

Cold water immersion offers no functional or perceptual benefit compared to a sham intervention during a resistance training program.

Brief running head: No benefit of CWI for resistance training adaptation

Authors: Laura J. Wilson¹, Lygeri Dimitriou², Frank A. Hills³, Marcela B. Gondek³, Aléchia van Wyk², Vlad Turek¹, Taylor Rivkin¹, Alex Villiere¹, Paul Jarvis¹, Stuart Miller⁴, Anthony Turner¹ & Emma Cockburn⁵

¹ London Sports Institute, Middlesex University, Allianz Park, London, UK.

² Department of Natural Sciences, Middlesex University, London, UK.

³ Biomarker Research Group, Department of Natural Sciences, Middlesex University, London, UK.

⁴ Sports and Exercise Medicine, Queen Mary University of London, London, UK.

⁵ Newcastle University, School of Biomedical, Nutritional and Sport Sciences, Newcastle upon Tyne, UK

Corresponding Author:

Laura Wilson

Middlesex University

The Burroughs, London, NW4 4BT

+44 (0)20 8411 5000

Email: L.Wilson@mdx.ac.uk

<https://orcid.org/0000-0002-3744-6132>

The authors received no specific funding for this work.

Cold water immersion offers no functional or perceptual benefit compared to a sham intervention during a resistance training program.

Abstract

Cold water immersion (CWI) is regularly employed by athletes as a post-exercise recovery strategy, but relatively little is understood about potential training adaptations associated with habitual use. The aim of this study was to investigate the influence of repeated CWI or a sham intervention on adaptations to a lower body resistance training program. Thirteen males (26 ± 6 years; 83.6 ± 15.7 kg) familiar with resistance training were allocated into a CWI (10 min at 10°C) or sham group and completed 2 x 4-week blocks of lower body resistance training. Participants completed a total of sixteen training sessions (2 x session/week), with each session immediately followed by their allocated recovery intervention. Measures of perceptual markers, muscle function, and muscle architecture were recorded at baseline, midpoint and post-training. Data were analysed using factorial ANOVAs. The training program resulted in significant increases in muscle fibre pennation angle ($p = 0.009$), isometric peak force ($p = 0.018$) and $\frac{1}{4}$ squat ($p < 0.001$) with no differences between groups (all $p > 0.05$). There were no differences in perceptual responses between groups. Despite the popularity of CWI as a post-exercise recovery intervention, the findings from the present study demonstrated no functional or perceptual benefit compared to a sham intervention during progressive strength and power training. Further, there was no detrimental impact of CWI on morphological adaptations after 16 exposures. These findings are important for athletes and practitioners wishing to utilise CWI as an acute recovery strategy after training, without blunting potential training adaptations.

Key words: Cryotherapy, Resistance Training Adaptation, Muscle Morphology

INTRODUCTION

Cryotherapy, and cold water immersion (CWI) particularly, is widely used as a recovery intervention following exercise (18). Previous research has demonstrated that CWI can positively influence perceptions of recovery and sleep quality post-exercise (14,15). Cryotherapy exposure modulates blood flow (21) and attenuates the inflammatory response (27,28), which may be beneficial for recovery in the short term, or tournament situations where repeated high level performance is vital. However, inflammation is a crucial part of the regeneration cycle required for adaptation to occur (12) and repeated blunting of this process could result in maladaptive responses to a specific training stimuli (12,32). With few longitudinal studies examining long term effects of repeated cryotherapy exposure, it is pertinent to examine the influence of habitual use on adaptations to training.

A recent systematic review with meta-analysis from Malta et al. (20) examined five studies (12,13,26,31,32) that investigated the impact of CWI on resistance training-induced changes and concluded that there is a deleterious effect of CWI on resistance training adaptations. Both of the studies from Yamane and colleagues (31,32) as well as the Fröhlich et al. (12) paper investigated strength adaptations between cooled and control limbs following a 4 week hand grip, and 5 week leg curl program respectively. The findings from all three studies showed that there were smaller performance gains in the CWI group compared to the control group. However, these studies are limited in that cooling was applied unilaterally and this may have impacted comparisons between cooled vs control limbs in the same individuals (24). Similarly,

the exercise protocols used in both Yamane et al. (31,32) studies lacked ecological validity and therefore it is difficult to extrapolate their findings to an applied setting.

