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

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1 **ABSTRACT**

2 **Purpose:** Cold-water immersion (CWI) and whole body cryotherapy (WBC) are

3 widely used recovery methods in an attempt to limit exercise-induced muscle damage,

4 soreness and functional deficits after strenuous exercise. The aim of this study was to

5 compare the effects of ecologically-valid CWI and WBC protocols on post-exercise

6 lower limb thermoregulatory, femoral artery and cutaneous blood flow responses.

7 **Methods:** Ten males completed a continuous cycle exercise protocol at 70% maximal

8 oxygen uptake until a rectal temperature of 38°C was attained. Participants were then

9 exposed to lower-body CWI (8°C) for 10 min, or WBC (-110°C) for 2 min, in a

10 randomized cross-over design. Rectal and thigh skin, deep and superficial muscle

11 temperatures, thigh and calf skin blood flow (laser Doppler flowmetry), superficial

12 femoral artery blood flow (duplex ultrasound) and arterial blood pressure were

13 measured prior to, and for 40 min post, cooling interventions. **Results:** Greater

14 reductions in thigh skin (CWI, -5.9±1.8°C; WBC, 0.2±0.5°C; $P < 0.001$) and superficial

15 (CWI, -4.4±1.3°C; WBC, -1.8±1.1°C; $P < 0.001$) and deep (CWI, -2.9±0.8°C; WBC, -

16 1.3±0.6°C; $P < 0.001$) muscle temperatures occurred immediately after CWI.

17 Decreases in femoral artery conductance were greater after CWI (CWI, -84±11%;

18 WBC, -59±21%, $P < 0.02$) and thigh (CWI, -80±5%; WBC, -59±14%, $P < 0.001$) and

19 calf (CWI, -73±13%; WBC, -45±17%, $P < 0.001$) cutaneous vasoconstriction was

20 greater following CWI. Reductions in rectal temperature were similar between

21 conditions after cooling (CWI, -0.6±0.4°C; WBC, -0.6±0.3°C; $P = 0.98$). **Conclusion:**

22 Greater reductions in blood flow and tissue temperature were observed after CWI in

23 comparison to WBC. These novel findings have practical and clinical implications for

24 the use of cooling in the recovery from exercise and injury.

25

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

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52 potential beneficial effects of WBC on hematological profiles (22), inflammatory

53 biomarkers (26, 40), muscle damage (13, 40), the autonomic nervous system (29), body

54 temperature (8), and tissue oxyhaemoglobin and oxygenation (31). Despite these

55 apparent favorable effects of WBC there is equivocal evidence for a positive impact of

56 WBC on functional recovery (7, 13, 14). Furthermore, the comparative physiological,

57 especially vascular, effects of WBC relative to CWI remain to be elucidated. Costello

58 *et* al (8) have previously shown that 4 minutes of exposure to either CWI or WBC

59 similarly decreased rectal and muscle temperatures for up to 60 minutes post exposure,

60 despite lower thigh skin temperatures after CWI. However, the CWI duration used in

61 that study was not typical of protocols used for recovery, i.e. $\geq$10 minutes (21, 36), the

62 cryotherapy modalities were applied under resting conditions and the vascular (blood

63 flow) and hemodynamic responses were not measured. It is therefore currently

64 unknown if the changes in blood flow of previously exercised limb(s) are different

65 between ecologically valid CWI and WBC protocols. This is important given that

66 reducing blood flow may represent an important mechanism through which cooling

67 influences post exercise muscle recovery.

68   The aim of the present study was to, therefore, examine the effects of

69 ecologically valid CWI and WBC protocols on femoral artery and cutaneous blood flow

70 and thermoregulatory responses after cycling exercise. We hypothesized that a longer

71 duration of CWI would decrease femoral artery and lower limb skin blood flow to a

72 greater extent, compared with WBC, and lead to a greater reduction in leg muscle

73 temperature.

