- 1 Exploring the Efficacy of a Safe Cryotherapy Alternative: Physiological Temperature
- 2 Changes from Cold Water Immersion vs Prolonged Phase Change Material Cooling
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- 20 Abstract
- 21 **Purpose**: To evaluate the effectiveness between cold water immersion (CWI) and phase change
- 22 material (PCM) cooling on intramuscular, core and skin temperature and cardiovascular
- 23 responses.
- 24 **Methods**: In a randomized, crossover design, 11 males completed 15 min of 15°C CWI to the
- 25 umbilicus and 2 h recovery or 3 h of 15°C PCM covering the quadriceps and 1 h of recovery,
- separated by 24 h. Vastus lateralis intramuscular temperature at 1 and 3 cm, core and skin
- 27 temperature, heart rate variability and thermal comfort were recorded at baseline, and 15 min
- 28 intervals throughout treatment and recovery.
- 29 **Results**: Intramuscular temperature decreased (P<0.001) during and after both treatments. A
- faster initial effect was observed from 15 min of CWI (Δ: 4.3±1.7°C 1 cm; 5.5±2.1°C 3 cm;
- 31 P=0.01). However, over time (2 h 15 min), greater effects were observed from prolonged PCM
- treatment (Δ : 4.2±1.9°C 1 cm; 2.2±2.2°C 3 cm; treatment × time P=0.0001). During the first
- 33 hour of recovery from both treatments, intramuscular temperature was higher from CWI at 1 cm
- 34 (P=0.013) but not 3 cm. Core temperature deceased 0.25±0.32 from CWI (P=0.001) and
- 35 0.28±0.27°C from PCM (P=0.0001) while heart rate variability increased during both treatments
- 36 (P=0.001), with no differences between treatments.
- 37 **Conclusions**: The magnitude of temperature reduction from CWI was comparable to PCM but
- 38 intramuscular temperature was decreased for longer during PCM. Utilizing PCM cooling packs
- offers an alternative for delivering prolonged cooling whenever application of CWI is
- 40 impractical while also exerting a central effect on core temperature and heart rate.
- 41 **Keywords**: cryotherapy; recovery; thermoregulation; cooling

Introduction

Cold water immersion (CWI) is a popular intervention utilized to facilitate recovery and improve function in the days following strenuous exercise. Two comprehensive reviews on CWI indicate some effectiveness at reducing soreness but inconclusive effects on other measures of recovery.^{1,2} Since typical CWI protocols involve a single post-exercise treatment for 10-15 min in water temperatures between 10-15°C,^{1,2} limited effectiveness might be a result of inadequate treatment temperature, duration, or a combination of the two. Low immersion temperatures may decrease tissue temperatures at a rate that may lead to excessive thermal stress and, if prolonged, are not well tolerated³ and are limited by individual thermal discomfort and risk of cold-related injury.⁴ Further, in practice, repeat treatments are impractical and present logistical challenges, but may be necessary if the goal is to decrease muscle⁵ and core⁶ temperature.

A longer duration of targeted post-exercise cooling can be provided using temperature controlled phase change material (PCM), whereby PCM packs are placed over specific muscle groups and worn inside of garments to hold them in place. From a practical perspective, this cryotherapy modality offers an attractive alternative to CWI as individuals can resume activities of daily living while simultaneously receiving cryotherapy treatment that maintains a constant temperature for an extended duration. A 6 h PCM application reduced pain and strength loss on the days after eccentric quadriceps exercise in recreational athletes. A 3 h PCM application after a professional soccer match also reduced pain and strength loss on subsequent days. In these studies, Participant thermal comfort was maintained while PCM packs were worn inside compression shorts and maintained a constant temperature of 15°C for at least 3 h before melting.

CWI has been shown to reduce muscle temperature, 9-12 core temperature, 6,13,14 and increase heart rate variability (HRV). 15-17 CWI is purported to enhance recovery following exercise primarily due to its ability to reduce tissue temperature and blood flow. Since the mechanism through which CWI is thought to be effective is through its anti-inflammatory effects, 18 prolonging the duration of physiological cooling in order to attenuate metabolic processes in tissues, slow the up-regulation of cytokines and myokines, and reduce the circulatory exposure of the tissue to inflammatory cells following exercise seems intuitive. As such, if the temperature of treatment remains physiologically favorable, then duration of exposure can be extended. It is unknown to what extent prolonged PCM cooling might exert effects similar to those from CWI. For this reason, it is important to understand the physiological temperature effects that occur during prolonged PCM cooling and to compare them with a CWI treatment of matched temperature. Therefore, the purpose of this study was to compare the physiological effects (muscle, core, skin temperature and HRV) of CWI versus PCM cooling. It was hypothesized that both CWI and PCM would decrease intramuscular temperature but with a prolonged effect from PCM due to its ability to deliver a longer cooling duration.