More recently, Fyfe et al. (13) and Roberts et al. (26) reported that strength and muscle mass increased to a greater extent after active recovery than CWI following 7 and 12 weeks of lower body resistance training respectively. In both studies, muscle biopsies demonstrated that CWI blunted the activation of key proteins and satellite cells following exercise, which may explain the improved strength and mass outcomes for participants in the thermoneutral immersion and active recovery groups respectively. Roberts et al. (26) postulated that suppression of satellite cell activity could lead to a cumulative detrimental response over time.

However, training cycles tend to be employed in shorter blocks, so the use of two distinct training blocks may more closely replicate the type of training cycles employed by athletes (3) and could indicate whether any detrimental impact of cryotherapy is evident after as little as 4 or 8 weeks of exposure. Another important facet noticeably absent from previous investigations is the effective blinding of participants to their intervention group, which may account for much of the positive anecdotal evidence associated with cryotherapy treatments (30). Of the five studies included in the review, only one (13) implemented a sham intervention (immersion at 23° C for 15 minutes), but participants were not blinded to their condition.

Therefore the aim of this study was to investigate the influence of repeated CWI or a sham intervention on perceptual, functional and structural adaptations to a 2 x 4 week block-periodization program, in which block one emphasised strength development and block two

emphasised power development; this training is in line with that regularly undertaken by athletes (3) and was derived by an accredited strength and conditioning coach. It was hypothesised that repeated CWI exposure would attenuate physiological and functional adaptations to the strength and power training program.

METHODS

Experimental approach to the problem

An overview of the study outline is provided in Table 1. At a familiarization session participants had their total lean mass assessed (via dual x-ray absorptiometry (DXA)), completed assessments of their repetition maximums (RMs) for all the exercises in the strength block of the program, and were then familiarized with all dependent variables (DV's) listed below. A ratio of back squat 4RM to total lean mass in kg was calculated in order to match participants into either the CWI ($n = 7$) or sham group ($n = 6$) based on relative strength (8). This study design was chosen as it was not possible to blind participants to their allocated intervention. One week following the familiarization session, baseline values were taken for muscle pennation angle (determined using ultrasound (US)) and all muscle function DV's, and these were repeated at mid-point and post. The training program consisted of 2 x 4-week blocks separated by one rest week in which mid-point data were collected. Participants trained 2 days/week, with at least 24 h between sessions, with the CWI or sham intervention conducted after each training session. On the morning of-, 24 and 48 h post the first and last training session of training block 1 and 2 (training sessions 1, 8, 9 and 16) participants completed muscle soreness and perceived recovery questionnaires. Lastly, the morning after the 2nd training session of the first and last week of each block, participants completed a Daily Analysis of the Lifestyle Demands of Athletes (DALDA) questionnaire.

Interventions

Sham

As it was not possible to blind participants to their allocated intervention, a sham intervention rather than a control group was employed. Leucine is an essential branched chain amino acid utilised in the synthesis of proteins, and a key nutrient ‘trigger’ for muscle anabolism, which has been shown to accelerate recovery following resistance exercise (23). Participants in the sham group were informed that their protein supplements contained an additional dose of leucine compared to the supplements received by participants in the cryotherapy group.

Cold Water Immersion (CWI)

Participants in the CWI group completed their allocated intervention following every training session (16 immersions total). Within 15 minutes of cessation of exercise participants sat in a mobile ice bath (iSprint Twin, iCool, Cranlea, UK) ensuring their lower limbs and iliac crest were fully immersed. Participants remained in the ice bath filled with water cooled to 10 degrees ($\pm 0.5^{\circ}\text{C}$) for 10 min. This protocol has been used previously to investigate the impact of CWI on acute recovery following resistance exercise (30).

***** INSERT TABLE 1 HERE*****

Subjects

All procedures were granted ethics approval by the Institutional committee according to the Declaration of Helsinki, and participants were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed consent document to

participate in the study. A convenience sample of thirteen healthy males volunteered to participate in the study (age 26 ± 6 years, height 1.8 ± 0.1 m and mass 83.6 ± 15.7 kg) (Table 2). Participants were recruited using targeted social media, and were familiar with strength training (minimum 1 years' experience). Participants were excluded from taking part in the study if they had; any injuries or illnesses that prevented them from completing any of the prescribed exercises, Raynaud's disease, circulatory problems, or needle phobia. Participants were able to maintain their normal sporting habits/routine, but were asked to refrain from any strength or power based training other than the prescribed sessions for the duration of the study. For the duration of the study period, participants were asked not to consume any nutritional supplements (e.g. creatine/protein; antioxidants) or non-steroidal anti-inflammatory drugs, and not to take part in any other recovery interventions (e.g. compression garments, massage, or electrostimulation).

