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Citation: Mawhinney, Chris, Jones, Helen, Low, David, Green, Daniel, Howatson, Glyn and Gregson, Warren (2017) Influence of Cold Water Immersion on Limb and Cutaneous Blood Flow after Resistance Exercise. European Journal of Sport Science. ISSN 1746-1391 (In Press)

Published by: Taylor & Francis

URL: <http://dx.doi.org/10.1080/17461391.2017.1279222>
<<http://dx.doi.org/10.1080/17461391.2017.1279222>>

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1 **Influence of Cold Water Immersion on Limb Blood Flow after Resistance**
2 **Exercise**

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41 The research study was undertaken at Liverpool John Moores University
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46 Word Count = 4,152
47

48 **ABSTRACT**

49 This study determined the influence of cold (8°C) and cool (22°C) water immersion
50 on lower limb and cutaneous blood flow following resistance exercise. Twelve
51 males completed 4-sets of 10-repetition maximum squat exercise and were then
52 immersed, semi-reclined, into 8°C or 22°C water for 10-min, or rested in a seated
53 position (control) in a randomized order on different days. Rectal and thigh skin
54 temperature, muscle temperature, thigh and calf skin blood flow and superficial
55 femoral artery blood flow were measured before and after immersion. Indices of
56 vascular conductance were calculated (flux and blood flow/mean arterial pressure).
57 The colder water reduced thigh skin temperature and deep muscle temperature to
58 the greatest extent ($P < 0.001$). Reductions in rectal temperature were similar
59 (0.2°C-0.4°C) in all three trials ($P = 0.69$). Femoral artery conductance was similar
60 after immersion in both cooling conditions, with both conditions significantly lower
61 (55%) than the control post-immersion ($P < 0.01$). Similarly, there was greater thigh
62 and calf cutaneous vasoconstriction (40-50%) after immersion in both cooling
63 conditions, relative to the control ($P < 0.01$), with no difference between cooling
64 conditions. These findings suggest that cold and cool water similarly reduce femoral
65 artery and cutaneous blood flow responses but not muscle temperature following
66 resistance exercise.

67 **Keywords:** blood flow; cooling; muscle damage; inflammation

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73 INTRODUCTION

74 Lower limb cold-water immersion (CWI) is a widely used recovery method
75 to reduce the negative symptoms associated with high-intensity or unaccustomed
76 exercise (Bailey et al., 2007; Leeder, Gissane, van Someren, Gregson & Howatson,
77 2012). Cooling of the exercised muscles is proposed to attenuate acute
78 inflammation, edema and swelling, thereby reducing the development of exercise-
79 induced muscle damage, function and soreness (Smith, 1991). Previous studies have
80 shown that CWI decreases limb muscle temperature and blood flow when applied at
81 rest (Gregson et al., 2011) and following continuous endurance exercise such as
82 cycling (Mawhinney et al., 2013; Vaile et al., 2011) and treadmill running (Ihsan,
83 Watson, Lipski & Abbiss, 2013). The effect of CWI on the physiological and
84 functional responses to resistance type exercise are less well known.

85 Recent research has shown that the chronic application of CWI ($2 \text{ d} \cdot \text{w}^{-1}$ over
86 12 weeks) after resistance exercise reduces resistance training-induced increases in
87 muscle strength and mass compared with an active cool-down due to the blunting of
88 cellular signaling (Roberts et al., 2015b). On the contrary, in the acute period, i.e.
89 hours, after CWI application, increases in muscle function relative to active
90 recovery have been reported (Roberts et al., 2015a). The improved recovery of
91 strength with acute CWI was modulated by muscle temperature and potentially
92 blood flow (muscle oxygenation) (Roberts et al., 2015a). Nevertheless, no study, to
93 date has directly examined the impact of CWI on limb blood flow following an
94 acute bout of resistance exercise. This is important to establish, since resistance
95 exercise can cause a different haemodynamic, thermoregulatory and mechanical
96 stress than endurance exercise. For example, the metabolic cost of muscle
97 contraction is prolonged during activities such as running and cycling, rather than

