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**Changes in Quadriceps Femoris Muscle Perfusion Following Different Degrees of Cold-Water Immersion**

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### Article

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1 **Changes in Quadriceps Femoris Muscle Perfusion Following Different Degrees of Cold-**  
2 **Water Immersion**

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15  
16 **Running Head:** Muscle perfusion and cold-water immersion

17 **Key Words:** muscle perfusion, cold water immersion, cooling

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28

29 **ABSTRACT**

30 We examined the influence of graded cold-water immersion (CWI) on global and regional  
31 quadriceps muscle perfusion using positron emission tomography (PET) and [<sup>15</sup>O]H<sub>2</sub>O. In  
32 thirty healthy males (33±8 yrs; 81±10 kg; 184±5 cm; percentage body fat: 13±5%;  $\dot{V}O_{2\text{peak}}$ :  
33 47±8 mL·kg<sup>-1</sup>·min<sup>-1</sup>) quadriceps perfusion, thigh and calf cutaneous vascular conductance  
34 (CVC), intestinal, muscle and local skin temperatures, thermal comfort, mean arterial pressure  
35 and heart rate were assessed prior to and following 10-min of CWI at 8°C, 15°C or 22°C.  
36 Global quadriceps perfusion did not change beyond a clinically relevant threshold (0.75  
37 mL·100g·min<sup>-1</sup>) in any condition, and was similar between conditions [range of the differences  
38 (95% confidence interval [CI]); 0.1 mL·100g·min<sup>-1</sup> (-0.9 to 1.2 mL·100g·min<sup>-1</sup>) to 0.9  
39 mL·100g·min<sup>-1</sup> (-0.2 to 1.9 mL·100g·min<sup>-1</sup>)]. Muscle perfusion was greater in vastus  
40 intermedius (VI) compared with vastus lateralis (VL) (2.2 mL·100g·min<sup>-1</sup>; 95%CI 1.5 to 3.0  
41 mL·100g·min<sup>-1</sup>) and rectus femoris (RF) (2.2 mL·100g·min<sup>-1</sup>; 1.4 to 2.9 mL·100g·min<sup>-1</sup>). A  
42 clinically relevant increase in VI muscle perfusion after immersion at 8°C and a decrease in  
43 RF muscle perfusion at 15°C were observed. A clinically relevant increase in perfusion was  
44 observed in the VI in 8°C compared with 22°C water (2.3 mL·100g·min<sup>-1</sup>; 1.1 to 3.5  
45 mL·100g·min<sup>-1</sup>). There were no clinically relevant between-condition differences in thigh CVC.  
46 Our findings suggest that CWI (8-22°C) does not reduce global quadriceps muscle perfusion  
47 to a clinically relevant extent, however, colder-water (8°C) increases deep muscle perfusion  
48 and reduces (15°C) superficial muscle (RF) perfusion in the quadriceps muscle.

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## 53 **NEW & NOTEWORTHY**

54 Using positron emission tomography, we report for the first time, muscle perfusion  
55 heterogeneity in the quadriceps femoris in response to different degrees of cold-water  
56 immersion (CWI). Noxious CWI temperatures (8°C) increases perfusion in the deep quadriceps  
57 muscle whilst superficial quadriceps muscle perfusion is reduced in cooler (15°C) water.  
58 Therefore, these data have important implications for the selection of CWI approaches used in  
59 the treatment of soft tissue injury, while also increasing our understanding of the potential  
60 mechanisms underpinning CWI.

61

## 62 **INTRODUCTION**

63 The application of cryotherapy (i.e., cold therapy) is widely used as a recovery modality  
64 in the treatment of soft tissue injuries (6, 18, 25). The proposed benefits of acute cryotherapy  
65 (e.g., cold-water immersion or extreme air-cooling) exposure are related to reductions in  
66 body/local temperatures, muscle microvascular blood flow, oedema, perceived soreness and  
67 possibly muscle damage (18). Therefore, understanding the change in muscle perfusion in  
68 response to cryotherapy is key in providing appropriate advice for effective intervention  
69 strategies.

70 The current theory that cooling causes reductions in lower limb muscle blood flow is  
71 based on studies employing techniques that only allow the inference of hemodynamic, e.g.,  
72 Doppler ultrasound alongside simultaneous cutaneous blood flow measures (14, 27, 28) or  
73 volume changes within the limb (9, 12, 19, 43). Positron emission tomography (PET) alongside  
74 oxygen-15 water radiotracer [<sup>15</sup>O]H<sub>2</sub>O kinetics, provides a unique tool for the direct  
75 measurement of skeletal muscle perfusion (35). With knowledge of [<sup>15</sup>O]H<sub>2</sub>O kinetics in the  
76 arterial blood and specific tissues, it is possible to provide quantitative perfusion measurements

77 in the muscles of interest (20, 36). PET and [<sup>15</sup>O]H<sub>2</sub>O has been employed previously to  
78 determine muscle perfusion responses of the lower limb to local and whole body heating (16),  
79 and thereby provides an excellent model to determine muscle perfusion changes during cooling.

80 Another key issue not yet considered when examining the impact of cooling on limb  
81 perfusion, is that individual skeletal muscles respond to cold differently (8, 42). For example,  
82 glucose metabolism, muscle perfusion and oxygen consumption have been shown to increase,  
83 particularly in deeper centrally located cervico-upper thoracic skeletal muscles compared to  
84 superficial muscles, as a response to cold-induced shivering thermogenesis (8, 42). This deep  
85 muscle activation, which cannot be investigated by surface electromyography (EMG), has been  
86 interpreted as a physiological response to maintain core temperature as a result of cold exposure  
87 (15). However, to date, the heterogeneity in the muscle perfusion response to cooling has only  
88 been documented in the upper body muscles as part of brown fat activation studies (42). While  
89 it has been shown that perfusion is spatially and heterogeneously distributed in the quadriceps  
90 femoris muscle at rest and during exercise (24), it remains unclear how cooling may influence  
91 the directional change in global and regional muscle perfusion in the lower body. Therefore,  
92 our aim was to examine the effects of lower body cooling with 8°C, 15°C and 22°C water on  
93 global and regional quadriceps muscle perfusion, using the PET-radiowater technique. We  
94 hypothesized that colder water would elicit the greatest reductions in global quadriceps muscle  
95 perfusion but would increase muscle perfusion within the deep lying quadriceps muscles.

