



Escola Superior de Saúde do Porto

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**Effect of cold-water immersion on  
muscle stiffness following exercise-  
induced muscle damage**

Orientador: Doutor Rui Torres

Mestrado em Fisioterapia  
Opção Desporto

Novembro de 2017



**Escola Superior de Saúde  
Instituto Politécnico do Porto**

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Dissertação submetida à Escola Superior de Saúde para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Fisioterapia – Opção Desporto, realizada sob a orientação científica do Professor Doutor Rui Manuel Tomé Torres.

**Novembro de 2017**



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## Resumo

**Contexto:** Enumeras estratégias são usadas na fisioterapia para reduzir o efeito do dano muscular após o exercício intenso. Estudos recentes focaram a atenção no papel da crioterapia após indução de dano muscular e a sua relevância dos marcadores de dano muscular. No entanto, existe falta de evidência sobre o efeito da crioterapia, nomeadamente da imersão em água fria na rigidez muscular, após a agressão muscular induzida pelo exercício. **Objetivo:** Analisar o efeito da imersão em água fria na rigidez muscular após exercício indutor de dano muscular. **Participantes:** Trinta jovens voluntários do sexo masculino, não atletas, ( $21.1 \pm 1.6$  anos,  $177.6 \pm 6.4$  cm,  $75.9 \pm 10,0$  kg,  $15.9 \pm 2.9\%$  massa gorda) foram aleatoriamente colocados no grupo controlo e no grupo da imersão em água fria. **Design do estudo:** Todos os participantes foram submetidos a um programa de exercícios pliométricos para induzir dano muscular; no entanto, o grupo da imersão em água fria foi submetido duas imersões em água a  $10^\circ\text{C}$  ( $\pm 1^\circ\text{C}$ ) durante 10 minutos. **Variáveis:** Limiar de dor à pressão, máxima contração isométrica voluntária, rigidez passiva longitudinal e rigidez passiva transversal foram avaliadas nos músculos solear e gêmeo em 4 momentos: antes do exercício, imediatamente após exercício, 24 e 72 horas após o protocolo de agressão. **Resultados:** Não foram observadas diferenças estatisticamente significativas entre grupos na variável máxima contração isométrica voluntária ( $p=0.529$ ), rigidez passiva longitudinal ( $p=0.315$ ), e rigidez passiva transversal ( $p=0.218$ ). Apenas na variável Limiar de dor à pressão foram observadas diferenças estatisticamente significativas entre grupos no músculo solear, imediatamente após exercício ( $p=0.040$ ). **Conclusão:** Os resultados apresentados demonstram que a crioterapia não teve influência na rigidez muscular. Contudo, a variável de limiar de dor à pressão foi influenciada pela crioterapia imediatamente após exercício.

**Palavras-chave:** crioterapia, tónus muscular, exercício pliométrico, dano muscular, exercício excêntrico



## **Abstract**

**Context:** Many strategies are used in physical therapy to reduce the effect of muscle damage following intense exercise. Recent studies have focused on the role of cryotherapy following exercise-induced muscle damage and its relevance for muscle-damage markers. Nevertheless, there is a lack of scientific evidence regarding the effect of this intervention on muscle stiffness. **Objective:** To analyze the effect of cryotherapy on muscle stiffness following exercise-induced muscle damage.

**Participants:** Thirty (30) untrained males ( $21.1 \pm 1.6$  y,  $177.6 \pm 6.4$  cm,  $75.9 \pm 10.0$  kg,  $15.9 \pm 2.9\%$  fat mass) were randomly allocated into a control group (CON) or a cold-water immersion group (CWI). **Design and settings:** All participants underwent a plyometric exercise program to induce muscle damage; however, the CWI group was subsequently subjected twice to cold-water immersion at  $10^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ) for 10 minutes. **Measures:** Pressure pain threshold (PPT), maximal voluntary isometric contraction, passive longitudinal stiffness and passive transverse stiffness were evaluated on the soleus and gastrocnemius muscles at four moments: pre-exercise, immediately post-exercise, 24 hours, and 72 hours after the damage protocol. **Results:** No significant differences between groups were observed in regard to maximal voluntary isometric contraction ( $p=0.529$ ), passive longitudinal stiffness ( $p=0.315$ ), and passive transverse stiffness ( $p=0.218$ ). Only a significant decrease was observed in PPT on the soleus muscle in the CWI group compared to the CON group immediately following exercise ( $p=0.040$ ). **Conclusion:** The results show that cryotherapy had no influence on muscle stiffness. However, PPT was influenced by CWI immediately following exercise.

**Keywords:** cryotherapy, muscle tone, plyometric exercise, muscle damage, eccentric exercise



## **Lista de Abreviaturas**

CWI – Cold-water immersion group.

CON – Control group.

DOMS – Delayed onset muscle soreness.

EIMD – Exercise-induced muscle damage.

MIVC – Maximal isometric voluntary contraction.

MVC – Maximal voluntary force.

PLS – Passive longitudinal stiffness.

PTS – Passive transverse stiffness.

PPT – Pressure pain threshold.



## **1.Introdução Geral**

Dos vários exercícios realizados para o aumento da performance desportiva, destacam-se o squat jump, countermovement jump e drop jump. Estes saltos são comumente realizados por compreenderem um ciclo de alongamento-encurtamento. Para que esse ciclo seja eficaz deve obedecer a três condições fundamentais: uma pré-ativação muscular antes da fase excêntrica, uma fase excêntrica curta e rápida e, por fim, uma fase de transição rápida entre a fase excêntrica (alongamento) e a fase concêntrica (encurtamento) (Komi & Golhofer, 1997).