Methods

Participants

Eleven active males (mean \pm SD; age, 27 ± 6 years; height, 183.6 ± 8.5 cm; body mass, 81.5 ± 12.4 kg) volunteered to participate in this study. All participants were free from lower leg injury for at least 1 month before the study and had no known vascular disease in the lower limbs, compromised circulation, allergy or hypersensitivity to cold. Participants were instructed to refrain from strenuous exercise for 72 h prior to, and for the duration of the study period. The

institutional ethics committee approved all procedures and participants gave written informed consent.

Experimental Design

In this repeated measures, crossover design study participants visited the laboratory on 3 consecutive days. First for a familiarization session before data collection commenced followed by two separate treatment sessions, all separated by 24 h. Participants were randomized to receive one treatment on day 1 and the other treatment on day 2. Vastus lateralis muscle temperature at 1 and 3 cm, skin temperature, core temperature, heart rate (HR), blood pressure (BP) and thermal comfort were recorded continuously throughout baseline, treatment (15 min CWI vs 3 h PCM) and recovery (2 h CWI vs 1 h PCM) during both treatments (Figure 1). Data collection during CWI treatment and recovery consisted of a shorter overall collection period compared to the PCM trial. Since both treatments were matched for temperature, it was impractical for participants to remain instrumented for the additional 1 h of recovery following CWI treatment, in order to match the duration of PCM treatment and recovery. During CWI treatment (iCool Sport, Australia), participants sat immersed to the umbilicus in an inflatable, temperature controlled (15 \pm 1°C) cold-water bath for 15 min and recovery of all variables was monitored for 2 h (2 h 15 min total time). During PCM treatment (Glacier Tek; USDA BioPreferred PureTemp, Plymouth, MN), two PCM blocks (864 cm2 area; 32.4 cm × 2 cm × 13.3 cm) frozen at 15°C were worn over the quadriceps muscles directly on the skin inside compression shorts (worn up to the knee) for 3 h of treatment, and recovery of all variables was monitored for 1 h (4 h total time). The PCM packs can maintain a constant temperature of 15°C for at least 3 h in a thermoneutral environment (as verified by the manufacturer and an independent quality assurance association, RAL; Quality and Testing Regulations for Phase Change Materials), until the substance is fully melted.

During data collection, participants remained in a semi-reclined seated position with legs outstretched on a bed except during the CWI treatment. Upon completion of each treatment, the dry shorts remained on the participant, while rolled up so that the skin remained exposed, for the duration of the recovery period. All testing was performed in a temperature-controlled laboratory $(24.9 \pm 3.4^{\circ}\text{C})$.

Intramuscular Temperature

To account for subcutaneous fat, skinfold at the exact site of thermocouple insertion on the quadriceps was measured using Skinfold Calipers (Harpenden, Baty International, West Sussex, UK) by the same individual. The vastus lateralis was then marked approximately 6 cm lateral to the mid-point between the superior pole of the patella and the anterior-superior iliac crest using a sterile pen. Additional markings were placed 1 cm inferior and superior to this point, one for each insertion depth. The area was cleaned with a povidone-iodine surgical scrub solution. Insertion depth was based upon halving the skinfold measure and adding this to the required depth (1 or 3 cm).