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101 **METHODS**

102 **Ethical Approval**

103 All procedures performed in the study were in accordance with the latest revision of the  
104 declaration of Helsinki, and was approved by the Ethical Committee of the Hospital District of  
105 South-Western Finland and National Agency for Medicines. The purpose, potential risks and  
106 nature of the study were fully explained to each participant before their written informed  
107 consent to participate was given.

108

109 **Participants**

110 Thirty recreationally active healthy males (age:  $33 \pm 8$  yrs; body mass:  $80.9 \pm 9.5$  kg;  
111 height:  $183.9 \pm 4.7$  cm; percentage body fat:  $12.9 \pm 5.3\%$ ;  $\dot{V}O_{2peak}$ :  $47.4 \pm 8.1$  mL·kg<sup>-1</sup>·min<sup>-1</sup>;  
112 peak power output on cycle ergometer (PPO):  $343 \pm 45$  W; means  $\pm$  standard deviation)  
113 volunteered to participate in this study. The participants were asked to abstain from alcohol  
114 and caffeine containing beverages for at least 24 h before the commencement of the  
115 experiments and asked to avoid strenuous exercise within 48 h of commencing the  
116 experimental protocol. Participants had no history of cardiovascular or neurological disease, or  
117 skeletal muscle abnormality, and were not currently taking any pharmacological medication.  
118 Given the exploratory nature of our study, a formal sample size estimation is not presented.  
119 Our sample of 10 participants per condition was chosen to be representative of the usual  
120 between-subject experiments in this domain (48).

121

122

123

124 ***Study Design***

125 Participants were randomly allocated to one of three conditions: 8°C water immersion,  
126 15°C water immersion, or 22°C water immersion (9, 43) using covariate adaptive  
127 randomization (40), after their first visit to the hospital. A within-subject crossover design was  
128 not permitted due to ethical restrictions concerning radioactive exposure limits and invasive  
129 arterial cannulation. The groups (n = 10) were matched for potentially confounding covariates  
130 which could influence changes in muscle perfusion, namely aerobic fitness ( $\dot{V}O_{2\text{peak}}$ ) and  
131 anthropometric indices (height, body mass, body surface area, muscle mass and thigh skinfold  
132 thickness).

133

134 **Experimental Protocol**

135 Each participant attended the hospital on two separate occasions. On the first visit, the  
136 participants were familiarised with the experimental protocol, had anthropometric  
137 measurements taken, and completed a peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) test. The participant's  
138 height was measured using a stadiometer (KaWe, Asperg, Germany) and body mass was  
139 obtained using electronic scales (Seca 703, Seca, Hamburg, Germany). Limb girths  
140 (circumferences) were then measured using a tape measure (Seca 201, Seca, Hamburg,  
141 Germany) placed around the participant's right mid-thigh, forearm and calf at pre-identified  
142 landmarks (38). These measurements enabled calculation of each participant's estimated  
143 muscle mass (26). Skinfold thickness measures using calipers (HSK BI; Baty International,  
144 West Sussex, U.K.) were then taken at seven body sites (21) to permit calculation of body fat  
145 percentage (%Bfat) (37). Following anthropometric assessments, each participant completed a  
146 maximal incremental cycling protocol on a cycle ergometer (Tunturi Ergometer E85, Tunturi,  
147 Finland) while simultaneous breath by breath ( $\dot{V}O_2$ ) measurements were recorded (Oxycon



148 Mobile, Jaeger, Germany). The cycling protocol commenced at 75 W and was increased 25 W  
149 every 2 min until volitional exhaustion was reached. Peak Power Output (PPO) was derived as  
150 the highest power output attained at this point.  $\dot{V}O_{2peak}$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) was recorded as the  
151 highest 30 s average recorded before volitional exhaustion.

152 On the second visit, each participant arrived at the hospital (0700-0800 h) in a fasted  
153 state and after having consumed 5 mL·kg bodyweight of water two hours prior to their arrival  
154 to help maintain hydration status (2). Each participant ingested a disposable temperature sensor  
155 pill (CorTemp, Human Technologies Inc., Florida, USA) on the evening (before sleeping) prior  
156 to arrival for experimental testing. The participant changed into a pair of shorts, and was fitted  
157 with a chest heart rate telemetry belt (Polar M400, Kempele, Finland) before resting in a semi-  
158 reclined position while laser Doppler probes and skin temperature thermistors were attached to  
159 the body. An anaesthesiologist then cannulated the radial artery under local anaesthesia to  
160 permit tracer administration and blood sampling during PET measurements. After resting semi-  
161 reclined for  $\geq 20$  min, to ensure physiological status was stabilised, baseline thermometry  
162 measures were taken. The skin thermistors were then unattached (laser Doppler probes  
163 remained affixed to skin), and the participant was taken by wheelchair to another room to  
164 undergo simultaneous PET/CT and laser Doppler measures. The participant was then immersed  
165 in a semi-reclined position up to the navel into an inflatable water bath (iSprint, iCool,  
166 Queensland, Australia) for a period of 10 min. The water temperature was pre-set to one of the  
167 three temperatures ( $8.7\pm 0.3^{\circ}\text{C}$ ,  $15.1\pm 0.3^{\circ}\text{C}$ ,  $22.0\pm 0.46^{\circ}\text{C}$ ) using a heating/chiller water system  
168 (Boyu CW Series, Guangdong, China) dependent on the participant's group allocation. The  
169 water temperature was continuously monitored using a skin thermistor (MHF-18050-A, Ellab,  
170 Rodovre, Denmark) to validate the water temperature. Upon completion of the immersion  
171 protocol, the participant's legs were dabbed dry (as not to stimulate blood flow) to enable the  
172 skin thermistors to be re-attached before being returned to the PET/CT room (via wheelchair)

173 to undergo PET and laser Doppler measures (commenced 10 min post-immersion). Our  
174 previous work has shown that CWI-induced (8°C & 22°C) decreases in deep muscle  
175 temperature, limb and cutaneous blood flows are further exacerbated over a 30 min recovery  
176 period following immersion under normal ambient temperatures (14). The 10 min period  
177 following CWI and the final PET and laser Doppler measures would therefore not have  
178 minimised the impact of CWI on these hemodynamic measures.

179 Heart rate, intestinal, skin and muscle temperatures were measured at baseline and after  
180 the post immersion PET/CT scan. Thigh and calf cutaneous blood flow and mean arterial  
181 pressure were measured during each PET/CT scan. Perceived thermal comfort, rated using a  
182 9-point Likert scale (0 = unbearably cold to 9 = very hot; (49), was recorded at baseline and  
183 during immersion.