Apesar do benefício deste exercício em termos de performance desportiva, devido à exigente contração excêntrica que inclui, este tornasse agressivo pela mecânica do salto e necessidade de produção de força assim como pela solicitação de fibras tipo II. A realização de exercícios que envolvam contrações excêntricas de alta intensidade está usualmente associada à produção de dano muscular (Chambers, Noakes, Lambert, & Lambert, 1998). As mudanças estruturais que ocorrem nas fibras musculares extrafusais, tais como irregularidades do tecido estriado e rutura do sarcómero, descritas como “popping sarcomere hypothesis” (Friden, Sjostrom, & Ekblom, 1983; Morgan & Proske, 2004), danos nos túbulos-t, retículo sarcoplasmático e sarcolema (Friden & Lieber, 2001), são induzidos por exercícios intensos e/ou não habituais, particularmente quando as contrações musculares excêntricas estão envolvidas (Armstrong, Warren, & Warren, 1991). Estas alterações na integridade das fibras musculares extrafusais, nomeadamente nas fibras de tipo II que possuem as linhas Z mais estreitas e fracas, e o tecido conjuntivo menos robusto do que as do Tipo I (Cheung, Hume, & Maxwell, 2003), levam à redução da força voluntária máxima (Torres, Pinho, Duarte, & Cabri, 2013) entre outros marcadores relevantes de dano muscular.

Um sinal comum presente após realização de exercícios que induzem dano muscular (EIDM) é a rigidez muscular, que aumenta logo após exercício e mantém-se elevada durante 5 dias (Janecki, Jarocka, Jaskolska, Marusiak, & Jaskolski, 2011). Esta alteração neuromuscular levará a lesões minor nas fibras musculares intrafusais durante o exercício agressivo tais como as verificadas nas fibras extrafusais (Algaflly & George, 2007). Consequentemente, se as fibras intrafusais são também danificadas alterações de sensibilidade dos fusos neuromusculares serão de esperar provocando alterações nos inputs sensoriais nomeadamente nas fibras aferentes Ia e II, e o aumento do recrutamento de

unidades motoras em repouso. No entanto, o distúrbio metabólico no músculo não pode ser excluído e, certamente, explicará parte do mecanismo relacionado ao EIDM (Armstrong, 1990; Warren, Ingalls, Lowe, & Armstrong, 2002). O aumento da temperatura do músculo, a respiração mitocondrial insuficiente, o pH reduzido e a produção de radicais livres que levam à disfunção da bomba de Na-K-ATPase e Ca<sup>2+</sup> -ATPase, resultando em perda de hemóstase dos íons Ca<sup>2+</sup> (Mattson, Gleichmann, & Cheng, 2008).

Paralelamente ao déficit de força e aumento de rigidez muscular, existem outros sinais e sintomas que aparecem após EIDM, tais como a dor muscular de início retardado, edema e aumento dos valores de creatina quinase (Bieuzen, Bleakley, & Costello, 2013). No sentido de recuperar os músculos após EIDM, diferentes intervenções são rotineiramente utilizadas. No entanto, a eficácia destas intervenções é ambígua relativamente a qual é a melhor das hipóteses. A massagem terapêutica, alongamento, exercício ativo e crioterapia são exemplos de técnicas de fisioterapia usadas no tratamento da dor e lesão tecidual (Torres, Ribeiro, Alberto Duarte, & Cabri, 2012).

Estudos recentes têm-se centrado no papel da crioterapia nos sinais e sintomas da EIDM e na sua relevância na alteração desses marcadores. No entanto, há alguma controvérsia relativamente ao melhor método de aplicação como em relação aos seus reais benefícios. A crioterapia, é comumente utilizada com o objetivo de aliviar e/ou minimizar as possíveis perturbações associadas à performance (Pointon, Duffield, Cannon, & Marino, 2011; Wilcock, Cronin, & Hing, 2006). Neste contexto, é mais eficaz quando comparada com a imersão em água quente (> 36°C), a imersão à temperatura neutra (entre 20 e 36°C) ou a recuperação passiva (Versey, Halson, & Dawson, 2013). Os efeitos associados à sua aplicação incluem as reduções do metabolismo celular, do fluxo sanguíneo local, da velocidade de condução nervosa e da atividade dos fusos neuromusculares (Bleakley, McDonough, & MacAuley, 2004; Nadler, Weingand, & Kruse, 2004; Versey et al., 2013). Uma revisão de 27 artigos revelou que o uso de crioterapia melhorou significativamente os sintomas provocados pelo exercício 24h após comparado com o grupo de controlo (Hohenauer, Taeymans, Baeyens, Clarys, & Clijsen, 2015). Não obstante, os efeitos da crioterapia em atividades desportivas e de reabilitação no músculo/tendão não estão esclarecidas (Kubo, Kanehisa, & Fukunaga, 2005).

Relativamente ao efeito da crioterapia na rigidez muscular a evidência científica é não é concreta. No entanto, estudos (Lum, Tan, Pang, & Barbosa, 2016; Muraoka et al., 2008) defendem que esta terapia aumenta a rigidez muscular, isto porque a aplicação de frio causa alterações nas propriedades mecânicas do músculo, tornando-o, mais tenso, mais rígido e com menor capacidade viscoelástica (Mustalampi, Ylinen, Kautiainen, Weir, & Hakkinen, 2012). Contudo, quando aplicada após o exercício excêntrico, a crioterapia parece reduzir a rigidez (Eston & Peters, 1999). Estes fatores são importantes no que diz respeito ao desempenho desportivo e à diminuição do risco de lesões, devendo existir mais evidência científica sobre a temática (McHugh & Cosgrave, 2010).

