Cold-Water Mediates Greater Reductions in Limb Blood Flow than Whole Body Cryotherapy

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ABSTRACT

Purpose: Cold-water immersion (CWI) and whole body cryotherapy (WBC) are widely used recovery methods in an attempt to limit exercise-induced muscle damage, soreness and functional deficits after strenuous exercise. The aim of this study was to compare the effects of ecologically-valid CWI and WBC protocols on post-exercise lower limb thermoregulatory, femoral artery and cutaneous blood flow responses. **Methods:** Ten males completed a continuous cycle exercise protocol at 70% maximal oxygen uptake until a rectal temperature of 38°C was attained. Participants were then exposed to lower-body CWI (8°C) for 10 min, or WBC (-110°C) for 2 min, in a randomized cross-over design. Rectal and thigh skin, deep and superficial muscle temperatures, thigh and calf skin blood flow (laser Doppler flowmetry), superficial femoral artery blood flow (duplex ultrasound) and arterial blood pressure were measured prior to, and for 40 min post, cooling interventions. **Results:** Greater reductions in thigh skin (CWI, -5.9±1.8°C; WBC, 0.2±0.5°C; $P < 0.001$) and superficial (CWI, -4.4±1.3°C; WBC, -1.8±1.1°C; $P < 0.001$) and deep (CWI, -2.9±0.8°C; WBC, -1.3±0.6°C; $P < 0.001$) muscle temperatures occurred immediately after CWI. Decreases in femoral artery conductance were greater after CWI (CWI, -84±11%; WBC, -59±21%, $P < 0.02$) and thigh (CWI, -80±5%; WBC, -59±14%, $P < 0.001$) and calf (CWI, -73±13%; WBC, -45±17%, $P < 0.001$) cutaneous vasoconstriction was greater following CWI. Reductions in rectal temperature were similar between conditions after cooling (CWI, -0.6±0.4°C; WBC, -0.6±0.3°C; $P = 0.98$). **Conclusion:** Greater reductions in blood flow and tissue temperature were observed after CWI in comparison to WBC. These novel findings have practical and clinical implications for the use of cooling in the recovery from exercise and injury.

Keywords: cooling; muscle damage; recovery; exercise

INTRODUCTION

Cold-water immersion (CWI) has become a widely used recovery method in sports performance in an attempt to enhance recovery following strenuous exercise (21). Despite its wide spread use, evidence that CWI accelerates functional recovery is currently equivocal (21, 27, 28). In contrast, CWI improves perceptions of fatigue and muscle soreness (10, 21) and reduces clinical signs of inflammation such as swelling/edema (11, 38) after strenuous exercise in humans. Indeed, a logic model proposed by Costello et al., (2013) suggests that beneficial physiological, neuromuscular, and perceptual effects following exposure to cryotherapy may interact to improve the recovery of performance (6).

One proposed physiological mechanism of cryotherapy is decreases in tissue temperature that mediate reductions in limb (23, 27) and deep muscle (20, 32) blood flow. It has been proposed that cooling induced reductions in limb blood flow are beneficial in limiting the inflammatory response to exercise in animal models (20, 30, 34). However, a recent study in humans has challenged this view by showing that CWI (10 min in 10ºC water) had no impact on the muscle inflammatory or cellular stress response compared with active recovery (25). It is possible therefore that CWI-induced reductions in muscle blood flow may benefit recovery from strenuous exercise by attenuating clinical signs of inflammation including edema and swelling *per se* (11, 38) and the associated pain (e.g. soreness) upon movement (10, 21).

Whilst the majority of the research literature investigating cryotherapy during recovery from exercise has employed CWI (18, 23, 27, 28, 33), the recent commercial availability of whole body cooling (WBC) facilities, which expose the body to very cold air (-110°C to -140°C) for short durations (2-4 min) (2), has led to further interest in the role of cryotherapy in exercise recovery (4). Various studies have reported

potential beneficial effects of WBC on hematological profiles (22), inflammatory biomarkers (26, 40), muscle damage (13, 40), the autonomic nervous system (29), body temperature (8), and tissue oxyhaemoglobin and oxygenation (31). Despite these apparent favorable effects of WBC there is equivocal evidence for a positive impact of WBC on functional recovery (7, 13, 14). Furthermore, the comparative physiological, especially vascular, effects of WBC relative to CWI remain to be elucidated. Costello *et* al (8) have previously shown that 4 minutes of exposure to either CWI or WBC similarly decreased rectal and muscle temperatures for up to 60 minutes post exposure, despite lower thigh skin temperatures after CWI. However, the CWI duration used in that study was not typical of protocols used for recovery, i.e. ≥10 minutes (21, 36), the cryotherapy modalities were applied under resting conditions and the vascular (blood flow) and hemodynamic responses were not measured. It is therefore currently unknown if the changes in blood flow of previously exercised limb(s) are different between ecologically valid CWI and WBC protocols. This is important given that reducing blood flow may represent an important mechanism through which cooling influences post exercise muscle recovery.

