- Regular Paper -

Local cryostimulation acutely preserves maximum isometric handgrip strength following fatigue in young women

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Abstract

Several types of cryostimulation have been recently proposed to rapidly lower skin temperature therefore gaining a possible neuro/muscular recovery after strenuous exercise or, more generally, in sports. Local crystimulation may be a viable and relatively portable tool to obtain physiological benefits in previously-efforted muscular districts. However, cohesive and standardized cryo-exposure protocols are lacking as well as the righteous procedure to efficaciously combine duration, treatments and temperature in relation to desirable effects on muscular strength. In this randomized-controlled study, fifty young women were tested for maximum isometric handgrip strength, before and after exhausting contractions.

Following the fatiguing protocol, the intervention group (cryo, n=25, 24.7 ± 2.5 years, BMI 21.7 ± 1.8 kg/m^2) underwent a 6-min local cryostimulation (-160 °C) on the extensor-flexor muscles of the dominant arm, while control-matched peers sat rested in a thermo-neutral room (22 ± 0.5 °C). Handgrip tests were repeated at baseline (T0), after cryostimulation (T1), and 15 min after T1 (T2). Throughout the protocol, the AUC of the strength performance was significantly higher in the cryo- compared to control group (P=0.006). In particular, following fatigue and cryostimulation, the cryo group preserved higher strength at T1 with respect to controls (26.8±2.8 vs 23.9±2.8 kg, Bonferroni's post-hoc, P<0.01). Likewise, ventral and dorsal temperature, recorded with a thermal camera, were lower in cryo- than control group (P<0.0001).

In conclusion, a brief session of local cryostimulation may acutely preserve maximal isometric force in young women following a fatiguing protocol. These findings may have implications in orchestrating strategies of district muscular recovery.

Keywords: cryostimulation, local cryotherapy, handgrip strength, recovery, maximal isometric force

Abbreviations: AUC, area under the curve; CWI, cold-water immersion; ES, effect size; ICC, intraclass correlation coefficient; LC, local cryotherapy; PBC, partial body cryotherapy, RM, repeated measures; 1-RM, one maximum repetition; SD, standard deviation, WBC, whole body cryotherapy

Introduction

An emerging body of literature has documented the development of cold application in different fields of medicine, health and sport sciences. Recent methods of cold stimulation, including whole-body cryotherapy (WBC), partial-body cryotherapy (PBC) and local cryotherapy (LC) are believed to rapidly cool down the skin temperature [42], inducing vasoconstriction and analgesia [20,43]. Cryostimulation, that is the exposure to very-low temperatures for a short period of time using specialized cooling device systems such as cryochamber (WBC), cryocabin (PBC) or vaporizer (LC), has been object of studies regarding several domains, from general health (physical functioning) to improvements in muscular recovery after exercise [9].

Cryocabins and vaporizers are mobile technologies, while cryochambers are larger and fixed devices, thus, according with Bouzigon and co-workers [9], over the past several years, PBC and LC have become trendy treatments, with LC usable directly over the regions of interest (ROIs) by athletes and teams during sport events. In some works, LC was even shown to attenuate joint inflammation and control articular swelling and temperature [38]. Despite the proliferation of scientific reports on these cooling technologies, there is still a lack of information concerning their effective benefits related to the optimal exposure protocols and the relationship with the treatments' supposed effects, especially regarding sport recovery.

In order to help fill this gap, in a previous study [33], we examined the effects of a single PBC session on the maximum handgrip strength values as measured by an hydraulic hand dynamometer, concluding that a single exposure in cryocabin (duration: 150s; temperature range: between -130 and -160°C) could have a significant and positive impact on isometric strength in healthy people. In that study we evaluated the handgrip strength: a simple and reliable method that has a multimodal application as a common field-based assessment. This tool reflects consistently the overall strength capacity [7], the nutritional status [23], the cardiovascular health in elderly obese women [41] and it is even used as a global functional indicator in various chronic diseases [2,14].

A recent study [36] showed that there were no differences between baseline, post-intervention and 3-month follow-up in pain-free handgrip strength values in patients with chronic lateral epicondilytis after 8 local cryostimulation sessions over a 4-week period.