Assim, o propósito deste estudo prende-se pela análise do efeito de um protocolo de imersão em água fria na rigidez muscular, após um protocolo de exercício indutor de dano muscular.

Deste modo, o presente documento é composto por 3 partes: A introdução geral, capítulo desenvolvido relativamente à temática das alterações da função neuro muscular e a da influência da crioterapia na sua recuperação; O artigo experimental, trabalho submetido à revista “Journal of Sport Rehabilitation” a 15 de novembro de 2017; e as conclusões gerais e perspetivas futuras.



## 2. Artigo Experimental

### Effect of cold-water immersion on muscle stiffness following exercise-induced muscle damage

João Pinto, António Rocha, Rui Torres

#### Abstract

**Context:** Many strategies are used in physical therapy to reduce the effect of muscle damage following intense exercise. Recent studies have focused on the role of cryotherapy following exercise-induced muscle damage and its relevance for muscle-damage markers. Nevertheless, there is a lack of scientific evidence regarding the effect of this intervention on muscle stiffness. **Objective:** To analyze the effect of cryotherapy on muscle stiffness following exercise-induced muscle damage. **Participants:** Thirty (30) untrained males ( $21.1 \pm 1.6$  y,  $177.6 \pm 6.4$  cm,  $75.9 \pm 10.0$  kg,  $15.9 \pm 2.9\%$  fat mass) were randomly allocated into a control group (CON) or a cold-water immersion group (CWI). **Design and settings:** All participants underwent a plyometric exercise program to induce muscle damage; however, the CWI group was subsequently subjected twice to cold-water immersion at  $10^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ) for 10 minutes. **Measures:** Pressure pain threshold (PPT), maximal voluntary isometric contraction, passive longitudinal stiffness and passive transverse stiffness were evaluated on the soleus and gastrocnemius muscles at four moments: pre-exercise, immediately post-exercise, 24 hours, and 72 hours after the damage protocol. **Results:** No significant differences between groups were observed in regard to maximal voluntary isometric contraction ( $p=0.529$ ), passive longitudinal stiffness ( $p=0.315$ ), and passive transverse stiffness ( $p=0.218$ ). Only a significant decrease was observed in PPT on the soleus muscle in the CWI group compared to the CON group immediately following exercise ( $p=0.040$ ). **Conclusion:** The results show that cryotherapy had no influence on muscle stiffness. However, PPT was influenced by CWI immediately following exercise.

**Keywords:** cryotherapy, muscle tone, plyometric exercise, muscle damage, recovery, eccentric exercise



## Introduction

Multiple exercises including eccentric muscle actions are generally performed to increase athletic performance; these include the squat jump, countermovement jump and drop jump. These exercises, containing a stretch–shortening cycle, comprise three fundamental conditions: a well-timed reactivation of the muscles before the eccentric phase, a short and fast eccentric phase, and an immediate transition (a very short delay) between the eccentric and concentric phases.<sup>1</sup>

Exercises involving high-intensity eccentric contractions are usually associated with muscle damage.<sup>2</sup> Indeed, structural and ultrastructural changes in the extrafusal muscle fiber are induced by intense and/or unaccustomed exercise,<sup>3</sup> namely irregularities of the cross-striated and sarcomere disruption described as the “popping sarcomere hypothesis”<sup>4,5</sup> damage t-tubules, sarcoplasmic reticulum and sarcolemma.<sup>6</sup> These alterations in the integrity of the extrafusal muscle fibers, particularly in type II fibers, which have the narrowest and weakest z-lines and less robust connective tissue in comparison to type I fibers,<sup>7</sup> lead to reduction in maximal voluntary force<sup>8</sup> and are accompanied by other relevant indirect markers of muscle damage.

A common sign observed after exercise-induced muscle damage (EIMD) is an increase in muscle stiffness, which can be observed immediately post-exercise and normally persists for 5 days.<sup>9</sup> This neuromuscular dysfunction is likely to lead to similar minor injuries in intrafusal muscle fibers during aggressive exercise as those verified in the extrafusal.<sup>10</sup> Hence, if this type of fiber is also damaged, changes in the sensitivity of neuromuscular spindles muscles should be expected, interfering in sensorial inputs, namely Ia and II afferent fibers, which consequently could increase the motor unit’s recruitment at rest. However, metabolic muscle disturbance cannot be excluded and, certainly, explains part of the mechanism related to EIMD.<sup>11,12</sup> The elevation in muscle temperature, insufficient mitochondrial respiration, lowered pH and free radical production lead to both Na-K-ATPase and Ca<sup>2+</sup>-ATPase pump dysfunction, resulting in loss of Ca<sup>2+</sup> ion homeostasis.<sup>13</sup> Parallel to the deficit of muscle strength and increment in muscle stiffness, other signals and symptoms appear following EIMD, such as delayed onset muscle soreness (DOMS), edema or an increase in levels of serum creatine kinase.<sup>14</sup>

To alleviate signs and symptoms of exercise-induced muscle damage (EIMD), different methods of cryotherapy intervention are routinely utilized.<sup>15</sup> Recent studies have focused on the role of cryotherapy in signs and symptoms of EIMD and its relevance for improving fast recovery. Nevertheless, some controversy exists regarding the best method of application when compared to its real benefits.

