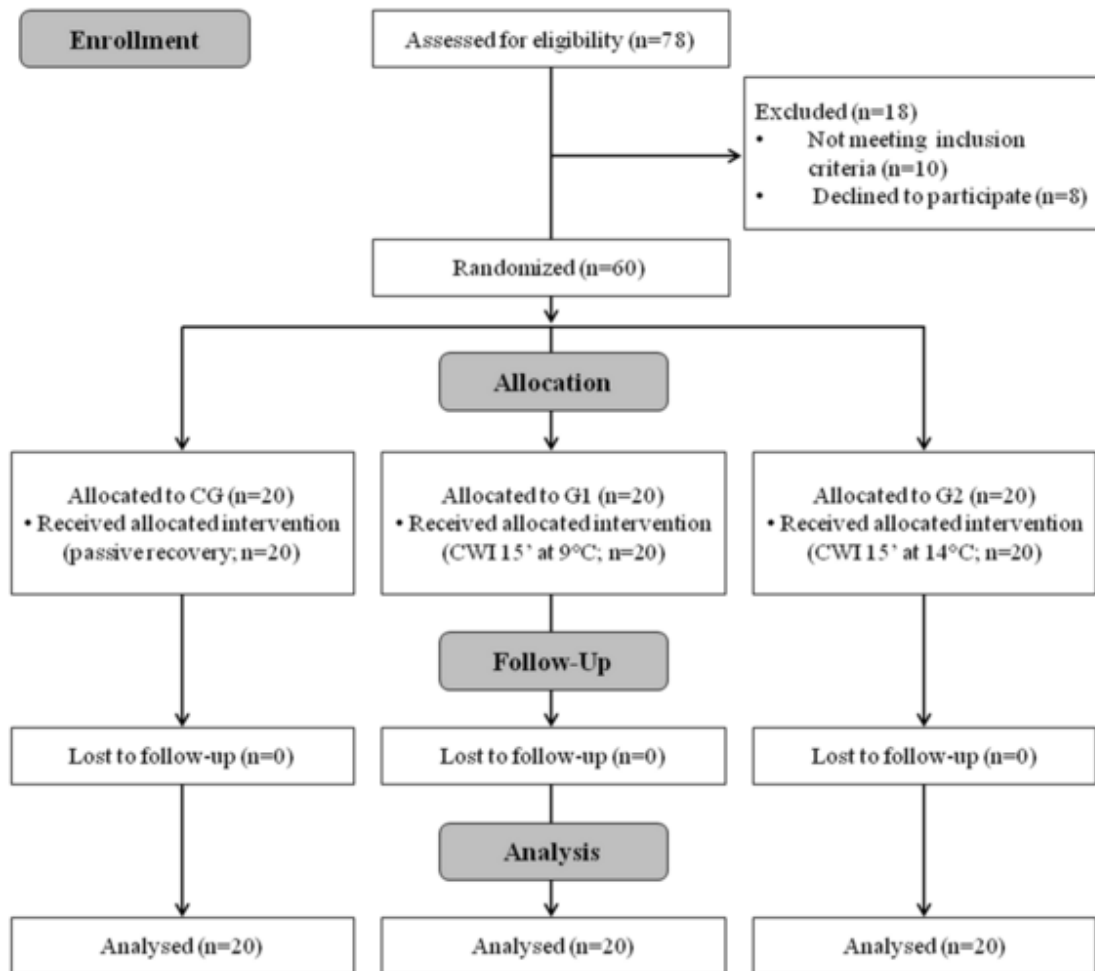


Fig 1. Flowchart of participants

To define the sample size a priori knowledge was used, based on the findings of Bailey *et al.*, 2007 [2] for concentration of creatine kinase (SD=200 U/L). A sample size of twenty participants per group was stipulated by a test of hypothesis (two-tail), with a 5% level of significance and 80% power. Thus, a randomization sequence was created using software (Microsoft Office Excel 2007) and a computer-generated random list was used for allocation.

The participants were allocated into three groups: control group (CG: passive recovery for 15 minutes) and two intervention groups (CWI1 – 15 minutes at $9\pm 1^{\circ}\text{C}$ and CWI2 – 15 minutes at $14\pm 1^{\circ}\text{C}$). During the study, the participants received no information about which intervention was considered therapeutic.

2.2 Ethics statement

The study had been previously approved by the Ethics Committee in Research of the Univ. Estadual Paulista (Faculty of Science and Technology – UNESP; protocol number: 2013/396.666). The participants received oral and written instructions and signed a Consent Form agreeing to the research procedures.

2.3 Study design

The study procedure was carried out between January and April 2014 at the Centre for Studies and Treatment in Physical Therapy and Rehabilitation – UNESP. All procedures were performed in standardized conditions (temperature: $21\text{-}23^{\circ}\text{C}$; relative humidity: 40-60%) [22].

Each participant attended the laboratory on five consecutive days. Prior to the procedures, the participants were assessed for anthropometric characteristics using a scale (*Tanita BC 554, Iron Man/Inner - Illinois, USA*) and a stadiometer (*Sany – American Medical do Brasil, São Paulo, Brazil*) (Table 1). The baseline assessment, eccentric exercise-induced muscle damage (EIMD), intervention and immediate assessment were performed on the first day. Subsequent visits were performed 24, 48, 72 and 96 hours after the EIMD, relating to the delayed effect. An overview of the study is presented in Fig 2.

Table 1. Comparison of sample characteristics (mean \pm SD)

	CG (n=20)	CW11 (n=20)	CW12 (n=20)	P-value
Age (years)	20.4 \pm 1.8	21.2 \pm 2.0	20.85 \pm 2.5	0.491
Height (m)	1.76 \pm 0.0	1.74 \pm 0.0	1.73 \pm 0.0	0.355
Body mass (kg)	76.7 \pm 10.0	71.5 \pm 8.8	74.9 \pm 13.7	0.260
Body mass index (kg . m ⁻²)	24.6 \pm 3.1	23.6 \pm 3.0	25.0 \pm 5.7	0.599

CWI: cold water immersion; SD: standard deviation

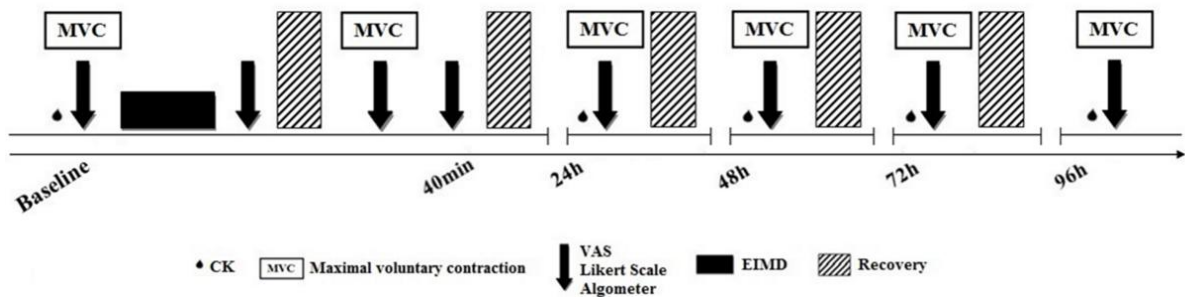


Fig 2. Study design. VAS: Visual Analogue Scale; EIMD: Exercise induced

muscle damage; CK: Creatine Kinase.

2.4 Procedures

2.4.1 Eccentric exercise-induced muscle damage protocol (EIMD)

For the eccentric protocol [23], muscle injury was induced in the dominant knee extensors using an isokinetic dynamometer (*Biodex System 4 Pro*, New York, USA). Prior to the protocol, the participants were familiarized with the eccentric exercise. The familiarization consisted of five sub-maximal eccentric contractions of knee extension. Before each repetition, the participant's dominant leg was positioned at 30° knee flexion (starting from 90° flexion). The participant was instructed to perform a knee extension while the dynamometer, with its resistance, returned the leg to 90° flexion (starting position), at a speed of 60° . s⁻¹ (1.04 rad . s⁻¹), performing a range of motion of 60° (30-90° knee flexion).

The protocol started five minutes after the familiarization and the participant was required to perform 75 maximal eccentric contractions of knee extension, divided into five

sets of 15 repetitions, separated by a rest period of 30s. The velocity and range of motion were similar to the familiarization.

2.4.2 Creatine kinase (CK)

Creatine kinase concentration was obtained from capillary blood, collected by finger-prick (32 μ L). The blood sample (total blood) was analyzed using a *Reflotron Plus* reader (*Roche Diagnostics*, Mannheim, Germany) in 37°C.