Moreover, Guilhem et al. [21] evaluated the effects of air-pulsed cryotherapy (-30°C) on neuromuscular recovery subsequent to a strenuous eccentric exercise. The authors found no improvements in the long-term recovery of muscle performance after three applications of air-pulsed cryotherapy (3 × 4 minutes at -30°C separated by 1 minute) in the 3 days after strenuous exercise.

To the best of our knowledge no study has investigated the effects of local cryostimulation, induced by vaporizing liquid nitrogen (-160°C) on muscular performance, even if LC has now reached greater diffusion through wellness centers and cryotherapy facilities globally.

In this context, the main purpose of the present study was to explore the effects of a single session of local cryostimulation (-160°C) on maximal isometric contraction of the extensor-flexor muscles, following fatiguing contractions, in a sample of young females.

Material and Methods

Subjects

Based on the *a priori* sample-sized determination, fifty young adult women were enrolled for this study. The present investigation was designed as a randomized-controlled trial. Using a restricted blocks randomization (computer-generated sequence), the participants were randomly allocated into a local cryostimulation- (cryo; n = 25) and control (n = 25) group. The allocation and the randomization were completed by one of the researchers without any contact or knowledge of the participants. Therefore, no allocation concealment mechanisms were necessary. All subjects were examined by a physician to exclude any contraindication to cryotherapy. Subjects were not accustomed to localized cryotherapy. To minimize the effects of circadian variation, measurements were consistently carried out at the same hour of the day (from 08:30 to 10:30). Subjects were also instructed to refrain from consuming alcohol, caffeine, theine, hot drinks nor undertaking exercise for 24 hours prior to the laboratory trial. In addition, subjects were also instructed not to take medications or supplements during the study. A physical activity questionnaire (Baecke's) [3] was administrated to participants in order to assess their physical activity levels.

Ethics statement

The study protocol, including each aspect of the design, was approved by the ethical board of the Università degli Studi di Milano in accordance with the Declaration of Helsinki. All subjects were given verbal and written information on the study and gave their written informed consent to participate.

Procedures

A diagram of the overall study-design is offered in Fig. 1.

On the day of the experiment, each participant arrived at the laboratory 30 min before the session so to acclimate to the room temperature (22 ± 0.5 °C). After acclimation, each participant familiarized with a portable JAMAR Hydraulic Hand dynamometer (Sammons Preston Rolyan Nottinghamshire, United Kingdom) using the dominant hand [31] as recommended for use in healthy people [6,8]. During individuals' adjustments, the hand dynamometer was regulated for each subject by fitting the hand and allowing flexion at the metacarpophalangeal joints. The scale of the dynamometer indicated handgrip strength in kilograms (kg). During the hand strength testing, the subjects sat upright against the back of an adjustable chair with feet flat on the floor [40]; the arm position was standardized with the shoulder adducted and neutrally rotated, elbow flexed to 90° [1]. The forearm and wrist were in a neutral position resting on the support surface [1,19,30,40]; the hand was maintained in line with the forearm holding the instrument upright on its base on the short side. When the individual adjustment operations were completed, each subject performed 3 submaximal voluntary isometric contractions maintained for 5 seconds as familiarization to the testing protocol. In our study, the handgrip strength testing showed an excellent reliability (α = 0.946).

In the warm-up period, each subject performed 10 submaximal voluntary isometric contractions at 25% of one maximum repetition (1-RM); 6 submaximal voluntary isometric contractions at 50% 1-RM and again10 submaximal voluntary isometric contractions at 25% 1-RM [46].

The testing protocol was administered after the warm-up period (T0), after the cryo-period (T1), and 15 min after T1 (T2). Instead of the cryostimulation, the control group rested for the equivalent period (6 min). The testing protocol consisted of 3 maximal voluntary isometric contractions maintained for 5 seconds with rest period of at least 60 seconds; the highest value was used for the determination of the maximal grip strength. The procedure and the methodology used during the handgrip strength test were performed according to the standards [4,5,29]. Specific verbal instructions were given to subjects before the evaluations and the experiments were performed with verbal encouragement [32].