A 45 and 32 mm sterile intravenous 20 gauge needle catheter was used for the 3 and 1 cm insertion, respectively. Insertion depth was verified by subtracting the total insertion depth (1 cm or 3cm plus half the skinfold) from the corresponding length of the needle. The difference (length of needle minus calculated insertion depth) was verified with a sterile ruler. Once at the correct insertion depth, the needle was removed and the flexible catheter remained inserted. A sterile flexible intramuscular Thermocouple Probe (Type T, IT-21; Physitemp Instruments,

Clifton, NJ) was threaded through the barrel of the catheter. The catheter was removed from the muscle while the thermocouple remained inserted. The thermocouple insertion site was secured in place with sterile tegaderm by bending the thermocouple flush with the skin. The procedure was then repeated for the 1 cm deep thermocouple. Once fully instrumented, the thermocouples were connected to a digital monitor (Bailey Instruments BAT-12, Physitemp Instruments, Inc) for continuous recording. Thermocouples remained inserted throughout the duration of treatment and recovery. At the conclusion of data collection, thermocouples were removed and 'actual' insertion depth was verified by measuring the inserted portion of the thermocouple against a sterile ruler. The left leg of each subject was instrumented with thermocouples for CWI, while the right leg of each subject was instrumented for PCM treatment.

Body Temperature and Cardiovascular Measures

Participants were provided with an activated ingestible core temperature sensor (VitalSense, Respironic Inc, Murrysville, PA, USA) during familiarization. Participants were instructed to ingest the capsule with water ~8 h prior to initial testing. Participants were given a second core temperature sensor following completion of testing on day 1 to ingest at the same time of day as done prior to the first visit.

On arrival to the lab for the treatments, participants were fitted with a wireless ambulatory chest strap heart rate monitor equipped with a Sensor Electronics Module (SEM; EQ02 LifeMonitor, Hidalgo Ltd, Cambridge, UK) that continuously recorded heart rate, core, and skin temperature and with an automated blood pressure cuff on their right arm (M10-IT; Omron Healthcare). A telemetric dermal patch temperature sensor (VitalSense, Respironic Inc, Murrysville, PA, USA) was applied to the quadriceps of the leg that was not being instrumented with intramuscular thermocouples to measure skin temperature.

Heart rate data were analyzed using Vivosense (Vivonoetics, San Diego, USA). Automatic artifact-marking algorithm was applied to the raw electrocadiogram (sensitivity level: medium noise filtering; minimal and maximal allowable heart rate limits: 30 and 220 beats per minutes respectively). R-wave markings were generated for HRV calculations. The square root of the mean squared differences of successive intervals (RMSSD) is reported. Research suggests that RMSSD provides the most reliable and practically applicable measure for day-to-day monitoring. Five min rolling averages were calculated for RMSSD, with the baseline measure taken prior to insertion of the intramuscular thermocouples.

Ratings of thermal comfort were recorded every 15 min. During CWI, thermal comfort was asked at the first and last minute of immersion. Participants were asked to rate their thermal comfort on a nine-point standard scale.²¹

Data Analysis

Prior to employing ANOVAs, normality of distribution of all data sets was verified using the Shapiro-Wilk test. Mauchly's test of sphericity was used to test assumptions of sphericity and, where necessary, Greenhouse-Giesser corrections were applied. Statistical analyses were performed using SPSS (v21 IBM, Armonk, NY). The comparison of treatments over time was assessed using a 2×10 , treatment by time, repeated measures analyses of variance (ANOVA). The levels for the treatment factor were group (CWI or PCM) and time (baseline [0 min], and every 15 min up to 2 h 15 min). For these analyses, the entire duration of CWI treatment (15 min) and recovery (2 h) was compared to the first 2 h 15 min of PCM treatment. Additionally,

recovery effect (return to baseline) from both treatments over time was assessed using a 2×5 , treatment by time repeated measures analyses of variance (ANOVA). The levels for the time factor were baseline (0 hr), 15, 30, 45 min, 1 h, and 1 h 15 min for CWI and baseline (0 hr), 3 h, 3 h 15 min, 3 h 30 min, 3 h 45 min, and 4 h for PCM. For these analyses, the first 1 h duration of recovery following each treatment was compared. Where there was a significant treatment effect, or treatment by time interaction, differences between treatments at any particular time interval were assessed using Bonferroni corrections for planned pairwise comparisons.

Within each treatment, the changes in dependent variables over time were assessed by a one factor ANOVA with differences versus baseline assessed using Bonferroni corrections for planned pairwise comparisons. Additionally, Pearson product-moment correlation coefficients were used to assess the relationship between thigh skinfold thickness and intramuscular temperature. A probability level < 0.05 was accepted to determine significance. All data are reported as group means \pm SD.