74

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·kg^{-1}·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·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

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125 protocols, which may have confounded post exercise femoral artery blood flow

126 measurements due to the protective effect of performing a single bout of muscle

127 damaging exercise (24).

128 In the CWI condition, participants were subsequently raised from the bed in a

129 semi-recumbent posture, using an electronic hoist (Bianca, Arjo Ltd, Gloucester,

130 United Kingdom), and lowered into the water bath (in the same position) until the thighs

131 were fully submerged for 10 minutes. This avoided the potential impact of any active

132 movement or muscular contraction on subsequent measures. In the WBC condition,

133 body sweat was lightly dabbed dry with a towel, and equipment was removed from the

134 body (skin temperature probes, heart rate monitor) for entering the WBC chamber. Skin

135 blood flow and rectal probes remained *in situ*, with connections covered and tucked

136 inside the participant's shorts and socks. Next, with the help of the researchers, the

137 participants donned the clothing to be worn inside the chamber (face mask, ear band,

138 gloves, socks and shoes) and were then transferred to, and pushed, in a chair to undergo

139 WBC exposure inside the chamber (seated on the chair). At the end of each separate

140 cooling trial, participants were returned to the bed using either the electronic hoist/or

141 via the chair, and remained in a supine position for a period of 40 min under the

142 temperature-controlled laboratory. A period of 10 min was permitted, before any post-

143 exposure measurements, for the reattachment of the skin temperature probes and heart

144 rate monitor equipment and removing clothing required in the chamber. The use of the

145 hoist to raise and lower the participants, and the chair to transfer the participants to and

146 from the chamber was important to avoid the effect of muscle activation on blood flow

147 and hemodynamic measures (15, 23).

148 Thermoregulatory variables were measured at baseline, pre-cooling and during

149 post-cooling period. Perceived thermal comfort, rated using a 9-point scale (0 =

150 unbearably cold, 1 = very cold, 2 = cold, 3 = cool, 4 = slightly cool, 5 = neutral, 6 =

151 slightly warm, 7 = warm, 8 = hot, 9 = very hot) (39) and shivering, rated using a 4 point

152 scale (1 = no shivering, 2 = slight shivering, 3 = moderate shivering, 4 = heavy

153 shivering) (35) were also recorded. All pre- and post-cooling measurements were made

154 in a supine position. A schematic illustration of the experimental design is shown in

155 Figure 1.

156

157 *Measurements*

158   *Rectal, Thigh Skin and Muscle Temperature*. A rectal probe (Rectal temperature

159 probe, adult, ELLAB, Rodovre, Denmark) was inserted 15 cm beyond the anal

160 sphincter for the assessment of rectal temperature. A skin thermistor (Surface

161 temperature probe, stationary, ELLAB, Rodovre, Denmark) was attached to the upper

162 thigh for the assessment of skin temperature. Muscle temperature was assessed using a

163 needle thermistor inserted into the vastus lateralis (Multi purpose needle probe,

164 ELLAB, Rodovre, Denmark) as previously described (8, 23). Briefly, thigh skinfold

165 thickness was measured using Harpenden skinfold calipers (HSK BI, Baty

166 International, West Sussex, United Kingdom) and divided by 2 to determine the

167 thickness of the thigh subcutaneous fat layer over each participant's vastus lateralis .

168 The needle thermistor was then placed at a depth of 3 cm plus one-half the skinfold

169 measurement for determination of deep muscle temperature (3 cm). The thermistor was

170 then withdrawn at 1 cm increments for determination of muscle temperature at 2 cm

171 and 1 cm below the subcutaneous layer. Rectal, skin and muscle temperatures were

172 recorded using an electronic measuring system (CTF 9004, ELAB).