As previously described by Veni and co-workers [46], the fatiguing protocol, lasting totally 5 min, was performed after 3 min of recovery from T1. It consisted of 60 maximal voluntary isometric contractions maintained for 4 seconds interleaved with 1-sec rest. Following fatigue, the cryo group underwent 6-min local cryostimulation at the level of the flexor and extensor muscles of the dominant hand/forearm previously used for maximal contractions. The time of exposure was in line with the manufacturer's recommendations. For local cryostimulation, a freezing nozzle was employed (Cryo Polar Bear, Vacuactiv, Slupsk, Poland), i.e. a portable nitrogen system which provides dry air at very low temperature (-160 °C). Cryostimulations were performed by the same and well-trained operator, which continuously made circular vaporizations above the forearm skin, as recommended by the manufacturer of the cryostimulation device used in this trial. The control group rested in sitting position, upright against the back of a chair with feet flat on the floor, in a room where the temperature was stabilized (22 ± 0.5 °C).

Skin temperatures of the ventral and dorsal regions of ROIs were assessed by means of a ThermoVision SC640 thermal imaging camera (Flir Systems, Danderyd, Sweden) in accordance with the standard protocol of infrared imaging in medicine [16,37]. Thermal images were taken prior to each testing protocol (T0, T1, T2). The camera, with the emissivity set in the range of 0.97 to 0.98, was connected to a personal computer with appropriate software (Thermacam Researcher Pro 2.10, version 5.13.18031.2002, Flir systems 2015, Danderyd, Sweden). The camera was mounted on a tripod and positioned in a way to focus on the dominant forearm and hand. The distance between the camera and the ROIs was kept constant at 1m. A mean temperature was calculated by averaging the skin temperature recorded for the ROIs.

Fig.1. *Flow-chart of the study*.

Statistical analysis

The test-retest reliability of the handgrip test was measured using an intraclass correlation coefficient (ICC, Cronbach-α) and interpreted as follows: $\alpha \geq 0.9$ = excellent; $0.9 > \alpha \geq 0.8$ = good; $0.8. > \alpha \geq 0.7$ = acceptable; $0.7 > \alpha \geq 0.6$ = questionable; $0.6 > \alpha \geq 0.5$ = poor [45]. The handgrip strength testing showed a Cronbach-α equal to 0.946.

The normality of the data distribution was assessed by Shapiro-Wilk test.

The maximal contractions performed during the fatiguing protocol had a non-parametric distribution, therefore they were shown as the maximum, the median and the minimum values, and they were compared with Mann-Whitney U test.

All other data (handgrip, temperature) met the gaussianity assumption and therefore they were parametrically analysed and represented as mean ± standard deviation (SD). The assumption of homogeneity of variance was checked with Bartlett's test. The handgrip strength performances at timepoints T0, T1, T2 and the relative thermographic measurements were analyzed with repeated measures (RM) two-way (time x treatment) analysis of variance (ANOVA) with Bonferroni's post-hoc. Time (T0, T1, T2) was the within-subjects factor, whereas treatment-group (control vs cryo) was the between-subjects factor. Eta squared (η^2) effect sizes (ES) [26] were determined and interpreted according to Cohen [15]: 0.01 = small; 0.06 = medium; 0.14 = large. The area under the curve (AUC) was used as summary measures of the strength- and thermal responses resulting from the experimental window T0-T2. Comparisons among means of the AUCs, anthropometric and demographic characteristics were performed using two-tailed, independent Student's *t* test. For all analyses, a p-value less than 0.05 was considered statistically significant.

Analyses were carried out with the Statistical Package SPSS version 25 for Mac (IBM Corp., Armonk, NY, USA), GraphPad Prism 5 (San Diego, CA, USA).

Results

Subjects' groups

All demographic and anthropometric characteristics of the participants are offered in Table 1.

All group subjects were matched-pairs as no statistical difference was registered per each of the characteristic listed in Table 1. According to the Baecke's questionnaire, volunteers resulted to be moderately active.