184

## 185 **Thermometry**

186 Upon arrival at the hospital, the ingestible core temperature sensor pill was immediately  
187 checked for location in the gastrointestinal tract by sipping 100 ml of cold water. If the  
188 temperature varied by <0.1°C, it was deemed that the ingestible sensor pill was sufficiently sited  
189 down the gastrointestinal tract to enable commencement of the experimental protocol (5). The  
190 sensor pill was remotely connected to a data logger worn around the waist of each participant  
191 during resting PET/CT measures and held next to the participant (umbilical level) during  
192 immersion. Local skin temperature was measured at four sites using skin thermistors (MHF-  
193 18050-A, Ellab, Rodovre, Denmark) affixed to the chest, forearm, thigh and calf using tape  
194 (Medipore, 3M). Mean skin temperature was subsequently calculated as a weighted average of  
195 these four measurement sites (34). Thigh muscle temperature was measured via insertion of a  
196 temperature thermistor (13050; Ellab, Rodovre, Denmark). The area of insertion was marked

197 over the muscle belly of the vastus lateralis by measuring half the length between the head of  
198 the femur and the lateral condyle. The depth of probe insertion was then determined by  
199 measuring skinfold thickness with calipers (HSK BI; Baly International, West Sussex, U.K.)  
200 and dividing by two to determine the subcutaneous fat layer. The probe was inserted to a depth  
201 of 3 cm, plus one-half of the skinfold measurement, for the determination of deep (3 cm) muscle  
202 temperature (11). The thermistor was then withdrawn at 1 cm decrements for the determination  
203 of muscle temperature at 2 cm and 1 cm below the subcutaneous layer. Muscle and skin  
204 temperature were recorded using an electronic measuring system (CTF-9004, Ellab, Rodovre,  
205 Denmark).

206

#### 207 **Blood flow measurements and analysis**

208 Radiowater positron emitting tracer [ $^{15}\text{O}$ ]H<sub>2</sub>O was produced using a Cyclone 3  
209 cyclotron (IBA Molecular, Belgium) and a PET/CT scanner (STE General Electric Medical  
210 systems, Milwaukee, USA) was used in three dimensional (3D) mode for image acquisition to  
211 measure muscle perfusion with [ $^{15}\text{O}$ ]H<sub>2</sub>O. A dynamic scan (6 min) was performed 20 seconds  
212 following an intravenous injection of ~455 MBq of [ $^{15}\text{O}$ ]H<sub>2</sub>O with dynamic scanning images  
213 performed in the following frames: 6x5 seconds, 12x10 seconds, 7x30 seconds and 12x10  
214 seconds.

215 Input function was obtained from arterial blood, which was continuously withdrawn  
216 using a pump during scanning (5 ml·min<sup>-1</sup>). Radioactivity concentration in blood was measured  
217 using a two-channel online detector system (Scanditronix, Uppsala, Sweden), cross-calibrated  
218 with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland) and the PET  
219 scanner. Arterial function was pre-processed with a delay correction. Muscle perfusion was  
220 subsequently measured using the 1-tissue compartment model. Data analysis were performed

221 using in-house developed programs (Carimas software, <http://www.turkupetcentre.fi/carimas>).  
222 Muscle perfusion was determined in a blinded fashion by the same individual for the specific  
223 regions of the right quadriceps muscle group, namely the rectus femoris (RF), vastus lateralis  
224 (VL), vastus intermedius (VI) and vastus medialis (VM; Figure 1). Blood pressure and MAP  
225 were recorded using a blood pressure monitor (Apteq AE701f, APTEQ, Finland) during the  
226 final 1 min of each PET scan.

227 Red blood cell flux was used as an index of skin blood flow using laser Doppler  
228 flowmetry (Periflux System 5001; Perimed Instruments, Jarfalla, Sweden). An integrated laser  
229 Doppler probe (Probe 455; Perimed, Suffolk, U.K) was positioned on the right anterior thigh  
230 halfway between the inguinal line and the patella, and on the calf in the region of the largest  
231 circumference. The probes remained in situ on the skin throughout the testing period.  
232 Cutaneous vascular conductance (CVC) was calculated as the ratio of laser Doppler flux to  
233 MAP. The data were transformed with natural logarithm using %CVC baseline and post-  
234 immersion data and expressed as percentage change from baseline values.

235

## 236 **Statistical Analysis**

237 We employed an ANCOVA model with the change score (post immersion minus  
238 baseline) as the dependent variable and baseline value as the covariate to control for any  
239 between-group imbalances (44). The least significant difference (LSD) test was used for post-  
240 hoc pairwise comparisons of the fixed effects. This ANCOVA model was used to examine the  
241 fixed effect of CWI Condition (8°C, 15°C, 22°C) under resting conditions on global muscle  
242 perfusion and skin blood flow (i.e., our primary outcomes measures), MAP, heart rate,  
243 intestinal temperature, mean and thigh skin temperature, muscle temperature, and thermal  
244 comfort (secondary outcomes measures). Following this, we employed an ANCOVA model,

245 again with the change score as the dependent variable and baseline as a covariate, and examined  
246 the fixed effect of CWI Condition (8°C, 15°C, 22°C) on muscle perfusion in each individual  
247 quadriceps muscle group (Muscle: rectus femoris (RF), vastus lateralis (VL), vastus  
248 intermedius (VI), vastus medialis (VM)). This model also assessed Condition\*Muscle group  
249 interactions. The same ANCOVA model assessed the fixed effect of Depth (3 cm, 2 cm, 1 cm)  
250 and Condition\*Depth interactions on muscle temperature. The LSD test was used for all post-  
251 hoc pairwise comparisons of the fixed effects and interactions.

252 For muscle perfusion, the fixed effects of CWI Condition, Muscle, and CWI  
253 Condition\*Muscle interactions, were assessed for clinical relevance against a minimal  
254 clinically important difference (MCID) of 0.75 mL·100g·min<sup>-1</sup>. This value was based on the  
255 comparable reduction of resting muscle perfusion with nitric oxide synthase inhibition (17).  
256 Changes in skin blood flow were assessed against an MCID of a 19% CVC reduction. This  
257 value was based on our previous work (27, 28, 29), with a ~6°C decrease in skin temperature  
258 after 22°C lower body cooling causing a reduction in thigh %CVC by ~19%. For our primary  
259 outcome measures (muscle perfusion and skin blood flow), statistical inference was then based  
260 on the disposition of the lower limit of the 95% confidence interval (95% CI) for the ANCOVA  
261 adjusted mean differences to our MCID's, with differences deemed clinically relevant when  
262 the lower confidence interval was equal to or exceeded the MCID. Differences not reaching  
263 this threshold were declared not clinically relevant. *P* values are also presented but not  
264 interpreted, as the *p*-value does not measure the size of an effect nor the practical importance  
265 of a result (13, 45). Interpretation of our cardiovascular and thermoregulatory responses  
266 (secondary outcome measures) were based on non-overlapping of 95% CI's for the ANCOVA  
267 adjusted change scores, with non-overlap of the CI's constituting a clear difference. Here, we  
268 purposefully placed less inferential emphasis on our secondary outcomes as these data were  
269 provided to describe the differential cardiovascular and thermoregulatory response of the lower

270 body cooling. Jamovi statistical software, version 0.9.2.8 (<https://www.jamovi.org>) was used  
271 for all statistical analysis. Data in the text are presented as means and 95% CI.