Indeed, cryotherapy is commonly used to minimize the potential detrimental effects of soreness and damage as a result of training and competition.<sup>16,17</sup> It is noteworthy that cryotherapy reduces inflammatory responses, thereby alleviating muscle spasm and pain,<sup>18,19</sup> as well as reducing nerve conduction velocity.<sup>20</sup> Moreover, it is associated with vasoconstriction and a relative decrease in blood flow.<sup>17,21</sup> A review of 27 articles revealed that the use of cryotherapy significantly improved symptoms caused by exercise at 24 hours compared to the control group.<sup>22</sup> However, Jakeman et al. (2009) reported that cryotherapy following damaging exercise had no beneficial effect on the recovery of maximal voluntary force (MVC) or perceived soreness. Sellwood et al. (2007) also reported that cryotherapy did not minimize markers of DOMS, including the perception of pain or tenderness. Apart from that, cryotherapy could interfere in muscle stiffness<sup>23,24</sup> by decreasing its viscoelastic capacity and making it more tense and rigid due to changes in the mechanical properties of the muscle.<sup>25</sup>

Furthermore, when applied following exercise, cryotherapy may also reduce stiffness,<sup>26</sup> which is important for sports performance and the reduced risk of injury. Therefore, there more scientific evidence regarding this subject is needed.<sup>27</sup> Thus, the aim of this study was to analyze the effect of cryotherapy on muscle stiffness following muscle damage caused by strenuous exercise.

## **Methods**

### **Settings and Participants**

Before the experimental study, a pilot study involving 15 participants ( $21.1 \pm 1.3$  years;  $76.6 \pm 12.5$  kg;  $178.8 \pm 8.1$  cm and  $14.3 \pm 3.4$  % of body fat) was conducted to determine the reliability of all variables used in the experimental study and to calculate the sample size. Aiming to establish intra-rater reliability, the same clinician assessed the

pressure pain threshold, maximal voluntary isometric contraction, passive longitudinal stiffness, and passive transverse stiffness twice, with a 24-hour interlude between each moment.

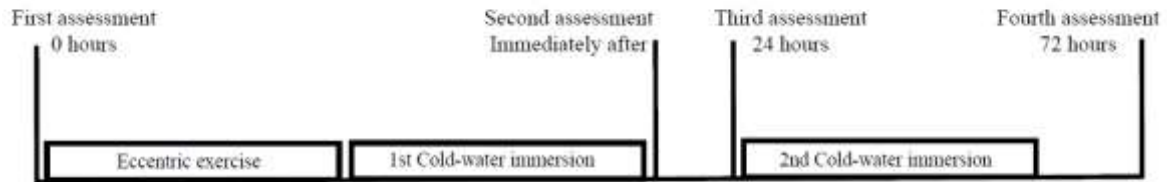
In the experimental study, 30 males ( $21.1 \pm 1.6$ y;  $177.6 \pm 6.4$ cm;  $75.9 \pm 10$  Kg and  $15.9 \pm 2.9\%$  of body fat), university students volunteered to participate after being informed about its aims, experimental protocol, and procedures. The subjects were all adults and non-athletes. Exclusion criteria included acute injury or pain in the lower limbs during the previous 6 months, skin lesions in the lower limbs, hypersensitivity to cold,<sup>28</sup> and previous and continuing use of steroids or nutritional supplements.<sup>29</sup> All participants read and signed the declaration of informed consent based on the Declaration of Helsinki before participating in the study.

The participants' lack of familiarity with eccentric training was ensured, and they were asked to refrain from doing any resistance training during the protocol.

### **Study design**

A leg-to-leg comparison model was used, i.e., all subjects participated in both groups, having their lower limbs equally and randomly being allocated to the cold-water immersion (CWI) and control (CON) groups. Thus, the lower limb that would be included in the control or experimental group was randomly chosen. The experimental protocol comprised four evaluation moments. The first assessment was performed before the eccentric exercise and the others immediately after the first cold-water immersion, at 24 and 72 hours following exercise.

Two cold-water immersions were selected as a treatment: immediately after exercise and at 24 hours (before the third assessment). At all evaluation moments, pressure pain threshold, maximal voluntary isometric contraction, passive longitudinal stiffness and passive transverse stiffness were measured.



**Figure 1** - Schematic representation of the study.

### **Muscle-damaging protocol**

Prior to performing the protocol to induce muscle damage, all subjects were given clear instructions about the exercise and the opportunity to experiment with the equipment involved in the procedures to ensure correct technique and maximal effort. Plyometric jumps were selected to induce muscle damage: participants performed 100 jumps (10 sets of 10 repetitions with one-minute intervals) from a box measuring 0.5m in height. Upon dropping and landing, subjects were instructed to jump as high as possible and land at the same place each time. This protocol had already been used successfully in previous studies.<sup>30,31</sup>

### **Cold-water immersion**

To carry out the cryotherapy protocol, the experimental leg was immersed and maintained in water at 10°C ( $\pm$  1°C) for 10 minutes. Ice cubes were added as needed to maintain the temperature within the desired range. Participants kept their leg underwater with their knee at 90° of flexion.<sup>31</sup>

### **Criterion measurements**

Pressure pain threshold was assessed using an algometer (Somedic Algometer Type II, Sollentuna, Sweden). Its reliability was calculated in the pilot study with good results (ICC=0,840). The device was placed perpendicularly on the soleus and medial gastrocnemius muscles. The precise reference point of the medial gastrocnemius muscle was marked in the most prominent region of the muscle.<sup>32</sup> Each individual was in a prone position with his knee slightly flexed, and a contraction of the muscle was requested to find its most prominent point. Relative to PPT assessment on the soleus muscle, each participant was in a supine position with his knee at 90° of flexion. The precise reference point on this muscle was marked two-thirds of the distance between the medial condyle of the femur and the

medial malleolus. Pressure was applied with the algometer on the tissue, and the individual was asked to say “Stop” when the pressure sensation reached the pain threshold.<sup>33</sup> Three measurements separated by 20 seconds were performed, and the mean value was calculated to describe the PPT.<sup>34</sup> This was previously performed on other muscles to familiarize participants with the procedure.