Results

Thermocouple Depth and Skinfold

Skinfolds were 10.1 ± 5.2 mm for the right leg of all participants, and 9.7 ± 5.5 mm for the left leg. Thermocouple depths, corrected for skinfolds were 3.0 ± 0.4 cm and 1.0 ± 0.3 cm for PCM and 3.1 ± 0.3 cm and 1.1 ± 0.3 cm for CWI. Decreases in intramuscular temperature were correlated with skinfold thickness with stronger effects at 1 cm (CWI r = 0.912, P < 0.001; PCM r = 0.853, P < 0.001) versus at 3 cm (CWI r = 0.727, P < 0.01; PCM r = 0.594, P = 0.05).

Intramuscular Temperature

Intramuscular temperature declined progressively during both treatments (time effect P = 0.0001, Table 1) and remained below baseline at the conclusion of the recovery period (all P < 0.01; Figure 2). CWI decreased intramuscular temperature more rapidly and was 14.0 and 16.1% lower at end of treatment vs 15 min into PCM treatment at both 1 and 3cm respectively (mean difference: 4.3 ± 1.7 °C at 1 cm and 5.5 ± 2.1 °C at 3 cm, both P = 0.01). Intramuscular temperature remained 10.6% lower 15 min into recovery following the CWI treatment vs 30 min into PCM treatment at 3 cm (difference: 3.4 ± 1.6 °C, P = 0.01) but no longer at 1 cm (2.1%; difference: 0.6 ± 1.8 °C, P = 0.99). Intramuscular temperature at 3 cm was 7.5% higher (difference: 2.4 ± 2.3 °C, P = 0.045; Figure 2) upon conclusion of CWI recovery (2 h 15 min total time) compared with 2 h 15 min into PCM treatment, while intramuscular temperature at 1 cm was on average 12.5% higher between CWI vs PCM treatment from 1 h (P = 0.003) to 2 h 15 min (P < 0.001; Figure 2). Over time, intramuscular temperature was lower from PCM treatment (treatment \times time P = 0.0001 at 3 and 1 cm; Figure 2). When comparing intramuscular temperature for the first 1 h of recovery from both treatments, intramuscular temperature at 1 cm was 4.1% higher from CWI averaging 28.6 ± 1.4 °C than from PCM averaging 27.7 ± 1.7 °C (treatment effect, P = 0.013; Figure 3), with no difference at 3 cm (2.2%; treatment effect P =0.35; Figure 3).

Core Temperature

Core temperature declined during the PCM and CWI treatments (time effect, P = 0.0001, Figure 4), with no difference between treatments (treatment × time P = 0.10) (Figure 4). The nadir of core temperature from PCM treatment occurred 45 min into the recovery period

- 217 (absolute time: 3 h 45 min; $0.28 \pm 0.27^{\circ}\text{C}$ below baseline) while the nadir of core temperature
- from CWI treatment occurred 1 h 30 min in to the recovery period (absolute time: 1 h 45 min;
- 219 0.25 ± 0.32 °C below baseline).
- 220 Skin Temperature
- 221 Skin temperature declined during both PCM and CWI treatments (time effect, P =
- 222 0.0001). CWI decreased skin temperature more rapidly (treatment x time, P = 0.0001) than PCM.
- Skin temperature immediately after CWI was 2.4 ± 1.7 °C lower than 15 min into the PCM
- treatment, however, at all subsequent time points, skin temperature was lower during the PCM
- treatment (P < 0.01). During CWI treatment, skin temperature dropped from 31.3 ± 1.1 °C at
- baseline to 23.6 ± 0.8 °C at 15 min and was 29.5 ± 1.2 °C 2 h after CWI. During PCM treatment,
- skin temperature averaged 24.1 ± 0.3 °C over the 3 h during which subjects wore the PCM and
- was 27.7 ± 1.1 °C 1 h after removal of PCM.
- 229 Perceived Thermal Comfort
- Thermal comfort was significantly different between treatments (treatment \times time P = 0.002) with greater thermal discomfort reported immediately post CWI (2.7 \pm 0.8 vs. 4.5 \pm 0.8
- 232 15 min into the PCM treatment, P = 0.01). This time point is also where thermal comfort reached
- 233 its nadir for both treatments. Upon conclusion of PCM treatment, thermal comfort was $(4.9 \pm$
- 234 1.0). Thermal comfort returned to baseline following 30 min of the recovery period post PCM
- 235 treatment.