272

## 273 **RESULTS**

### 274 **Muscle Perfusion**

275 Baseline and post-immersion muscle perfusion and temperature data (absolute values)  
276 are included in Table 1. The change in global quadriceps muscle perfusion was not clinically  
277 relevant in any CWI condition when compared to the  $0.75 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$  MCID ( $p = 0.233$ ;  
278 Figure 2). The differences in global quadriceps muscle perfusion between cooling conditions  
279 also failed to reach clinical relevance ( $p = 0.174$  to  $0.791$ ; Figure 2).

280 The change in muscle perfusion in VI compared to VL and RF was clinically relevant  
281 (Figure 3A). The CWI Condition\*Muscle interactions also revealed a clinically relevant  
282 increase in VI muscle perfusion after immersion at  $8^{\circ}\text{C}$  ( $2.15 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ;  $1.28$  to  $3.02$   
283  $\text{mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ) and a decrease in RF muscle perfusion at  $15^{\circ}\text{C}$  ( $-1.61 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ;  $-2.47$  to  
284  $-0.75 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ , Figure 3B), respectively. In the  $8^{\circ}\text{C}$  group, clinically relevant differences  
285 in muscle perfusion were found between the VI and RF ( $3.1 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ;  $1.9$  to  $4.4$   
286  $\text{mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ,  $p < 0.001$ ) and VI and VL ( $3.5 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ;  $2.3$  to  $4.7 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ,  
287  $p < 0.001$ ). Similarly, after  $15^{\circ}\text{C}$  CWI, clinically relevant differences in muscle perfusion were  
288 found between the VI and RF ( $2.4 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ;  $1.1$  to  $3.6 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ,  $p < 0.001$ ) and VI  
289 and VL ( $2.2 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ;  $1.0$  to  $3.5 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ,  $p < 0.001$ ; Figure 3B). The change in  
290 muscle perfusion in the VI was greater after  $8^{\circ}\text{C}$  CWI when compared to  $22^{\circ}\text{C}$  ( $2.3$   
291  $\text{mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ;  $1.1$  to  $3.5 \text{ mL}\cdot 100\text{g}\cdot \text{min}^{-1}$ ,  $p < 0.001$ ). All other differences in muscle perfusion  
292 between individual muscles effects did not reach clinical relevance, with the differences

293 ranging from 0.1 mL·100g·min<sup>-1</sup> (95% CI, -1.2 to 1.1 mL·100g·min<sup>-1</sup>,  $p=0.937$ ) to 1.8  
294 mL·100g·min<sup>-1</sup> (0.7 to 3.0 mL·100g·min<sup>-1</sup>,  $p=0.003$ ).

295

## 296 **Skin Blood Flow**

297         There was a clinically relevant reduction in CVC at the thigh (Figure 4A) and calf  
298 (Figure 4B) in each cooling condition. However, there were no clinically relevant between-  
299 condition differences in CVC at either site (Figure 4C & 4D).

300

## 301 **Thermoregulatory and Cardiovascular Responses**

### 302 *Muscle Temperature*

303         There were clear differences in the changes in muscle temperature for the fixed effect  
304 of Depth, with greater muscle temperature decreases at 1 cm and 2 cm depths compared with  
305 3 cm (Figure 5A). At a depth of 1 cm, a clear difference in the change in muscle temperature  
306 was observed in the 8°C and 15°C conditions compared with 22°C (Figure 5B). However, there  
307 were no clear differences in the change in muscle temperature between conditions at depths of  
308 2 cm or 3 cm (Figure 5C & 5D).

309

### 310 *Intestinal and Skin Temperature*

311         There were no clear differences in intestinal temperature between conditions (Figure  
312 6A). A clear difference in mean skin temperature was observed in the 8°C condition compared  
313 with 22°C (Figure 6A). A clear difference in local thigh skin temperature was also found in the  
314 8°C and 15°C conditions compared with 22°C (Figure 6A).

315

### 316 *Thermal Comfort*

317 A clear difference was observed in thermal comfort ratings between the 8°C and 22°C  
318 conditions (Figure 6B).

319

### 320 *Mean Arterial Pressure and Heart Rate*

321 There were no clear differences observed for either MAP or heart rate responses  
322 between conditions (Figure 6C).

323

## 324 **DISCUSSION**

325 We show for the first time that CWI temperatures between 8°C and 22°C did not reduce  
326 global quadriceps muscle perfusion beyond a clinically relevant threshold. However, the  
327 change in muscle perfusion was not uniform across the individual muscles of the quadriceps.  
328 A clinically relevant increase in muscle perfusion was observed in the deeper vastus  
329 intermedius (VI) in the 8°C group, while muscle perfusion decreased in the more superficial  
330 rectus femoris (RF) muscle after 15°C. Taken together, our findings provide new insights  
331 regarding the influence of CWI on quadriceps femoris muscle perfusion.

332 Muscle perfusion responses to local and whole-body heating have previously been  
333 investigated (16), but this is the first study to quantitatively determine lower limb muscle  
334 perfusion responses to cooling. The observation of similar changes in global quadriceps muscle  
335 perfusion ( $<0.75 \text{ mL}\cdot 100\text{g}\cdot\text{min}^{-1}$ ), from baseline, and between CWI trials (see Figure 2)  
336 contrasts with previous work from our laboratory (14) and others that assessed forearm blood  
337 flow (4) under resting conditions. Using simultaneous Doppler ultrasound and cutaneous blood  
338 flow measurements, to provide indirect estimates of muscle perfusion, we reported that total



339 leg blood flow decreased after both 8 and 22°C CWI with greater blood flow reductions in the  
340 colder water. The contrast of the present study's findings with our previous work most likely  
341 relate to the methods used to index muscle perfusion. Nonetheless, our current observations  
342 are partly in agreement with other previous studies, which have qualitatively examined the limb  
343 blood volume/flow response to different CWI temperatures after exercise using various  
344 measurement techniques (9, 27, 29). In line with the current investigation, these studies  
345 reported similar reductions in limb blood flow/volume (clinical relevance not determined)  
346 between the different cooling conditions (range: 8 to 22°C).