***** INSERT TABLE 2 HERE*****

Calculation of Repetition Maximums (RMs)

The calculation of RMs was completed during the familiarization session based on the protocol of the National Strength and Conditioning Association (2). 4RM was determined for the $\frac{1}{4}$ squat and split squat and 6RM was determined for hip thrusts and Romanian deadlifts, in order to provide the training loads for the first session of the strength training block. Testing was carried out by an accredited strength and conditioning coach. Repeat assessments of $\frac{1}{4}$ squat 4RM were also repeated in session 9 and 16 to provide the mid- and post values respectively for analysis.

Training Program

The training program was split into two periodized blocks each lasting 4 weeks: block 1 emphasised strength development and block 2 emphasised power development. The strength training program comprised 5 exercises (jump shrugs (4 sets x 2 reps), $\frac{1}{4}$ squat (4 x 4), split squat (4 x 4 per leg), barbell hip thrusts (3 x 6) and Romanian deadlift (3 x 6)). All exercises (except for jump shrugs) were performed at RM for the number of reps in each set, e.g. split squat at 4RM. Jump shrugs were performed at the load that elicited peak power during the familiarization.

The power training program also comprised 5 exercises ($\frac{1}{4}$ squat, jump shrugs, box jumps, squat jumps and drop jumps). All exercises were performed for 5 sets of 3 repetitions, with the exception of the $\frac{1}{4}$ squats which were completed in 4 sets of 2. The $\frac{1}{4}$ squat and jump shrugs for the first session of the power block used the loads achieved in the final session of the strength block. Squat jumps were initially performed at the same load as the jump shrugs. Drop jumps were performed using a 30 cm box.

Loads for each exercise were progressed when participants achieved the prescribed sets and reps with the scheduled load. In order to be included in the final analysis, participants were required to attend 15 out of the 16 training sessions. This type of training program has previously been used successfully to investigate adaptations to training (8).

Session Rating of Perceived Exertion (sRPE)

Session rating of Perceived Exertion (sRPE) was collected post the first and last training session of training blocks 1 and 2 (training sessions 1, 8, 9 and 16). Participants were asked to record an sRPE fifteen minutes after cessation of exercise. Analysis revealed that there was no change in sRPE over time ($F_{(1, 10)} = 0.022, p = 0.884; \eta^2 = 0.002$) and there were no differences between groups ($F_{(1, 10)} = 0.100, p = 0.758; \eta^2 = 0.010$).

Nutritional Supplementation

In order to minimise potential variation in training responses, participants were provided with individualised nutritional supplements. All participants were given individual servings of a whey protein isolate (The Protein Works, Cheshire, UK) equivalent to $0.4 \text{ g}\cdot\text{kg}^{-1}$ body mass to consume ~ 1 h before, and within 15 min following completion of each training session. They were also given two recovery bars (Protein Grazers, The Protein Works, Cheshire, UK) each containing 7.4 g protein and 14.2 g carbohydrate to consume 2 hours after each training session. The nutritional supplementation strategy utilised in this study is similar to that used by Roberts et al., (2015), however, rather than providing a standardised serving of whey protein, doses were individualised to take into account large variations in body mass. By using $2 \times 0.4 \text{ g}\cdot\text{kg}^{-1}$ doses on training days, it was expected that all participants would reach the minimum $1.4\text{-}2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ protein recommended for physically active individuals (6). From 1 h before, until 2 h post each training session, participants were only allowed to consume the supplements provided, and water. Participants were instructed to avoid consuming any additional dietary supplements and to follow their habitual diet for the duration of the study. Dietary intake was monitored using 7-day food diaries, completed during the first and last week of training, and analysed using Nutritics (Nutritics Ltd., Co. Dublin, Ireland) software. When adjusted for body

mass, there were no differences in total energy (kcal), carbohydrate (g) or protein (g) intake between groups at either time point (all $p > 0.05$).

Dependent variables

Muscle Soreness and Perceived Recovery

Delayed Onset Muscle Soreness (DOMS) and perceived recovery (PREC) scores were collected in accordance with the procedures outlined by Wilson et al. (29). These measures were recorded the morning of-, 24 and 48 h following the first and last exercise session of each training block (Table 1). Participants were emailed unique links to online versions of the scales so they could be completed on a computer or smart phone. Participants completed the questionnaire within 60 minutes of waking on the relevant days.

Daily Analysis of the Lifestyle Demands of Athletes (DALDA)

The questionnaire is used to identify potential sources of stress (part A) as well as stress reaction symptoms (part B). Participants completed an online version of the DALDA questionnaire the morning after the 2nd training session of the first and last week of each training block. Previous research has shown that there are only significant changes in part B following a period of intensified training (16,30), therefore only results for part 'B' are reported herein.