173   *Heart Rate and Blood Pressure.* Heart rate was continuously measured using a

174 heart rate monitor (S610; Polar Electro Oy, Kempele, Finland). Blood pressure was

175 measured noninvasively via automated brachial auscultation (Dinamap, GE Pro 300V2,

176 Tampa, Florida, USA).

177 *Femoral Artery Blood Flow*. A 15 MHz linear array transducer attached to a

178 high-resolution ultrasound machine (Acuson P50, Siemens, Germany) was used to

179 measure superficial femoral artery diameter and velocity (3 cm distal to the bifurcation)

180 as previously described (23). This position was marked on the skin such that the

181 ultrasound head could be accurately repositioning during subsequent measures.

182 Analysis of diameter and velocity was performed using custom designed edge-

183 detection and wall-tracking software (37) which is considerably more repeatable than

184 manual methods and associated with less observer error (37). Resting diameter, blood

185 velocity and blood flow were calculated as the mean of the data collected over a 20 s

186 period of each 2 min recoding for statistical analysis. Femoral vascular conductance

187 was calculated as the ratio of blood flow/mean arterial pressure.

188 *Cutaneous Blood Flow*. Red blood cell flux was used as an index of skin blood

189 flow via laser Doppler flowmetry (Periflux System 5001, Perimed Instruments, Jarfalla,

190 Sweden). A laser Doppler probe (455, Perimed, Suffolk, United Kingdom) was

191 attached to the mid-anterior thigh, midline, halfway between the inguinal line and the

192 patella, and on the calf, left of the midline, in the region of the largest circumference.

193 Once affixed, the probes were not removed until the completion of each trial. Cutaneous

194 vascular conductance was calculated as the ratio of laser Doppler flux to mean arterial

195 blood pressure (cutaneous vascular conductance = laser Doppler flux/mean arterial

196 blood pressure x 100) and expressed as a percentage change from pre immersion values.

197 When expressed as a percentage change from baseline to maximum, cutaneous blood

198 flow has a coefficient variation of 4% in our laboratory with a coefficient of variation

199   of 10% observed for resting cutaneous blood flow. Thigh and calf skin conductance are

200   expressed as percentage change from pre immersion (zero)

201

202   *Statistical Analysis*

203   Using our previous data (23), 8°C water immersion mediates a reduction from

204   pre-exercise baseline in femoral artery blood flow of 60 mL·min^{-1}. To replicate this

205   reduction in femoral artery blood flow with 80% power and an α of 0.05, a sample size

206   of 9 participants is required. Similarly, we utilized a previous study (8) to estimate a

207   minimum clinically important difference in thigh skin temperature of 3.4 ± 2.4°C

208   immediately following CWI compared to WBC. To detect this difference with 80%

209   power and an α of 0.05, a sample size of 7 participants is required.

210   A two-factor [condition (CWI & WBC) x time (baseline, post-exercise/pre

211   cooling, post cooling 10, 20, 30, 40 min)] general linear model (GLM) was used to

212   evaluate treatment differences between the CWI and WBC conditions. A three-way

213   GLM (condition x depth x time) was used to analyse muscle temperature. Where a

214   significant interaction between condition and time was observed, differences were

215   followed up with Newman-Keuls multiple contrasts.

216   Simple effect size (ES), estimated from the ratio of the mean difference to the

217   pooled standard deviation (Hedges' *g*), were also calculated. The ES magnitude was

218   classified as trivial (<0.2), small (>0.2-0.6), moderate (>0.6-1.2), large (>1.2-2.0) and

219   very large (>2.0-4.0) (17). SPSS version 20, Statistical Package for the Social Sciences

220   was employed for all statistical analysis (Chicago, IL). The statistical significance was

221   set at $P < 0.05$. Data are presented as mean ± SD.