Table 1. **Anthropometric and demographic characteristics of the women studied.**

	control (n = 25)	**cryo** (n = 25)
Age (years)	24.3 ± 3.3	24.7 ± 2.5
Weight (kg)	60 ± 9.2	59.3 ± 5.5
Height (m)	1.63 ± 0.05	1.65 ± 0.05
BMI (kg/m^2)	22.4 ± 2.9	21.7 ± 1.8
Total hand length (cm)	17.6 ± 0.9	17.8 ± 0.9
Palm length (cm)	9.9 ± 0.5	9.9 ± 0.5
Spam length (cm)	19.5 ± 1	19.8 ± 1.3
Physical Activity Index (AU)	8.77 ± 1.3	8.37 ± 1.2

Data are expressed as means ± SD.

Fatiguing protocol

No differences were found as to the median, minimum and maximum values obtained throughout the fatiguing protocol, in both control and cryo groups (Fig. 2).

Strength responses

Overall, the results of the two-way RM-ANOVA analysis revealed a significant main effect of cryotherapy ($F = 6.436$, $P = 0.0145$, $\eta^2 = 0.118$) and time ($F = 70.5$, $P < 0.0001$, $\eta^2 = 0.254$) on handgrip strength performance (Fig. 3A). The AUC of the strength performance was significantly higher in the cryo group compared to control group (841.9 ± 17.4 vs 770.6 ± 17.7 kg · min, $P = 0.006$, Fig. 3B). In particular, following fatigue and cryostimulation (or rest, for the controls), the cryo group preserved higher strength at T1 with respect to control group (26.8 ± 2.8 vs 23.9 ± 2.8 kg, Bonferroni's post-hoc, P<0.01, Fig. 3A). In fact, after the fatiguing protocol, the strength decrease of the control group was of a greater extent compared to that one of the cryo group (Δ_{T1-T0} = - 4.99 ± 2.66 vs - 3.58 ± 3.16 kg, respectively). Endline values of the cryo group's strength were closer to baseline with respect to controls (Δ_{T2-T0} = - 2.04 ± 2.35 vs - 2.76 ± 2.19 kg, respectively).

Thermal responses

Skin temperature analysed by means of thermal images reflected a pattern similar to that one registered for the strength responses (Fig. 4). As to the ventral temperature effects, a significant interaction "time x treatment" was documented (F = 173.6, P < 0.0001, η^2 = 0.717). In detail, ventral temperature (Fig. 4A) was significantly affected by cryotherapy (F = 62.24, P < 0.0001, η^2 = 0.564) and time (F = 125.5, P = 0.0001, η^2 = 0.646). Post-hoc comparisons revealed that ventral temperature was significantly lower at T1 in the cryo group with respect to the control group (24.5 ± 2.2 vs 33 ± 1.5 °C, Bonferroni's, P<0.0001, Fig. 4A). The AUC of the ventral temperature was significantly lower in the cryo group than in control group (851.1 ± 9 vs 979.6 ± 7.5 °C · min, P < 0.0001, Fig. 4B). A significant interaction "time x treatment" was also found for dorsal temperature (F = 69.93, P < 0.0001, η^2 = 0.393). Likewise, cryotherapy (F = 8.763, P < 0.001, η^2 = 0.152) and time (F = 26.83, P < 0.0001, η^2 = 0.199) significantly impacted on dorsal temperature (Fig. 4C). Again, at T1, dorsal temperature of the cryo group was significantly lower than that one of the controls (28.2 ± 2.3 vs 32.6 ± 1.6 °C, Bonferroni's post-hoc, P < 0.0001, Fig. 4C). The AUC of the dorsal temperature was significantly lower in cryo group than control group (904 ± 9.8 vs 964.5 ± 8.8 °C · min, P < 0.0001, Fig. 4D).

