Effects of Partial-body Cryotherapy (−110°C) on Muscle Recovery between High-intensity Exercise Bouts

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Abstract
The aim of this study was to evaluate the effects of a single partial-body cryotherapy bout between training sessions on strength recovery. Twelve young men (23.9 ± 5.9 years) were randomly exposed to 2 different conditions separated by 7 days: 1) Partial-body cryotherapy (subjects were exposed to 3 min of partial-body cryotherapy at −110°C between 2 high-intensity training sessions); 2) Control (subjects were not exposed to partial-body cryotherapy between 2 high-intensity training sessions). Subjects were exposed to partial-body cryotherapy after the first training session. The 2 knee extension high-intensity training sessions were separated by a 40-min rest interval. Knee extension training consisted of 6 sets of 10 repetitions at 60°.s−1 for concentric actions and 6 sets of 10 at 180°.s−1 for eccentric actions. The decrease in eccentric peak torque and total work was significantly (p<0.05) less after partial-body cryotherapy (5.6 and 2%, respectively) when compared to control (16 and 11.6%, respectively). However, the decrease in concentric peak torque and total work was not different (p>0.05) between partial-body cryotherapy (9.4 and 6.5%, respectively) and control (7.5 and 5.2%, respectively). These results indicate that the use of partial-body cryotherapy between-training sessions can enhance eccentric muscle performance recovery.

Introduction
High-level athletes or physically active individuals may temporarily experience impaired muscular performance following high-intensity exercise bouts or competition. According to Barnett [4], the decline in strength performance might be temporary, lasting minutes, hours or several days. Short-term neuromuscular performance impairment may result from several factors, such as: 1) decreased muscle pH [10, 37, 38], 2) depletion of phosphocreatine [11, 37, 39], ATP [11, 16] and muscle glycogen stores [1, 3], and 3) metabolic products accumulation from muscle contractions [16, 27, 39] or 4) reduction in brain signals to muscle fibers [6, 41]. In this way, several recovery strategies between training sessions or competitions have been used to accelerate muscle recovery, such as massage, active recovery, compression garments, and cryotherapy, among others [4, 6]. A relatively novel modality of cryotherapy is whole-body cryotherapy (WBC), in which the head is not exposed to cold, have also been used as a similar modality of WBC [21]. In addition, some studies have reported temperatures as low as −160 °C [20] and −195 °C [17]. WBC was used for the first time in the treatment of rheumatoid arthritis in the late 1970s by Toshiro Yamauchi [36]. Since then, it has been reported to reduce spasticity in some neurological diseases [24], pain, edema, inflammation, as well as promote musculoskeletal relaxation and increase range of motion in patients with rheumatic diseases [24, 26].

Furthermore, studies have reported that WBC improves muscle damage recovery after exercise [2, 20, 33]. Banfi et al. [3] showed that 5 WBC sessions decreased muscular enzymes related to muscle damage, such as creatine kinase and lactate dehydrogenase, reduced prostaglandin PGE2, cytokines IL-8, IL-2, and increased cytokine IL-10. However, Costello et al. [13] showed that one session of WBC did not enhance muscle recovery from exercise-induced muscle damage. Moreover, the results reported by Fonda and Sarabon [17] did not completely support the use...
of WBC as a recovery modality. It is important to note that these studies aimed to evaluate the effects of WBC on muscle damage recovery. Moreover, cryotherapy between training sessions has been used to improve recovery for athletes who sometimes do work out twice daily [4,6]. Similarly, competitive sports with heats and finals in the same day have also been using cryotherapy to accelerate muscle recovery [4,6]. However, to the best of our knowledge, only one study investigated the effects of WBC on recovery between 2 training sessions on the same day. Schaal et al. [40] examined the effect of WBC on the parasympathetic reactivation and metabolic parameters of recovery between 2 synchronized swimmers simulated competition separated by 70 min. The effects of WBC on neuromuscular recovery between successive same-day training sessions therefore require further investigation.