222

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,

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298   which may infer that CWI reduces muscle blood flow to a greater extent and has a

299   superior impact upon reducing edema (32). Greater CWI-induced reductions in limb

300   blood flow suggest that CWI may limit the inflammatory response after exercise to a

301   greater extent compared to WBC based on previous animal (20, 30) and human (26,

302   40) studies that reported blunted increases in inflammatory markers after local/whole-

303   body cryotherapy. The purported relationship of blood flow and inflammation after

304   exercise has recently been challenged in a study that reported no impact of CWI (10

305   min at 10ºC) on the muscle inflammatory or cellular stress response compared with an

306   active recovery after lower body resistance exercise (25). A reduction in muscle blood

307   flow may therefore provide benefits to the acute recovery from exercise by attenuating

308   the clinical signs of inflammation such as edema and swelling per se (11, 38) and

309   associated pain (e.g. soreness) upon movement. Indeed, recent work reported that CWI

310   was more effective than WBC in accelerating recovery kinetics and reducing muscle

311   soreness post exercise (1).

312   The interpretation of the magnitude of change in post-cooling limb blood flow

313   with regards to the therapeutic benefit to recovery is difficult to ascertain. In practical

314   terms, the difference of femoral artery blood flow of ~50 mL·min^1 between WBC and

315   CWI conditions is of physiological relevance, particularly when it is evident over the

316   entire 40 min and perhaps longer. To our knowledge, no study has directly addressed

317   the cooling-induced minimally important clinical difference in limb/muscle blood flow

318   required to influence muscle soreness and clinical signs of inflammation such as

319   swelling/edema following exercise. Past studies have largely focused on the effects of

320   cooling on functional/performance measures and/or markers of muscle damage, but

321   have not related the desired outcome measures with changes in limb blood flow. More

322   work is required to relate changes in limb blood flow with the measured outcome

323   variable of interest after post-exercise cooling.

324   The reduction in femoral artery blood flow is mediated via activation of

325   thermos-nociceptors during skin cooling, which leads to a reflex increase in

326   sympathetic nerve activity (19). The differences in arterial blood flow between CWI

327   and WBC may therefore be related to the different thermal input, e.g., core and local

328   tissue temperatures, associated with skin cooling in both recovery modalities. To date,

329   only one study (8) has compared the thermoregulatory responses (i.e., core, muscle and

330   skin temperatures) between CWI and WBC recovery modalities. In that study, the

331   duration of exposures was matched to delineate the impact of the different modalities.

332   However, the duration of the CWI (4 min) protocol was not representative of the CWI

333   protocol typically used for recovery in various sporting environments, i.e. $\geq$ 10 min (21,

334   36) and neither modality was applied after exercise. In the current study, we observed

335   no difference in recovery rectal temperatures between cooling modalities and noted a

336   lower skin temperature after CWI throughout the recovery period in agreement with

337   Costello *et al.* (8). In contrast, our findings of greater reductions in deep and superficial

338   muscle temperatures after CWI are not consistent with the findings of Costello *et al.*

339   (8). These findings are likely related to the greater conductance of tissue heat

340   transfer/loss in water compared with air (4) and/or the greater duration of CWI cooling

341   used after exercise in the current study.

342   The decreases in deep muscle temperature after CWI likely contributed to the

343   larger reduction in femoral artery conductance after CWI (3). The temporal pattern in

344   femoral artery conductance mirrored that of deep muscle temperature in that the

345   differences between CWI and WBC became larger as the post-cooling recovery period

346   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

372   match typical durations of WBC protocols. Previous research suggests that colder

373   temperatures e.g. -135°C may be better for recovery (31), therefore colder WBC

374   temperatures and/or longer exposure durations may have a greater impact on deep tissue

375   temperature, which may lead to greater reductions in limb blood flow than presently

376   observed. Further work is required to explore the potential benefits of lower WBC

377   temperatures and/or increased durations on the limb blood flow response after exercise.

378   Nevertheless, despite a greater thermal gradient between the colder air temperatures

379   and skin during WBC exposure, the greater thermal conductance and/or duration of

380   CWI promoted greater changes in tissue temperature and limb blood flow in the present

381   study. In light of the current findings, the physiological rationale for using WBC instead

382   of CWI, in addition to the associated logistical and cost implications, is questionable.