Dual X-ray Absorptiometry (DXA)

Total lean body mass of all subjects was assessed via DXA (fan beam, Lunar Prodigy 4, GE Medical Systems, Lunar, Madison, WI, USA) at baseline, mid and post-training. Participants

were required to attend the lab in a euhydrated state to ensure accurate measurements. Whilst it was not appropriate to conduct test–retest reliability of DXA anthropometric measures as part of this study, data have previously been derived from repeated scans, demonstrating excellent precision for fat-free soft tissue mass (CV = 0.3%) and acceptable reliability for fat measures (CV: fat mass = 2.5%, percent body fat = 2.5%) (4).

Ultrasound (US)

Ultrasound images of the vastus lateralis (VL) of the right leg were taken at baseline, mid and post. Portable ultrasound equipment (MyLab™ 30Gold Cardiovascular, ESAOTE, Bracco UK, Buckinghamshire, UK) was used to acquire longitudinal images of the VL. The probe was placed at 50% of thigh length, defined as the distance from the greater trochanter to the popliteal crease. Using Image-J software, muscle fibre pennation angle was calculated as the acute angle formed between the deep aponeurosis and a muscle fascicle (9) (Figure 1). The US scans had an intraclass correlation coefficient (ICC) of 0.91.

*****INSERT FIGURE 1 HERE*****

Reactive Strength Index (RSI)

Participants performed drop jumps from a platform at a height of 30 cm on to a portable force plate (Kistler, Switzerland). Emphasis was placed on minimum ground contact time, whilst maintaining maximum jump height. Participants were required to keep their hands on their hips for the duration of the movement, and perform 3 maximal jumps at each testing point. Reactive strength index for each effort was calculated by dividing flight time by ground contact time and peak RSI values were used for statistical analysis. The RSI scores had an ICC of 0.84.

Peak Force and Rate of Force Development (RFD)

Isometric squat parameters were measured using a portable force platform (Kistler, Switzerland) placed inside a custom designed rack (Absolute Performance, Cardiff, UK). Data were collected in accordance with the procedures outlined by Wilson et al. (30), with participants maintaining a knee joint angle of 90° for each trial. Three trials were completed at each testing session with 3 minutes rest between efforts. Average RFD was calculated from 50 to 100 and 100 to 200 ms and peak isometric force was determined as the maximal force recorded from each trial minus body weight. The peak RFD value from 100-200 ms was used for analysis as well as the corresponding 50-100 ms value from the same trial. Initiation of the contraction was identified as the first force value greater than 5 standard deviations of the baseline period (7). Peak force and RFD had ICCs of 0.98 and 0.74 respectively.

¼ Squat 4RM

Baseline values for the ¼ squat were determined during the familiarization session where 4RM was assessed. The loads achieved during repeat testing in training sessions 9 and 16 were then used for the mid and post values respectively.

Statistical Analyses

Perceptions of soreness and recovery were collected at multiple time points over multiple weeks. Therefore, when analysing these variables, delta scores were calculated for baseline to 24 h and baseline to 48 h and then compared from week 1 to week 4 and from week 5 to week 8 in order to investigate group differences. Baseline values for all dependent variables are presented as raw mean \pm SD. Mixed model ANOVAs were used to derive *p*-values for all

dependent variables. If the ANOVA indicated a significant interaction effect (time \times group), a Bonferroni adjusted post-hoc test was applied. Homogeneity of variance was checked with Mauchly's test of sphericity, and in the event of a significant result, Greenhouse-Geisser adjustments were used. Adjusted values are denoted in the text with the use of the suffix $_{GG}$. Statistical significance for all analyses was set at $p = 0.05$ and all data were analysed using IBM SPSS Statistics 25 for Windows (Surrey, UK). Partial-eta² (η^2) effect size statistics were considered small (0.01–0.05), medium (0.06–0.13) or large (≥ 0.14). Confidence Intervals (CIs) for all delta scores are provided as supplementary material (Supplement 1).