2.4.3 Muscle soreness (VAS) and pain threshold (algometer)

The participants were instructed to assess the soreness in their leg (induced by the damage), using a Visual Analogue Scale (VAS) ranging from 0 “no soreness” to 10 “extreme soreness” [2]. During the assessment, the participant performed a maximal isometric contraction on an isokinetic dynamometer with the knee flexed at 60° during the assessment.

Pain threshold was assessed using a pressure algometer (*FPX 50/220*, *Wagner Instruments*, Greenwich, USA). The participant indicated specific painful points during the isometric contraction (60° knee flexion). The algometer was then applied to the indicated point until the participant reported discomfort. The pain threshold was defined in Kgf and did not exceed 2.55 Kgf, as suggested by Jönhagen *et al.*, 2009 [24]. The assessment was performed with the leg at 60° knee flexion, with muscles relaxed.

2.4.4 Perception of recovery (Likert Scale)

Perception of recovery was obtained using a 10-point Likert Scale, with a rating of 1 indicating the feeling “not recovered” and a rating of 10 the feeling “fully recovered” [25]. The participant was asked the following question to assess muscle function: “If you had to perform the MVIC now, how recovered do you feel?”

2.4.5 Maximal Voluntary Isometric Contraction (MVIC)

Prior to the assessment of muscle function, the participants performed a warm-up consisting of 10 concentric repetitions of knee flexion-extension at $180^\circ \cdot s^{-1}$ ($3.14 \text{ rad} \cdot s^{-1}$) throughout the range of motion, as suggested by Baroni *et al.*, 2010 [23].

Muscle function was determined as the highest torque value of three repetitions of 5 seconds of MVIC at 60° knee flexion (0° corresponding to the maximal extension). The repetitions were separated by a rest interval of 2 minutes to minimize possible effects of fatigue. The participants were instructed to perform maximal isometric contractions and were verbally encouraged by the researcher throughout the assessment.

2.4.6 Recovery strategies

Immediately following the EIMD protocol (post-exercise), the participants from the intervention groups were immersed in water for 15 minutes, up to the height of the iliac crest at temperatures of $9 \pm 1^\circ\text{C}$ or $14 \pm 1^\circ\text{C}$, controlled by a thermometer of 0.1°C with an accuracy of 0.1°C . During this period, the control group participants remained seated for passive recovery.

The same recovery strategies were performed at the end of each visit, including the following moments after the eccentric protocol (40 minutes, 24, 48 and 72 hours post-exercise) to minimize the potential effects caused by stress during MVIC.

2.4.7 Statistical analysis

We used SPSS (version 18; SPSS Inc, Chicago, IL) to conduct the analysis. Initially, sphericity of the data was tested by Mauchly's test. In case of violation of the sphericity assumption, the Greenhouse-Geisser corrections were used. Thus, the mean and standard deviation were considered. Data were analyzed using Repeated Measures Analysis

of Variance (Bonferroni's test was performed when required), which provide information of time, group and interaction effects. All statistical analysis assumed a significance level of 5%.

3. RESULTS

Values of Table 2 are presented as mean and standard deviation values and shows a significant effect for time for all outcomes ($P < 0.001$). There were no significant group and interaction (Group*time) effects.

Creatine kinase activity had increased significantly at 24 hours and continued increasing until 96 hours post-exercise ($P < 0.05$). The CK peak occurred between 72 and 96 hours post-exercise.

The exercise protocol resulted in severe muscle soreness that peaked immediately after the protocol and 48 hours post-exercise. Both intervention groups demonstrated reduced ratings of muscle soreness immediately post-recovery and 40 minutes post-exercise. All groups reported a reduced rating of soreness at 72 hours post-exercise ($P > 0.05$). Ratings of perception of recovery decreased significantly post-exercise ($P < 0.05$) and began to increase at 72 hours post-exercise.

All groups presented reduced MVIC post-recovery ($P < 0.05$). However, significant muscle function losses were recorded 24 hours post-exercise for the control and CWI 1 groups, while the CWI 2 demonstrated recovered values and presented no difference to baseline after the first day (Fig 3).

Table 2. Creatine kinase activity, muscle soreness, pain threshold, perception of recovery and maximal voluntary isometric contraction. Values are presented as mean±SD.

<i>Follow up</i>									
	Baseline	EIMD	Recovery	40min	24h	48h	72h	96h	Summary of effects
<i>Creatine Kinase (U/L)</i>									
Control group	145.4±28.2	n/a	n/a	n/a	549.5±448.4*	807.6±516.7*	843.90±540.2*	822.4±549.0*	Time ($p=0.001$)
15 min at 9°C	143.6±39.7	n/a	n/a	n/a	538.8±499.6*	786.7±564.8*	881.4±588.6*	896.6±661.5*	Group*time ($p=0.270$)
15 min at 14°C	142.0±46.7	n/a	n/a	n/a	420.0±317.2*	860.2±543.3*	1118.0±549.6*	1015.6±555.7*	Group ($p=0.784$)
<i>Muscle soreness (VAS)</i>									
Control group	1.3± 1.8	5.7±2.5*	4.5±2.2*	3.4± 2.5*#	3.3±1.7*	4.3±2.9*	2.7±2.6	1.4±1.6#	Time ($p=0.001$)
15 min at 9°C	1.5±2.2	5.5±2.7*	2± 2.5#	2.6±2.6#	3.1±1.9#	4.6±2.9*	2.2±2.1#	0.6±0.8#	Group*time ($p=0.257$)
15 min at 14°C	1.7±2.1	6.0±2.7*	2.7±2.2#	3.4±2.6#	3.9±1.7*	4.8±2.4*	2.9±2.5#	1.7±1.6#	Group ($p=0.299$)
<i>Recovery (Likert Scale)</i>									
Control group	10±0	3.1±1.5*	4.8±1.7*	4.4±1.4*#	5.9±1.7*#	4.9±2.8*	5.7±2.5*#	7.0±2.1*#	Time ($p=0.001$)
15 min at 9°C	10±0	3.7±2.5*	4.1±2.2*	5.0±2.1*	5.6±2.2*	5.7±3.1*	7.0±2.5*#	8.4±1.8*#	Group*time ($p=0.319$)
15 min at 14°C	10±0	3.4±1.8*	4.0±2.4*	5.3±1.9*#	5.8±2.2*#	5.1±2.9*	6.9±2.5*#	7.1±2.2*#	Group ($p=0.495$)
<i>MVIC (N. m)</i>									
Control group	285.2± 46.9	n/a	234.6±68.9*	n/a	249.3±69.8*	247.3±95.1	252.5±95.5	264.3±84.1	Time ($p=0.001$)
15 min at 9°C	284.9±44.8	n/a	230.0±58.6*	n/a	250.1±58.7*	250.0±67.9	260.0±59.6	269.9±45.5	Group*time ($p=0.980$)
15 min at 14°C	281.6±51.1	n/a	227.9±44.5*	n/a	248.3±59.9	243.1± 69.9	246.1±56.5	257.2±47.5	Group ($p=0.931$)

$n = 20$ per group

n/a: not applicable

EIMD: Exercise induced-muscle damage; MVIC: Maximal voluntary isometric contraction; Recovery: immediately post-recovery.

* Statistically significant difference ($P<0.05$) from baseline

Statistically significant difference ($P<0.05$) from EIMD

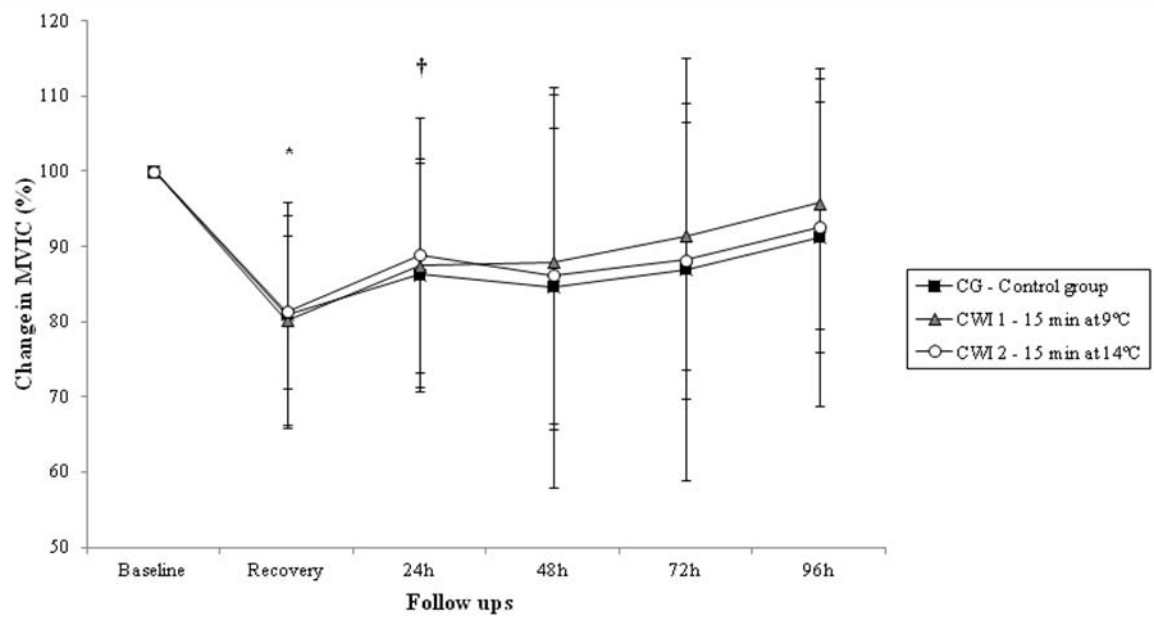


Fig 3. Change in maximal voluntary isometric contraction (%; mean and SD).
 *Statistically significant difference ($P < 0.05$) from baseline for all groups; †Statistically significant difference ($P < 0.05$) from baseline for control group and CWI 1 (15 min at $9 \pm 1^\circ\text{C}$).

