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Effects of Cold Water Immersion on Muscle Oxygenation During Repeated Bouts of Fatiguing Exercise

A Randomized Controlled Study

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Abstract: Postexercise cold water immersion has been advocated to athletes as a means of accelerating recovery and improving performance. Given the effects of cold water immersion on blood flow, evaluating *in vivo* changes in tissue oxygenation during cold water immersion may help further our understanding of this recovery modality. This study aimed to investigate the effects of cold water immersion on muscle oxygenation and performance during repeated bouts of fatiguing exercise in a group of healthy young adults.

Twenty healthy subjects performed 2 fatiguing bouts of maximal dynamic knee extension and flexion contractions both concentrically on an isokinetic dynamometer with a 10-min recovery period in between. Subjects were randomly assigned to either a cold water immersion (treatment) or passive recovery (control) group. Changes in muscle oxygenation were monitored continuously using near-infrared spectroscopy. Muscle performance was measured with isokinetic dynamometry during each fatiguing bout. Skin temperature, heart rate, blood pressure, and muscle soreness ratings were also assessed. Repeated measures ANOVA analysis was used to evaluate treatment effects.

The treatment group had a significantly lower mean heart rate and lower skin temperature compared to the control group ($P < 0.05$). Cold water immersion attenuated a reduction in tissue oxygenation in the second fatiguing bout by 4% when compared with control. Muscle soreness was rated lower 1 day post-testing ($P < 0.05$). However, cold water immersion had no significant effect on muscle performance in subsequent exercise.

As the results show that cold water immersion attenuated decreased tissue oxygenation in subsequent exercise performance, the metabolic response to exercise after cold water immersion is worthy of further exploration.

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Abbreviations: HbO = oxyhaemoglobin, Hb_{total} = total haemoglobin, HHb = deoxyhaemoglobin, TOI = tissue oxygenation index.

INTRODUCTION

Various passive and active recovery techniques have been developed to reduce fatigue and enhance performance in athletes. The use of cold water immersion in temperatures $<15^{\circ}C$ is an increasingly popular recovery strategy. It has been proposed that the hydrostatic pressure exerted on the body when immersed in cold water causes intracellular fluid shifts, reducing inflammation and edema, and thereby preserving muscle function and maintaining muscle performance.[1–3] In particular, it is well documented that eccentric-biased exercises are associated with muscle damage, swelling, stiffness, and pain.[4] Team sports such as rugby, football have a high component of high-intensity eccentric muscle contractions, which lead to muscle damage. Thus, cold water immersion may be considered as a modality to reduce inflammatory response as well as decrease associated swelling and pain.

There is an emerging body of evidence supporting the use of cold water immersion between repeated bouts of high-intensity exercise occurring over several days. In a study investigating the effects of postmatch cold water immersion during a 4-day soccer tournament, cold water immersion attenuated decrements in running performance and moderated heart rate during a subsequent match.[5] In another study on basketball players, postmatch cold water immersion improved jump performance 24 h after the match.[6] In both studies, cold water immersion led to lower perceptions of overall fatigue and leg soreness the next day compared to other recovery interventions. Additionally, cold water immersion immediately after a high-intensity training session results in better next-day run performance when compared with delayed cold water immersion performed 3 h postexercise.[7] In contrast, other studies show no obvious beneficial effect of cold water immersion on repeated performance.[8,9]

Cold water immersion causes peripheral vasoconstriction that results in a central pooling of blood, followed by peripheral vasodilation immediately after emerging from the cold water.[10,11] This mechanism may improve the rate at which muscles become reoxygenated. Near-infrared spectroscopy is a noninvasive and direct method to study local tissue oxygenation and hemodynamic by monitoring changes in oxy- and deoxyhemoglobin. This technique can determine local information about muscle oxygen consumption and blood flow. Several studies have looked specifically at the effects of cold water immersion on muscle oxygenation.[12–15] Tseng et al[12] reported that cold water immersion elicited higher muscle oxygenation

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during immersion, whereas Ihsan et al[13] extended the investigation by examining the effect of cold water immersion on muscle oxygenation and blood flow postexercise. More recently, Roberts et al[14] reported that muscle hemodynamics was reduced during the hour after cold water immersion following resistance exercise. However, these studies did not examine the effects of cold water immersion on subsequent exercise. More recently, Stanley and colleagues[15] reported that cold water immersion following high-intensity interval training reduced oxygen delivery and utilization by muscle, and this response increased anaerobic metabolism during subsequent high-intensity exercise. Many team sports (such as soccer, field hockey, and rugby) use cold water immersion during halftime of the competition as a strategy to enhance second-half performance[16–18]; it is therefore important to evaluate the impact of cold water immersion on muscle oxygenation during subsequent exercise performance. The aim of the present study was to investigate the effects of cold water immersion compared to passive recovery on local muscle oxygenation and muscle performance in a subsequent bout of resistance exercise on the same day.