We hypothesized that the physiological responses to cold exposure from WBC will improve neuromuscular recovery in subjects or athletes who compete or train more than once on the same day. The rationale for our hypothesis is that cryotherapy causes a vasoconstriction associated with decreased muscle temperature [23]. A secondary vasodilation occurs after the muscles have been exposed to cryotherapy [23]. This vasodilation incrases capillary blood flow, oxygen and nutrients into the muscles and enhances removal of metabolic products from muscle contractions [23]. In addition, hardening muscular recovery is especially important when subjects perform more than one training session per day [4,6] or when the sport requires several maximal performances in the same day. For instance, athletes of various sports, such as swimming, athletics, judo, jiujitsu, among others frequently perform several bouts with 30 min rest interval between matches. Therefore, the aim of this study was to evaluate the effect of a single PBC treatment between 2 high-intensity resistance training sessions on strength recovery.

Materials and Methods

Subjects
The sample size test for both WBC and control experimental condition was determined in GPower (version 3.1.2; Franz Faul, Universität Kiel, Germany). The following design specifications were taken into account: α=0.05; (1-β)=0.8; effect size f=0.4; test family=F test and statistical test = ANOVA repeated measures, within-between interaction. The sample size estimated according to these specifications was 10 subjects. 12 well-trained young men (23.9±5.9 years, 92.5±10.4 kg and 180.5±6.8 cm) volunteered to participate in this study. Their training routine included 4–6 resistance training sessions per week, performing 6–10 sets per muscle group, and 6–12 maximum repetitions with 60–120 s of rest interval between sets. The minimum overall resistance training exercise required to enter the study was one year. They were informed of the purpose, procedures, possible discomforts, risks and benefits of the study prior to signing the written informed consent term. Subjects were considered healthy and fit for physical exercise by answering no to all the PAR-Q questions [42]. Additionally, the following exclusion criteria were adopted based on Pobdielska et al. [32]; untreated arterial hypertension, cardiovascular or respiratory diseases, angina, peripheral artery occlusive disease, venous thrombosis, urinary tract diseases, severe anemia, allergy to cold, tumor diseases, viral and bacterial infections, Raynaud’s syndrome, claustrophobia or convulsions. The present study was performed in accordance with the ethical standards of the IJSM [19] and approved by the local Ethics Committee (Ethics Committee from Catholic University of Brasilia, Brazil; Protocol: 71484/2012).

Experimental design
In order to test the hypothesis that a single PBC treatment between 2 high-intensity resistance exercise sessions would improve muscle recovery, subjects were asked to visit the laboratory on 3 occasions. The first visit consisted of a familiarization of experimental procedures and anthropometric assessment. Familiarization was performed one week prior to the second visit. Thereafter, volunteers participated in 2 experimental conditions: 1) PBC (subjects were exposed to 3 min of PBC at −110 °C between 2 high-intensity resistance training sessions); 2) Control (CON, subjects were not exposed to a WBC between 2 high-intensity resistance training sessions). Subjects were exposed to partial-body cryotherapy immediately after the first training session, and 37 min after PBC performed second training session. Thus, the 2 knee extension training sessions were separated by a 40-min rest interval. Order of conditions was randomized with 7 days between conditions. A diagram of the experimental design for both CON and WBC is presented in Fig. 1. To avoid circadian influence, subjects performed both conditions at the same time of day. Volunteers were instructed to avoid caffeine and alcohol intake for 24 h before testing and to not perform lower-limb exercises for 72 h before experimental exercise sessions. Additionally, they were instructed to not take supplements, nonsteroidal anti-inflammatory drugs or similar products over the course of the study.