4. DISCUSSION

The present study aimed to compare the immediate and delayed effects of two CWI strategies, at different water temperatures, on the management of some markers of muscle damage after eccentric exercise and to examine the possible deleterious effects on subsequent days post-exercise. The main findings of this study were that all recovery strategies acted in a similar way concerning creatine kinase activity and pain threshold and the CWI groups acted more efficiently for soreness and muscle function. The findings regarding soreness demonstrated that the CWI groups presented lower ratings immediately post-recovery. For the delayed effects, all groups reported a decrease in perception of pain at 72

hours post-exercise. For muscle function, the application of CWI for 15 minutes at 14°C presented earlier recovery compared with other CWI and control conditions.

Severe changes in markers of muscle damage corroborate the findings of Baroni *et al.*, 2010 [23] who used the same protocol. These changes provide evidence that the objective of causing muscle damage through this exercise protocol was successful. Different protocols have been used to induce damage such as high intensity and eccentric exercise [8]. Baroni *et al.*, 2010 [23] claimed that the intensity of exercise, number of repetitions, velocity and range of motion can influence the magnitude of damage. Glasgow *et al.*, 2014 [1] used a protocol based on 3 sets of eccentric exercises which resulted in a considerably lower soreness peak than that found in the present study. This may explain the small differences found between the CWI groups and control condition.

The ratings of soreness increased after the eccentric exercise protocol and presented peaks post-exercise and at 48 hours for all groups. Crystal *et al.*, 2013 [3] also found a double peak; however, the second peak occurred at 24 hours post-exercise. An interesting finding is the soreness reduction after CWI. These findings could be due to the analgesic effect of CWI and inhibition of muscle damage [2, 26]. Analgesia is present in the short term, but the precise time is still uncertain [2, 17]. It is speculated that this period can vary from between 3 minutes to 3 hours [26], which may explain the immediate effect results. The improvement in ratings of soreness found in the CWI when compared with the control condition immediately post-recovery and 40 minutes post-exercise is similar to a previous study [27], yet demonstrated no significant differences. The delayed effects are in agreement with other studies of the same nature, demonstrating the attenuation of soreness after 48 hours post-exercise [3, 6, 17]. In addition, in accordance with a previous study, Crystal *et al.*, 2013 [3] found improvement in soreness for all groups at 72 hours post-exercise compared with

pre-exercise. No effects were observed for pain threshold, which corroborates with Sellwood's study [11].

Perception of recovery has been presented as an important tool for evaluating the effectiveness of different techniques and plays a crucial role in the adaptive process [28, 29]. The improvement in perception of recovery has a direct relationship with the benefits of CWI [13, 25, 28, 30]. No evidence of an improvement in the perception of recovery in the immediate effects, unlike the studies of Parouty *et al.* [30] and Buchheit *et al.* [25]. Some authors [28, 31] have pointed out that there is a contribution from the psychological mechanism and athletes often present better performances when they believe they have received a beneficial treatment, which was not observed in this study.

Another commonly employed marker of muscle damage is the blood concentration of creatine kinase. It was observed that all groups similar responses in this respect and that the period of 96 hours post-exercise was not sufficient for recovery to baseline concentration levels, demonstrating a time effect as in the studies of Glasgow *et al.* [1] and Ingram *et al.* [32]. Eston and Peters, 1999 [33] claimed that the actual mechanisms involved in the alterations of CK are still unclear and some researchers have questioned whether CK levels accurately assess the severity of muscle damage [32-34]. Warren and Lowe and Armstrong, 1999 [34] further claimed that CK levels are dissociated from histological signs of damage and suggest, as do Morton *et al.*, 2005 [35], that the assessment of maximal voluntary contraction is a more relevant marker.

The possible effects of the techniques on muscle function were also verified. Studies claim that the electrical activity of the muscle is considerably lower after cooling techniques, although the relationship between muscle cooling and subsequent performance remains unclear [20]. Crowe *et al.*, 2007 [36] claim that decreases in blood flow after the application of CWI can be detrimental to performance when the athlete needs to compete

again and a decrease in muscle temperature has been related to a decrease in muscle power and strength [30, 36-39]. However, the hypothesis that CWI can worsen performance was not upheld in this study and, in fact, contradictorily, the findings reflected performance improvements in the time function for CWI applied for 15 minutes at 14°C, indicating recovery at 24 hours, while the other groups recovered from 48 hours post-exercise. As previously cited, performance reduction after CWI is proportionally dependent on the temperature at which the muscle is cooled [21], supporting the findings and demonstrating that lower temperatures (CWI applied at 9°C), which further reduced the muscle temperature, were not able to more efficiently restore the isometric contraction values.

The current study presents high methodological quality due to being a randomized controlled trial with parallel groups and allocation concealment. As stated previously, we ensured that the participants were not informed about the benefits of each technique [1]. However, it is not possible to fully blind the participants due to the control condition. Despite its widespread use and the large body of research involving CWI, there are few studies reporting the effects of different methodologies of CWI on immediate and delayed responses, the dose-response and deleterious effects of CWI. From the findings of the present study, no dose-response relationship was observed for any outcome based on the application of the temperatures used. Nevertheless, it was observed that CWI for 15 minutes at 14°C was the most appropriate dosage of application, represented by the early recovery of MVIC, which contradicts many studies. A potential limitation of the present study was the characteristics of the participants. Although the study sample consisted of young, healthy participants, it was not possible to ensure the exercise specificity of each participant, such as level or type of exercise, which could have influenced the responses. Therefore, we suggest further investigations which consider dose-response and a wider range of temperatures and durations of immersion, in addition to investigating the responses of high performance athletes.

5. CONCLUSIONS

From the findings of the present study, the application of CWI, independent of water temperature, presents an earlier recovery for soreness at immediate effect. It was noted that CWI for 15 minutes at 14°C represented the best dosage when the aim was performance recovery, presenting an anticipated recovery for maximal voluntary isometric contraction. However, no evidence was found to suggest a dose-response relationship for any outcome. It was also observed that there were no deleterious effects on performance after application of CWI.

Thus, the use of CWI is recommended as a post-exercise recovery technique; however the application should be appropriate to the specific intended outcome.

ACKNOWLEDGMENTS

The authors would like to thank the volunteers for their participation in the study.

6. REFERENCE

1. Glasgow PD, Ferris R, Bleakley CM. Cold water immersion in the management of delayed-onset muscle soreness: Is dose important? A randomised controlled trial. *Phys Ther Sport*. 2014.
2. Bailey DM, Erith SJ, Griffin PJ, et al. Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running. *J Sports Sci*. 2007;25(11):1163-70.
3. Crystal NJ, Towson DH, Cook SB, LaRoche DP. Effect of cryotherapy on muscle recovery and inflammation following a bout of damaging exercise. *Eur J Appl Physiol*. 2013;113(10):2577-86.
4. Bleakley C, McDonough S, Gardner E, et al. Cold-water immersion (cryotherapy) for preventing and treating muscle soreness after exercise (Review). *Cochrane Database Syst Rev*. 2012;(2).
5. Vaile J, Halson S, Gill N, et al. Effect of hydrotherapy on the signs and symptoms of delayed onset muscle soreness. *Eur J Appl Physiol*. 2008;102(4):447-55.
6. Howatson G, Goodall S, van Someren KA. The influence of cold water immersions on adaptation following a single bout of damaging exercise. *Eur J Appl Physiol*. 2009;105(4):615-21.
7. Pastre CM, Bastos FN, Netto Junior, et al. Métodos de recuperação pós-exercício: uma revisão sistemática. *Rev Bras Med Esporte*. 2009;15(2):138-44.
8. Leeder J, Gissane C, van Someren K, et al. Cold water immersion and recovery from strenuous exercise: a meta-analysis. *Br J Sports Med*. 2012;46(4):233-40.
9. Bleakley CM, Davison GW. What is the biochemical and physiological rationale for using cold-water immersion in sports recovery? A systematic review. *Br J Sports Med*. 2010;44(3):179-87.