The aim of the present study was to, therefore, examine the effects of ecologically valid CWI and WBC protocols on femoral artery and cutaneous blood flow and thermoregulatory responses after cycling exercise. We hypothesized that a longer duration of CWI would decrease femoral artery and lower limb skin blood flow to a greater extent, compared with WBC, and lead to a greater reduction in leg muscle temperature.

MATERIALS AND METHODS

Participants

Ten recreationally active men (mean±SD: age, 22.3±3.4 yrs; height, 1.8±0.1 m; mass, 81.1±8.3 kg; $\dot{V}O_{2max}$, 45.0 ± 9.0 $mL \cdot kg^{-1} \cdot min^{-1}$; Peak Power Output, 177±32 W) free from cardiovascular, metabolic and respiratory disease were studied. The experiment conformed to the Declaration of Helsinki and was approved by the Institutional Ethics Committee. Following written informed consent, participants were familiarized with the experimental procedures and interventions. On the day of the experimental trials, participants arrived at the laboratory at least 3 hours post-prandial, having refrained from exercise, alcohol, tobacco and caffeine during the previous 24 hours. Nutritional and fluid intake were recorded across this period and returned to the participant so that they could repeat their preparation at the subsequent trial. They also consumed 5 $mL \cdot kg^{-1}$ of water 2 hours before arriving at the laboratory.

Experimental Design

Following familiarization each participant attended the laboratory on two occasions during which they completed an identical submaximal cycle ergometer protocol, followed by exposure to either WBC or CWI (Figure 1). The conditions were conducted in a randomized and counterbalanced order, at least 1 week apart and at the same time of day. The CWI exposure consisted of 10 minutes of immersion to the iliac crest in 8ºC water in a temperature-controlled bath; dimensions = 1.34 m width x 1.64 m length x 1.20 m length (ECB Ltd, Gloucester, U.K.). The WBC exposure consisted of 2 min exposure at a temperature of -110ºC in a specialized mobile cryotherapy unit; approx. dimensions = 2.40 m width x 2.90 m height x 1.20 m length (KrioSystem, Wroclaw, Poland). Entry to the main chamber was preceded by a 30 *s* adaptation period

100 in a pre-chamber at a temperature of minus 60ºC. The CWI and WBC protocols were
101 based on methods and durations frequently reported in the literature and commonly
102 used in applied sports science practice (16, 23).
103
104 *Experimental Protocol*
105 Prior to any experimental trials, each participant completed a maximal
106 incremental cycling protocol on a cycle ergometer (Lode, Corival, Netherlands) while
107 simultaneous breath-by-breath ($\dot{V}O_2$) measurements were recorded (Oxycon Pro,
108 Jaeger, Germany). The cycling protocol commenced at 75 W and was increased 25 W
109 every 2 min until volitional exhaustion was reached. Peak power output was derived as
110 the highest power output attained at this point. Maximal oxygen uptake ($\dot{V}O_{2max}$)
111 ($mL \cdot kg^{-1} \cdot min^{-1}$) was recorded as the highest 30 *s* average recorded prior to volitional
112 exhaustion.
113 On arrival at the laboratory for each experimental trial, the participant's nude
114 body mass (kg) was obtained using digital scales (Seca, Hamburg, Germany). A rectal
115 probe was self-inserted and a heart rate monitor was positioned across the chest.
116 Participants were then placed in a supine position for 30 min on a bed for the attachment
117 of instrumentation and to stabilise physiological status, wearing shorts where the
118 ambient temperature was maintained at 22-24°C (~40% relative humidity) throughout
119 the protocol. Following baseline measurements, participants cycled at 70% $\dot{V}O_{2max}$ until
120 a rectal temperature of 38°C was attained. Participants were not allowed to consume
121 any food or fluid during or after exercise. Participants then returned to a supine position
122 for 10 min to enable pre-cooling measurements to be taken. This protocol was selected
123 in line with our previous study (23) to minimize muscle damage compared to other
124 forms of exercise such as resistance training or other specific muscle damaging

protocols, which may have confounded post exercise femoral artery blood flow measurements due to the protective effect of performing a single bout of muscle damaging exercise (24).