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Cardiovascular Measures

There were technical issues with heart rate signals for 2 participants during the entire PCM treatment and for one participant after 2 h of the PCM treatment. Thus only 9 participants were included in the treatment by time analysis of heart rate data and the time analysis only included data up to 2 h. heart rate declined during both treatments (time effect P = 0.0001) with no interaction effects (P > 0.05; Table 2). Overall there was an increase in RMSSD during treatments (Time effect P = 0.0001) with no interaction effect (P = 0.155; Table 2). For the PCM treatment there was a trend for an increase in RMSSD (Time effect P = 0.069) while for the CWI treatment there was a clear increase in RMSSD (Time effect P = 0.0001). Blood pressure was unaffected by either treatment (P = 0.15-0.95) and there were no differences between treatments (P = 0.62 for systolic, P = 0.84 for diastolic).

Discussion

The main finding in this study was that 15 min of CWI was comparable to PCM packs applied directly to the skin overlying the quadriceps for 3 hours in terms of the magnitude of reduction in vastus lateralis intramuscular temperature. Ultimately PCM treatment provided a sustained decrease in intramuscular temperature that was maintained for the 3 h of application (Figure 2), and a more gradual recovery (Figure 3). However, the initial reduction in intramuscular temperature was more rapid during the CWI treatment. In addition to the local effects on muscle temperature, both treatments provided local and systemic effects by decreasing core temperature, heart rate and increasing HRV. Importantly, the systemic effects were observed despite there being no exercise intervention to induce cardiovascular stress prior to the treatments. The combined local and systemic effects likely explain the accelerated recovery from strenuous exercise that have recently been demonstrated with PCM cooling.^{7,8} This study

provides the first evidence that the application of this novel cooling modality, PCM, elicits comparable physiological effects to those from CWI treatment.

In the present study, average vastus lateralis temperature at 1 cm for the total PCM trial period (4 h) was 7% lower than the average temperature at 1 cm for the total CWI trial (2 h 15 min).. Thus, not only can PCM provide prolonged cooling, it can also provide a greater magnitude of cooling to the peripheral musculature. This may have implications for use in exercise recovery. Since the damage that occurs following strenuous exercise is bimodal, involving both the initial mechanical and/or metabolic muscle injury and a secondary phase that involves a disruption in intracellular homeostasis followed by an inflammatory response which initiates 2-6 hrs post damaging exercise. A prolonged cooling intervention during this timeframe has potential to blunt the inflammatory process that occurs following exercise, thereby mitigating any additional damage caused by the inflammatory response, limiting further hemorrhage and cell death. In support of this rationale, it has previously been demonstrated in an animal model that local cooling at 8°C for 6 hrs after closed soft tissue injury limited subsequent tissue damage.

An interesting aspect of the current results is that CWI can induce a rapid drop in muscle temperature while PCM cooling provides a gradual prolonged decrease in muscle temperature with a slower rise in muscle temperature at 1 cm during recovery (Figure 3). Therefore, if the goal is to maximize the tolerable decline in muscle temperature for a sustained period of time, athletes might opt to combine the treatments. In practice, once an athlete completed a CWI treatment, quickly decreasing their intramuscular and core temperature, they could apply PCM over muscle groups they wish to keep coolin order to maintain the reduction of temperature while returning to normal post exercise activities (e.g. meal, relaxation, recreational activities). This could allow the athlete to sustain the treatment effect from CWI for a longer period of time in the immediate post-exercise period.

The systemic effects observed from PCM cooling in this study are surprising considering PCM application was localized while CWI involved submerging the lower half of the body. The longer treatment duration from PCM provided a progressive decline in core temperature so that ultimately the effects on core temperature and HRV were comparable between treatments. Previous studies have shown that the rate of core temperature reduction during post-exercise CWI is dependent on temperature, duration, and the time from the end of exercise to commencement of CWI treatment. However, few studies have examined the impact of CWI on resting core temperature where there is no exercise-induced temperature elevation prior to CWI treatment. Costello et al (2012) reported a 0.4 ± 0.2 °C reduction in resting rectal temperature 60 min after a 4 min CWI at 8°C with subjects submerged to the sternum. Gregson et al (2011) reported a 0.2 ± 0.1 °C drop in core temperature following two 5 min, 8°C CWI treatments, separated by 2 min with subjects submerged to the waist. Comparable reductions in core temperature during treatment were evident in the present study and core temperature remained depressed for the duration of recovery from both treatments.