The passive transverse stiffness variable was evaluated using Myoton technology (Myoton AS, Tallinn, Estonia), which demonstrated good reliability in our pilot study (ICC=0,813). Muscle stiffness is a myotonometric parameter that reflects a muscle’s resistance to the force that changes its shape. The Myoton Pro III is a device that enables the measurement of muscle properties including stiffness. The device enables the recording of the mechanical oscillations of the tissue provoked by mechanical impact (0.4 N) made by the testing-end component (with mass  $m=20\text{g}$  and area of the testing-end 3mm in diameter). By means of a switch, the device’s electromagnet produces a short (15ms) constant force impulse that is forwarded via the testing-end to the contact area. This causes tissue deformation that is recorded by an acceleration-transducer situated on the testing-end. Myoton Pro III was programmed to produce 6 consecutive measurements at each point, and the mean of these measurements was automatically calculated by the software incorporated in the device. The points used to assess the pressure pain threshold variable were also used to evaluate muscle stiffness. The Myoton Pro III was placed perpendicularly to the muscular references previously marked.<sup>35</sup> The subject was asked to relax during measurements.

Apart from transverse stiffness, passive longitudinal stiffness was also measured through an Isokinetic Dynamometer Biodex System 3 (Biodex Medical Systems, Inc, New York, USA), the reliability of which was assessed in the pilot study (ICC=0,801). The values of longitudinal stiffness were calculated by the variation of the passive torque divided by the amplitude and presented in  $\text{N.m} / ^\circ$ . An arc of movement of  $20^\circ$ , from  $5^\circ$  to  $25^\circ$  starting at the maximal stretching of the sural triceps, was defined to reach this variable.

The participants were familiarized with the instrument and informed regarding all procedures. Each individual was correctly positioned in the isokinetic chair, taking into account the  $90^\circ$  of knee flexion and the ankle being in a neutral position. Starting from the maximal position of stretching of the sural triceps, the passive torque of  $70^\circ$  of a range of

motion was assessed, first, in the direction of plantar flexion and afterwards in the opposite direction. This movement was repeated three times.

The Biodex System 3 was also used to evaluate the maximal isometric voluntary contraction (MIVC) of the muscles of the sural triceps; this equipment is frequently used in neuromuscular muscle-function studies. Briefly, after a warm-up set of four submaximal muscle actions, participants completed three maximal isometric contractions of 5s each separated by 5s of rest. Maximal voluntary isometric torque of the sural triceps was assessed with 90° of the knee flexion and the ankle in a neutral position. Participants were given verbal encouragement, and the best performance of the three contractions, as measured by the dynamometer software (Biodex System 3 Advantage Software, Biodex Medical System, Inc., Shirley, NY), was collected.

### **Statistics**

The Shapiro–Wilk test for normality revealed that the majority of the variables were not normally distributed. Nonparametric analysis was carried out using the Friedman test to determine whether changes in scores differed across the 4 moments of evaluation, with the alpha level set at  $p \leq .05$ . The Wilcoxon signed-rank test was used to compare individual differences between moments. The Mann–Whitney U test was performed to analyze the results between groups. Statistical significance was set at  $p < 0.05$ . SPSS version 24.0 was used.

## Results

**Table 1** - Intragroup comparisons over time in for Pressure pain threshold, maximal voluntary isometric contraction, passive longitudinal stiffness and passive transversal stiffness (values are expressed as median and interquartile range),  $p < 0.05$ . (CON – Control group; CWI – Cold-water immersion group).

Variable	Muscle	Group	Before	1 hour	24 hours	72 hours	p value	
<b>Maximal Voluntary Isometric Contraction (N.m)</b>	Triceps Surae	CON	85.2 (79.6 - 98.4)	79.6 (74.7 - 84.9) a	80.1 (63.3 - 93.1) a	88.3 (72.8 - 93.1)	0.008	
		CWI	86.4 (77.5 - 93.9)	74.1 (65.2 - 94.7)	83.2 (67.9 - 91.6)	85.3 (74.9 - 96.1)	0.564	
		p < 0.05: a vs Before						
		<hr/>						
<b>Passive Longitudinal Stiffness (N.m<sup>2</sup>)</b>	Triceps Surae	CON	1.2 (1.1 - 1.9)	1.2 (0.9 - 1.2)	1.3 (0.9 - 1.57)	1.0 (0.9 - 1.2)	0.392	
		CWI	1.2 (0.9 - 1.3)	1.01 (0.9 - 1.2)	1.01 (0.9 - 1.2)	1.0 (0.9 - 1.3)	0.401	
		<hr/>						
		<hr/>						
<b>Passive Transversal Stiffness (N/m)</b>	Triceps Surae	CON	353.7 (307.5 - 394.3)	378.5 (337.0 - 406.3)	394.5 (343.5 - 448.8) a	379.5 (338.3 - 412.8)	0.024	
		CWI	364.3 (345.8 - 417.0)	400.8 (349.8 - 438)	396.5 (374.5 - 438.8) a	370.5 (355.5 - 435.3)	0.151	
	Internal Gastrocnemius	CON	316.1 (311.5 - 326.3)	358.2 (343.0 - 373.3)	356.5 (330.5 - 380.8) a	350.2 (313.8 - 394.3)	0.105	
		CWI	316.8 (303.0 - 338.8)	340.5 (318.5 - 414.8)	367.4 (325.5 - 387.3) a	361.7 (312.8 - 389.5)	0.279	
	p < 0.05: a vs Before							
	<hr/>							
<b>Pressure Pain Threshold (KPa)</b>	Triceps Surae	CON	559.2 (420.4 - 952.2)	460.0 (369.1 - 949.7)	438.2 (365.7 - 894.3) a	425.7 (376.8 - 885.4) a	0.034	
		CWI	545.5 (400.7 - 906.3)	594.8 (494.0 - 999.6)	417.0 (357.8 - 889.4) b	492.0 (377.7 - 984.8)	0.001	
	Internal Gastrocnemius	CON	539.7 (416.6 - 917.4)	504.2 (361.8 - 800.9)	487.5 (323.7 - 797.6) a,b	488.5 (400.3 - 811.5)	0.004	
		CWI	959.3 (907.3 - 1087.9)	1021.1 (863.8 - 1141.8)	858.7 (777.9 - 932.9) b	912.2 (763.2 - 1008.2) b	0.034	
	p < 0.05: a vs Before; b vs 1h							
	<hr/>							