MATERIALS AND METHODS

Subjects

Twenty subjects (10 M:10F, 22.0 ± 0.52 years) were recruited for the study (Figure 1). The study was approved by the Human Ethics Committee of the Hong Kong Polytechnic University (HSEARS20120716001) and complied with the Declaration of Helsinki. All subjects gave their written informed consent before participation. Subjects were excluded from the study if they were unable to perform knee exercise pain-free, had contraindications to the use of cold therapy, or had adipose tissue thickness of >6 mm at the position of near-infrared spectroscopy probe placement.[19]

Sample Size

Sample size calculations were based on near-infrared spectroscopy-derived data from a previous cold water immersion study.[13] The mean percentage difference during cold water immersion compared with control for total hemoglobin was $20 \pm 12\%$ (mean $\pm$ SD). Power analysis for independent samples *t* test was used to determine the sample size. Assuming

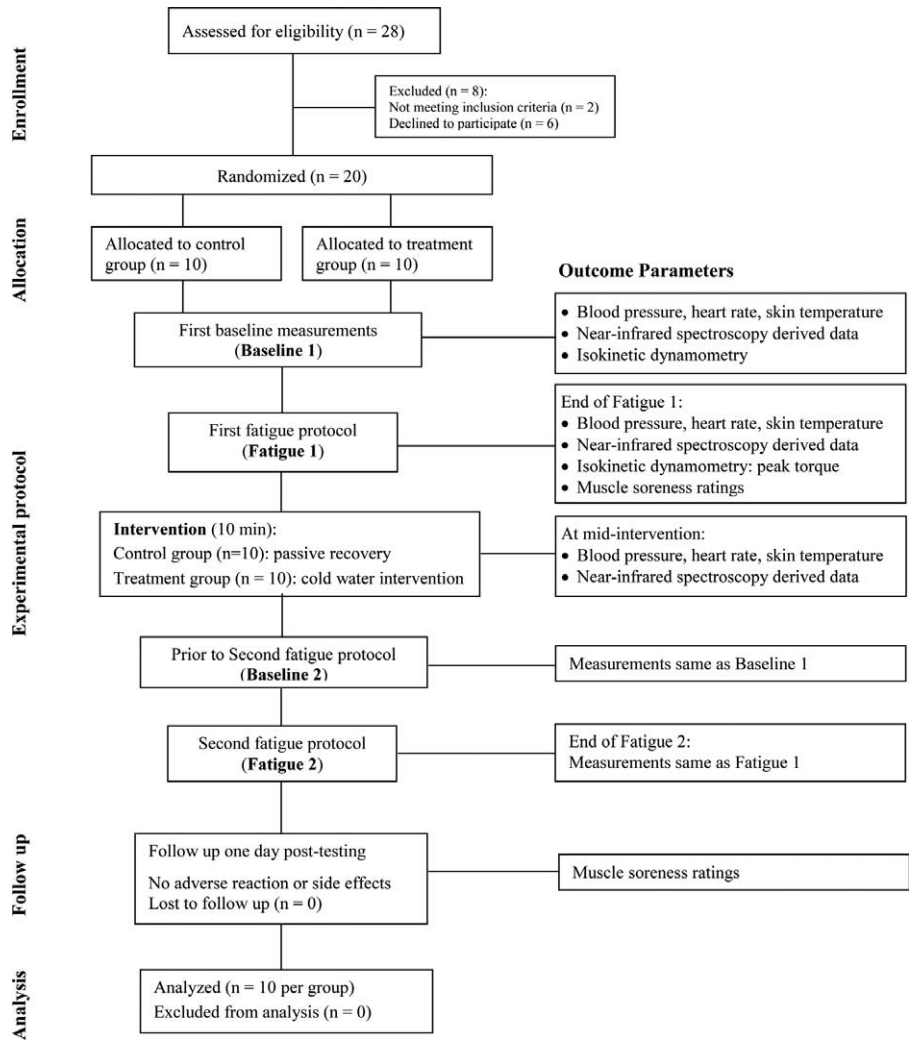

FIGURE 1. Subject selection and schematic flow diagram of the experimental protocol.

a type I error of 0.05 and power of 0.80, 7 subjects were needed to show statistically significant differences in tissue oxygenation between intervention and control (G*Power 3.1.9 software). Ten subjects per group were recruited with consideration of dropouts.