347         Skin blood flow also contributes to total limb blood flow and was consistently reduced  
348 in all experimental conditions in the present study. Indeed, our novel findings demonstrate that  
349 cold-induced reductions in limb blood flow are likely mediated through reduced flow to the  
350 skin, superficial skeletal muscles and other tissues (i.e., subcutaneous fat). Under resting  
351 conditions, we have previously reported (14) a higher cutaneous blood flow response to  
352 noxious (8°C) versus non-noxious (22°C) cooling despite lower skin temperatures at 8°C. We  
353 speculated that this higher cutaneous blood flow response may have been due to the occurrence  
354 of cold-induced vasodilation, which could have potentially redistributed blood from the  
355 underlying muscle. In the present study, the graded decrease in skin blood flow between the  
356 cold (8°C-15°C) and cool (22°C) conditions provided no evidence of cold-induced vasodilation  
357 (Figure 4A & B). The discrepancy with our present findings may be related to our experimental  
358 design, with the group design (and selected measurement time points) utilised in this study  
359 potentially masking the identification of any cold-induced vasodilation due to the inter-  
360 individual nature of skin blood flow responses (33).

361         Despite not finding a change in global muscle perfusion after cooling, we observed a  
362 directionally different muscle perfusion response in the deep VI muscle compared with the  
363 superficial VL and RF muscles (see Figure 3A). The differences in the changes in perfusion

364 between these individual muscles were only evident with exposure to the colder water  
365 temperatures (8°C-15°C; see Figure 3B). The 8°C water also induced a clinically relevant  
366 increase in VI muscle perfusion compared with 22°C cooling (see Figure 3B). Our findings  
367 suggest that colder water temperatures modulate specific muscle perfusion responses across  
368 individual quadriceps muscles. Indeed, a spatially and heterogeneous distribution of quadriceps  
369 muscle perfusion has previously been reported at rest and after exercise (24). The observation  
370 of greater perfusion in the VI under these conditions were thought to be related to the higher  
371 proportion of slow oxidative fibres within this muscle. In addition, our findings also support  
372 the observation of greater muscle perfusion within deeper centrally located upper body skeletal  
373 muscles during cold exposure (8, 42). Therefore, our novel findings subsequently extend  
374 previous observations (8, 42) to support the view that in response to relatively intense cold  
375 exposure (8°C-15°C), deep muscle perfusion is also elevated in the lower body.

376         The deep lying VI muscle, located next to the femoral bone, has a higher proportion of  
377 type 1 fibres in comparison to the three other superficial muscles in the quadriceps (23). It may  
378 be speculated that shivering was responsible for the increase in VI muscle perfusion in the  
379 colder water, since burst shivering rates have been related to differences in muscle fiber  
380 compositions between individuals (7), with low intensity shivering in particular associated with  
381 type 1 fibers (15, 30). It has been proposed that this benign shivering response begins from  
382 deep muscles to maintain core temperature (8). Slight twitching of muscle fibers stimulates  
383 metabolism and oxygen consumption, with more blood supply in the form of blood flow needed  
384 to meet the increased metabolic demands (1, 22, 32) of the largely type I muscle fibers (10, 23).  
385 Nevertheless, it is difficult to ascertain with certainty that the increase in VI muscle perfusion  
386 in the 8°C condition was related to shivering thermogenesis since responses were not  
387 objectively measured. Surface electromyography (EMG) cannot be used to assess the shivering  
388 contribution in deeper muscles and limits interpretation of surface EMG signals in superficial

389 muscles which are in close proximity to each other (3). The use of EMG would, however, have  
390 provided an indication of the degree of shivering in superficial muscles and therefore the  
391 absence of EMG measures represents a study limitation. Blondin *et al's.*, (8) seminal work  
392 indicated that EMG measures of shivering are strongly associated with PET measures of  
393 fludeoxyglucose (<sup>18</sup>FDG) uptake in superficial muscle. Future work may consider  
394 extrapolating this method to determine the relationship between the shivering and perfusion  
395 response in superficial and deeper muscles in response to cooling to confirm our present  
396 findings.

397         In the present study, the generally lower magnitude of muscle temperature reduction in  
398 the deeper tissue (3 cm depth; see Figure 5A) was associated with higher muscle perfusion in  
399 the VI compared with the RF and VL muscles across the conditions. This finding suggests that  
400 after cooling the legs with CWI (independent of water temperature), perfusion in the deeper  
401 and superficial muscle tissue does not respond in a similar manner to reductions in muscle  
402 temperature across the quadriceps musculature. Another key finding was the greater increase  
403 in VI muscle perfusion in the colder water (8°C) compared with 22°C immersion. This  
404 difference in muscle perfusion was evident despite similar changes in deep muscle  
405 temperatures (2 & 3 cm) across the conditions (Figure 5B & C). It would perhaps be expected  
406 that a difference in muscle temperature of sufficient magnitude would be required to modify  
407 the observed perfusion response between the cooling conditions (4, 29). However, it must be  
408 noted that muscle temperature was only measured at different depths within the VL muscle and  
409 therefore does not necessarily represent tissue temperature changes within other quadriceps  
410 muscles, in particular the deeper muscles (i.e., VI muscle).

411         Cryotherapy is widely administered in clinical and applied sport settings in the acute  
412 treatment of soft tissue injuries and exercise induced muscle damage. It is proposed that a  
413 cooling induced reduction in muscle perfusion may limit infiltration of leucocytes,

414 macrophages and other pro-inflammatory cells to better preserve cellular oxygen supply, which  
415 may be otherwise compromised by local swelling, oedema and capillary constriction (39, 41,  
416 46). This may limit hypoxic cell death and damage and minimize secondary tissue damage (31,  
417 41, 46). We demonstrate for the first time, that 10 min of lower body CWI, can lead to a  
418 clinically relevant reduction in muscle perfusion in superficial areas of the quadriceps femoris  
419 muscle. This reduction appears to be dependent on water temperature with the decline in RF  
420 muscle perfusion observed in 15°C water (Figure 3B). Nevertheless, in contrast to deep  
421 muscle(s), there was a trend for perfusion to decrease in the three superficial muscles (RF, VL  
422 and VM) across all experimental conditions. Since superficial muscles still contribute to a large  
423 part of the bulk skeletal muscle mass, our findings suggest that cold-induced reductions in  
424 superficial perfusion and skin blood flow play an important role in mediating reductions in  
425 total limb blood flow previously reported (9, 14, 27, 28, 29, 43). Taken together, our data  
426 indicates that a less noxious water temperature (15°C) may be the most viable option as a  
427 treatment for soft tissue injury by promoting a clinically relevant decrease in superficial muscle  
428 perfusion whilst minimising increases in deep (VI) muscle perfusion (Figure 3B). Moreover,  
429 the increase in deep muscle perfusion (VI) in the 8°C condition suggests that more noxious  
430 CWI cooling may potentially accentuate the inflammatory response in deeper tissues. This  
431 inference, however, warrants further investigation.