In the CWI condition, participants were subsequently raised from the bed in a semi-recumbent posture, using an electronic hoist (Bianca, Arjo Ltd, Gloucester, United Kingdom), and lowered into the water bath (in the same position) until the thighs were fully submerged for 10 minutes. This avoided the potential impact of any active movement or muscular contraction on subsequent measures. In the WBC condition, body sweat was lightly dabbed dry with a towel, and equipment was removed from the body (skin temperature probes, heart rate monitor) for entering the WBC chamber. Skin blood flow and rectal probes remained *in situ*, with connections covered and tucked inside the participant's shorts and socks. Next, with the help of the researchers, the participants donned the clothing to be worn inside the chamber (face mask, ear band, gloves, socks and shoes) and were then transferred to, and pushed, in a chair to undergo WBC exposure inside the chamber (seated on the chair). At the end of each separate cooling trial, participants were returned to the bed using either the electronic hoist/or via the chair, and remained in a supine position for a period of 40 min under the temperature-controlled laboratory. A period of 10 min was permitted, before any post-exposure measurements, for the reattachment of the skin temperature probes and heart rate monitor equipment and removing clothing required in the chamber. The use of the hoist to raise and lower the participants, and the chair to transfer the participants to and from the chamber was important to avoid the effect of muscle activation on blood flow and hemodynamic measures (15, 23).

Thermoregulatory variables were measured at baseline, pre-cooling and during post-cooling period. Perceived thermal comfort, rated using a 9-point scale (0 =

unbearably cold, 1 = very cold, 2 = cold, 3 = cool, 4 = slightly cool, 5 = neutral, 6 = slightly warm, 7 = warm, 8 = hot, 9 = very hot) (39) and shivering, rated using a 4 point scale (1 = no shivering, 2 = slight shivering, 3 = moderate shivering, 4 = heavy shivering) (35) were also recorded. All pre- and post-cooling measurements were made in a supine position. A schematic illustration of the experimental design is shown in Figure 1.

Measurements

Rectal, Thigh Skin and Muscle Temperature. A rectal probe (Rectal temperature probe, adult, ELLAB, Rodovre, Denmark) was inserted 15 cm beyond the anal sphincter for the assessment of rectal temperature. A skin thermistor (Surface temperature probe, stationary, ELLAB, Rodovre, Denmark) was attached to the upper thigh for the assessment of skin temperature. Muscle temperature was assessed using a needle thermistor inserted into the vastus lateralis (Multi purpose needle probe, ELLAB, Rodovre, Denmark) as previously described (8, 23). Briefly, thigh skinfold thickness was measured using Harpenden skinfold calipers (HSK BI, Baty International, West Sussex, United Kingdom) and divided by 2 to determine the thickness of the thigh subcutaneous fat layer over each participant's vastus lateralis . The needle thermistor was then placed at a depth of 3 cm plus one-half the skinfold measurement for determination of deep muscle temperature (3 cm). The thermistor was then withdrawn at 1 cm increments for determination of muscle temperature at 2 cm and 1 cm below the subcutaneous layer. Rectal, skin and muscle temperatures were recorded using an electronic measuring system (CTF 9004, ELAB).

Heart Rate and Blood Pressure. Heart rate was continuously measured using a heart rate monitor (S610; Polar Electro Oy, Kempele, Finland). Blood pressure was

measured noninvasively via automated brachial auscultation (Dinamap, GE Pro 300V2, Tampa, Florida, USA).

Femoral Artery Blood Flow. A 15 MHz linear array transducer attached to a high-resolution ultrasound machine (Acuson P50, Siemens, Germany) was used to measure superficial femoral artery diameter and velocity (3 cm distal to the bifurcation) as previously described (23). This position was marked on the skin such that the ultrasound head could be accurately repositioning during subsequent measures. Analysis of diameter and velocity was performed using custom designed edge-detection and wall-tracking software (37) which is considerably more repeatable than manual methods and associated with less observer error (37). Resting diameter, blood velocity and blood flow were calculated as the mean of the data collected over a 20 s period of each 2 min recoding for statistical analysis. Femoral vascular conductance was calculated as the ratio of blood flow/mean arterial pressure.