Randomization

Subjects were randomly allocated to either a passive recovery (control, n = 10) or cold water immersion (treatment, n = 10) group based on a computer-generated block randomization code. This method of randomization was used to ensure that an equal number of subjects were allocated to each group. Each subject was asked to choose the date/session of the testing schedule, which would then determine the group assignment. The subjects were blinded to the intervention when selecting the testing schedule. There was no gender preference for the allocation. The investigators had no influence on the group allocation. The nature of the exercise protocol and intervention made it impossible to conceal group allocation to the subjects or the investigators.

Experimental Procedures

The experimental protocol is schematically illustrated in Figure 1.

Fatiguing Protocols

The fatiguing protocols were performed using an isokinetic dynamometer (version 2.04, Cybex Humac Norm, Henly Healthcare). The subject was comfortably seated with the hip joint at ~85° flexion. The distal shin pad of the dynamometer was attached 2 to 3 cm proximal to the lateral malleolus by a strap. Straps were applied across the chest, pelvis, and midthigh to minimize extra body movements during the fatigue protocol. The subject was asked to position his/her arms across the chest with each hand clasping the opposite shoulder during testing. To become familiarized with the testing procedure, the subject performed 5 sub-maximal knee extension contractions at an angular velocity of $60^{o}\ s^{-1}$. After a 1 min rest, the first fatigue protocol was performed (Fatigue 1). The subject was asked to perform maximal knee extension and flexion contractions in the concentric-concentric mode. Fatigue was determined as the point at which the last 10 contractions decreased <60% of peak torque. Peak torque is defined as the highest torque obtained from the first 10 contractions at baseline. Consistent verbal encouragements were given to the subjects throughout the test. Each subject repeated a second fatigue protocol (Fatigue 2) within 1 min following either cold water immersion or passive recovery.

Recovery Interventions

At the end of the first fatigue protocol, subjects were seated for 3 min for near-infrared spectroscopy recording before the intervention. The intervention for both groups was 10 min duration. For the treatment group, subjects were rested in a semireclined position in a cold water pool (12–15°C) with both legs extended and water up to the level of the iliac crest. The water temperature was monitored continuously with a mercury-in-glass thermometer and adjusted by addition of crushed ice. Subjects in the control group rested on a mat in the same posture as the treatment group. Physiological data and near-infrared spectroscopy data were collected 5 to 7 min into the intervention. For the intervention group, the leg for probe placement was taken out of water for muscle oxygenation measurements. All testing and recovery sessions were performed in a temperature-controlled laboratory (25°C, relative humidity 75%).

Outcomes

The primary outcomes were physiological responses, muscle oxygenation, and muscle performance. The secondary outcome was muscle soreness ratings.

Physiological Responses

Blood pressure, heart rate, and skin temperature were measured: (i) at rest (Baseline 1); (ii) after the first fatigue protocol (Fatigue 1); (iii) at 5 to 7 min into intervention of either passive recovery or cold water immersion (intervention); (iv) before the second fatigue protocol (Baseline 2); and (v) after the second fatigue protocol (Fatigue 2). Blood pressure and heart rate were measured with an automatic device (Press-Mate BP-8800, Colin Electronics Co. Ltd, San Antonio, TX). A copper temperature sensor was used (Jockey Club Rehabilitation Engineering Centre, Hong Kong) to monitor skin surface temperature.

Muscle Oxygenation

Muscle oxygenation was assessed with ISS Imagent (ISS, Champaign, IL) using a frequency-domain near-infrared spectroscopy technique with 2 wavelengths of near-infrared light (690 and 830 nm) at a sampling rate of 25 Hz. A multidistance optical probe, configured with 1 optical detector and 8 optical source fibers, was attached to muscle. The fibers were positioned on the probe such that there are 4 source and detector separation distances (2.0, 2.5, 3.0, 3.5 cm) for each wavelength. Before the testing procedure, skinfold thickness at the site of near-infrared spectroscopy optode placement was measured by a skinfold calliper (Harpenden, Baty International, West Sussex, UK) to determine adipose tissue thickness, defined as skinfold thickness/2.[19] The probe was positioned on the belly of the right vastus lateralis muscle (~10 cm from the centre of the patella and 25° lateral to the midline of the thigh).[20] Pen-marks were made around the margins of the probe to enable reproduction of the placement position in the subsequent procedure. The probe was secured with tape and black elastic bandages were wrapped around the leg to block background light.