432         Our experimental design, using CWI as the cooling stimulus, was used to simulate real-  
433 world practice (construct validity), which required the logistics of moving participants from  
434 the bed/cold water bath to the PET scan room to undertake muscle blood perfusion  
435 measurements. We therefore used a wheelchair to move the participants from either location  
436 to try and control any muscle activation and limit any confounding of perfusion measurements.  
437 Whilst we endeavoured to limit any unnecessary muscle activation, it is important to note that  
438 participants briefly had to stand out of the wheelchair to position themselves onto the PET

439 scanner in a supine position. However, there was a 10 min period prior to commencing PET  
440 scans after lying supine, which is likely to have limited any potential confounding of muscle  
441 perfusion. Indeed, another limitation of the present study was that PET scan perfusion measures  
442 were only measured at one time point after cooling. We have documented (14, 27, 29)  
443 prolonged decreases in deep muscle temperatures during extended post cooling periods (30  
444 min) due to sustained tissue heat loss via thermal conduction. In addition, the magnitude of this  
445 deep muscle temperature decrease is related to the CWI water temperature (14, 27, 29).  
446 Therefore, if tissue temperature change is of sufficient magnitude to modify muscle perfusion  
447 *per se*, it is possible that a greater change in muscle perfusion may have been observed over a  
448 longer duration post-cooling.

449         The semi-reclined immersion protocol utilized in this study is only one of several that  
450 can be chosen, for example, CWI protocols can be undertaken at a variety of depths (navel,  
451 chest, neck), positions (seated or standing), temperatures, and/or durations. In the current  
452 protocol, the hydrostatic pressure acting on the legs (whilst seated) was minimal, due to the  
453 pressure that acts on a body part being dependent on its depth in the water (46). However,  
454 changes in central hemodynamic responses and muscle perfusion associated with hydrostatic  
455 pressure will need to be accounted for when adopting greater water depths. Additionally, CWI  
456 is often used immediately after intense or muscle damaging exercise (47), when tissue  
457 temperature, and skin and muscle blood flow, are elevated. It remains to be elucidated if any  
458 potential differences in muscle perfusion would be noted when CWI is applied under these  
459 conditions. Therefore, there is greater scope for work in this area by utilizing different cooling  
460 protocols and examining perfusion responses across different muscle groups at rest and after  
461 exercise.

462         In summary, we used PET and [<sup>15</sup>O]H<sub>2</sub>O to quantitatively measure muscle perfusion in  
463 the quadriceps muscle after different degrees of CWI cooling. CWI (8-22°C) did not reduce

464 global quadriceps muscle perfusion to a clinically relevant extent, however, the muscle  
465 perfusion response to cooling was not uniform across the individual muscles composing the  
466 quadriceps. Our findings suggest that colder-water (8°C) increases deep muscle perfusion,  
467 while 15°C water reduces superficial muscle (RF) perfusion in the quadriceps muscle.  
468 Therefore, a less noxious water temperature (15°C) may be considered a viable option as a  
469 treatment for soft tissue injury.

470

## 471 **ADDITIONAL INFORMATION**

### 472 **Conflict of Interest**

473 The authors declare no conflict of interest.

### 474 **Author Contributions**

475 WG, NTC, DAL, HJ, IH, JK and KKK conceived and designed the study. CM and IH were  
476 responsible for all data collection. JK was the responsible physician of the study and AK was  
477 responsible for the radiotracer production. MW and CM performed the statistical analysis. CM,  
478 IH, WG, DAL, HJ and MW contributed to writing the paper. IH, CH, KKK, AK and JK  
479 provided expertise for data acquisition for and from PET scans. CH, IH, KKK and CM  
480 performed PET scan analysis. All authors have approved the final version of this manuscript.

481

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489

490

491 **References**

492

493 1. **Alexander G, Bell AW, Setchell BP.** Regional distribution of cardiac output in young  
494 lambs: effect of cold exposure and treatment with catecholamines. *J Physiol* 220: 511-  
495 528, 1972.

496

497 2. **American College of Sports Medicine, Sawka MN, Burke LM, Eichner ER,**  
498 **Maughan RJ, Montain SJ, Stachenfeld NS.** American College of Sports Medicine  
499 position stand. Exercise and fluid replacement. *Med Sci Sports Exerc* 39: 377-390, 2007.

500

501 3. **Arnold JT, Hemsley Z, Hodder SG, Havenith G, Lloyd LB.** Reliability and validity  
502 of methods in the assessment of cold-induced shivering thermogenesis. *Eur J Appl*  
503 *Physiol* 120: 591-601, 2020.

504

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

507

- 508       **5. Barr D, Gregson W, Sutton L, Reilly T.** A practical cooling strategy for reducing the  
509           physiological strain associated with firefighting activity in the heat. *Ergonomics* 52:  
510           413-420, 2009.
- 511
- 512       **6. Bleakley CM, Davison GW.** What is the biochemical and physiological rationale for  
513           using cold-water immersion in sports recovery? A systematic review. *Br J Sports Med*  
514           44: 179-187, 2010.
- 515
- 516       **7. Blondin DP, Frisch F, Phoenix S, Guerin B, Turcotte EE, Haman F, Richard D,**  
517           **Carpentier AC.** Inhibition of Intracellular Triglyceride Lipolysis Suppresses Cold-  
518           Induced Brown Adipose Tissue Metabolism and Increases Shivering in Humans. *Cell*  
519           *Metab* 25: 438-447, 2017.
- 520
- 521       **8. Blondin DP, Labbe SM, Phoenix S, Guerin B, Turcotte EE, Richard D, Carpentier**  
522           **AC, Haman F.** Contributions of white and brown adipose tissues and skeletal muscles  
523           to acute cold-induced metabolic responses in healthy men. *J Physiol* 593: 701-714,  
524           2015.
- 525
- 526       **9. Choo HC, Nosaka K, Peiffer JJ, Ihsan M, Yeo CC, Abbiss CR.** Peripheral blood flow  
527           changes in response to postexercise cold water immersion. *Clin Physiol Funct Imaging*  
528           38: 46-55, 2018.
- 529
- 530       **10. Edgerton VR, Smith JL, Simpson DR.** Muscle fibre type populations of human leg  
531           muscles. *Histochem J* 7: 259-266, 1975.