Training session protocol
Each knee extension exercise session consisted of 6 sets of 10 repetitions at 60·s−1 for concentric actions and 6 sets of 10 repetitions at 180·s−1 for eccentric actions, with 1 min interset rest interval. All exercise was performed in a Biodex System 3 Isokinetic Dynamometer (Biodex Medical Inc., Shirley, NY, USA). Subjects were positioned comfortably on the dynamometer seat with belts fastened across the trunk, pelvis and thigh to minimize extraneous body movements which could affect peak torque and power values. The lateral epicondyle of the femur was used to align the knee with the dynamometer’s lever arm, allowing free and comfortable knee flexion and extension from 80° flexion to full extension. With the participants positioned on the seat, the following measures were recorded to standardize the test position for each participant: seat height, backrest
Inclination, dynamometer height and lever arm length in order. Gravity correction was obtained at full extension by measuring the torque exerted by the lever arm and the participant’s relaxed leg. All isokinetic variables were automatically adjusted for gravity within the Biodex Advantage software. All procedures were in accordance with Bottaro et al. [9].

Calibration of the dynamometer was carried out according to manufacturer’s specifications. During training sessions, participants were asked to cross their arms across the chest. Moreover, they received verbal encouragement throughout the training sessions, and all training procedures were performed by the same examiner. Baseline test/retest reliability ICC values for knee extension concentric and eccentric peak torque were 0.91 and 0.93, respectively. Additionally, baseline test/retest reliability ICC values for knee extension concentric and eccentric total work were 0.8 and 0.87, respectively.

Recovery modalities
During the PBC condition (Fig. 2), subjects stood in a head-out cryochamber using gaseous nitrogen (Kryos Tecnologia, Brasília, Brazil) at −110°C for 3 min. The temperature and duration of PBC exposure were based on Banfi et al. [2]. They wore bathing suits, gloves, socks and shoes with thermic protection to protect their extremities. The CON condition consisted of passive recovery, during which subjects stood in the cryochamber for 3 min at 21°C. Thigh temperature (anterior central area) was measured by an infrared thermometer (Fluke, 566, China) before and immediately after PBC and CON condition. The measurement area was marked with a pen (Pilot 2 mm, Brazil) before each condition.

Statistical analyses
Data are presented as mean ± standard deviation. The Shapiro-Wilk test was used to check data for normal distribution. Considering that data presented normal distribution, 2 × 6 [condition (PBC and CON) × training sessions (1st, 2nd, 3rd, 4th, 5th and 6th)] 2-way repeated measures ANOVAs were used to analyze knee extension concentric and eccentric peak torque and total work. In the case of significant differences, a Tukey’ post hoc test was used. Significance level was set a priori at P < 0.05.

Results
There were no significant differences in baseline values (1st training session) between PBC and CON conditions for any of the variables measured (Table 1). Normalized concentric peak torque and total work throughout 2nd training session after each condition (PBC and CON) are presented in Fig. 3, 4, respectively. Baseline relative strength (throughout 1st training session) was expressed as 100 %. There were no significant interactions for knee extension concentric peak torque (F = 0.53, p = 0.48) or total work (F = 0.22, p = 0.64) and no significant main effect for training sets for knee extension concentric total work (F = 1.72, p = 0.15). However, there was a main effect for training sets for knee extension concentric peak torque (F = 7.6, p < 0.001). In both conditions, knee extension peak torque was higher in the 4th, 5th and 6th sets when compared to the 1st set (p < 0.001). Additionally, the 5th set was higher when compared to the 2nd set (p < 0.001).

Table 1 Mean ± SD of knee extension concentric and eccentric peak torque and total work throughout 1st training session before WBC and CON conditions.