Cutaneous Blood Flow. Red blood cell flux was used as an index of skin blood flow via laser Doppler flowmetry (Periflux System 5001, Perimed Instruments, Jarfalla, Sweden). A laser Doppler probe (455, Perimed, Suffolk, United Kingdom) was attached to the mid-anterior thigh, midline, halfway between the inguinal line and the patella, and on the calf, left of the midline, in the region of the largest circumference. Once affixed, the probes were not removed until the completion of each trial. Cutaneous vascular conductance was calculated as the ratio of laser Doppler flux to mean arterial blood pressure (cutaneous vascular conductance = laser Doppler flux/mean arterial blood pressure x 100) and expressed as a percentage change from pre immersion values. When expressed as a percentage change from baseline to maximum, cutaneous blood flow has a coefficient variation of 4% in our laboratory with a coefficient of variation

of 10% observed for resting cutaneous blood flow. Thigh and calf skin conductance are expressed as percentage change from pre immersion (zero)

Statistical Analysis

Using our previous data (23), 8°C water immersion mediates a reduction from pre-exercise baseline in femoral artery blood flow of 60 $mL \cdot min^{-1}$. To replicate this reduction in femoral artery blood flow with 80% power and an α of 0.05, a sample size of 9 participants is required. Similarly, we utilized a previous study (8) to estimate a minimum clinically important difference in thigh skin temperature of 3.4 ± 2.4°C immediately following CWI compared to WBC. To detect this difference with 80% power and an α of 0.05, a sample size of 7 participants is required.

A two-factor [condition (CWI & WBC) x time (baseline, post-exercise/pre cooling, post cooling 10, 20, 30, 40 min)] general linear model (GLM) was used to evaluate treatment differences between the CWI and WBC conditions. A three-way GLM (condition x depth x time) was used to analyse muscle temperature. Where a significant interaction between condition and time was observed, differences were followed up with Newman-Keuls multiple contrasts.

Simple effect size (ES), estimated from the ratio of the mean difference to the pooled standard deviation (Hedges' *g*), were also calculated. The ES magnitude was classified as trivial (<0.2), small (>0.2-0.6), moderate (>0.6-1.2), large (>1.2-2.0) and very large (>2.0-4.0) (17). SPSS version 20, Statistical Package for the Social Sciences was employed for all statistical analysis (Chicago, IL). The statistical significance was set at $P < 0.05$. Data are presented as mean ± SD.

RESULTS

Baseline vs Post-exercise/Pre-cooling

All ten participants completed the experiment and no adverse events were recorded. The exercise time necessary to achieve a rectal temperature of 38 °C was ~45 min for both trials. The cycling protocol elicited similar increases in heart rate, rectal and muscle temperatures and thermal discomfort between CWI and WBC (Table 1). Thigh skin temperature also increased with exercise in both trials but was higher in the CWI trial (P = 0.01). Systolic, diastolic and mean arterial pressure were unchanged after exercise and were similar between conditions (all P > 0.05). Exercise increased arterial blood flow and conductance by ~65-70% (P < 0.001) with no difference between conditions (P > 0.05). Cutaneous vascular conductance increased after exercise and was similar between conditions at the thigh but was lower at the calf in WBC (Table 1).

Pre-cooling vs Post-cooling

Thermoregulatory responses. Rectal temperature decreased over the post cooling recovery period (P < 0.001) and was similar between conditions (P = 0.98, ES = 0.3) (Figure 2). Thigh skin temperature was lower throughout the post-cooling period in CWI compared with WBC (P < 0.001, ES = 3.6; Figure 2**)** with the largest difference occurring 10 min post-cooling (6.0±2.4°C, ES = 4.3).

Muscle temperature was reduced following cooling in both conditions at all depths (P < 0.001; Figure 3). The reduction in muscle temperature at each depth was greater after CWI compared with WBC at 10 min (1 cm: 3.6±1.0°C, ES= 2.9; 2 cm: 2.8±1.0°C, ES = 2.5; 3 cm: 1.1±0.4°C, ES = 2.8) and 40 min time points (1 cm: 2.2±1.2°C, ES = 1.7; 2 cm: 2.2±1.1°C, ES = 1.9; 3 cm: 1.6±0.8°C, ES = 2.1). Decreases

248 in thermal comfort were lower (1±1 a.u., ES = 1.0) after CWI at 10 min and (1±1 a.u.,
249 ES = 1.0) 20 min post cooling compared with WBC. There was no shivering observed
250 throughout the post immersion period in either experimental condition.