Before data acquisition, the system was calibrated with a factory-manufactured calibration block with known optical properties (absorption and scattering coefficients). Changes in oxyhemoglobin (HbO), deoxyhemoglobin (HHb), and total hemoglobin (Hb_{total}) concentration in the muscle were monitored continuously through the testing procedure. The tissue oxygenation index ($TOI = (HbO/Hb_{total}) \times 100$) was calculated based on these data.[21] All signals were recorded and stored for subsequent analysis. The near-infrared spectroscopy derived data were time-aligned and averaged over a 2-min period to obtain a single response for each subject. Data was extracted (i) at baseline (Baseline 1); (ii) at the end of first fatigue protocol (Fatigue 1); (iii) during midintervention period (intervention); (iv) before the second fatigue protocol (Baseline 2); and (vi) at the end of second fatigue protocol (Fatigue 2). Values are presented as mean change from the baseline value (ΔHbO_{mean}, $\Delta_{HHbmean}$, ΔHb_{total}, and ΔTOI). To examine the rate of recovery of muscle oxygenation after the cessation of exercise, half-recovery time was calculated by extracting the first 2 min of HbO data immediately after the fatigue protocol and fitting it to a mono-exponential curve. This variable is defined as the time

required for half recovery of HbO from maximal deoxygenation to maximal reoxygenation.[22]

Muscle Performance

Muscle performance during the fatiguing protocol was evaluated by peak torque adjusted to body weight (peak torque$_{(BW)}$), fatigue rate, and total work done. Fatigue rate, expressed as a percentage, was defined as the difference between the mean force production of the first 10 and last 10 contractions divided by the mean of the first 10 contractions. Total work, quantified as the area under the torque curve × angular displacement over the contraction period, was recorded and analyzed by the custom script software (LabView version 8.6, National Instruments Corporation, Austin) and data acquisition device (NI-USB6211, National Instruments Corporation, TX) at a sampling rate of 1000 Hz.

Muscle Soreness Ratings

Self-ratings of muscle soreness were evaluated using a 10-point numerical rating scale with "0" as no soreness and "10" as extremely intense soreness. The ratings were taken after each fatigue protocol, and on the following day through follow-up phone calls.

Statistical Analysis

Subjects' baseline characteristics were analyzed using independent samples *t* tests. Comparisons for the dependent variables between and within groups were analyzed using 2-way repeated ANOVA. Post-hoc Bonferonni correction analyses were used to compare pairs of means. Where appropriate, Cohen's *d* effect size was calculated to quantify differences between groups. Data was analyzed using the Graphpad Prism software (version 5.0, GraphPad, La Jolla, CA). Values are presented as mean ± standard error (SE). Statistical significance was set at $P < 0.05$.

RESULTS

Baseline Characteristics

Table 1 shows the characteristics of the subjects. The 2 groups did not differ at baseline. None of the subjects reported any adverse reactions or side effects within 48 h after testing.

Physiological Responses

Both fatiguing protocols resulted in a significantly higher systolic ($P < 0.01$) and diastolic blood pressure ($P < 0.01$), but there was no significant difference between groups. The heart rate was significantly changed during fatiguing exercise ($P < 0.01$). Post-hoc analysis revealed that cold water immersion led to a lower heart rate (88.80 ± 5.52 beats min^{-1}) after the second fatigue protocol than passive recovery (105.20 ± 5.96 beats min^{-1}, $P < 0.05$; $d = -0.28$). The cold water immersion maneuver reduced skin surface temperature by ~ 4 °C (control: 28.2 ± 0.31°C; treatment: 24.3 ± 0.32°C; $d = 3.91$) before the

TABLE 1. Descriptive Characteristics of the Subjects

Characteristics	Control	Treatment	*P*
Subjects	n = 10 (5M:5F)	n = 10 (5M:5F)	–
Age, y	21.6 ± 0.5	22.4 ± 0.9	0.45
Weight, kg	54.5 ± 2.4	57.9 ± 3.0	0.39
Height, cm	163.8 ± 2.6	167.2 ± 2.9	0.39
Body mass index, kg m^{-2}	20.3 ± 0.5	20.8 ± 0.6	0.53
Adipose tissue thickness, mm	4.8 ± 0.4	4.5 ± 0.5	0.65