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>WBC</th>
<th>P-value</th>
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<tbody>
<tr>
<td>CC PT</td>
<td></td>
<td></td>
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<tr>
<td>1st set</td>
<td>299 ± 34* □</td>
<td></td>
<td>294 ± 28* □</td>
</tr>
<tr>
<td>2nd set</td>
<td>254 ± 29* □</td>
<td></td>
<td>256 ± 37* □</td>
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<tr>
<td>3rd set</td>
<td>226 ± 37* □</td>
<td></td>
<td>230 ± 35* □</td>
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<tr>
<td>4th set</td>
<td>212 ± 40* □</td>
<td></td>
<td>203 ± 40* □</td>
</tr>
<tr>
<td>5th set</td>
<td>196 ± 36</td>
<td>190 ± 31</td>
<td></td>
</tr>
<tr>
<td>6th set</td>
<td>188 ± 37</td>
<td>182 ± 26</td>
<td></td>
</tr>
<tr>
<td>CC TW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st set</td>
<td>2324 ± 411* □</td>
<td></td>
<td>2397 ± 314* □</td>
</tr>
<tr>
<td>2nd set</td>
<td>1925 ± 297* □</td>
<td></td>
<td>2028 ± 327* □</td>
</tr>
<tr>
<td>3rd set</td>
<td>1721 ± 283* □</td>
<td></td>
<td>1724 ± 320* □</td>
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<td>4th set</td>
<td>1605 ± 341</td>
<td>1538 ± 299</td>
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<td>5th set</td>
<td>1452 ± 322</td>
<td>1437 ± 263</td>
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<tr>
<td>6th set</td>
<td>1365 ± 282</td>
<td>1362 ± 261</td>
<td></td>
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<tr>
<td>EC PT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st set</td>
<td>340 ± 43* □</td>
<td></td>
<td>311 ± 71* □</td>
</tr>
<tr>
<td>2nd set</td>
<td>325 ± 47* □</td>
<td></td>
<td>302 ± 74* □</td>
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<tr>
<td>3rd set</td>
<td>294 ± 73* □</td>
<td></td>
<td>282 ± 77* □</td>
</tr>
<tr>
<td>4th set</td>
<td>289 ± 78</td>
<td>267 ± 77</td>
<td></td>
</tr>
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<td>5th set</td>
<td>279 ± 79</td>
<td>268 ± 74</td>
<td></td>
</tr>
<tr>
<td>6th set</td>
<td>275 ± 81</td>
<td>249 ± 77</td>
<td></td>
</tr>
<tr>
<td>EC TW</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1st set</td>
<td>2193 ± 610* □</td>
<td></td>
<td>1937 ± 637* □</td>
</tr>
<tr>
<td>2nd set</td>
<td>2115 ± 573* □</td>
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<tr>
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<td>1857 ± 661* □</td>
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<td>1750 ± 671* □</td>
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<tr>
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<tr>
<td>6th set</td>
<td>1557 ± 651</td>
<td>1308 ± 544</td>
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CC PT, concentric peak torque. CC TW, concentric total work. EC PT, eccentric peak torque. EC TW, eccentric total work. CON, control condition. WBC, whole body cryotherapy condition. Set*condition, Set*condition interaction. (*) p < 0.05, higher than 6th set. (#) p < 0.05, higher than 5th set. (†) p < 0.05, higher than 4th set. (§) p < 0.05, higher than 3rd set. (‡) p < 0.05, higher than 2nd set.
Normalized knee extension eccentric peak torque and total work throughout the 2nd training session after PBC and CON conditions are presented in Fig. 5, 6, respectively. Baseline relative strength (throughout 1st training session) was expressed as 100%. There were no significant interactions for knee extension eccentric peak torque (F = 0.36, p = 0.87) or total work (F = 0.77, p = 0.57). There were also no significant main effects for training sets for knee extension eccentric peak torque (F = 1.06, p = 0.39) or total work (F = 0.58, p = 0.71). However, there were main effects for condition for knee extension eccentric peak torque (F = 9.26, p = 0.011) and total work (F = 4.92, p = 0.048), which were higher in the PBC condition when compared to the CON condition.