251 *Heart rate, blood pressure and arterial blood pressure.* Heart rate decreased
252 throughout the recovery period in both conditions ($P < 0.001$; see Table 2). There was
253 a significant interaction of time and condition ($P < 0.001$). Heart rate returned to pre-
254 exercise baseline during CWI at 10 min post-cooling whereas heart rate remained
255 higher throughout post-cooling recovery during WBC. Furthermore, relative to WBC,
256 heart rate was higher at 10 and 20 min post CWI. Systolic blood pressure was similar
257 to pre-exercise throughout the recovery period with no difference between conditions
258 ($P > 0.05$ for main effects of time and condition; see Table 2). There was a significant
259 interaction effect of time and condition for diastolic ($P < 0.001$) and mean arterial
260 pressure ($P = 0.002$). Diastolic and mean arterial pressure were similar to pre-exercise
261 throughout the recovery period in WBC, whereas, during CWI, diastolic and mean
262 arterial pressure were higher at 10 and 40 min post-cooling during CWI relative to pre-
263 exercise baseline.

264 *Femoral artery and cutaneous blood flow responses.* The decrease in femoral
265 artery blood flow ($P < 0.001$; ES = >0.7) and femoral vascular conductance ($P < 0.001$;
266 ES = >1.0) was greater in the CWI condition throughout the post-cooling period (Figure
267 4). At 40 min post recovery, femoral artery blood flow and femoral artery conductance
268 were (~45-50%) lower in CWI compared with WBC (Figure 4). A greater skin
269 vasoconstriction was observed after CWI at the thigh (~75% *vs.* ~55%; $P < 0.001$, ES
270 = 1.9) and calf (~70% *vs.* ~45%; $P < 0.001$, ES = 1.6) throughout the recovery period
271 (Figure 5).

272

DISCUSSION

The major finding of the present study is that, relative to WBC, CWI led to greater reductions in femoral artery and cutaneous blood flow, as well as deep and superficial muscle temperature, during the post-exercise recovery period. Collectively, our novel data provide evidence that post-exercise CWI may potentially reduce muscle blood flow to a greater extent than WBC. These findings provide important insights into the relative efficacy of, and the possible mechanisms that underpin, distinct cryotherapy recovery modalities commonly used in clinical and sporting environments.

To our knowledge, only one study has previously attempted to document the limb blood flow response to WBC cooling, using near-infrared spectroscopy (NIRS) (31). On the morning after exercise (a rugby league match) reductions in tissue oxyhaemoglobin and tissue oxygenation index of the vastus lateralis were evident immediately after 3 min of WBC, which caused a reduction in mean skin temperature of a maximum of ~9 °C (31). The NIRS method provides indirect estimates of relative changes in blood volume within the muscle microcirculation, but is associated with a number of limitations (12), including that tissue oxygenation indices are confounded when marked changes in skin blood flow arise (e.g. exercise, heating, cooling) (9). In the present investigation, we continuously measured changes in lower limb cutaneous blood flow using laser Doppler flowmetry while simultaneously measuring femoral artery blood flow via conduit artery high-resolution duplex ultrasound. Cutaneous blood flow was reduced throughout the recovery period relative to pre-immersion in both CWI (~70-75%) and WBC (~45-55%) conditions, with a greater vasoconstriction observed after CWI (ES = 1.6–1.9).

Alongside the changes in cutaneous blood flow there was a ~50% greater reduction in femoral artery conductance after CWI at the end of the recovery period,

which may infer that CWI reduces muscle blood flow to a greater extent and has a superior impact upon reducing edema (32). Greater CWI-induced reductions in limb blood flow suggest that CWI may limit the inflammatory response after exercise to a greater extent compared to WBC based on previous animal (20, 30) and human (26, 40) studies that reported blunted increases in inflammatory markers after local/whole-body cryotherapy. The purported relationship of blood flow and inflammation after exercise has recently been challenged in a study that reported no impact of CWI (10 min at 10ºC) on the muscle inflammatory or cellular stress response compared with an active recovery after lower body resistance exercise (25). A reduction in muscle blood flow may therefore provide benefits to the acute recovery from exercise by attenuating the clinical signs of inflammation such as edema and swelling per se (11, 38) and associated pain (e.g. soreness) upon movement. Indeed, recent work reported that CWI was more effective than WBC in accelerating recovery kinetics and reducing muscle soreness post exercise (1).