In line with the reduction in core temperature, there was a decrease in heart rate and an increase in HRV, from both treatments. Restoration of cardiovascular homeostasis is an important component of overall recovery and interventions that increase HRV are thought to be advantageous to exercise recovery.²⁴ Monitoring indices of HRV has been of increasing interest among athletes.²⁵ Post-exercise CWI has been shown to accelerate recovery of HRV.^{15,16} The present data indicate that CWI and PCM are capable of elevating HRV from a resting condition.

Since this study did not utilize an exercise intervention prior to the treatments, both the magnitude and duration of the physiological effects cannot be extrapolated to what might occur in a post-exercise condition. It remains imperative to mention the paradox between the use of cryotherapy for acute reduction in inflammation to facilitate recovery and the potential negative effects that may be caused by blunting the stress response. 18 Since some degree of inflammation, which plays a crucial role in the remodeling and adaptation of skeletal muscle, is required for the resolution of muscle fiber damage resulting from an exercise insult. However, since the recovery benefits of CWI have been extensively studied, and preliminarily studies utilizing PCM cooling with durations between 3-6 h illustrate beneficial effects on recovery of strength, in addition to soreness, ^{7,8} the present results serve primarily to demonstrate the capacity of both CWI and PCM cooling to have local and systemic effects. The shorter overall CWI data collection period compared with PCM data collection complicated the comparisons between treatments. However, it was not practical to have study participants remain instrumented for an additional 1 h 45 min following CWI to match the PCM duration, especially since it was a crossover design. A post CWI duration of 2 h was sufficient to demonstrate the magnitude and duration of effects on recovery, especially since it has been demonstrated that intramuscular temperature does not return to baseline for up to 4 h following CWI administered after exercise. ²⁶ The 3 h PCM duration was chosen to replicate the treatment time in field testing, 8 and the 1 h recovery time was deemed sufficient and practical for study participants who were sitting for more than 4 hours. Previous studies have demonstrated that the cooling effect in calf muscles is maintained for 3-4 h following CWI in normothermic individuals^{27,28} due to inactivity. Therefore, it was not feasible to keep study participants instrumented to monitor temperatures that likely would not have returned to baseline.

This study utilized a cohort of male participants with thigh skinfolds averaging 9.9 ± 5.2 mm., Decreases in intramuscular temperature were correlated with skinfold thickness during both treatments, due to the insulating effect of adiposity. ^{29,30} It has previously been shown that body composition influences the magnitude of change in skin, muscle, and core temperature during and after CWI²⁶. It has also been suggested that muscle mass and its regional distribution, body surface area to mass ratio, age and ethnicity influence thermal and physiological responses to water immersion³¹ Therefore, the results of this study should be cautiously interpreted when relating them to a more heterogeneous group. This study should be repeated in a female population since women generally have greater subcutaneous body fat compared to men, and because for a given change in body temperature, as occurs during and from exercise, females require a greater cooling stimulus to maintain thermal comfort levels.³²The results from this study may further differ in a female population due to the added variable of sex hormone-related fluctuations in body temperature and some thermoregulatory processes during the menstrual cycle. 33 Consequently, the results of this study should be extrapolated with a degree of caution to the effect of CWI or PCM on intramuscular and core temperature in females, and following exercise in both genders. Future research should examine PCM application in a more heterogeneous group as well as following exercise.

Practical Applications

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PCM cooling packs applied directly to the skin underneath garments to hold them in place is an efficacious alternative to CWI, especially if the athlete is seeking a prolonged cooling exposure. PCM cooling may be more practical than CWI, because individuals can continue with their activities of daily living while simultaneously receiving a cryotherapy dose.

Conclusions

This is the first examination of the effect of PCM cooling on intramuscular temperature, core temperature and cardiovascular function. The magnitude of temperature reduction with prolonged PCM application was similar to the CWI treatment, but critically, the PCM provided a sustained cooling effect that was better tolerated than CWI. These physiological effects may explain the previously reported benefits of PCM cooling in reducing muscle damage in recreational athletes⁷ and accelerating recovery in professional soccer players.⁸

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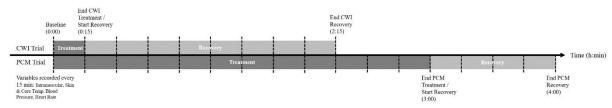


Figure 1: Experimental protocol of treatment and recovery during both conditions over time.