RESULTS

Muscle Soreness

Changes in perceptions of soreness were assessed by examining delta scores from pre-24 h and pre-48 h from week 1 to week 4 and week 5 to week 8. A decrease in scores indicates a reduction in the soreness response post-exercise. For weeks 1 to 4 from pre-24 h there was no significant time ($p = 0.43$; $\eta^2 = 0.080$) or interaction effect ($p = 0.32$; $\eta^2 = 0.125$). For weeks 1 to 4 from pre-48 h there was a significant decrease in scores over time ($p = 0.009$; $\eta^2 = 0.474$), however there was no significant interaction effect ($p = 0.41$; $\eta^2 = 0.063$). For weeks 5 to 8 from pre-24 h there was no significant time ($p = 0.27$; $\eta^2 = 0.122$) or interaction effect ($p = 0.60$; $\eta^2 = 0.028$). For weeks 5 to 8 from pre-48 h there was no significant time ($p = 0.23$; $\eta^2 = 0.172$) or interaction effect ($p = 0.37$; $\eta^2 = 0.100$).

Perceived Recovery

PREC scores were analysed in the same manner as perceptions of soreness as described above. However, in contrast to the soreness data, an increase in scores indicates an improved perception of recovery. For weeks 1 to 4 from pre-24 h there was no significant time ($p = 0.06$; $\eta p^2 = 0.413$) or interaction effect ($p = 0.13$; $\eta p^2 = 0.299$). For weeks 1 to 4 from pre-48 h there was a significant improvement in scores over time ($p = 0.002$; $\eta p^2 = 0.682$), however there was no significant interaction effect ($p = 0.77$; $\eta p^2 = 0.010$). For weeks 5 to 8 from pre-24 h there was no significant time ($p = 1.00$; $\eta p^2 = 0.000$) or interaction effect ($p = 0.45$; $\eta p^2 = 0.058$). For weeks 5 to 8 from pre-48 h there was no significant time ($p = 0.97$; $\eta p^2 = 0.000$) or interaction effect ($p = 0.64$; $\eta p^2 = 0.025$).

DALDA

At baseline, DALDA scores marked worse than normal were 3 ± 3 for both the sham and CWI groups. Delta scores were calculated from week 1 to 4 and from week 5 to 8. A decrease in scores would indicate a reduction in stress symptoms. Analysis revealed no significant time ($p = 0.22$; $\eta p^2 = 0.281$) or interaction effect ($p = 0.94$; $\eta p^2 = 0.001$).

DXA and Ultrasound

Analysis revealed that there was no significant effect of time ($p = 0.52$; $\eta p^2 = 0.057$) and no interaction effect ($p = 0.72$; $\eta p^2 = 0.029$) for lean mass assessed via DXA (Table 3). Pennation angle increased from baseline in both groups at both time points ($p = 0.009$; $\eta p^2 = 0.447$), however, there was no significant interaction effect ($p = 0.39$; $\eta p^2 = 0.111$) (Figure 2 and Table 3).

*****INSERT FIGURE 2 HERE*****

Muscle Function and ¼ Squat 4RM

Analysis revealed that there was no significant effect over time ($p = 0.12$; $\eta p^2 = 0.178$) and no significant interaction effect ($p = 0.95$; $\eta p^2 = 0.005$) for RSI (Table 3). Isometric peak force values increased in both groups at both time points ($p = 0.018$; $\eta p^2 = 0.306$), however, there was no significant interaction effect ($p = 0.80$; $\eta p^2 = 0.021$) (Figure 3 and Table 3). Analysis revealed that there was neither a significant time ($p = 0.10$; $\eta p^2 = 0.187$ and $p = 0.33$; $\eta p^2 = 0.097$) nor interaction ($p = 0.48$; $\eta p^2 = 0.065$ and $p = 0.71$; $\eta p^2 = 0.031$) effect for RFD at 50 to 100 ms and 100 to 200 ms respectively (Table 3). There was a significant increase in ¼ Squat 4RM performance over time ($p < 0.001_{GG}$; $\eta p^2 = 0.644$) however, there was no significant interaction effect ($p = 0.83_{GG}$; $\eta p^2 = 0.009$) (Table 3).

INSERT FIGURE 3 HERE

INSERT TABLE 3 HERE

DISCUSSION

The aim of this study was to investigate the influence of repeated CWI or a sham intervention on adaptations to a lower body strength and power training program. It was anticipated that the use of 2 distinct training blocks and assessment of muscle architecture via ultrasound would offer novel additions to the current body of cryotherapy literature. Analysis of data revealed that the training program resulted in significant improvements in ¼ squat 4RM and peak force alongside increases in muscle fibre pennation angle. Conversely, there were no changes in any of the measured perceptual responses to training. The findings from the present study demonstrate that there were no differences between conditions, indicating neither a beneficial

nor detrimental impact of CWI on functional, morphological or perceptual responses compared to a sham intervention during a lower body strength and power training program.