Analyzing the intragroup results, a significant decrease in pressure pain threshold 24h after exercise in both muscles was observed, meaning that pain increases 24h after exercise ( $p < 0.05$ ). In fact, the PPT decreased from 559.17 kPa to 438.17 kPa in the soleus muscle and from 539.67 kPa to 487.50 kPa in the internal gastrocnemius.

In regard to maximal voluntary isometric contractions, the control group had a significant decrease from 85.2 N.m to 80.1 N.m, 24h after exercise (Table 1). These values suggest that the exercise protocol selected caused muscle damage to the muscles that were studied. Moreover, there was an increase of stiffness in passive longitudinal stiffness (PLS) and passive transverse stiffness (PTS) 24h after exercise. In PLS, we observed an increase of 8.6% and in PTS an increase of 11.4%.

Figure 2A - Maximal voluntary isometric contraction

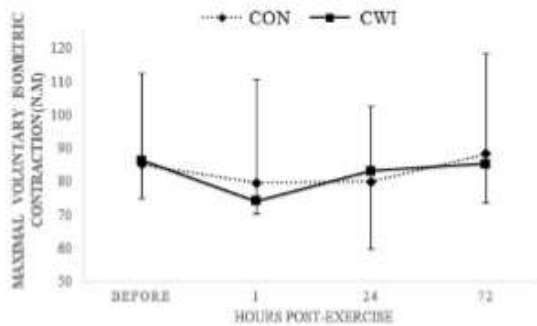


Figure 2B - Passive longitudinal stiffness

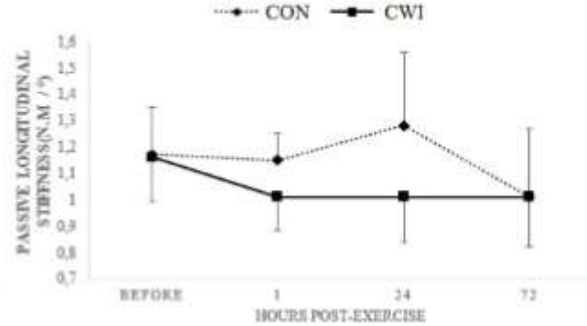


Figure 2C - Passive transverse stiffness

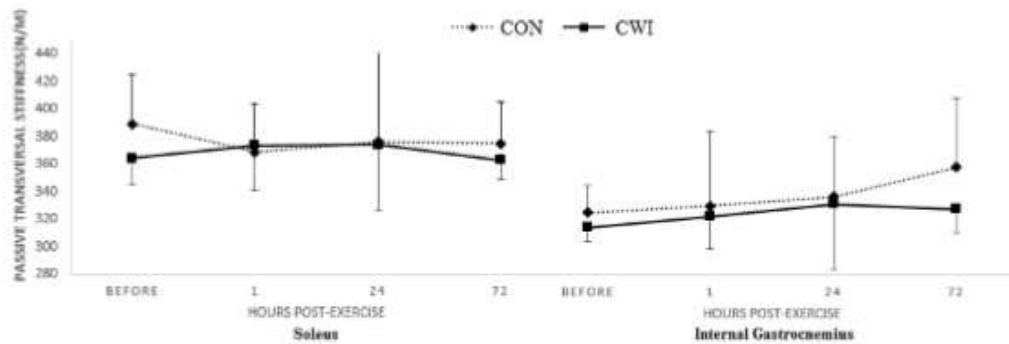
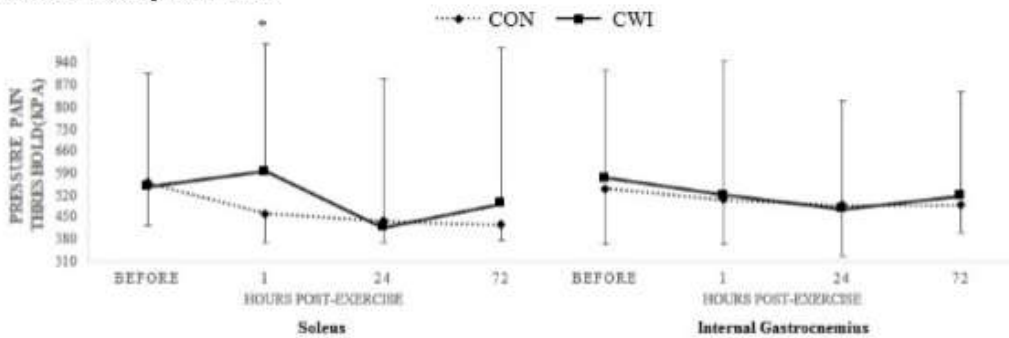


Figure 2D - Pressure pain threshold



**Figure 2 A-D** - Intergroup comparisons for each measure: maximal voluntary isometric contraction, passive 418 longitudinal stiffness, passive transverse stiffness and pressure pain threshold (values expressed as median 419 and interquartile range). \*Indicates a significant difference,  $p < 0.05$ . (CON – Control group; CWI – Cold-420 water immersion group).