10. Brophy-Williams N, Landers G, Wallman K. Effect of Immediate and Delayed Cold Water Immersion After a High Intensity Exercise Session on Subsequent Run Performance. *J Sports Sci Med*. 2011;10(4):665-70.
11. Sellwood KL, Brukner P, Williams D, et al. Ice-water immersion and delayed-onset muscle soreness: a randomised controlled trial. *Br J Sports Med*. 2007;41(6):392-97.
12. Ascensão A, Leite M, Rebelo AN, et al. Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. *J Sports Sci*. 2011;29(3):217-25.
13. Versey NG, Halson SL, Dawson BT. Water immersion recovery for athletes: effect on exercise performance and practical recommendations. *Sports Med*. 2013;43(11):1101-30.
14. Elias GP, Wyckelsma VL, Varley MC, et al. Effectiveness of water immersion on postmatch recovery in elite professional footballers. *Int J Sports Physiol Perform*. 2013;8(3):243-53.
15. Elias GP, Varley MC, Wyckelsma VL, et al. Effects of water immersion on posttraining recovery in Australian footballers. *Int J Sports Physiol Perform*. 2012;7(4):357-66.
16. Pournot H, Bieuzen F, Duffield R, et al. Short term effects of various water immersions on recovery from exhaustive intermittent exercise. *Eur J Appl Physiol*. 2011;111(7):1287-95.
17. Jakeman JR, Macrae R, Eston R. A single 10-min bout of cold-water immersion therapy after strenuous plyometric exercise has no beneficial effect on recovery from the symptoms of exercise-induced muscle damage. *Ergonomics*. 2009;52(4):456-60.
18. Bieuzen F, Bleakley CM, Costello JT. Contrast water therapy and exercise induced muscle damage: a systematic review and meta-analysis. *PLoS One*. 2013;8(4).

19. Herrera E, Sandoval MC, Camargo DM, et al. Effect of walking and resting after three cryotherapy modalities on the recovery of sensory and motor nerve conduction velocity in healthy subjects. *Rev Bras Fisioter.* 2011;15(3):233-40.
20. Vieira A, Oliveira AB, Costa JR, et al. Cold modalities with different thermodynamic properties have similar effects on muscular performance and activation. *Int J Sports Med.* 2013;34(10):873-80.
21. Ranatunga KW, Sharpe B, Turnbull B. Contractions of a human skeletal muscle at different temperatures. *J Physiol.* 1987;390:383-95.
22. Bastos FN, Vanderlei LC, Nakamura FY, et al. Effects of cold water immersion and active recovery on post-exercise heart rate variability. *Int J Sports Med.* 2012;33(11):873-79.
23. Baroni BM, Leal Junior EC, De Marchi T, et al. Low level laser therapy before eccentric exercise reduces muscle damage markers in humans. *Eur J Appl Physiol.* 2010;110(4):789-96.
24. Jönhagen S, Ackermann P, Saartok T. Forward lunge: a training study of eccentric exercises of the lower limbs. *J Strength Cond Res.* 2009;23(3):972-78.
25. Buchheit M, Peiffer JJ, Abbiss CR, et al. Effect of cold water immersion on postexercise parasympathetic reactivation. *Am J Physiol Heart Circ Physiol.* 2009;296(2):H421-27.
26. Meeusen R, Lievens P. The use of cryotherapy in sports injuries. *Sports Med.* 1986;3(6):398-414.
27. Getto CN, Golden G. Comparison of active recovery in water and cold-water immersion after exhaustive exercise. *Athletic Training & Sports Health Care.* 2013;5(4):169-76.


28. Stanley J, Buchheit M, Peake JM. The effect of post-exercise hydrotherapy on subsequent exercise performance and heart rate variability. *Eur J Appl Physiol.* 2012;112(3):951-61.
29. Cook CJ, Beaven CM. Individual perception of recovery is related to subsequent sprint performance. *Br J Sports Med* 2013;47:705-09.
30. Parouty J, Al Haddad H, Quod M, et al. Effect of cold water immersion on 100-m sprint performance in well-trained swimmers. *Eur J Appl Physiol.* 2010;109(3):483-90.
31. Higgins TR, Heazlewood IT, Climstein M. A random control trial of contrast baths and ice baths for recovery during competition in U/20 rugby union. *J Strength Cond Res.* 2011;25(4):1046-51.
32. Ingram J, Dawson B, Goodman C, et al. Effect of water immersion methods on post-exercise recovery from simulated team sport exercise. *J Sci Med Sport.* 2009;12(3):417-21
33. Eston R, Peters D. Effects of cold water immersion on the symptoms of exercise-induced muscle damage. *J Sports Sci.* 1999;17(3):231-38.
34. Warren GL, Lowe DA, Armstrong RB. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 1999;27(1):43-59.
35. Morton JP, Atkinson G, MacLaren DP, et al. Reliability of maximal muscle force and voluntary activation as markers of exercise-induced muscle damage. *Eur J Appl Physiol.* 2005;94(5-6):541-48.
36. Crowe MJ, O'Connor D, Rudd D. Cold water immersion reduces anaerobic performance. *Int J Sports Med.* 2007;28:994–8.
37. Yamane M, Teruya H, Nakano M, et al. Post-exercise leg and forearm flexor muscle cooling in humans attenuates endurance and resistance training effects on muscle performance and on circulatory adaptation. *Eur J Appl Physiol.* 2006;96(5):572–80.

38. Schniepp J, Campbell TS, Powell KI, et al. The effects of cold water immersion on power output and heart rate in elite cyclists. *J Strength Cond Res.* 2002;16(4):561–6.

39. Peiffer JJ, Abbiss CR, Nosaka K, et al. Effect of cold water immersion after exercise in the heat on muscle function, body temperatures, and vessel diameter. *J Sci Med Sport.* 2009;12(1):91–6.

Appendix: Registration REBEC (Registro Brasileiro de Ensaios Clínicos). Available:

<http://www.ensaiosclinicos.gov.br/rg/RBR-2bz8rj/>



USUÁRIO SENHA ENTRAR [Esqueceu a senha?](#)
[Registrar-se](#)

[PT](#) | [ES](#) | [EN](#)

[NOTÍCIAS](#) | [SOBRE](#) | [AJUDA](#) | [CONTATO](#)

Buscar ensaios
[BUSCA AVANÇADA](#)

[HOME](#) / [ENSAIOS REGISTRADOS](#) /

RBR-2bz8rj

Efeitos imediatos e tardios da criomersão após protocolo de exercício excêntrico

Data de registro: 5 de Set. de 2013 às 10:52
Last Update: 23 de Jan. de 2015 às 11:48

Tipo do estudo:
Intervenções

Título científico:

PT-BR Efeitos imediatos e tardios da criomersão após protocolo de exercício excêntrico	EN Immediate and delayed effects of cold water immersion after eccentric exercise protocol
---	---

Identificação do ensaio

Número do UTN: U1111-1147-6348

Título público:

PT-BR Efeitos da imersão em água fria após exercício	EN Effects of cold water immersion after exercise
---	--

Acrônimo científico:

Acrônimo público:

Identificadores secundários:
Número do parecer: 396.666
Órgão emissor: Comitê de Ética em Pesquisa - Universidade Estadual Paulista "Júlio de Mesquita Filho"

CAAE: 22229913.0.0000.5402
Órgão emissor: Plataforma Brasil

Patrocinadores

Patrocinador primário: Universidade Estadual Julio de Mesquita Filho

Patrocinadores secundários:
Instituição: Universidade Estadual Julio de Mesquita Filho
Instituição: Faculdade de Ciências e Tecnologia - UNESP

Fontes de apoio financeiro ou material:
Instituição: Fundação de Amparo à Pesquisa do Estado de São Paulo

Condições de saúde

Condições de saúde ou problemas:

PT-BR	EN
Lesões músculo esqueléticas da perna	Leg's musculoskeletal injuries

Descritores gerais para as condições de saúde:

PT-BR	ES	EN
C05: Doenças musculoesqueléticas	C05: Enfermedades musculoesqueléticas	C05: Musculoskeletal diseases
PT-BR	ES	EN
C26: Ferimentos e lesões	C26: Heridas y traumatismos	C26: Wounds and injuries

Descritores específicos para as condições de saúde:

PT-BR	ES	EN
C26.558: Traumatismos da Perna	C26.558: Traumatismos de la Pierna	C26.558: Leg Injuries
PT-BR	ES	EN
A02.633.567: Músculo Esquelético	A02.633.567: Músculo Esquelético	A02.633.567: Muscle, Skeletal

Intervenções

<u>Categorias das intervenções</u>
Other

Intervenções:

PT-BR	EN
Os participantes serão divididos em 3 grupos, sendo 2 grupos intervenção (G1 e G2) e um grupo controle (GC). Cada grupo contará com 20 participantes.	The participants will be divided into 3 groups: 2 intervention groups (G1 and G2) and control group (CG). Each group will have 20 participants.
Serão os grupos: Criomersão - G1 (15 minutos; 9±1°C) Criomersão - G2 (15 minutos; 14±1°C) Controle (15 minutos; sentado)	Groups: Cold water immersion - G1 (15 minutes; 9±1°C) Cold water immersion - G2 (15 minutes; 14±1°C) Control - CG (15 minutes; setead)
Imediatamente após o protocolo de exercício excêntrico, os participantes do grupo intervenção serão imersos até a altura da crista íliaca por 15 minutos à temperatura de 9±1°C ou 14±1°C. Durante esse período, os participantes do grupo controle permanecerão sentados para recuperação passiva.	Immediately after the eccentric exercise protocol, participants in the intervention group will be immersed to the level of the iliac crest for 15 minutes at a temperature of 9±1°C or 14±1°C. During this period, participants in the control group will remain seated for passive recovery.
A temperatura da água será controlada por meio de um termômetro de 0.3° de acurácia. As mesmas estratégias de recuperação ocorrerão no final de cada visita (40 minutos, 24, 48 e 72 horas pós-exercício) a fim de minimizar os potenciais efeitos causados pelo estresse durante a contração voluntária máxima.	Water temperature is controlled by a thermometer accuracy of 0.3°. The same recovery strategies will occur at the end of each visit (40 minutes, 24, 48 and 72 hours post-exercise) in order to minimize the potential effects caused by stress during maximal voluntary contraction.

Descritores para as intervenções:

E02.258: Crioterapia	PT-BR	E02.258: Crioterapia	ES
E05.466: Imersão	PT-BR	E05.466: Inmersión	ES
D01.455.900.400: Gelo	PT-BR	D01.455.900.400: Hielo	ES

Recrutamento

Situação de recrutamento: Recruitment completed

Pais de recrutamento

Brazil

Data prevista do primeiro recrutamento: 2013-09-20

Data prevista do último recrutamento: 2013-12-10

Tamanho da amostra alvo:	Gênero para inclusão:	Idade mínima para inclusão:	Idade máxima para inclusão:
48	M	18 Y	30 Y

Crítérios de inclusão:

Sexo masculino; aparentemente saudáveis; fisicamente ativos	PT-BR	Male; apparently healthy; physically active	EN
---	-------	---	----

Crítérios de exclusão:

Ser etilista; consumir drogas; fumo ou medicamentos anti-inflamatórios de forma crônica; apresentar anemia; processo inflamatório, diabetes; doença cardiovascular; problemas no fígado; episódio de lesão músculo-tendinea ou osteoarticular nos membros inferiores e/ou coluna nos últimos seis meses.	PT-BR	Be alcoholics; drug use; smoking or anti-inflammatory medications chronically; present anemia; inflammation; diabetes; cardiovascular disease; liver problems; injury episode musculotendinous osteoarticular or lower limbs and / or spine in the last six months.	EN
--	-------	---	----

Tipo do estudo

Desenho do estudo:

Ensaio Clínico de tratamento, randomizado, factorial, aberto, com três braços	PT-BR	Clinical trial treatment, randomized, factorial, open, with 3 arms	EN
---	-------	--	----

Programa de acesso expandido	Enfoque do estudo	Desenho da intervenção	Número de braços	Tipo de mascaramento	Tipo de alocação	Fase do estudo
False	Treatment	Factorial	3	Abrir	Randomized-controlled	N/A

Desfechos

Desfechos primários:

PT-BR
Desfecho esperado: Redução da dor muscular, avaliada por meio de uma Escala Visual Análoga de Dor (EVA). Considerou-se o valor respondido compreendido entre 0 – 10. Avaliação nos momentos: basal, 40 minutos, 24h, 48h, 72h e 96h após exercício.

EN
Expected outcome: Reduction of muscle soreness, assessed by a visual analog scale of pain (VAS). It was considered the value between 0 - 10. Time points to evaluation: baseline, 40 minutes, 24h, 48h, 72h and 96h post-exercise.

PT-BR
Desfecho encontrado: Redução da dor muscular, avaliada por meio de uma Escala Visual Análoga de Dor (EVA). Considerou-se o valor respondido compreendido entre 0 – 10, durante a contração voluntária máxima. Foi realizada análise comparativa entre os grupos e entre os momentos. Avaliação nos momentos: basal, 40 minutos, 24h, 48h, 72h e 96h após exercício.

EN
Outcome found: Reduction of muscle soreness, assessed by a visual analog scale of pain (VAS). It was considered the value between 0-10, during maximal voluntary contraction. Comparative analysis was performed between groups and between times. Assessment in moments: Time points to evaluation: baseline, 40 minutes, 24h, 48h, 72h and 96h post-exercise.

Desfechos secundários:

PT-BR
Desfecho esperado (1): alteração de sensibilidade, avaliada por meio do algômetro de pressão, dado em Kgf. Considerou-se o valor de mínimo desconforto sentido. Avaliação nos momentos: basal, 40 minutos, 24h, 48h, 72h e 96h após exercício. Desfecho esperado (2): Análise da concentração de creatina quinase, avaliada por meio do Reflotron Plus. Avaliação nos momentos: basal, 24h, 48h, 72h e 96h após exercício. Desfecho esperado (3): diminuição da força muscular, avaliada por meio do dinamômetro isocinético, na condição isométrica em 3 tentativas de 5 segundos cada. Avaliação nos momentos: basal, 40 minutos, 24h, 48h, 72h e 96h após exercício. Desfecho esperado (4): percepção de recuperação, avaliada por meio de uma escala Likert. Considerou-se o valor respondido compreendido entre 0 – 10. Avaliação nos momentos: basal, 40 minutos, 24h, 48h, 72h e 96h após exercício.

EN
Expected outcome (1): sensitivity alteration, assessed by pressure algometer given in kgf. It was considered the minimum discomfort value. Time points to evaluation: baseline, 40 minutes, 24h, 48h, 72h and 96h after exercise. Expected outcome (2): Analysis of creatine kinase concentration, assessed using the Reflotron Plus. Time points to evaluation: baseline, 24h, 48h, 72h and 96h after exercise. Expected outcome (3): decreased muscle strength, evaluated by means of isokinetic dynamometer, in isometric condition in 3 repetition of 5 seconds. Time points to evaluation: baseline, 40 minutes, 24h, 48h, 72h and 96h after exercise. Expected outcome (4): perception of recovery evaluated using a Likert scale. It was considered the value between 0 - 10. Time points to evaluation: baseline, 40 minutes, 24h, 48h, 72h and 96h after exercise.

PT-BR
Desfecho encontrado (1): alteração de sensibilidade, avaliada por meio do algômetro de pressão, dado em Kgf.

EN
Outcome found (1): sensitivity alteration, assessed by pressure algometer given in kgf. It was considered the minimum

Considerou-se o valor de mínimo desconforto sentido no membro inferior com o músculo relaxado. Foi realizada análise comparativa entre os grupos e entre os momentos. Avaliação nos momentos: basal, 40 minutos, 24h, 48h, 72h e 96h após exercício. Desfecho encontrado (2): Análise da concentração de creatina quinase, avaliada fibas de CK no Reflotron Plus, por meio de punção digital. Foi realizada análise comparativa entre os grupos e entre os momentos. Avaliação nos momentos: basal, 24h, 48h, 72h e 96h após exercício. Desfecho encontrado (3): diminuição da força muscular, avaliada por meio do dinamômetro isocinético, na condição isométrica. O valor foi dado pela melhor das 3 tentativas de 5 segundos cada. Foi realizada análise comparativa entre os grupos e entre os momentos. Avaliação nos momentos: basal, 40 minutos, 24h, 48h, 72h e 96h após exercício. Desfecho encontrado (4): percepção de recuperação, avaliada por meio de uma escala Likert. Considerou-se o valor respondido compreendido entre 0 – 10, sendo 10 totalmente recuperado. Foi realizada análise comparativa entre os grupos e entre os momentos. Avaliação nos momentos: basal, 40 minutos, 24h, 48h, 72h e 96h após exercício.

discomfort value in the lower limb with the relaxed muscle. Comparative analysis was performed between groups and between times. Time points to evaluation: baseline, 40 minutes, 24h, 48h, 72h and 96h after exercise. Outcome found (2): Analysis of creatine kinase concentration evaluated CK tapes in Reflotron Plus, by a finger prick. Comparative analysis was performed between groups and between times. Time points to evaluation: baseline, 24h, 48h, 72h and 96h after exercise. Outcome found (3): decreased muscle strength, evaluated by means of isokinetic dynamometer, in isometric condition. The value given was the best of three repetition of 5 seconds each. Comparative analysis was performed between groups and between times. Time points to evaluation: baseline, 40 minutes, 24h, 48h, 72h and 96h after exercise. Outcome found(4): perception of recovery evaluated using a Likert scale. It was considered the value answered between 0-10, 10 being fully recovered. Comparative analysis was performed between groups and between times. Time points to evaluation: baseline, 40 minutes, 24h, 48h, 72h and 96h after exercise.