Perceptions of soreness and recovery were recorded pre, and 24 h and 48 h post sessions 1, 8, 9 and 16. Although scores increased as a result of each training session, there was no significant change in this pattern over time or between the groups. These findings are also supported by the sRPE scores which showed no change over time or difference between groups. The finding that CWI did not attenuate the magnitude of soreness experienced by participants was unexpected and not in agreement with the majority of acute studies that show improved perceptual recovery following cryotherapy compared to control interventions (11,14,17). This potentially lends further weight to the argument that previously reported perceptual benefits of cryotherapy treatment may be largely ascribed to a placebo effect (5,30). Similarly, DALDA data revealed no significant change over time or group effect. Collectively, these data suggest that whilst repeated cryotherapy does not negatively impact perceptual responses to resistance training, it does not offer any benefit over a sham intervention.

Previous research has demonstrated that increases in fibre pennation angle are usually closely associated with an increase in physiological and anatomical cross-sectional area in the quadriceps (1). The angle of fibre pennation in the VL increased in both groups at both time points, although there was no difference between groups. The findings from the present study are therefore in contrast to previous research; Roberts et al. (26) demonstrated that type II muscle fibre cross-sectional area increased in the active recovery group ($p < 0.05$), but not the CWI group following a 12 week strength training intervention. Muscle biopsy data from a follow up study revealed that CWI attenuated acute changes in satellite cell numbers and

activity of kinases (NCAM⁺ and Pax7⁺) that regulate muscle hypertrophy, which may have resulted in the diminished long-term training gains in muscle hypertrophy for the CWI group (10). More recently Fyfe et al. (13) reported that increases in type II muscle fibre cross sectional area were attenuated with CWI treatment compared to a passive recovery control intervention following 7 weeks of resistance training. The differing results may be explained in part by methodological differences; the training program utilised in the present study comprised one strength and one power training block (each lasting 4 weeks), whereas the other studies focused on strength training for the duration of the program (12 and 7 weeks respectively) which may have led to greater structural changes. Secondly, participants were exposed to fewer CWI treatments in the present study (16 exposures) compared to the other investigations (24 and 21 exposures respectively) and CWI may have a cumulative impact on architectural changes.

In terms of the more dynamic and sport specific markers, there were no significant changes in RSI or RFD throughout the training intervention and there were no differences between groups. However, peak force derived from the isometric squats demonstrated a significant increase over time. Whilst full range squats may be considered generally superior for the development of strength, ¼ squats were chosen for the training programme for two main reasons; firstly, the depth replicated the position for the isometric squat allowing for maximal joint-angle specific increases in strength (25), and secondly, not all participants could safely squat, under maximal load, to a full depth position, therefore ¼ squats offered a safe alternative. Whilst neural adaptations are largely responsible for strength gains during the first three to five weeks of a training program (22), it is possible that increases in peak force were also a product of the observed increases in muscle fibre pennation angle. The findings from the present study

support previous investigations suggesting that there is no marked improvement in neuromuscular performance following cold habituation (19).

Potential limitations of the current study should be addressed. Participants were matched into groups based on a ratio of lean mass and 4RM, resulting in differences in absolute strength between groups, however there was no interaction effect despite baseline group differences. Additionally, although participants had previous strength training experience, they were not actively weight training prior to study participation. As such, findings from this study may not be directly applicable to highly trained or elite individuals. Although muscle fibre pennation angle was included to assess changes in muscle architecture, the training program implemented was targeted towards strength and power. As such, a hypertrophy program may have been more efficacious in affecting morphological changes. CWI presents a specific challenge in terms of blinding participants to their treatment interventions. Whilst a thermoneutral water immersion would have provided a true placebo, a sham intervention was utilised for the second group in order to maintain a level of deception. There was no direct measure of expectancy effect or treatment belief in the present study, which could have strengthened the findings. However, anecdotal evidence from the present study suggests that the sham intervention was administered effectively, and that participants believed in its efficacy.

PRACTICAL APPLICATIONS

The findings from this study suggest that there was neither a beneficial nor detrimental impact of repeated CWI on perceptual, functional or morphological adaptations to an 8 week strength and power training program compared to a sham intervention. The shorter training program (2

x 4 weeks) utilised in the present study is a novel addition to the cryotherapy literature. However, where a longer training program of 12 weeks (26) or greater number of CWI exposures (>16) (13) has been utilised, CWI attenuated long term gains in muscle mass and/or strength (20). These data, in addition to the current findings, suggest that there is a cumulative impact of cryotherapy on adaptation, and that decrements may be seen as a result of habitual use.