532

533 11. **Enwemeka CS, Allen C, Avila P, Bina J, Konrade J, Munns S.** Soft tissue  
534 thermodynamics before, during, and after cold pack therapy. *Med Sci Sports Exerc* 34,  
535 45-50: 2002.

536

537 12. **Fiscus KA, Kaminski TW, Powers ME.** Changes in lower-leg blood flow during  
538 warm-, cold-, and contrast-water therapy. *Arch Phys Med Rehabil* 86: 1404-1410, 2005.

539

540 13. **Greenland S, Senn SJ, Rothman KJ, Carlin JB, Poole C, Goodman SN, Altman**  
541 **DG.** Statistical tests, P values, confidence intervals, and power: a guide to  
542 misinterpretations. *Eur J Epidemiol* 31: 337-350, 2016.

543

544 14. **Gregson W, Black MA, Jones H, Milson J, Morton J, Dawson B, Atkinson G,**  
545 **Green DJ.** Influence of cold water immersion on limb and cutaneous blood flow at rest.  
546 *Am J Sports Med* 39: 1316-1323, 2011.

547

548 15. **Haman F, Blondin DP.** Shivering thermogenesis in humans: Origin, contribution and  
549 metabolic requirement. *Temperature (Austin)* 4: 217-226, 2017.

550

551 16. **Heinonen I, Brothers RM, Kemppainen J, Knuuti J, Kalliokoski KK, Crandall**  
552 **CG.** Local heating, but not indirect whole body heating, increases human skeletal  
553 muscle blood flow. *J Appl Physiol (1985)* 111: 818-824, 2011.

554

- 555 17. **Heinonen I, Saltin B, Kemppainen J, Sipila HT, Oikonen V, Nuutila P, Knuuti J,**  
556 **Kalliokoski K, Hellsten Y.** Skeletal muscle blood flow and oxygen uptake at rest and  
557 during exercise in humans: a pet study with nitric oxide and cyclooxygenase inhibition.  
558 *Am J Physiol Heart Circ Physiol* 300: H1510-1517, 2011.
- 559
- 560 18. **Ihsan M, Watson G, Abbiss CR.** What are the Physiological Mechanisms for Post-  
561 Exercise Cold Water Immersion in the Recovery from Prolonged Endurance and  
562 Intermittent Exercise? *Sports Med* 46: 1095-1109, 2016.
- 563
- 564 19. **Ihsan M, Watson G, Lipski M, Abbiss CR.** Influence of postexercise cooling on  
565 muscle oxygenation and blood volume changes. *Med Sci Sports Exerc* 45: 876-882,  
566 2013.
- 567
- 568 20. **Iida H, Kanno I, Miura S, Murakami M, Takahashi K, Uemura K.** Error analysis  
569 of a quantitative cerebral blood flow measurement using H<sub>2</sub>(<sup>15</sup>O) autoradiography and  
570 positron emission tomography, with respect to the dispersion of the input function. *J*  
571 *Cereb Blood Flow Metab* 6: 536-545, 1986.
- 572
- 573 21. **Jackson AS, Pollock ML, Gettman LR.** Intertester reliability of selected skinfold and  
574 circumference measurements and percent fat estimates. *Res Q* 49: 546-551, 1978.
- 575
- 576 22. **Jansky L, Hart JS.** Cardiac output and organ blood flow in warm- and cold-acclimated  
577 rats exposed to cold. *Can J Physiol Pharmacol* 46: 653-659, 1968.
- 578

- 579 23. **Johnson MA, Polgar J, Weightman D, Appleton D.** Data on the distribution of fibre  
580 types in thirty-six human muscles. An autopsy study. *J Neurol Sci* 18: 111-129, 1973.  
581
- 582 24. **Kalliokoski KK, Kemppainen J, Larmola K, Takala TO, Peltoniemi P, Oksanen**  
583 **A, Ruotsalainen U, Cobelli C, Knuuti J, Nuutila P.** Muscle blood flow and flow  
584 heterogeneity during exercise studied with positron emission tomography in humans.  
585 *Eur J Appl Physiol* 83: 395-401, 2000.  
586
- 587 25. **Leeder J, Gissane C, van Someren K, Gregson W, Howatson G.** Cold water  
588 immersion and recovery from strenuous exercise: a meta-analysis. *Br J Sports Med* 46:  
589 233-240, 2012.  
590  
591
- 592 26. **Martin AD, Spenst LF, Drinkwater DT, Clarys JP.** Anthropometric estimation of  
593 muscle mass in men. *Med Sci Sports Exerc* 22: 729-733, 1990.  
594
- 595 27. **Mawhinney C, Jones H, Joo CH, Low DA, Green DJ, Gregson W.** Influence of  
596 cold-water immersion on limb and cutaneous blood flow after exercise. *Med Sci Sports*  
597 *Exerc* 45: 2277-2285, 2013.  
598
- 599 28. **Mawhinney C, Jones H, Low DA, Green DJ, Howatson G, Gregson W.** Influence  
600 of cold-water immersion on limb blood flow after resistance exercise. *Eur J Sport Sci*  
601 17: 519-529, 2017.  
602

- 603 29. **Mawhinney C, Low DA, Jones H, Green DJ, Costello JT, Gregson W.** Cold Water  
604 Mediates Greater Reductions in Limb Blood Flow than Whole Body Cryotherapy. *Med*  
605 *Sci Sports Exerc* 49: 1252-1260, 2017.
- 606
- 607 30. **Meigal A.** Gross and fine neuromuscular performance at cold shivering. *Int J*  
608 *Circumpolar Health* 61: 163-172, 2002.
- 609
- 610 31. **Merrick MA, McBrier NM.** Progression of secondary injury after musculoskeletal  
611 trauma-a window of opportunity? *J Sport Rehabil* 19: 380-388, 2010.
- 612
- 613 32. **Murrant CL, Sarelus IH.** Local and remote arteriolar dilations initiated by skeletal  
614 muscle contraction. *Am J Physiol Heart Circ Physiol* 279: H2285-2294, 2000.
- 615
- 616 33. **Petrofsky JS.** Resting blood flow in the skin: does it exist, and what is the influence of  
617 temperature, aging, and diabetes? *J Diabetes Sci Technol* 6: 674-685, 2012.
- 618
- 619 34. **Ramanathan NL.** A new weighting system for mean surface temperature of the human  
620 body. *J Appl Physiol* 19: 531-533, 1964.
- 621
- 622 35. **Rudroff T, Weissman JA, Bucci M, Seppanen M, Kaskinoro K, Heinonen I,**  
623 **Kalliokoski KK.** Positron emission tomography detects greater blood flow and less  
624 blood flow heterogeneity in the exercising skeletal muscles of old compared with young  
625 men during fatiguing contractions. *J Physiol* 592: 337-349, 2014.