Contatos

Contatos para questões públicas

Nome completo: Aryane Flauzino Machado

Endereço: Rua Felipe Carnevale, 150

Cidade: Presidente Prudente / Brazil

CEP: 19060-220

Fone: +55(18)981422543

E-mail: aryfmachado@yahoo.com.br

Afiliação: Universidade Estadual Paulista
FCT/UNESP

Contatos para questões científicas

Nome completo: Aryane Flauzino Machado

Endereço: Rua Felipe Carnevale, 150

Cidade: Presidente Prudente / Brazil

CEP: 19060-220

Fone: +55(18)981422543

E-mail: aryfmachado@yahoo.com.br

Afiliação: Universidade Estadual Paulista

FCT/UNESP

Contatos para informação sobre os centros de pesquisa

Nome completo: Aryane Flauzino Machado

Endereço: Rua Felipe Carnevale, 150

Cidade: Presidente Prudente / Brazil

CEP: 19060-220

Fone: +55(18)981422543

E-mail: aryfmachado@yahoo.com.br

Filiação: Universidade Estadual Paulista
FCT/UNESP

Anexos

http://www.ensaiosclinicos.gov.br/static/attachments/parecer-comite-de-etica_3.pdf (Parecer - Comitê de Ética em Pesquisa Documento gerado pela Plataforma Brasil)


Links adicionais:

[Download no formato ICTRP](#)

[Download no formato XML OpenTrials](#)

Appendix: Ethical approval – Human Research Ethics Committee

Available: http://www.ensaiosclinicos.gov.br/static/attachments/parecer-comite-de-etica_3.pdf

FACULDADE DE CIÊNCIAS E TECNOLOGIA - UNESP/ CAMPUS DE PRESIDENTE		
PARECER CONSUBSTANCIADO DO CEP		
DADOS DO PROJETO DE PESQUISA		
Título da Pesquisa: EFEITOS IMEDIATOS E TARDIOS DA CRIOMERSÃO APÓS PROTOCOLO DE EXERCÍCIO EXCÊNTRICO		
Pesquisador: Aryane Flauzino Machado		
Área Temática:		
Versão: 1		
CAAE: 22229913.0.0000.5402		
Instituição Proponente: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO		
Patrocinador Principal: FUNDAÇÃO DE AMPARO A PESQUISA DO ESTADO DE SÃO PAULO		
DADOS DO PARECER		
Número do Parecer: 396.666		
Data da Relatoria: 13/09/2013		
Apresentação do Projeto:		
O projeto apresenta boa revisão literária. É assunto relevante e importante para as lesões musculares advindas de atividades físicas e a recuperação da lesão muscular. Os protocolos estão bem explicados e fundamentados e não ferem a ética com relação aos sujeitos do estudo.		
Objetivo da Pesquisa:		
O objetivo do presente estudo será analisar e comparar o efeito da criomersão após protocolo de exercício excêntrico a partir de diferentes temperaturas sobre aspectos clínicos (percepção de dor, de recuperação e alteração de sensibilidade), bioquímicos (creatina cinase) e funcionais		
Avaliação dos Riscos e Benefícios:		
os sujeitos poderão apresentar algum desconforto após protocolo de contração excêntrica e recuperação mas receberão cuidados e orientações de profissional médico.		
Comentários e Considerações sobre a Pesquisa:		
nada a relatar		
Considerações sobre os Termos de apresentação obrigatória:		
Estão apresentados e adequados		
Endereço: Rua Roberto Simonsen, 305		
Bairro: Centro Educacional	CEP: 19.060-900	
UF: SP	Município: PRESIDENTE PRUDENTE	
Telefone: (18)3229-5315	Fax: (18)3229-5353	E-mail: cep@fct.unesp.br

FACULDADE DE CIÊNCIAS E
TECNOLOGIA - UNESP/
CAMPUS DE PRESIDENTE



Continuação do Parecer: 396.666

Recomendações:

nada relatar

Conclusões ou Pendências e Lista de Inadequações:

Diante de toda apresentação de documentos e a forma apresentada do projeto de pesquisa e a declaração de profissional na realização e acompanhamento do estudo o projeto será conduzido adequadamente respeitando éticamente os sujeitos da pesquisa

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

O Comitê de Ética em Pesquisa aprovou ad referendum em 16/09/2013

PRESIDENTE PRUDENTE, 16 de Setembro de 2013

Assinador por:
Edna Maria do Carmo
(Coordenador)

Endereço: Rua Roberto Simonsen, 305
Bairro: Centro Educacional **CEP:** 19.060-900
UF: SP **Município:** PRESIDENTE PRUDENTE
Telefone: (18)3229-5315 **Fax:** (18)3229-5353 **E-mail:** cep@fct.unesp.br

A partir da pesquisa realizada pode-se concluir que os resultados da aplicação da imersão em água fria sobre os marcadores de dano muscular ainda não estão plenamente elucidados, variando quanto aos desfechos analisados. Entretanto, ambos os estudos apontam uma dose de aplicação similar.

Ainda que a revisão sistemática seja composta por artigos de baixa qualidade metodológica, a meta-análise realizada sugere uma aplicação com água à temperatura em torno de 11 a 15°C com o tempo de imersão variando entre 11 e 15 minutos. Corroborando os achados, o ensaio clínico randomizado, apresenta uma melhor dose quando aplicada a 14°C por 15 minutos, ainda que seja para recuperação antecipada da *performance*. Sob outro enfoque, conclui-se também que ambos os estudos apontam que a imersão em água fria, quando não produz efeitos que minimizam os marcadores de dano muscular, também não provoca efeitos deletérios.

1. Bleakley CM, Davison GW. What is the biochemical and physiological rationale for using cold-water immersion in sports recovery? A systematic review. *Br J Sports Med.* 2010 Feb;44(3):179–87.
2. Brophy-Williams N, Landers G, Wallman K. Effect of immediate and delayed cold water immersion after a high intensity exercise session on subsequent run performance. *J Sports Sci Med.* 2011 Jan;10(4):665–70.
3. Bailey DM, Erith SJ, Griffin PJ, Dowson A, Brewer DS, Gant N, et al. Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running. *J Sports Sci.* 2007 Sep;25(11):1163–70.
4. Sellwood KL, Brukner P, Williams D, Nicol A, Hinman R. Ice-water immersion and delayed-onset muscle soreness: a randomised controlled trial. *Br J Sports Med.* 2007 Jun;41(6):392–7.
5. Ascensão A, Leite M, Rebelo AN, Magalhães S, Magalhães J. Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. *J Sports Sci.* 2011 Feb;29(3):217–25.
6. Bleakley C, McDonough S, Gardner E, Baxter GD, Hopkins JT, Davison GW. Cold-water immersion (cryotherapy) for preventing and treating muscle soreness after exercise. *Cochrane Database Syst Rev.* 2012 Jan;2:CD008262.
7. Pournot H, Bieuzen F, Duffield R, Lepretre P-M, Cozzolino C, Hausswirth C. Short term effects of various water immersions on recovery from exhaustive intermittent exercise. *Eur J Appl Physiol.* 2011 Jul;111(7):1287–95.

8. Ingram J, Dawson B, Goodman C, Wallman K, Beilby J. Effect of water immersion methods on post-exercise recovery from simulated team sport exercise. *J Sci Med Sport*. 2009 May;12(3):417–21.
9. Glasgow PD, Ferris R, Bleakley CM. Cold water immersion in the management of delayed-onset muscle soreness: Is dose important? A randomised controlled trial. *Phys Ther Sport*. 2014 Jan 29.
10. Getto CN, Golden G. Comparison of Active Recovery in Water and Cold-Water Immersion After Exhaustive Exercise. *Athl Train Sport Heal Care*. 2013 Jul 2;5(4):169–76.
11. Jakeman JR, Macrae R, Eston R. A single 10-min bout of cold-water immersion therapy after strenuous plyometric exercise has no beneficial effect on recovery from the symptoms of exercise-induced muscle damage. *Ergonomics*. 2009 Apr;52(4):456–60.
12. Wilcock IM, Cronin JB, Hing WA. Physiological Response to Water Immersion A Method for Sport Recovery? *Sports Med*. 2006;36(9):1–18.
13. Eston R, Peters D. Effects of cold water immersion on the symptoms of exercise-induced muscle damage. *J Sports Sci*. 1999 Mar;17(3):231–8.
14. Andrews JR, Harrelson GL, Wilk KE. Reabilitação física nas lesões desportivas. 2.ed. Guanabara Koogan, 2000.
15. Pastre CM, Bastos FN, Netto Junior, J, Vanderlei LCM, Hoshi RA. Métodos de Recuperação Pós-exercício: uma Revisão Sistemática. *Rev Bras Med Esporte*. 2009;15:138–44.