In the intergroup analysis, a significant reduction of 22.67% was observed in PPT right after exercise in the soleus muscle (fig. 2D). This difference suggests that the group that performed the cryotherapy protocol after 1h had less pain in comparison to the control group. Although there were several differences between groups in the remaining variables (fig. 2A; fig. 2B; fig. 2C), such as PLS reduction of stiffness of about 21% at 24h hours, no significant statistical differences were found ( $p > .218$ ).

## **Discussion**

Several studies have focused on the role of cryotherapy role for symptoms and signals of EIDM, analyzing its influence in MVIC and PPT.<sup>29-31</sup> In the present study, PPT decreased at 24h after exercise, and the MVIC decreased at 24h after exercise, showing that the drop jump exercise was successful in causing damage, which is in accordance with previous literature using a similar protocol.<sup>29-31</sup>

Significant differences in PPTs right after exercise were observed between groups. This reduction of pain immediately after exercise has already been demonstrated by other authors.<sup>36,37</sup> Some attribute this reduction in muscle pain to the analgesic effects of cooling the surface rather than treating muscle damage.<sup>38-40</sup> The application of cold to muscle tissues, with temperatures around 10–15°C, reduces nerve conduction velocity, muscle spindle activity, the stretch-reflex response, and spasticity, thereby inhibiting the pain–spasm cycle. However, the duration of this analgesia is limited to 1–3 hours.<sup>40</sup> This mechanism can explain the reduction of muscle pain observed just 1h after exercise.

The production of force assessed through the MVIC showed no differences between groups, indicating that the cryotherapy protocol was not effective as a recovery strategy. Those results contrast to the findings of other researchers, who have found beneficial effects using different protocols of cryotherapy.<sup>37,41</sup> However, it is noteworthy that Gill and Beaven and Cook<sup>41</sup> used a protocol with contrast water therapy as an intervention, and Bailey et al., in 2007,<sup>37</sup> observed beneficial effects in knee flexors but no effects in knee extensors. In addition, many studies used pressure pain threshold and maximal voluntary isokinetic contraction variables, but just a few focused their goal on the behavior of muscle stiffness using a cryotherapy protocol following muscle damage. After eccentric exercise, muscle

stiffness is expected to increase<sup>42,43</sup> due to neuromuscular dysfunction<sup>44</sup> and/or metabolic disturbance,<sup>13</sup> corroborating our findings showing significant differences in passive transverse stiffness 24 hours after exercise, with an increase of 10.41% in the soleus muscle and 11.36% in the internal gastrocnemius.

When cryotherapy is applied to superficial tissues, muscle stiffness increases<sup>24,45</sup> due to changes in the mechanical properties of the muscle, making it more tense and rigid and decreasing its viscoelastic capacity.<sup>25</sup> In PTS and PLS variables, no significant differences between groups were found. Although Goodall and Howatson<sup>46</sup> and Howatson, Goodall, and van Someren<sup>29</sup> used different methodologies, their results support our findings.

Contrary to our results are those obtained by Eston and Peters,<sup>26</sup> which showed a decrease in stiffness after the cryotherapy protocol. Despite these differences, time and temperature of cold-water immersion are comparable between studies. However, in the Eston and Peters<sup>26</sup> study, the application of cryotherapy is performed immediately following exercise and every 12 hours for 3 days, while in our study and others, cryotherapy was applied less frequently (following exercise and at 24h). It is possible that cryotherapy, after muscle-damaging exercise, has a greater benefit in decreasing muscle stiffness if applied more frequently and if there is a shorter interval between applications, which will enhance its effects.

## **Conclusion**

The aim of this study was to assess the effectiveness of cold-water immersion on muscle stiffness following strenuous exercise. Although cryotherapy reduces muscle pain 24h after exercise, the results of this study on PTS and PLS variables suggest that cryotherapy has no effect on muscle stiffness.

## **Limitations and further research**

The small number of participants might be an aspect of the present study that contributes to several limitations regarding our results. As well, the subjects in our study, i.e. university students, may make extrapolation of the results to other populations difficult. Another potential limitation is that we analyzed only calf muscles, which may make it difficult to compare our results to other studies because the behavior of variables might change according to different muscles. Further research should investigate alternative modes of inducing muscle damage, varying temperatures and durations of cold-water immersion, as well as the effect of such an intervention in thermocomfortable conditions.



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### **3. Conclusões Gerais**

Tendo como base os resultados obtidos, é possível concluir que a crioterapia após exercício indutor de dano muscular promoveu:

- Aumento da dor 24h após exercício;
- Diminuição da força 24h após exercício;
- Diminuição da dor no grupo CWI comparativamente com o grupo CON 1h após exercício;
- Moderado aumento da rigidez muscular 24h após exercício no grupo CON na variável PTS;

As duas primeiras conclusões advém do protocolo de agressão e do que este pretendia inculir, dano muscular. Este aumento da dor e diminuição da força foi de igual forma verificado por outros autores (Howatson, Goodall, & van Someren, 2009; Jakeman, Macrae, & Eston, 2009; Twist & Eston, 2005) e corroborado com o nosso estudo. Este conhecimento torna-se fundamental no que toca à avaliação do comportamento dos sinais e sintomas do EIDM e, posteriormente, no papel do fisioterapeuta na recuperação destes sintomas.