The interpretation of the magnitude of change in post-cooling limb blood flow with regards to the therapeutic benefit to recovery is difficult to ascertain. In practical terms, the difference of femoral artery blood flow of ~50 $mL \cdot min^{1}$ between WBC and CWI conditions is of physiological relevance, particularly when it is evident over the entire 40 min and perhaps longer. To our knowledge, no study has directly addressed the cooling-induced minimally important clinical difference in limb/muscle blood flow required to influence muscle soreness and clinical signs of inflammation such as swelling/edema following exercise. Past studies have largely focused on the effects of cooling on functional/performance measures and/or markers of muscle damage, but have not related the desired outcome measures with changes in limb blood flow. More

work is required to relate changes in limb blood flow with the measured outcome variable of interest after post-exercise cooling.

The reduction in femoral artery blood flow is mediated via activation of thermos-nociceptors during skin cooling, which leads to a reflex increase in sympathetic nerve activity (19). The differences in arterial blood flow between CWI and WBC may therefore be related to the different thermal input, e.g., core and local tissue temperatures, associated with skin cooling in both recovery modalities. To date, only one study (8) has compared the thermoregulatory responses (i.e., core, muscle and skin temperatures) between CWI and WBC recovery modalities. In that study, the duration of exposures was matched to delineate the impact of the different modalities. However, the duration of the CWI (4 min) protocol was not representative of the CWI protocol typically used for recovery in various sporting environments, i.e. $\geq$ 10 min (21, 36) and neither modality was applied after exercise. In the current study, we observed no difference in recovery rectal temperatures between cooling modalities and noted a lower skin temperature after CWI throughout the recovery period in agreement with Costello *et al.* (8). In contrast, our findings of greater reductions in deep and superficial muscle temperatures after CWI are not consistent with the findings of Costello *et al.* (8). These findings are likely related to the greater conductance of tissue heat transfer/loss in water compared with air (4) and/or the greater duration of CWI cooling used after exercise in the current study.

The decreases in deep muscle temperature after CWI likely contributed to the larger reduction in femoral artery conductance after CWI (3). The temporal pattern in femoral artery conductance mirrored that of deep muscle temperature in that the differences between CWI and WBC became larger as the post-cooling recovery period progressed. Previous work from our laboratory (23) has shown that relatively small

changes in deep muscle temperature (~0.5°C) do not influence femoral artery conductance. Our findings indicate that relative to WBC, lower deep muscle temperature is evident during CWI recovery, which may suggest deep muscle temperature differences of >1.0°C likely modulated limb, and perhaps muscle, blood flow.

Cold stress can also induce pain via noxious stimulation (19). Immersing the hands in 28, 21 and 14 °C water temperatures decreased hand skin temperature to 20-24°C and pain sensations ranged from not painful to somewhat painful, but, muscle sympathetic nerve activity was unchanged (19). During 7 and 0 °C water hand immersion, which decreased skin temperature below 15°C, perceived pain was rated as intensively painful and muscle sympathetic nerve activity greatly increased. It is therefore possible that CWI could induce pain and elevations in sympathetic nerve activity, independent of the thermal stimulus, depending on the magnitude of reduction in skin temperature. In the present study, the lowest skin temperatures were approx. 24 °C after CWI. Therefore, despite a likely minor increase in pain sensation after CWI in the present study sympathetic nerve activity directed to the musculature was likely not increased above that caused by the cold thermal stimulus alone.

Although there are no definitive guidelines regarding the effective and safe use of WBC (6), it is common that individuals continuously move their arms and legs and/or walk around the inside of the cryotherapy chamber during relatively short exposure durations (5, 8). Methodologically, this is problematic in the assessment of limb blood flow, due to muscle activation confounding measurements. We were therefore cautious to select a less severe WBC temperature and duration to limit the prospect of any adverse skin reactions/cold burn injury whilst seated inside the cryotherapy chamber (no adverse skin reactions were noted in the present study) and to

match typical durations of WBC protocols. Previous research suggests that colder temperatures e.g. -135°C may be better for recovery (31), therefore colder WBC temperatures and/or longer exposure durations may have a greater impact on deep tissue temperature, which may lead to greater reductions in limb blood flow than presently observed. Further work is required to explore the potential benefits of lower WBC temperatures and/or increased durations on the limb blood flow response after exercise. Nevertheless, despite a greater thermal gradient between the colder air temperatures and skin during WBC exposure, the greater thermal conductance and/or duration of CWI promoted greater changes in tissue temperature and limb blood flow in the present study. In light of the current findings, the physiological rationale for using WBC instead of CWI, in addition to the associated logistical and cost implications, is questionable.