Instructions for Author – Sports Medicine

Types of papers

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

- **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 refer to Integrity of Research and Reporting below for more information on reporting requirements.

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all coauthors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both

the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Authors should submit their manuscripts online. Electronic submission substantially reduces the editorial processing and reviewing times and shortens overall publication times. Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.

Internal Review by Editorial Staff

The journal editor will perform an initial appraisal of each manuscript. If your paper has been peer reviewed by another journal as part of a prior submission, the journal editor will also assess any previous editorial/referee comments and how these have been dealt with as part of the appraisal process. If your manuscript is considered unsuitable for the journal to which it has been submitted, it may be assessed for suitability for publication in other Adis journals by appropriate editors. However, it will not be progressed to external peer review for an alternative journal without your permission.

Title page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)

- The email address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references. For manuscripts reporting the results of a systematic review with or without a meta-analysis, the abstract should be structured as described in the PRISMA statement. For these types of manuscripts the abstract length can be increased from the 250 word limit to allow full compliance with PRISMA / CONSORT.

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- 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.

Abbreviations

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

Footnotes

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

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lowercase 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 before the reference list. 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 [13,7].

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; doi: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

Tables

- 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.
- Footnotes to tables should be indicated by superscript lowercase letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and illustrations guidelines

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

Halftone Art

- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art

- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8pt type on an axis and 20pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

When preparing your figures, size figures to fit in the column width.

For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.

For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that. All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1

Submission

Supply all supplementary material in standard file formats.

Please include in each file the following information: article title, journal name, author names; affiliation and email address of the corresponding author.

To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability. A collection of figures may also be combined in a PDF file.

Spreadsheets

Spreadsheets should be converted to PDF if no interaction with the data is intended. If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

Specialized Formats

Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables. Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", "... additional data are given in Online Resource 4". Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

After acceptance

Upon acceptance of your article you will receive a link to the special Author Query Application at Springer’s web page where you can sign the Copyright Transfer Statement online and indicate whether you wish to order OpenChoice and offprints.

Once the Author Query Application has been completed, your article will be processed and you will receive the proofs.

Open Choice

In addition to the normal publication process (whereby an article is submitted to the journal and access to that article is granted to customers who have purchased a

subscription), Springer now provides an alternative publishing option: Springer Open Choice. A Springer Open Choice article receives all the benefits of a regular subscription-based article, but in addition is made available publicly through Springer's online platform SpringerLink.

Springer Open Choice

Copyright transfer: Authors will be asked to transfer copyright of the article to the Publisher (or grant the Publisher exclusive publication and dissemination rights). This will ensure the widest possible protection and dissemination of information under copyright laws. Open Choice articles do not require transfer of copyright as the copyright remains with the author. In opting for open access, the author(s) agree to publish the article under the Creative Commons Attribution License.

Color illustrations: Publication of color illustrations is free of charge.

Proof reading: The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor. After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First: The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

Integrity of research and reporting

Ethical standards

Manuscripts submitted for publication must contain a statement to the effect that all human and animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. It should also be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted.

These statements should be added in a separate section before the reference list. If these statements are not applicable, authors should state: The manuscript does not contain clinical studies or patient data.

The editors reserve the right to reject manuscripts that do not comply with the above-mentioned requirements. The author will be held responsible for false statements or failure to fulfill the above-mentioned requirements.

Conflict of interest

Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research. They should also state that they have full control of all primary data and that they agree to allow the journal to review their data if requested. Therefore the manuscript must be accompanied by the “Conflict of Interest Disclosure Form”.

General

Adis journals endorse the ‘Uniform Requirements for Manuscripts Submitted to Biomedical Journals,’ issued by the International Committee for Medical Journal Editors and are members of the Committee on Publication Ethics.

Ethics/Institutional Review Board Approval of Research

Authors should be able to submit, upon request, a statement from the research ethics committee or institutional review board indicating approval of the research. If the study was not submitted to a research ethics committee or institutional review board, authors may need to provide documentation to prove that not seeking review for the study was in accordance with the policy of their institution. Details of the ethical approval status of the research must be described in the methods section of the paper.

Health Research Reporting Guidelines

Systematic reviews (with or without a meta-analysis) should follow the reporting guidelines of PRISMA. Authors must provide a completed PRISMA flowchart and checklist. Meta-analysis of observational studies in epidemiology should follow the reporting guidelines of MOOSE. Authors must provide a completed MOOSE checklist.

Use of Personal Communications and Unpublished Data

Authors must include a signed statement of permission from each individual identified as a source of information in a personal communication or as a source for unpublished data (this includes papers that have been submitted, but not yet accepted for publication), and specify the date of communication and whether the communication was written or oral.

Duplicate Publication and Duplicate Submission

Manuscripts are considered with the understanding that they have not been published previously and are not under consideration by another publication. Copies of possibly duplicative materials (i.e. those containing substantially similar content or using the

same or similar data) that have been previously published or are being considered elsewhere must be provided at the time of manuscript submission. Submitted or published manuscripts that are found to be duplicated in any substantive way will be addressed in accordance with the guidelines of the Committee on Publication Ethics.

Committee on Publication Ethics

The journal will, however, consider republication of a paper previously published in a language other than English, or simultaneous publication of a paper in multiple journals with different audiences, if the specific circumstances warrant this action. This will be done with full and prominent disclosure of the original source and with any necessary permission. The journal does not consider posting of protocols and results in clinical trial registries to be prior publication. Press releases of studies presented at scientific meetings are also not considered prior publication and will not compromise an author's ability to write up a full study provided the release does not disclose results beyond those presented in the meeting abstract or poster.

Plagiarism

Plagiarism is the use of others' published and unpublished ideas or words (or other intellectual property) without attribution or permission, and presenting them as new and original rather than derived from an existing source. Plagiarism is scientific misconduct and will be addressed as such following the Committee on Publication Ethics guidelines.

Instructions for Author: PLoS ONE

1. Format Requirements

PLOS ONE does not consider pre-submission inquiries. All submissions should be prepared with the following files:

- Cover letter
- Manuscript, including tables and figure legends
- Figures

Prior to submission, authors who believe their manuscripts would benefit from professional editing are encouraged to use language-editing and copyediting services. Obtaining this service is the responsibility of the author, and should be done before initial submission. These services can be found on the web using search terms like "scientific editing service" or "manuscript editing service." Submissions are not copyedited before publication.

Submissions that do not meet the PLOS ONE Publication Criterion for language standards may be rejected.

Cover Letter

You should supply an approximately one page cover letter that:

- Concisely summarizes why your paper is a valuable addition to the scientific literature
- Briefly relates your study to previously published work
- Specifies the type of article you are submitting (for example, research article, systematic review, meta-analysis, clinical trial)
- Describes any prior interactions with PLOS regarding the submitted manuscript

- Suggests appropriate PLOS ONE Academic Editors to handle your manuscript
- Lists any opposed reviewers

Your cover letter should not include requests to reduce or waive publication fees.

Should your manuscript be accepted, you will have the opportunity to include your requests at that time. See PLOS ONE Editorial Policy for more information regarding publication fees.

Manuscript Organization

PLOS ONE considers manuscripts of any length. There are no explicit restrictions for the number of words, figures, or the length of the supporting information, although we encourage a concise and accessible writing style. We will not consider monographs.

All manuscripts should be double-spaced and include line numbers and page numbers.

Manuscripts should begin with the ordered sections:

- Title
- Authors
- Affiliations
- Abstract
- Introduction

And end with the sections of:

- Acknowledgments
- References
- Supporting Information Captions

Figures should be cited in ascending numeric order upon first appearance. Each figure caption should then be inserted immediately after the first paragraph in which it is cited in the article file.

Figures should not be included in the main manuscript file. Each figure must be prepared and submitted as an individual file. Tables should be cited in ascending numeric order upon first appearance. Each table should then be inserted immediately after the first paragraph in which it is cited in the article file.