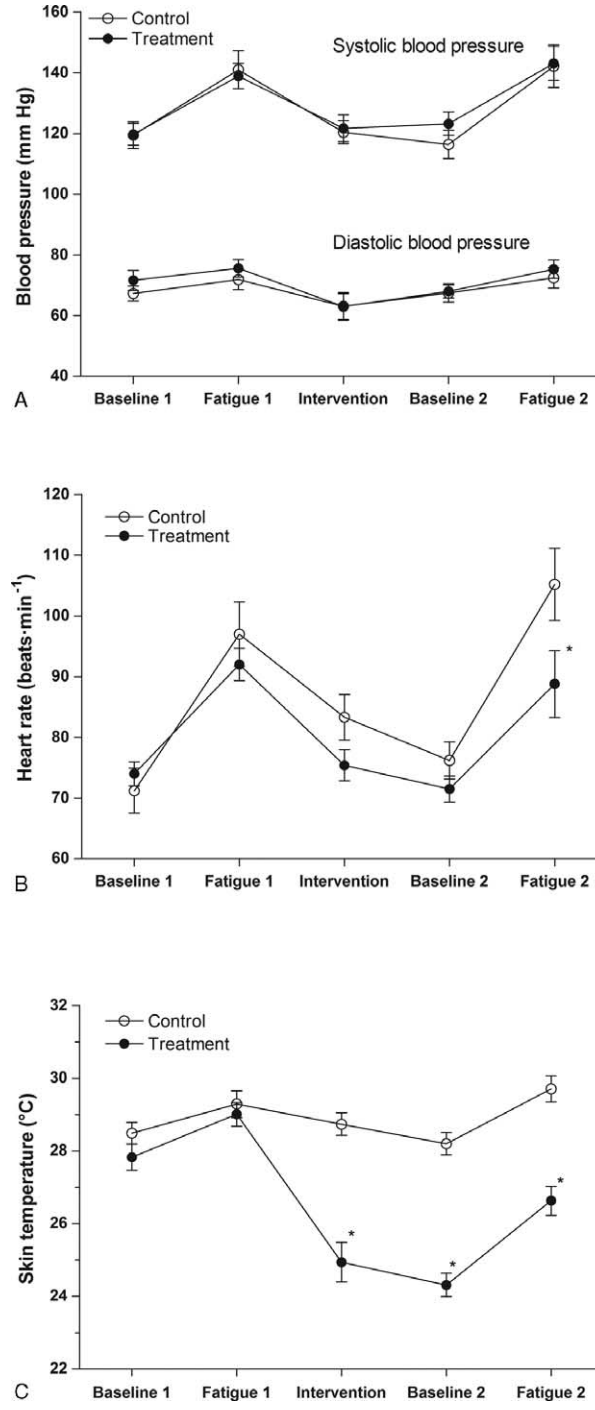

FIGURE 2. Changes in (A) blood pressure, (B) heart rate, and (C) skin temperature before and after the first fatigue protocol, in the middle of intervention, and before and after the second fatigue protocol. Asterisks (*) indicates significant differences between control (passive recovery) and treatment (cold water immersion) groups. Values are mean ± SE. SE = standard error.

second fatigue protocol (ie Baseline 2) and skin temperature remained significantly lower after the second fatigue protocol when compared to control treatment ($P < 0.01$; $d = 2.58$). Changes in blood pressure, heart rate, and skin temperature over stages of the experiment are shown in Figure 2.

Muscle Oxygenation

The mean changes in ΔTOI, ΔHbO_{mean}, and ΔHHb_{mean} at the end of the 2 fatiguing protocols normalized to its corresponding baseline. There was a significant change over time for ΔTOI, ΔHbO_{mean}, and ΔHHb_{mean} ($P < 0.01$ for all, Figure 3). In both groups, ΔTOI and ΔHbO_{mean} significantly decreased during the first fatigue protocol, and this was accompanied by a rise in ΔHHb_{mean}. However the decrease in ΔTOI (control: $73.1 \pm 3.07\%$; treatment: $83.5 \pm 2.91\%$, $P < 0.05$; $d = -1.11$) and ΔHbO_{mean} (control: $73.9 \pm 4.77\%$; treatment: $87.6 \pm 4.84\%$; $P < 0.05$; $d = -0.90$) during the second fatigue protocol was significantly attenuated following cold water immersion intervention reflecting greater reperfusion and an increase in oxygen delivery to the muscle. There was a trend for lower muscle deoxygenation (as reflected by ΔHHb_{mean}) in the second bout of exercise after cold water immersion, but it did not reach statistical significance (control: $167 \pm 11.4\%$; treatment: $142 \pm 6.42\%$; $P = 0.07$; $d = 0.89$). Though the half-recovery time for HbO was not significantly different between the 2 groups, the cold water immersion group also showed a trend of improvement ($P = 0.07$; $d = 0.61$) in reoxygenation recovery after the second fatigue protocol (control: Fatigue 1: 36.5 ± 5.73 s, Fatigue 2: 35.8 ± 4.97 s; intervention: Fatigue 1: 36.7 ± 6.20 s, Fatigue 2: 27.9 ± 3.77 s). Figure 3D shows the raw tracings of HbO for the first 2 min of recovery following fatigue protocol 1 and 2.