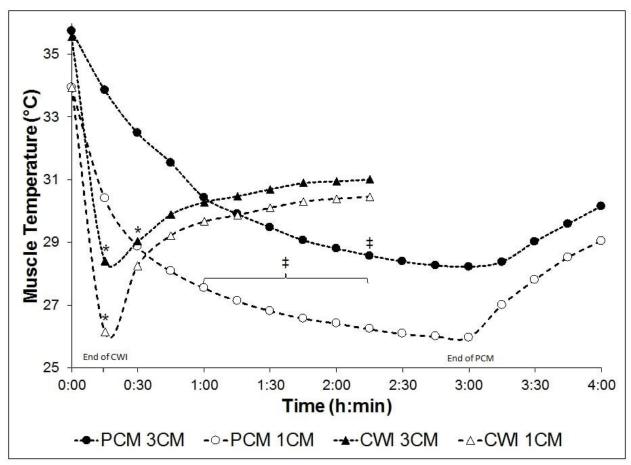


Figure 2: Vastus lateralis intramuscular temperature. Intramuscular temperature declined progressively during the PCM treatment (time effect, P < 0.0001) and remained below baseline after 1:00 of recovery at both depths (P < 0.01). CWI treatment decreased intramuscular temperature from a baseline to immediately post treatment (time effect, P < 0.0001) and remained below baseline after 2 h of recovery at both depths (P < 0.01). Intramuscular temperature was lower with the PCM treatment (treatment x time, P < 0.0001 at 3 and 1 cm). Intramuscular temperature was lower with CWI vs PCM at 3 cm from 0:00 to 0:30 but only from 0:00 to 0:15 at 1 cm (*, P < 0.01). Intramuscular temperature was lower at 2:15 for PCM vs CWI treatment at 3 cm and at from 1:00 to 2:15 at 1 cm (‡, P < 0.05).

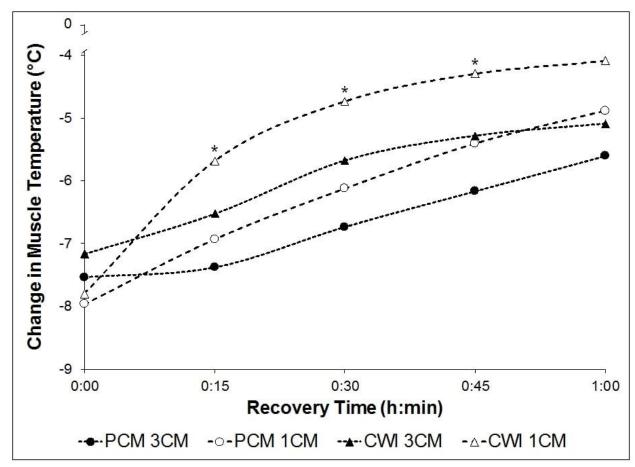


Figure 3: Change in vastus lateralis intramuscular temperature from baseline during recovery. Recovery time is displayed for the 1 hour immediately following conclusion of both treatments. Absolute time displayed for CWI is 0:15 through 1:15, and for PCM is 3:00 through 4:00. Intramuscular temperature was lower (treatment effect, P = 0.013) with PCM vs. CWI at 1 cm (*, P < 0.05).

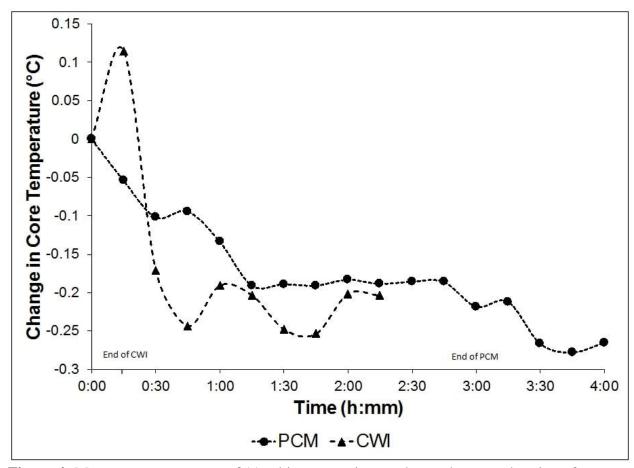


Figure 4: Mean core temperature of 11 subjects superimposed over the same duration of treatment and recovery after both PCM and CWI treatment. Core temperature declined during the PCM and CWI treatments (time effect, P < 0.0001) with no difference between treatments (treatment x time, P = 0.10).