Discussion

A widespread literature encompasses a multitude of cryo-exposure protocols [49]. Nevertheless, cohesive and standardized procedures are lacking as to achieving desirable effects on muscular strength. One challenge is represented by efficaciously combining duration, treatments and temperature as a potential recovery technique in diverse muscular efforts and in different sports. In this study we investigated whether six minutes of local cryostimulation affected maximum isometric handgrip strength after fatigue in a sample of young women. Our results showed that the AUC of the strength performance was significantly higher in the cryo group (~ +10%) compared to the AUC of the strength performance of the control group. In particular, following the fatiguing protocol and a 6-min session of cryosimulation (T1), the cryo group preserved higher strength values respect to the control group.

These results are in line with earlier studies evaluating the effects of cold stimulation on the maximal isometric force [24,33,34]. In fact, in a recent study, Kodejška and co-workers [24] demonstrated that cold-water immersion (CWI) significantly increased intermittent handgrip performance in rock climbers as compared to a passive recovery routine. In that work participants completed two different protocols of CWI recovery session: one group immersed the dominant forearm in water at 8°C, whereas the other group immersed the arm at 15°C. Each protocol was repeated twice and lasted for 18 minutes (three x 6-minute cycles composed by 4-min immersion and 2-min rest out of water). The authors concluded that cooling in water at 15°C temperature is an effective procedure to increase recovery from climbing-specific intermittent handgrip performance. We have previously tested the hypothesis that a single PBC session would not significantly worsen the handgrip maximum isometric strength, founding a remarkable increase in the strength performance, compared to baseline and the control group, after a 150-s partial-body cryostimulation session [33]. Nodehi Moghadami et al. [34] measured the maximal isometric forces of elbow flexion before and after placing ice and hot packs over the arm. They showed no differences between pre and post maximal isometric force scores in control and heat groups, and a significant improvement between pre and post scores, following a 15-min cold pack treatment. The authors measured skin temperature of the forearm by means of a thermometer, reaching 14.7 °C at the elbow in the cold exposure group. In our study, at the end of the 6-min period of cryostimulation, the mean temperature of the ventral area of the forearm was 24.5°C while the mean temperature of the dorsal area was 28.2°C (during cryo-sessions we recorded skin temperature values below 10°C). Interestingly, this skin temperature reduction (-13%) was paralleled by a gain in strength performance, of a same entity (+12%), at T1. Although there is no consensus concerning the ideal skin temperature reductions, one study [11] reported that a temperature below 12°C is required to obtain a 10% decrease in nerve conduction velocity, which is relieving in inflammatory conditions owing to analgesic effects.

Consistently with literature, isometric force production starts to decrease when muscle temperature falls below 25°C due to peripheral muscle cooling [39]. As a limitation of the present study, temperature was not measured directly at the level of the elbow flexor muscles. Nonetheless, it is credible that our exposure protocol was not capable in detecting a robust reduction of isometric force because the threshold temperature of 25°C was not reached at the muscular site.

One of the strengths of this study is that skin temperature was measured by means of an infrared thermal camera that is the gold standard in assessing skin temperature after cryostimulation [16]. Instead, the disparity of achieved results among other studies might be explained by methodological differences. Often the discrepancy can be due to the use of vague and/or inadequate ways of measurements (e.g. thermometers, not-reported models of thermometers, distance to the skin, emissivity, ROI, etc.). In other studies, skin- and muscle temperatures were not even measured. Instead, thermal imaging may be also useful in sports medicine as a helpful method in endurance evaluation [12,44].

Our design was not cross-sectional and further insights could be gathered by studies enrolling different gender, different athletes, and different sport practitioners. On another hand, theoretical models on tissue cooling efficiency suggested choosing shorter cryotherapy sessions when considering women compared to men [35]. Additionally, ultrastructural data on neuromuscular recovery might help describing the obtained results, regarding the expendability of this recovery modality. However, giving the homogeneity of the two groups in terms of both anthropometric characteristics and maximal force-generating capacity at baseline (T0), we were able to detect clear differences with a very small error, and with a very large effect size, implying the proposed protocol was efficacious.