Force

No significant interaction effect between groups (cold water immersion and passive recovery) and conditions (Fatigue 1 and Fatigue 2) was found for peak $torque_{(BW)}$ ($P = 0.81$), work done ($P = 0.37$) and fatigue rate ($P = 0.54$). There was no

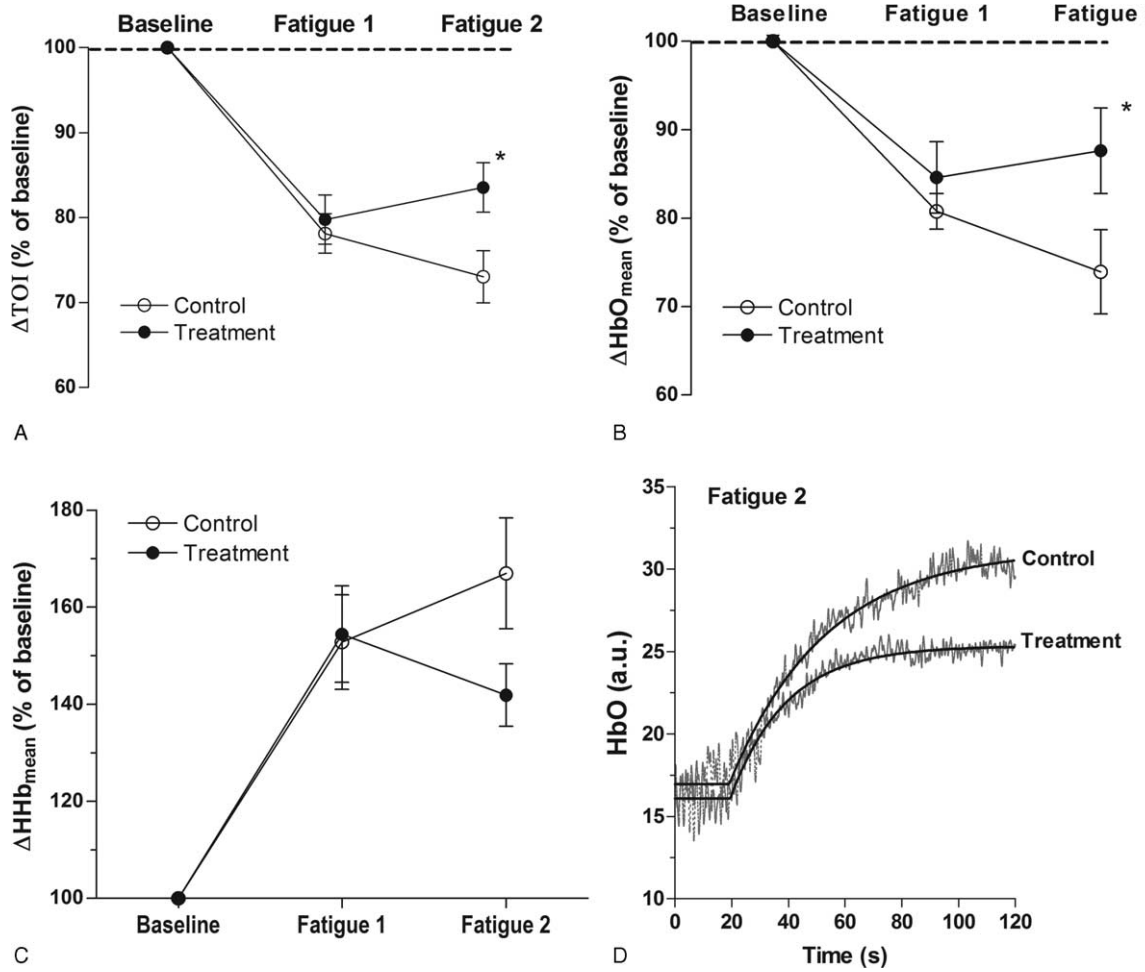

FIGURE 3. Mean changes in (A) tissue oxygenation index (ΔTOI), (B) oxyhemoglobin (ΔHbO_{mean}), and (C) deoxyhemoglobin (ΔHHb_{mean}) during the first and second fatigue protocols compared with baseline. Baseline was determined as the mean value >2 min before the onset of each fatigue protocol. Representative tracings (D) of the first 2 min of HbO data immediately after fatigue protocol 2 (Fatigue 2) in 1 subject from the control group and a subject from the treatment group. The solid lines represent the monoexponential model fits of reoxygenation recovery. Asterisk (*) indicates significant differences between treatment (cold water immersion) and control (passive recovery) groups. Values are mean ± SE. a.u. = arbitrary units, SE = standard error.

significant main effects between groups for peak torque$_{(BW)}$ ($P = 0.96$), work done ($P = 0.68$), and fatigue rate ($P = 0.98$) (Figure 4).