383   It is also important to acknowledge that CWI will result in increased hydrostatic

384   pressure and potentially increased central blood volume, which could affect vascular

385   responses independent of the water temperature. More specifically, baroreceptor

386   mediated peripheral vasodilation could occur. Nevertheless, previous research has

387   reported no change in total peripheral resistance during hip-level (the same level used

388   for CWI in the present study) thermoneutral water immersion (36). Moreover, any

389   baroreflex-mediated vasodilation from immersion *per se* would have blunted the

390   sympathetic peripheral vasoconstriction from cold-water stimulation rather than

391   contributed to/exacerbated the clear differences in vascular responses between CWI

392   and WBC observed in the present study. Finally, the aim of the present practically

393   oriented study was to compare the thermoregulatory and vascular responses to two

394   commonly used/ecologically valid but very different recovery methods, rather than

395   investigate the effects of each intervention independently. Due to the repeated measures

396   design of the present study moderate intensity cycling was employed as the exercise

397   stimulus prior to the cooling interventions, which would likely have not induced

398   significant muscle damage. It would be logical to further investigate the vascular and

399   thermoregulatory responses to CWI vs. WBC after high-intensity endurance exercise

400   that results in pronounced muscle damage.

401   In summary, this study demonstrates that an ecologically valid CWI protocol

402   decreases both femoral artery and cutaneous blood flow and muscle temperature to a

403   greater extent compared with a typical WBC protocol after endurance exercise. CWI

404   may therefore be a more effective cooling modality due, in part, to the hydrostatic

405   pressure of water and the greater ability of water to conduct heat. These findings have

406   practical implications in athletic and clinical settings where cryotherapy is employed

407   with the aim to accelerate recovery from exercise. Further studies are necessary to

408   evaluate if, relative to WBC, CWI-induced greater decreases in conduit and

409   microvascular blood flow and muscle temperature result in greater therapeutic benefits

410   post exercise.

411

421

422   The results of the present study do not constitute endorsement by the American College

423   of Sports Medicine.

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425   The results of this study are presented clearly, honestly, and without fabrication,

426   falsification, or inappropriate data manipulation.

427

428   Whole body cryotherapy is not yet approved by the FDA and is not labeled for the use

429   under discussion.

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REFERENCES

1. Abaidia AE, Lamblin J, Delecroix B et al. Recovery From Exercise-Induced Muscle Damage: Cold Water Immersion Versus Whole Body Cryotherapy. *Int J Sports Physiol Perform*. 2016:1-23.

2. Banfi G, Lombardi G, Colombini A, Melegati G. Whole-body cryotherapy in athletes. *Sports Med*. 2010;40(6):509-17.

3. Barcroft H, Edholm OG. The effect of temperature on blood flow and deep temperature in the human forearm. *J. Physiol*. 1943;102:5-20.

4. Bleakley CM, Bieuzen F, Davison GW, Costello JT. Whole-body cryotherapy: empirical evidence and theoretical perspectives. *Open Access J Sports Med*. 2014;5:25-36.

5. Costello JT, Algar LA, Donnelly AE. Effects of whole-body cryotherapy (-110 degrees C) on proprioception and indices of muscle damage. *Scand J Med Sci Sports*. 2012;22(2):190-8.

6. Costello JT, Baker PR, Minett GM, Bieuzen F, Stewart IB, Bleakley C. Whole-body cryotherapy (extreme cold air exposure) for preventing and treating muscle soreness after exercise in adults (Protocol). *Cochrane Database Syst Rev*. 2013;(10):CD010789.

7. Costello JT, Baker PR, Minett GM, Bieuzen F, Stewart IB, Bleakley C. Whole-body cryotherapy (extreme cold air exposure) for preventing and treating muscle soreness after exercise in adults. *Cochrane Database Syst Rev*. 2015;(9):Cd010789.