ACKNOWLEDGEMENTS

The authors are grateful to Eleftheria Panayiotou for her assistance in analysing the food diaries. This study was undertaken with no external financial support, and there are no conflicts of interest to declare.

REFERENCES

1. Aagaard, P, Andersen, JL, Dyhre, P, Leffers, AM, Wagner, A, Peter Magnusson, S, et al. A mechanism for increased contractile strength of human pennate muscle in response to strength training: Changes in muscle architecture. *J Physiol* 534: 613–623, 2001.
2. Baechle, TR, Earle, RW, and Wathen, D. Essentials of Strength and Conditioning: National Strength and Conditioning Association (NSCA). 2000.
3. Bartolomei, S, Hoffman, JR, Merni, F, and Stout, JR. A comparison of traditional and block periodized strength training programs in trained athletes. *J Strength Cond Res* 28: 990–997, 2014.
4. Billsborough, JC, Greenway, K, Opar, D, Livingstone, S, Cordy, J, and Coutts, AJ. The accuracy and precision of DXA for assessing body composition in team sport athletes. *J Sports Sci* 32: 1821–1828, 2014.
5. Broatch, JR, Petersen, A, and Bishop, DJ. Postexercise Cold Water Immersion Benefits Are Not Greater than the Placebo Effect. *Med Sci Sport Exerc* 46: 2139–2147, 2014. Available from:
<http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00005768-201411000-00014>
6. Campbell, B, Kreider, RB, Ziegenfuss, T, La Bounty, P, Roberts, M, Burke, D, et al. International Society of Sports Nutrition position stand: protein and exercise. *J Int Soc Sport Nutr* 4: 8–10, 2007.
7. Chavda, S, Bromley, T, Jarvis, P, Williams, S, Bishop, C, Turner, AN, et al. Force-time Characteristics of the Countermovement Jump: Analyzing the Curve in Excel. *Strength Cond J* 40: 67–77, 2018.
8. Cormie, P, McGuigan, MR, and Newton, RU. Influence of strength on magnitude and mechanisms of adaptation to power training. *Med Sci Sports Exerc* 42: 1566–1581, 2010.
9. e Lima, KM, Carneiro, SP, Alves, DDS, Peixinho, CC, and de Oliveira, LF. Assessment of muscle architecture of the biceps femoris and vastus lateralis by

- ultrasound after a chronic stretching program. *Clin J Sport Med* 25: 55–60, 2015.
10. Figueiredo, V, Roberts, LA, Markworth, JF, and Barnett, MPG. Impact of resistance exercise on ribosome biogenesis is acutely regulated by post-exercise recovery strategies. *Physiol Rep* 4: 1–12, 2016.
 11. Fonda, B and Sarabon, N. Effects of whole-body cryotherapy on recovery after hamstring damaging exercise: A crossover study. *Scand J Med Sci Sports* n/a-n/a, 2013. Available from: <http://doi.wiley.com/10.1111/sms.12074>
 12. Fröhlich, M, Faude, O, Klein, M, Pieter, A, Emrich, E, and Meyer, T. Strength training adaptations after cold water immersion. *J Strength Cond* 28: 2628–2633, 2014. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24552795>
 13. Fyfe, JJ, Broatch, JR, Trewin, AJ, Hanson, ED, Argus, CK, Garnham, AP, et al. Cold water immersion attenuates anabolic signaling and skeletal muscle fiber hypertrophy, but not strength gain, following whole-body resistance training. *J Appl Physiol* 127: 1403–1418, 2019.
 14. Garcia, CA, Ribeiro, G, Mota, D, Hida, RA, and Júnior, MM. Evidence of cold water immersion in team sports recovery: a systematic review. *Arq Cien Esp* 44: 20–26, 2016. Available from: <http://seer.uftm.edu.br/revistaelectronica/index.php/aces>
 15. Al Haddad, H, Parouty, J, and Buchheit, M. Effect of daily cold water immersion on heart rate variability and subjective ratings of well-being in highly trained swimmers. *Int J Sports Physiol Perform* 7: 33–8, 2012. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21941017>
 16. Halson, SL, Bridge, MW, Meeusen, R, Busschaert, B, Gleeson, M, Jones, DA, et al. Time course of performance changes and fatigue markers during intensified training in trained cyclists. *J Appl Physiol* 93: 947–956, 2002.
 17. Hausswirth, C, Louis, J, Bieuzen, F, Pournot, H, Fournier, J, Filliard, J-R, et al. Effects of Whole-Body Cryotherapy vs. Far-Infrared vs. Passive Modalities on Recovery from Exercise-Induced Muscle Damage in Highly-Trained Runners. *PLoS One* 6: e27749, 2011. Available from: <http://dx.plos.org/10.1371/journal.pone.0027749>
 18. Holmes, M and Willoughby, D. The Effectiveness of Whole Body Cryotherapy