Muscle Soreness Rating

Muscle soreness was significantly increased after each fatigue protocol ($P < 0.01$). The treatment group had a lower muscle soreness rating compared to the control group 1 day post-testing (control: 3.10 ± 0.64; treatment: 2.20 ± 0.64, $P < 0.05$; $d = 0.44$).

DISCUSSION

The aim of this study was to investigate the effects of cold water immersion as compared to passive recovery on physiological measures, muscle oxygenation, force production, and muscle soreness in 2 subsequent bouts of exercise on the same day. We showed a decrease in heart rate and skin temperature, and an increase in muscle oxygenation in the cold water immersion group. Cold water immersion also decreased the perception of muscle soreness the day following exercise. However, there was no significant effect on muscle performance.

We observed a significant decrease in heart rate and skin temperature after cold water immersion as reported in other studies.[11,23,24] It has been suggested that cold water immersion leads to cutaneous vasoconstriction, resulting in a decrease in peripheral blood flow. This redistribution of blood from the periphery to the core augments venous return and increases stroke volume. The net effect of these changes is to enhance blood and oxygen delivery to working muscles, and possibly to enhance exercise performance.[1,25] However, this explanation assumes that muscle blood flow is the main limiting factor during exercise.

We observed a decrease in HbO concomitant with an increase in HHb at the end of the fatigue protocol in both groups. In addition, the TOI was significantly decreased. TOI primarily reflects the dynamic balance between oxygen supply and demand in muscle. These responses reflect tissue extraction of oxygen to meet the increased metabolic demands during exercise.[13,26]

Several studies have used near-infrared spectroscopy to examine muscle oxygenation and hemodynamics after cold water immersion.[13–15] Using a single-leg cold water immersion design with near-infrared spectroscopy-derived data, Ihsan et al[13] reported a decrease in Hb_{total} (~20%) and an increase in TOI (~ 3%) during cold water immersion. Here we have extended those findings to show that cold water immersion attenuates the reduction in HbO and TOI at the end of the subsequent fatigue bout. Specifically, the cold water immersion group showed an attenuation of TOI of ~ 4% in the second fatiguing protocol compared with the first fatigue bout, indicating a possible increase in muscle oxygen availability and enhancement of oxidative capacity after cold water immersion. In contrast, Stanley et al[15] showed a trend toward reduced muscle blood flow in a subsequent high-intensity interval cycling exercise. This is probably due to differences in the fatigue protocols that may impose different metabolic demands. Half recovery time measured by near-infrared spectroscopy is an interesting parameter for examining postexercise muscle reoxygenation kinetics.[22,27,28] This variable includes recovery of both vascular and muscle oxygen desaturation. We noted a trend toward quicker recovery with cold water immersion intervention post-fatigue, but the difference was insignificant. In a similar experimental setup, Roberts and colleagues[14] showed that cold water immersion reduced resting muscle blood volume after unilateral knee extension