626

627 36. **Ruotsalainen U, Raitakari M, Nuutila P, Oikonen V, Sipila H, Teras M, Knuuti**

628 **MJ, Bloomfield PM, Iida H.** Quantitative blood flow measurement of skeletal muscle

629 using oxygen-15-water and PET. *J Nucl Med* 38: 314-319, 1997.

630

631 37. **Siri WE.** The gross composition of the body. *Adv Biol Med Phys* 4: 239-280, 1956.

632

633 38. **Stewart A, Marfell-Jones M, Olds T, De Ridder H.** *International Standards for*

634 *Anthropometric Assessment.* The International Society for the Advancement of

635 Kinanthropometry, Potchefstroom, South Africa, 2011.

636

637 39. **Swenson C, Sward L, Karlsson J.** Cryotherapy in sports medicine. *Scand J Med Sci*

638 *Sports* 6: 193-200, 1996.

639

640 40. **Taves DR.** Minimization: a new method of assigning patients to treatment and control

641 groups. *Clin Pharmacol Ther* 15: 443-453, 1974.

642

643 41. **Tipton MJ, Collier N, Massey H, Corbett J, Harper M.** Cold water immersion: kill

644 or cure? *Exp Physiol* 102: 1335-1355, 2017.

645

646 42. **U Din M, Raiko J, Saari T, Kudomi N, Tolvanen T, Oikonen V, Teuvo J, Sipila**

647 **HT, Savisto N, Parkkola R, Nuutila P, Virtanen KA.** Human brown adipose tissue

648 [(15)O]O<sub>2</sub> PET imaging in the presence and absence of cold stimulus. *Eur J Nucl Med*  
649 *Mol Imaging* 43: 1878-1886, 2016.

650

651 43. **Vaile J, O'Hagan C, Stefanovic B, Walker M, Gill N, Askew CD.** Effect of cold  
652 water immersion on repeated cycling performance and limb blood flow. *Br J Sports*  
653 *Med* 45: 825-829, 2011.

654

655 44. **Vickers AJ.** The use of percentage change from baseline as an outcome in a controlled  
656 trial is statistically inefficient: a simulation study. *BMC Med Res Methodol* 1: 6, 2001.

657

658

659 45. **Wasserstein R, Lazar N.** The ASA's Statement on p-Values: Context, Process, and  
660 Purpose. *Am Stat* 70: 129-131, 2016.

661

662 46. **Wilcock IM, Cronin JB, Hing WA.** Physiological response to water immersion: a  
663 method for sport recovery? *Sports Med* 36: 747-765, 2006.

664

665 47. **Wilcock IM, Cronin JB, Hing WA.** Water immersion: does it enhance recovery from  
666 exercise? *Int J Sports Physiol Perform* 1: 195-206, 2006.

667

668 48. **Yeung SS, Ting KH, Hon M, Fung NY, Choi MM, Cheng JC, Yeung EW.** Effects  
669 of Cold Water Immersion on Muscle Oxygenation During Repeated Bouts of Fatiguing  
670 Exercise: A Randomized Controlled Study. *Medicine (Baltimore)* 95: e2455, 2016.

671

672 49. **Young AJ, Sawka MN, Epstein Y, Decristofano B, Pandolf KB.** Cooling different  
673 body surfaces during upper and lower body exercise. *J Appl Physiol (1985)* 63: 1218-  
674 1223, 1987.

675

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685 **Table 1.** Baseline and post immersion absolute values of muscle perfusion and temperature  
686 variables (mean  $\pm$  SD).

687

688 **Figure 1.** Representative cross-sectional computed tomography (CT) image of a participant's  
689 right quadriceps femoris muscle (left). The specified region of interests (ROI) are shown on  
690 the CT image (middle), which were fused with the positron emission tomography (PET) image  
691 to calculate muscle blood flow (right).

692

693 **Figure 2.** The mean  $\Delta$  in global quadriceps muscle perfusion after 8°C, 15°C and 22°C cooling  
694 (mean  $\pm$  95% CI). Clinical relevance was assessed against a minimally clinically important  
695 difference (MCID) in muscle perfusion of  $\pm 0.75$  mL $\cdot$ 100g $\cdot$ min<sup>-1</sup> (shaded area).

696

697 **Figure 3.** The mean difference in muscle perfusion between individual muscles independent  
698 of the cooling condition (A) and the mean  $\Delta$  in perfusion in each quadriceps muscle after 8°C,  
699 15°C and 22°C cooling, respectively (B) (mean  $\pm$  95% CI). Clinical relevance was assessed  
700 against a minimally clinically important difference (MCID) in muscle perfusion of  $\pm 0.75$   
701 mL $\cdot$ 100g $\cdot$ min<sup>-1</sup> (shaded area).

702

703 **Figure 4.** The mean  $\Delta$  in thigh (A) and calf (B) cutaneous vascular conductance (CVC) from  
704 baseline and the mean differences in thigh (C) and calf (D) CVC between the 8°C, 15°C and  
705 22°C conditions, respectively (mean  $\pm$  95% CI). Clinical relevance was assessed against a  
706 minimally clinically important difference (MCID) in CVC of  $\pm 19.0\%$  (shaded area).

707

708 **Figure 5.** The mean  $\Delta$  in muscle temperature for the fixed effect of depth (A) and at 1 cm (B),  
709 2 cm (C) and 3 cm (D) depths in the 8°C, 15°C and 22°C cooling conditions (mean  $\pm$  95% CI).  
710 None overlap of  $\pm 95\%$  CI's represents clear difference between conditions.

711

712 **Figure 6.** Forest plot displaying condition main effects of secondary outcome variables:  
713 temperature (A), subjective measures (B) and cardiovascular measures (C). Symbols represent



714 mean differences: 8°C (●), 15°C (■) and 22°C (▲) ± 95% CI. None overlap of ±95% CI's

715 represents clear difference between conditions.

716