Discussion

The aim of this study was to evaluate the effects of a single PBC session (3 min at −110 ºC) on strength recovery between training sessions on the same day in young men. The main finding was that PBC improved eccentric muscle recovery 40 min after a high-intensity exercise bout. In contrast, concentric muscle recovery between high-intensity training sessions was not hastened by PBC. While it was hypothesized that a PBC session would improve concentric and eccentric muscle recovery, improvement was observed only during eccentric actions. Klimek et al. [23] suggested that cryotherapy causes an increase in blood flow, oxygen and nutrients to the muscles and also enhances removal of metabolic products from muscle contrac-
tions due to a secondary vasodilation that occurs after exposure to cryotherapy. However, PBC did not improve concentric recovery in the present study. A possible reason for similar decreases in concentric muscle performance from the first to the second training sessions in both conditions may be related to the thermoregulatory effects (drop in muscle temperature) of WBC [14]. Additionally, cold exposure may cause deleterious effects due to decrease in nerve conduction and reduced rate of enzymatic activity, which would cause a decrease in cross-bridge attachment/detachment during muscle contraction [8]. Thus, any possible positive effects of PBC on concentric muscle recovery could have been masked by detrimental effects of body cooling. Indeed, some studies have reported a decrease in muscle performance due to body cooling [5, 12, 15, 25, 31]. Costello et al. [14] measured muscle temperature of the vastus lateralis before and 60 min after WBC (20 s at −60 ± 3 °C followed by 3 min and 40 s at −110 ± 3°C). They observed that the vastus lateralis temperature decreased 20 min after WBC exposure with the greatest reduction observed 60 min after treatment (1.6 ± 0.6 °C). Thus, according to Costello et al. [14] it appears that muscle temperature starts to decrease 20 min after WBC. Because our second training session was performed 30 min after WBC exposure, we could speculate that quadriceps temperature might have been decreased at the onset of the second training session, which could have influenced concentric muscle performance. Alternatively, the thermoregulatory effects of PBC exposure might be responsible for hastening eccentric muscle recovery. Eccentric muscle actions produce greater torque and require less energy compared to concentric and isometric muscle actions because they also include passive tension from the elastic properties of the muscle, not just active tension from cross-bridge cycling [28, 35]. Increases in muscle-tendon stiffness resulting from cold exposure have been well documented [18, 29, 34]. García-Manso et al. [18] evaluated the vastus lateralis in 12 professional soccer players who were exposed to 4 cold-water immersion sessions at 4 °C lasting 4 min each. They observed a decrease in vastus lateralis muscle deformation from the control condition (5.12 ± 2.27 mm) to the 3rd (3.64 ± 2.27 mm) and 4th immersions (3.38 ± 1.34 mm). The authors concluded that muscle stiffness increased after cold exposure. In addition, it was demonstrated in a systematic review that cold exposure could increase both elastic and viscous tissue stiffness [7].

The mechanisms underlying the effects of cold on muscle-tendon stiffness may be related to an increase in viscoelastic properties of the muscle [18, 30, 34] and titin protein stiffness during eccentric actions [22]. Price and Lehmann [34] found an increase in passive resistance of muscle caused by a decrease in muscle viscosity in response to cooling. According to Herzog et al. [22], there is an increase in stiffness of the titin protein during eccentric actions. Although muscle temperature was not measured, skin thigh temperature in the present study dropped 11.9 ± 1.3 °C immediately after and 3.7 ± 1.5 °C 12 min after PBC compared to the basal level.

Thus, the improvement in eccentric muscle performance observed may be due to increases in muscle-tendon stiffness associated with PBC (−110 °C). However, future studies are needed to elucidate the mechanisms involved in eccentric and concentric muscle actions after cryotherapy. A major limitation of the present study was that quadriceps muscle temperature and metabolic products from muscle contractions were not measured.

In summary, a single dose of PBC (3 min at −110 °C) enhanced recovery of eccentric muscle performance between 2 resistance training sessions in young men. However, PBC did not affect concentric muscle recovery. From a practical standpoint, PBC might be applied in those athletes who compete or train more than once in the same day in order to improve eccentric muscle recovery and performance. However, further studies on this topic are necessary in order to arrive at more precise conclusions and an improved understanding of the effects of PBC on muscle recovery between successive workouts or competitions.

Conflicts of interest: The authors have no conflict of interest to declare.

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