Table 1 A Comparison of Intramuscular Temperatures (1cm and 3cm) during Baseline, Treatment, and Recovery of the 2 Cryotherapy Treatments (CWI vs PCM), Mean \pm SD

Temperature at Protocol Times	CWI 1cm	CWI 3cm	PCM 1cm	PCM 3cm
Baseline	34.0 ± 1.1 °C	$35.6 \pm 0.6^{\circ}$ C	$33.9 \pm 1.5^{\circ}\text{C}$	35.8 ± 0.5 °C
End of Treatment	26.2 ± 2.9 °C	$28.4 \pm 2.7^{\circ}$ C	26.0 ± 2.2 °C	$28.2 \pm 2.8^{\circ}$ C
End of Recovery	$30.5 \pm 1.0^{\circ}\text{C*\&}$	$31.0 \pm 1.0^{\circ}$ C*	29.0 ± 1.6 °C*	$30.1 \pm 2.1^{\circ}\text{C*}$
Average	29.4 ± 1.1 °C	$30.2 \pm 1.2^{\circ}\mathrm{C}$	$27.4 \pm 2.1^{\circ}$ C\$	$29.8 \pm 2.4 ^{\circ}\mathrm{C}$

Intramuscular temperature remained significantly below baseline at the end of recovery for all conditions (*P < 0.01). Intramuscular temperature during the first hour of recovery was higher from CWI vs PCM at 1cm (treatment effect, &P = 0.013) but not at 3cm (treatment effect P = 0.35). Average intramuscular temperature at 1cm was significantly lower from PCM treatment than CWI (\$P < 0.001) but there was no difference at 3cm (P=0.46).

Table 2 Fifteen min rolling average HR and RMSSD data during 2 h of PCM application and 15 min of CWI followed by 1 h 45 min of recovery, Mean \pm SD

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PCM HR	CWI HR	PCM RMSSD	CWI RMSSD
68 ± 7	68 ± 8	60 ± 22	62 ± 30
$62 \pm 9^*$	61 ± 11	63 ± 24	67 ± 28
63 ± 7	61 ± 8 *	61 ± 24	79 ± 25 *
64 ± 6	57 ± 8 *	65 ± 22	74 ± 26
61 ± 7 *	59 ± 8 *	65 ± 26	70 ± 31
62 ± 9	57 ± 8 *	70 ± 26	71 ± 27
60 ± 5 *	59 ± 11	75 ± 25	75 ± 31
$59\pm7^*$	56 ± 9 *	70 ± 25	79 ± 32
61 ± 9	57 ± 8 *	73 ± 23	84 ± 33 *
	68 ± 7 $62 \pm 9^*$ 63 ± 7 64 ± 6 $61 \pm 7^*$ 62 ± 9 $60 \pm 5^*$ $59 \pm 7^*$	68 ± 7 68 ± 8 $62 \pm 9^*$ 61 ± 11 63 ± 7 $61 \pm 8^*$ 64 ± 6 $57 \pm 8^*$ $61 \pm 7^*$ $59 \pm 8^*$ 62 ± 9 $57 \pm 8^*$ $60 \pm 5^*$ 59 ± 11 $59 \pm 7^*$ $56 \pm 9^*$	68 ± 7 68 ± 8 60 ± 22 $62 \pm 9^*$ 61 ± 11 63 ± 24 63 ± 7 $61 \pm 8^*$ 61 ± 24 64 ± 6 $57 \pm 8^*$ 65 ± 22 $61 \pm 7^*$ $59 \pm 8^*$ 65 ± 26 62 ± 9 $57 \pm 8^*$ 70 ± 26 $60 \pm 5^*$ 59 ± 11 75 ± 25 $59 \pm 7^*$ $56 \pm 9^*$ 70 ± 25

474 HR was elevated immediately following CWI treatment (15 min) but was reduced over time during both treatments (time effect, P < .0001). There was a trend for an increase in RMSSD

during PCM treatment (Time effect, P = 0.069) and a clear increase in RMSSD during and

following CWI (Time effect, P < .0001). * = significant difference from baseline (P < .05)