- Compared to Cold Water Immersion: Implications for Sport and Exercise Recovery. *Int J Kinesiol Sport Sci* 4, 2016. Available from: <http://www.journals.aiac.org.au/index.php/IJKSS/article/view/3001/2496>
19. Mäkinen, TM. Human cold exposure, adaptation, and performance in high latitude environments. *Am J Hum Biol Off J Hum Biol Assoc* 19: 155–164, 2007.
 20. Malta, ES, Dutra, YM, Broatch, JR, Bishop, DJ, and Zagatto, AM. The Effects of Regular Cold-Water Immersion Use on Training-Induced Changes in Strength and Endurance Performance: A Systematic Review with Meta-Analysis. *Sport Med* 51: 161–174, 2021. Available from: <https://doi.org/10.1007/s40279-020-01362-0>
 21. Mawhinney, C, Low, DA, Jones, H, Green, DJ, Costello, JT, and Gregson, W. Water Mediates Greater Reductions in Limb Blood Flow than Whole Body Cryotherapy. *Med Sci Sport Exerc* , 2017.
 22. Moritani, T and DeVries, HA. Neural factors versus hypertrophy in the time course of muscle strength gain. *Am J Phys Med Rehabil* 58: 115–130, 1979.
 23. Norton, LE and Layman, DK. Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. *J Nutr* 136: 533S-537S, 2006.
 24. Peake, JM, Roberts, LA, Figueiredo, VC, Egner, I, Krog, S, Aas, SN, et al. The effects of cold water immersion and active recovery on inflammation and cell stress responses in human skeletal muscle after resistance exercise. *J Physiol* 595: 695–711, 2017. Available from: <http://doi.wiley.com/10.1113/JP272881>
 25. Rhea, MR, Kenn, JG, Peterson, MD, Massey, D, Simão, R, Marin, PJ, et al. Joint-Angle Specific Strength Adaptations Influence Improvements in Power in Highly Trained Athletes. *Hum Mov* 17: 43–49, 2016.
 26. Roberts, LA, Raastad, T, Markworth, JF, Figueiredo, VC, Egner, IM, Shield, A, et al. Post-exercise cold water immersion attenuates acute anabolic signalling and long-term adaptations in muscle to strength training. *J Physiol* 593: 4285–4301, 2015.
 27. Tipton, MJ, Collier, N, Massey, H, Corbett, J, and Harper, M. Cold water immersion: kill or cure? *Exp Physiol* 102: 1335–1355, 2017.
 28. Vieira Ramos, G, Pinheiro, CM, Messa, SP, Delfino, GB, Marqueti, R de C, Salvini, T

- de F, et al. Cryotherapy Reduces Inflammatory Response Without Altering Muscle Regeneration Process and Extracellular Matrix Remodeling of Rat Muscle. *Sci Rep* 6: 18525, 2016. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4698758&tool=pmcentrez&rendertype=abstract>
29. Wilson, LJ, Cockburn, E, Paice, K, Sinclair, S, Faki, T, Hills, FA, et al. Recovery following a marathon: a comparison of cold water immersion, whole body cryotherapy and a placebo control. *Eur J Appl Physiol* 118: 153–163, 2018.
30. Wilson, LJ, Dimitriou, L, Hills, FA, Gondek, MB, and Cockburn, E. Whole body cryotherapy, cold water immersion, or a placebo following resistance exercise: a case of mind over matter? *Eur J Appl Physiol* 119: 135–147, 2019.
31. Yamane, AM, Ohnishi, N, and Matsumoto, T. Does Regular Post-exercise Cold Application Attenuate Trained Muscle Adaptation? *d-nb.info* 36: 647–653, 2015. Available from: <http://dx.doi.org/>
32. Yamane, M, Teruya, H, Nakano, M, Ogai, R, Ohnishi, N, and Kosaka, M. 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* 96: 572–580, 2006.

Figure legends

Figure 1. Ultrasound images of the vastus lateralis (VL) showing alterations in fibre pennation angle from baseline (*left*) to post (*right*).

Figure 2. Muscle fibre pennation angle (°). Individual responses are plotted using dashed lines, and the group mean response is represented as a solid bold line.

Figure 3. Isometric peak force (N). Individual responses are plotted using dashed lines, and the group mean response is represented as a solid bold line.