Hand-held dinamometry is used to measure the muscular force generated by flexor mechanism of the hand and forearm. It should be noted that handgrip test is an indirect indicator of overall and peripheral fatigue [7,48]. The testing protocols need to be consistent, controlling manifold variables which could affect the performance: a) the time of day, since grip strength shows its peak in the afternoon [10]; b) the posture, considering that the lower the flexion at the elbow, the greater the grip strength [25]; c) the anthropometric measures which need dynamometer adjustments [47]. In the present study, we cautiously scrutinize all these variables, in order to stringently estimate the effectiveness of local cryostimulation in enhancing recovery of the forearm muscles after fatigue. We found that local cryostimulation allowed to express greater isometric strength after the fatiguing protocol compared to controls (T1). Furthermore, at endline (T2), the cryo group reached strength values closer to baseline with respect to those of control group. These findings are in agreement with those of our previous research on acute isometric strength performance following a single PBC session [33], i.e. a cold-based technology that has been receiving an increasingly attention in the field of performance recovery [28]. Therefore, the effects of a single LC session on isometric strength performance are comparable to those obtained with a single PBC session, opening a new scenario on the utilization of LC as a recovery tool in sports disciplines like climbing, racket sports, or gymnastics, in which hand isometric strength is critically required. In rock climbing, for example, athletes' isometric strength recovery is determinant when attempting multiple isometric efforts.

To the best of our knowledge, the present study is the first one investigating the effects of local cryostimulation induced by vaporizing liquid nitrogen (-160°C) on muscular performance. Several avenues of investigations can be opened by differently-arranged cryostimulation interventions: potential fields of research may be expanded to therapeutic strategies in the management of overweight and obesity [27]. In fact, a growing body of literature includes only a few studies on the physiological [22], clinical [36] and neuromuscular [21] effects of local cryotherapy devices inducing low-temperature decrease, i.e. gaseous cryotherapy (-78°C) [17,36] and air-pulsed cryotherapy (-30°C) [21]. Besides, conflicting results are reported in literature about the actual benefits of cryotherapy on muscular strength performance and the related recovery. For instance, repeated air-pulsed cryotherapy was incapable of gaining evident benefits on the recovery of muscle function after a severe mono-articular eccentric exercise [21]. However, in the study of Guilhem and colleagues [21], a 4-min session of repeated (3 sets) air-pulsed cryotherapy was used at -30 °C: a temperature definitively higher in comparison with our study. There are still numerous factors to be examined for the feasibility and the efficacy of cryostimulation in order to accelerate muscular recovery. Body mass index seems to influence the effects of cryostimulation [11]. The concept of “fatigue” is complex, and certainly deserves further multi-level research [18].

Here, we showed a modality of isolated cryostimulation, usable to maintain muscular performance after repeated maximal exercise bouts. It is a relatively portable technique, directly exploitable in the sports field, between two intense training sessions or competitions. Besides, previous studies showed that LC and WBC register similar temperature differences between before and after body cooling in patients with spinal diseases, confirming a convenient and lower-cost use of LC [13]. Future research should explore the influence of cryotherapy in a wider range of motor patterns including evaluation of either range of motion or muscle functioning. As it stands, although widely used, grip strength may not be translated into a full spectrum of sport performance.

In conclusion, a brief session of local cryostimulation at -160°C may acutely preserve maximal isometric force in young women following a fatiguing protocol. These findings may have implications in orchestrating strategies of district muscular recovery.

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Declaration of interest

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Captions

Figure 1. Flow-chart of the study.

Figure 2. Fatiguing protocol.

Handgrip strength performances (maximal contractions = 60) during the 5-min fatigue protocol in the control (A) and cryo (B) group. Data are plotted as maximum (grey line), median (black line), minimum (silver line) values per contraction.

Figure 3. Strength test responses

Maxium isometric handgrip strength test (A) and relative area under the curve (AUC) of the performance (B) in the control and cryo group.

Data are expressed as means ± SD. ** $P < 0.01$

Figure 4. Thermal responses

Timecourses of skin ventral temperature measurements (A) and relative area under the curve (B) in the control and cryo group. Corresponding skin dorsal temperature measurements are shown (C), along with respective AUC (D) in the control and cryo group.

Data are expressed as means ± SD. *** $P < 0.0001$