8. Costello JT, Culligan K, Selfe J, Donnelly AE. Muscle, skin and core temperature after -110 degrees c cold air and 8 degrees c water treatment. *PLoS One*. 2012;7(11):e48190.

9. Davis SL, Fadel PJ, Cui J, Thomas GD, Crandall CG. Skin blood flow influences near-infrared spectroscopy-derived measurements of tissue oxygenation during heat stress. *J Appl Physiol*. 2006;100(1):221-4.

10. Diong J, Kamper SJ. Cold water immersion (cryotherapy) for preventing muscle soreness after exercise. *Br J Sports Med*. 2014;48(18):1388-9.

11. Dolan MG, Thornton RM, Fish DR, Mendel FC. Effects of cold water immersion on edema formation after blunt injury to the hind limbs of rats. *J Athl Train*. 1997;32(3):233-7.

12. Ferrari M, Mottola L, Quaresima V. Principles, techniques, and limitations of near infrared spectroscopy. *Can J Appl Physiol*. 2004;29(4):463-87.

13. Ferreira-Junior JB, Bottaro M, Vieira A et al. One session of partial-body cryotherapy (-110 degrees C) improves muscle damage recovery. *Scand J Med Sci Sports*. 2015;25(5):e524-30.

14. Fonda B, Sarabon N. Effects of whole-body cryotherapy on recovery after hamstring damaging exercise: a crossover study. *Scand J Med Sci Sports*. 2013;23(5):e270-8.

15. Gregson W, Black MA, Jones H et al. Influence of cold water immersion on limb and cutaneous blood flow at rest. *Am J Sports Med*. 2011;39(6):1316-23.

16. Hausswirth C, Louis J, Bieuzen F et al. Effects of whole-body cryotherapy vs. far-infrared vs. passive modalities on recovery from exercise-induced muscle damage in highly-trained runners. *PLoS One*. 2011;6(12):e27749.

17. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*. 2009;41(1):3-13.