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

There are no explicit requirements for section organization between these beginning and ending sections. Articles may be organized in different ways and with different section titles, according to the authors' preference. In most cases, internal sections include:

- Materials and Methods
- Results
- Discussion
- Conclusions (optional)

PLOS ONE has no specific requirements for the order of these sections, and in some cases it may be appropriate to combine sections.

Abbreviations should be kept to a minimum and defined upon first use in the text. Non-standard abbreviations should not be used unless they appear at least three times in the text.

Standardized nomenclature should be used as appropriate, including appropriate usage of species names and SI units.

PLOS articles do not support text footnotes. If your accepted submission contains footnotes, you will be asked to move that material into either the main text or the reference list, depending on the content.

Manuscript File Requirements

Authors may submit their manuscript files in Word (as .doc or .docx), LaTeX (as .pdf), or RTF format. Word files must not be protected.

2. Guidelines for Standard Sections

Title

Manuscripts must be submitted with both a full title and a short title, which will appear at the top of the PDF upon publication if accepted. Only the full title should be included in the manuscript file; the short title will be entered during the online submission process.

The full title must be 250 characters or fewer. It should be specific, descriptive, concise, and comprehensible to readers outside the subject field. Avoid abbreviations if possible. Where appropriate, authors should include the species or model system used (for biological papers) or type of study design (for clinical papers).

The short title must be 50 characters or fewer and should state the topic of the paper.

Authors and Affiliations

All author names should be listed in the following order:

- First names (or initials, if used),

- Middle names (or initials, if used), and
- Last names (surname, family name)

Each author should list an associated department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. If the article has been submitted on behalf of a consortium, all author names and affiliations should be listed at the end of the article.

This information cannot be changed after initial submission, so please ensure that it is correct.

To qualify for authorship, one should contribute to all of the following:

1. Conception and design of the work, acquisition of data, or analysis and interpretation of data
2. Drafting the article or revising it critically for important intellectual content
3. Final approval of the version to be published
4. Agreement to be accountable for all aspects of the work

All persons designated as authors should qualify for authorship, and all those who qualify should be listed. Each author must have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgments.

When a large group or center has conducted the work, the author list should include the individuals whose contributions meet the criteria defined above, as well as the group name.

All authors must approve the final manuscript before submission. PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission of the manuscript.

One author should be designated as the corresponding author, and his or her email address or other contact information should be included on the manuscript cover page. This information will be published with the article if accepted.

Abstract

The abstract should:

- Describe the main objective(s) of the study
- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

Abstracts should not include:

- Citations
- Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

Materials and Methods

This section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

We encourage authors to submit detailed protocols for newer or less well-established methods as Supporting Information.

Methods sections of papers on research using human or animal subjects and/or tissue or field sampling must include required ethics statements.

Methods sections of papers with data that should be deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers and version numbers, if appropriate. Accession numbers should be provided in parentheses after the entity on first use. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

Results, Discussion, and Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section or a mixed Discussion/Conclusions section. These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn. Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a

succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions.

Acknowledgments

People who contributed to the work but do not fit the PLOS ONE authorship criteria should be listed in the acknowledgments, along with their contributions. You must ensure that anyone named in the acknowledgments agrees to being so named.

Funding sources should not be included in the acknowledgments, or anywhere in the manuscript file. You will provide this information during the manuscript submission process.

References

General guidelines

- PLOS uses the reference style as outlined in the ICMJE sample references, also referred to as the “Vancouver” style.
- References must be listed at the end of the manuscript and numbered in the order that they appear in the text.
- In the text, citations should be indicated by the reference number in brackets.
- Authors may cite any and all available works in the reference list.
- Authors may not cite unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., “unpublished work,” “data not shown”).
- If an article is submitted to a journal and also publicly available as a pre-print, the pre-print may be cited.

- If related work has been submitted to PLOS ONE or elsewhere, authors should include a copy with the submitted article as confidential supplementary information, for review purposes only.

- Authors should not state 'unpublished work' or 'data not shown,' but instead include those data as supplementary material or deposit the data in a publicly available database.

- Journal name abbreviations should be those found in the NCBI databases.

Reference formatting

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial. References should be formatted as follows:

- Published papers: Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun, B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (*Ailuropoda melanoleuca*). Genet Mol Res. 2011;10: 1576-1588.

- Accepted, unpublished papers: Same as above, but “In press” appears instead of the page numbers or DOI.

- Websites or online articles: Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available: <http://www.globalizationandhealth.com/content/1/1/14>.

- Books: Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.

- Book chapters: Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
- Deposited articles (preprints, e-prints, or arXiv): Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity; 1991. Preprint. Available: arXiv:1403.3301v1. Accessed 17 March 2014.
- Published media (print or online newspapers and magazine articles): Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 29 Jan 2014. Available: <http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html>. Accessed 17 March 2014.
- New media (blogs, websites, or other written works): Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available: <http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/>.
- Masters' theses or doctoral dissertations: Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available: <http://cumincad.scix.net/cgi-bin/works/Show?2e09>.
- Databases and repositories (Figshare, arXiv): Roberts SB. QPX Genome Browser Feature Tracks; 2013. Database: figshare [Internet]. Accessed: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214.
- Multimedia (videos, movies, or TV shows): Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

Figure Legends

Figures should not be included in the manuscript file, but figure legends should be. Figure legends should describe the key messages of a figure. Legends should have a short title of 15 words or less. The full legend should have a description of the figure and allow readers to understand the figure without referring to the text. The legend itself should be succinct, avoid lengthy descriptions of methods, and define all non-standard symbols and abbreviations.

Figures should be cited in ascending numeric order upon first appearance. Each figure caption should be inserted immediately after the first paragraph in which they are cited in the article file.

Supporting Information Captions

Because Supporting Information is accessed via a hyperlink attached to its captions, captions must be listed in the article file. Do not submit a separate caption file. It is acceptable to have them in the file itself in addition, but they must be in the article file for access to be possible in the published version.

The file category name and number is required, and a one-line title is highly recommended. A legend can also be included but is not required. Supporting Information captions should be formatted as follows.

Data Reporting Guidelines

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article. Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data

types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific, general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CCBY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include: Dryad and figshare. Please contact data@plos.org to make recommendations for further partnerships.

Accession Numbers

All appropriate datasets, images, and information should be deposited in public resources. Please provide the relevant accession numbers (and version numbers, if appropriate). In addition, as much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database. Providing accession numbers allows linking to and from established databases and integrates your article with a broader collection of scientific information.

Striking Images

Authors are encouraged to upload a "striking image" that may be used to represent their paper online in places like the journal homepage or in search results. The striking image must be derived from a figure or supporting information file from the paper, ie. a cropped

portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows. If no striking image is uploaded, a figure from the paper will be designated as the striking image.

Please keep in mind that PLOS's Creative Commons Attribution License applies to striking images. As such, do not submit any figures or photos that have been previously copyrighted unless you have express written permission from the copyright holder to publish under the CCAL license. Note that all published materials in PLOS ONE are freely available online, and any third party is permitted to read, download, copy, distribute, and use these materials in any way, even commercially, with proper attribution.

Care should be taken with the following image types in particular:

1. PLOS ONE is unable to publish any images generated by Google software (Google Maps, Street View, and Earth)
2. Maps in general are usually copyrighted, especially satellite maps
3. Photographs
4. Commercial or government images, slogans, or logos
5. Images from Facebook or Twitter

Authors must also take special care when submitting manuscripts that contain potentially identifying images of people. Identifying information should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF).

Tables

Tables should be cited in ascending numeric order upon first appearance. Each table should be inserted immediately after the first paragraph in which it is cited in the article

file. All tables should have a concise title. Footnotes can be used to explain abbreviations. Citations should be indicated using the same style as outlined above. Tables occupying more than one printed page should be avoided, if possible. Larger tables can be published as Supporting Information.

3. Specific Reporting Guidelines

Human Subject Research

Methods sections of papers on research using human subject or samples must include ethics statements that specify:

- The name of the approving institutional review board or equivalent committee(s). If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed

- Whether informed consent was written or oral. If informed consent was oral, it must be stated in the manuscript:

- Why written consent could not be obtained
- That the Institutional Review Board (IRB) approved use of oral consent
- How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example

whether any rules of human categorization were required by their funding agency

- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis.

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: "Caucasian" should be changed to "white" or "of [Western] European descent" (as appropriate); "cancer victims" should be changed to "patients with cancer."

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal (PDF), which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

Clinical Trials

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement website. Before the paper can enter peer review, authors must:

1. Provide the registry name and number in the methods section of the manuscript
2. Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as Supporting Information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.

3. Include the CONSORT flow diagram as the manuscript's "Fig. 1"

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The methods section must include the name of the registry, the registry number, and the URL of your trial in the registry database for each location in which the trial is registered.