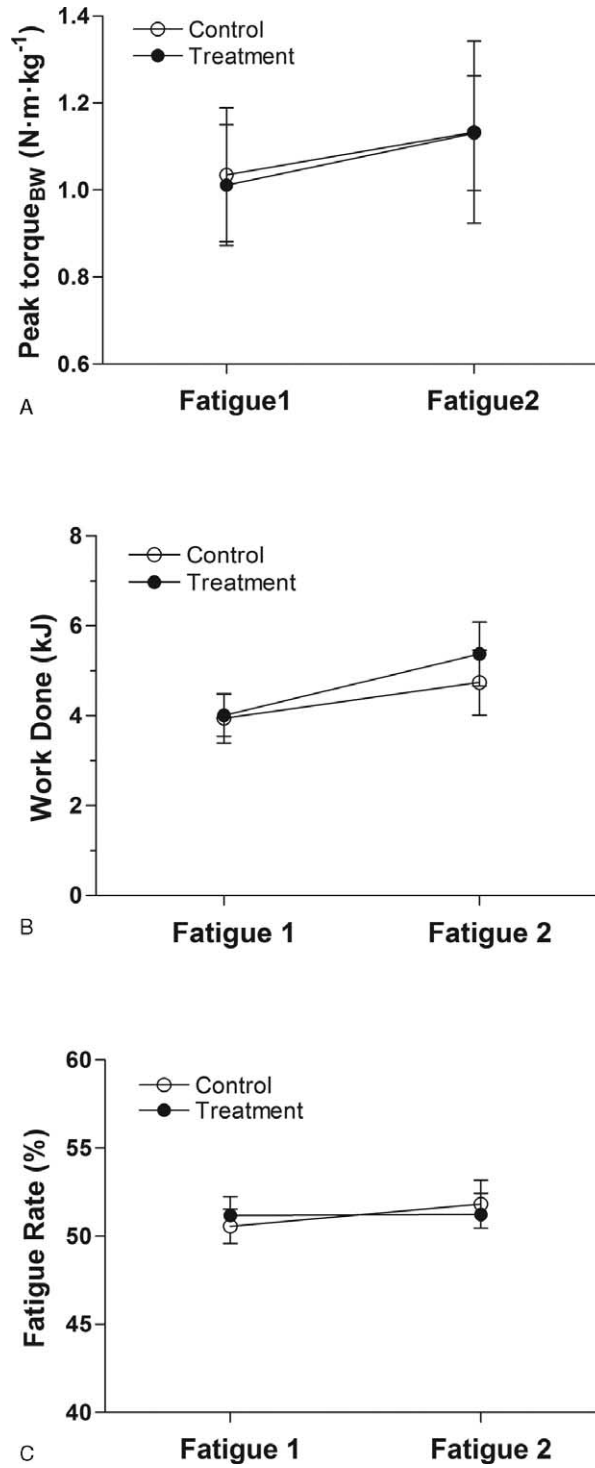

FIGURE 4. Changes in (A) peak torque adjusted by body weight, (B) work done, and (C) fatigue rate between the 2 fatigue protocols. Values are mean ± SE. SE = standard error.

exercise. However reoxygenation recovery time was not significantly different from the pre-exercise baseline at 5, 20, and 40 min postimmersion when subjects performed a single bout of maximal isometric knee extension. Whether half-recovery time is dependent on exercise intensity requires further investigation.

Although we observed effects of cold water immersion on heart rate, skin temperature, and muscle oxygenation, the effect was not reflected in muscle performance. Studies that examine the effect of cold water immersion on endurance exercises (up to 40 min) show favorable results,[29] whereas studies on intermittent or short duration exercise are less consistent.[26,30–32] Furthermore, the beneficial effect seems to be less obvious in local muscular fatigue protocols.[33,34] The perception of muscle soreness was significantly lower in our cold water immersion group 24 h postfatiguing exercise. This finding supports previously reports that cold water immersion improves perception of recovery after exercise.[35–38] The reduction in skin temperature after cold water immersion may be a plausible explanation for a diminished perception of muscle soreness if cold water immersion reduces an inflammatory response and minimizes secondary muscle damage.[39] Studies have examined the effect of cold water immersion in reducing muscle damage produced by the eccentric exercise. Based on the findings from several meta-analyses, it appears that cold water immersion has beneficial effects in reducing delayed-onset muscle soreness[3] as well as on recovery of exercise performance.[40] Furthermore, studies reported that cold water immersion has some beneficial effects in reducing indices of muscle damage such as plasma creatine kinase activity[39] or myoglobin[37,41] after eccentric exercises, although some controversy remains.[12,42] Our study uses concentric mode of contraction as the fatigue protocol. Further studies on the effect of cold water immersion in aiding recovery from eccentric-induced muscle damage are clearly needed.

There are several limitations to this study. Near-infrared spectroscopy only monitors oxygenation changes in a small area of the muscle, but perfusion heterogeneity may exist in different regions within a muscle. Cold water immersion induces varied physiological responses depending on the exercise mode, duration, and intensity. The present study examines the effect of cold water immersion on muscle oxygenation in the localized muscle fatigue protocol, future studies using cold water immersion recovery in actual athletic practice or competition should be considered.

CONCLUSIONS

This study evaluated the effects of cold water immersion on subsequent exercise performance. The results show that cold water immersion reduces heart rate and skin temperature and attenuates the decrease in tissue oxygenation during subsequent exercise. The perceptual rating of muscle soreness in subjects treated with cold water immersion was also lower 24 h postfatigue. It would be interesting to characterize the effect of cold water immersion on muscle oxygenation for high-intensity endurance exercises and to analyze its possible influence on reoxygenation recovery times in the subsequent performances.

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