It is also important to acknowledge that CWI will result in increased hydrostatic pressure and potentially increased central blood volume, which could affect vascular responses independent of the water temperature. More specifically, baroreceptor mediated peripheral vasodilation could occur. Nevertheless, previous research has reported no change in total peripheral resistance during hip-level (the same level used for CWI in the present study) thermoneutral water immersion (36). Moreover, any baroreflex-mediated vasodilation from immersion *per se* would have blunted the sympathetic peripheral vasoconstriction from cold-water stimulation rather than contributed to/exacerbated the clear differences in vascular responses between CWI and WBC observed in the present study. Finally, the aim of the present practically oriented study was to compare the thermoregulatory and vascular responses to two commonly used/ecologically valid but very different recovery methods, rather than investigate the effects of each intervention independently. Due to the repeated measures design of the present study moderate intensity cycling was employed as the exercise

stimulus prior to the cooling interventions, which would likely have not induced significant muscle damage. It would be logical to further investigate the vascular and thermoregulatory responses to CWI vs. WBC after high-intensity endurance exercise that results in pronounced muscle damage.

In summary, this study demonstrates that an ecologically valid CWI protocol decreases both femoral artery and cutaneous blood flow and muscle temperature to a greater extent compared with a typical WBC protocol after endurance exercise. CWI may therefore be a more effective cooling modality due, in part, to the hydrostatic pressure of water and the greater ability of water to conduct heat. These findings have practical implications in athletic and clinical settings where cryotherapy is employed with the aim to accelerate recovery from exercise. Further studies are necessary to evaluate if, relative to WBC, CWI-induced greater decreases in conduit and microvascular blood flow and muscle temperature result in greater therapeutic benefits post exercise.

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Conflict of Interest

WG has received funding from ECB Cold Spa Ltd for the cold-water immersion facility and from UK Sport for part funding of a Ph.D. program. CM, DL, HJ, DG and JC have no conflicts of interest.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Whole body cryotherapy is not yet approved by the FDA and is not labeled for the use under discussion.

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Figure captions

Figure 1. The experimental design

Figure 2. Thigh skin temperature (A) and rectal temperature (B) pre and post cooling in CWI and WBC (n = 10, mean ± SD). Main effects for condition ($P < 0.001$) and time ($P < 0.001$) alongside a significant interaction between condition and time ($P < 0.001$) were found for thigh skin temperature. Main effects for time ($P < 0.001$) and a significant interaction between condition and time ($P < 0.001$) were found for thigh skin temperature. * Significant difference from Baseline ($P < 0.05$). + Significant difference between cooling conditions ($P < 0.05$).

Figure 3. Muscle temperature pre and post cooling at temperature probe depths of 3 cm (A), 2 cm (B), and 1cm (C) in CWI and WBC (n =10, mean ± SD). Main effects for condition ($P < 0.001$) and time ($P < 0.001$) were found along with a significant interaction between condition, time and probe depth ($P < 0.001$) at each depth. * Significant difference from Baseline ($P < 0.01$). + Significant difference between cooling conditions ($P < 0.05$).

Figure 4. Femoral artery blood flow (A) and conductance (B) pre and post cooling in CWI and WBC (n = 10, mean ± SD). A main effect for time ($P = < 0.001$) alongside a significant interaction between condition and time ($P < 0.01$) was found for both artery flow and conductance. * Significant difference from Baseline ($P < 0.001$). + Significant difference between cooling conditions ($P < 0.05$).

Figure 5. Percentage change in thigh cutaneous vascular conductance (A) and calf vascular conductance (B) from pre immersion in CWI and WBC (n =10, mean ± SD). Main effects for condition ($P < 0.001$) were found for both thigh and calf cutaneous vascular conductance. A main effect for time ($P < 0.01$) was also found for thigh conductance. There were no interactions between condition and time in thigh ($P = 0.44$) or calf vascular conductance ($P = 0.52$). * Significant difference from pre cooling ($P < 0.001$). + Significant difference between cooling conditions ($P < 0.001$).