Relativamente à diminuição da dor no grupo CWI comparativamente com o grupo CON 1h após exercício, isto revela-nos como a crioterapia se assume como uma técnica de recuperação na dor logo após exercício, o que se torna fundamental para o papel do fisioterapeuta na recuperação dos seus atletas após exercício.

Apesar de não terem existido diferenças entre grupos nas variáveis PTS e PLS, o aumento da rigidez provocado pelo exercício corrobora com a literatura existente e revela-nos o comportamento deste sintoma no EIDM.

#### **Perspetivas futuras**

Novos estudos deverão ser realizados tendo como objetivo principal a alteração da rigidez muscular após indução de dano muscular, utilizando um protocolo de recuperação tal como a crioterapia. Como sugestão, achamos pertinente investigar formas alternativas de indução de dano muscular e/ou variar temperatura e duração do protocolo de crioterapia. Sugerimos ainda a alteração do método de recuperação.



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## 5. Anexos

### Termo de consentimento informado

#### TERMO DE CONSENTIMENTO INFORMADO

Trabalho de investigação: **Effect of cold-water immersion on muscle stiffness following exercise-induced muscle damage.**

Investigador: João Pinto

Eu, \_\_\_\_\_, noticio que o aluno de Mestrado em Fisioterapia João Pinto me traçou o projeto que pretende conduzir na Escola Superior de Saúde do Vale do Sousa (um estudo cujo objetivo consiste em verificar o “*Effect of cold-water immersion on muscle stiffness following exercise-induced muscle damage*”) que decorrerá de Novembro de 2016 a Julho de 2017, solicitando a minha integração na amostra que participará no projeto.

Foi-me dada a oportunidade de fazer as perguntas que julguei necessárias e a todas obtive resposta satisfatória.

Pela garantia de que é salvaguardado a confidencialidade, aceito participar neste estudo e autorizo o processamento dos dados que forem gerados pela minha participação no questionário.

Assim, com vista a permitir a normal evolução deste projeto de investigação, responsabilizo-me a cooperar com o estudo.



## Questionário de seleção e caracterização da amostra



ESCOLA  
SUPERIOR  
DE SAÚDE



### QUESTIONÁRIO DE SELEÇÃO E CARACTERIZAÇÃO DA AMOSTRA

Este questionário tem como objetivo selecionar participantes para um estudo de obtenção do grau de Mestrado em Fisioterapia, intitulado de “*Effect of cold-water immersion on muscle stiffness following exercise-induced muscle damage*”.

Assim, venho solicitar a sua colaboração no preenchimento deste questionário, tendo como foco recolher dados importantes para a participação no estudo.

O preenchimento do questionário demora cerca de 2 minutos. Nos espaços em branco deve completar com a informação solicitada e nas questões de opção, deve assinalar com uma cruz (x) a sua resposta. Os dados recolhidos são confidenciais e serão usados apenas para o efeito da investigação.

Nome: \_\_\_\_\_

Idade: \_\_\_\_\_ anos

1. Pratica atividade física regularmente?

Sim

Não

2. Nos últimos 6 meses, participou em algum estudo que implicasse a realização de protocolos de exercício pliométrico ou de resistência?

Sim

Não

3. No último ano, teve alguma lesão músculo-esquelética nos membros inferiores?

Sim

Não

Se sim, qual? \_\_\_\_\_

4. Apresenta alterações de sensibilidade nos membros inferiores (Ex: intolerância ao frio, fenómeno de Raynaud)?

Sim

Não

Se sim, qual? \_\_\_\_\_

5. Apresenta alguma doença cardiorrespiratória que limite a realização de exercício físico?

Sim

Não

Se sim, qual? \_\_\_\_\_

6. Toma habitualmente fármacos anti-inflamatórios, esteroides ou suplementos nutricionais?

Sim

Não

Se sim, qual? \_\_\_\_\_

Obrigado pelo tempo despendido no preenchimento deste questionário!



Folha de recolha de dados

**FOLHA DE RECOLHA DE DADOS**

Nome: \_\_\_\_\_

Membro controlo – \_\_\_\_\_

Membro experimental – \_\_\_\_\_

Dados da Balança digital:

- ▶ Peso (kg): \_\_\_\_\_
- ▶ % Massa gorda: \_\_\_\_\_

Altura (m):

- ▶ \_\_\_\_\_

**MOMENTO 1 (antes do protocolo de agressão e de crioterapia):**

Dor (Algómetro):

<b>M1</b>	<b>M. l. direito</b>		<b>M. l. esquerdo</b>	
	Repetições	Média	Repetições	Média
Gémeo interno	/ /	=	/ /	=
Solear	/ /	=	/ /	=

**MOMENTO 2 (após a crioterapia):**

Dor (Algómetro):

<b>M2</b>	<b>M. l. direito</b>		<b>M. l. esquerdo</b>	
	Repetições	Média	Repetições	Média
Gémeo interno	/ /	=	/ /	=
Solear	/ /	=	/ /	=

**MOMENTO 3 (às 24h e após 1 sessão de crioterapia):**

Dor (Algómetro):

<b>M3</b>	<b>M. l. direito</b>		<b>M. l. esquerdo</b>	
	Repetições	Média	Repetições	Média
Gémeo interno	/ /	=	/ /	=
Solear	/ /	=	/ /	=

**MOMENTO 4 (às 72h e após 2 sessões de crioterapia):**

Dor (Algómetro):

<b>M4</b>	<b>M. l. direito</b>		<b>M. l. esquerdo</b>	
	Repetições	Média	Repetições	Média
Gémeo interno	/ /	=	/ /	=
Solear	/ /	=	/ /	=



XXXVIII