481   18.   Ihsan M, Watson G, Lipski M, Abbiss CR. Influence of postexercise cooling
482   on muscle oxygenation and blood volume changes. *Med Sci Sports Exerc*.
483   2013;45(5):876-82.
484   19.   Kregel KC, Seals DR, Callister R. Sympathetic nervous system activity during
485   skin cooling in humans: relationship to stimulus intensity and pain sensation. *J*
486   *Physiol*. 1992;454:359-71.
487   20.   Lee H, Natsui H, Akimoto T, Yanagi K, Ohshima N, Kono I. Effects of
488   cryotherapy after contusion using real-time intravital microscopy. *Med Sci*
489   *Sports Exerc*. 2005;37(7):1093-8.
490   21.   Leeder J, Gissane C, van Someren K, Gregson W, Howatson G. Cold water
491   immersion and recovery from strenuous exercise: a meta-analysis. *Br J Sports*
492   *Med*. 2012;46(4):233-40.
493   22.   Lombardi G, Lanteri P, Porcelli S et al. Hematological profile and martial
494   status in rugby players during whole body cryostimulation. *PLoS One*.
495   2013;8(2):e55803.
496   23.   Mawhinney C, Jones H, Joo CH, Low DA, Green DJ, Gregson W. Influence
497   of cold-water immersion on limb and cutaneous blood flow after exercise.
498   *Med Sci Sports Exerc*. 2013;45(12):2277-85.
499   24.   McHugh MP. Recent advances in the understanding of the repeated bout
500   effect: the protective effect against muscle damage from a single bout of
501   eccentric exercise. *Scand J Med Sci Sports*. 2003;13(2):88-97.
502   25.   Peake JM, Roberts LA, Figueiredo VC et al. The effects of cold water
503   immersion and active recovery on inflammation and cell stress responses in
504   human skeletal muscle after resistance exercise. *J Physiol*. 2016.
505   26.   Pournot H, Bieuzen F, Louis J et al. Time-course of changes in inflammatory
506   response after whole-body cryotherapy multi exposures following severe
507   exercise. *PLoS One*. 2011;6(7):e22748.
508   27.   Roberts LA, Muthalib M, Stanley J et al. Effects of cold water immersion and
509   active recovery on hemodynamics and recovery of muscle strength following
510   resistance exercise. *Am J Physiol Regul Integr Comp Physiol*.
511   2015;309(4):R389-98.
512   28.   Roberts LA, Nosaka K, Coombes JS, Peake JM. Cold water immersion
513   enhances recovery of submaximal muscle function after resistance exercise.
514   *Am J Physiol Regul Integr Comp Physiol*. 2014;307(8):R998-R1008.
515   29.   Schaal K, Le Meur Y, Bieuzen F et al. Effect of recovery mode on
516   postexercise vagal reactivation in elite synchronized swimmers. *Appl Physiol*
517   *Nutr Metab*. 2013;38(2):126-33.
518   30.   Schaser KD, Disch AC, Stover JF, Lauffer A, Bail HJ, Mittlmeier T.
519   Prolonged superficial local cryotherapy attenuates microcirculatory
520   impairment, regional inflammation, and muscle necrosis after closed soft
521   tissue injury in rats. *Am J Sports Med*. 2007;35(1):93-102.
522   31.   Selfe J, Alexander J, Costello JT et al. The effect of three different (-135
523   degrees C) whole body cryotherapy exposure durations on elite rugby league
524   players. *PLoS One*. 2014;9(1):e86420.
525   32.   Thorlacius H, Vollmar B, Westermann S, Torkvist L, Menger MD. Effects of
526   local cooling on microvascular hemodynamics and leukocyte adhesion in the
527   striated muscle of hamsters. *J Trauma*. 1998;45(4):715-9.
528   33.   Vaile J, O'Hagan C, Stefanovic B, Walker M, Gill N, Askew CD. Effect of
529   cold water immersion on repeated cycling performance and limb blood flow.
530   *Br J Sports Med*. 2011;45(10):825-9.

531 34. Vieira Ramos G, Pinheiro CM, Messa SP et al. Cryotherapy Reduces
532 Inflammatory Response Without Altering Muscle Regeneration Process and
533 Extracellular Matrix Remodeling of Rat Muscle. *Sci Rep*. 2016;6:18525.
534 35. Wakabayashi H, Hanai A, Yokoyama S, Nomura T. Thermal insulation and
535 body temperature wearing a thermal swimsuit during water immersion. *J
536 Physiol Anthropol*. 2006;25(5):331-8.
537 36. Wilcock IM, Cronin JB, Hing WA. Physiological response to water
538 immersion: a method for sport recovery? *Sports Med*. 2006;36(9):747-65.
539 37. Woodman RJ, Playford DA, Watts GF et al. Improved analysis of brachial
540 artery ultrasound using a novel edge-detection software system. *J Appl
541 Physiol*. 2001;91(2):929-37.
542 38. Yanagisawa O, Niitsu M, Yoshioka H, Goto K, Kudo H, Itai Y. The use of
543 magnetic resonance imaging to evaluate the effects of cooling on skeletal
544 muscle after strenuous exercise. *Eur J Appl Physiol*. 2003;89(1):53-62.
545 39. Young AJ, Sawka MN, Epstein Y, Decristofano B, Pandolf KB. Cooling
546 different body surfaces during upper and lower body exercise. *J Appl Physiol*.
547 1987;63(3):1218-23.
548 40. Ziemann E, Olek RA, Kujach S et al. Five-day whole-body cryostimulation,
549 blood inflammatory markers, and performance in high-ranking professional
550 tennis players. *J Athl Train*. 2012;47(6):664-72.
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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 ($P < 0.001$).