98 intermittent during resistance exercise, with skeletal muscle blood flow matched to
99 the metabolic demands of the contracting muscle (Joyner & Casey, 2015).
100 Similarly, the intermittent nature and potential for breath holding in resistance
101 exercise contrasts the linear increase and plateau in limb blood flow in endurance
102 exercise (MacDougall et al., 1992; Mortensen, Damsgaard, Dawson, Secher &
103 Gonzalez-Alonso, 2008). It is also possible that resistance exercise does not cause
104 increases in core body temperature of the same magnitude as endurance exercise
105 (Deschenes et al., 1998). A higher core body temperature may increase tissue-
106 cooling rate due to a greater temperature gradient between the body and the water
107 (Stephens, Halson, Miller, Slater & Askew, 2016). Moreover, resistance exercise
108 stimulates greater muscle damage compared with other modes of exercise, such as
109 cycling and running (Dolezal, Potteiger, Jacobsen & Benedict, 2000; Howatson et
110 al., 2012).

111 We have previously shown that CWI of various water temperatures similarly
112 decreases post-cycling lower limb blood flow despite greater reductions in muscle
113 and thigh skin temperatures in colder water (Mawhinney et al., 2013). It is currently
114 unknown if the differences in hemodynamic and temperature responses mediated by
115 resistance, relative to endurance, exercise, would impact upon post-resistance
116 exercise responses to CWI and if different water temperatures of CWI would result
117 in similar or graded decreases in limb blood flow after resistance exercise.
118 Therefore, the aim of this study was to examine the effects of cold (8°C) and cool
119 (22°C) water immersion on lower limb blood flow and muscle temperature changes,
120 after a typical bout of resistance exercise.

121

122 **MATERIALS AND METHODS**

123 **Participants**

124 Twelve recreationally active men who were non-smokers and free from
125 cardiovascular, respiratory and metabolic disease were studied (mean±s: age, 26±6
126 yrs; height, 1.8±0.1 m; mass, 77.5±11.2 kg; 10-repetition maximum (10 RM),
127 50.4±13.4 kg). The participants typically performed resistance exercise at least three
128 times per week and performed squat exercise at least once per week in their training
129 regime (self-report questionnaire). The participants were familiarized with the
130 experimental procedure and associated risks and gave their written informed
131 consent to participate. The study was approved by the Institutional Ethics
132 Committee and conformed to the 1964 Declaration of Helsinki and its later
133 amendments for research using human participants.

134

135 **Experimental Design**

136 Two weeks prior to the commencement of the experimental trials, each participant
137 completed a 10 RM parallel depth squat assessment using a Smith machine
138 (Familiarization 1). The squat protocol consisted of a warm up set, using only the
139 bar, followed by progressive increases in load until the attainment of the 10 RM
140 within five attempts (Baechle & Earle, 2000). The following week, participants
141 completed 4 sets of the predetermined 10 RM squat exercise interspersed with 2
142 min rest periods (Familiarization 2). This second familiarization trial was performed
143 to reduce the magnitude of any subsequent muscle damage and inflammation from
144 the exercise stimulus in the proceeding trials, e.g., reduce an order effect, that might
145 influence blood flow, which is commonly known as the protective repeated bout
146 effect (Howatson & van Someren, 2008).

147 The experimental trials were performed in a randomized counterbalanced
148 order, at least 7-days following the second familiarization session and at least 7-
149 days apart. For each trial, participants arrived at the laboratory at least 3 h
150 postprandial, having refrained from exercise, alcohol, tobacco and caffeine during
151 the previous 24 h and having consumed 5 ml·kg⁻¹ of water 2 h before arrival. All
152 participants recorded their nutritional and fluid intake for 24 h prior to their first
153 experimental trial. This record was photocopied and returned to them to repeat for
154 their remaining trials. All trials were conducted under an ambient temperature of
155 22-24°C to control variability in cutaneous blood flow (Cracowski, Minson, Salvat-
156 Melis & Halliwill, 2006) and at the same time of day in order to avoid the circadian
157 variation in internal body temperature.

158 Each participant was required to complete 4 sets of 10 RM squats followed
159 by a 10 min period of immersion in either 8°C or 22°C water or seated rest
160 (Control). The water temperatures and immersion protocol was based on our
161 previous studies (Gregson et al., 2011; Mawhinney et al., 2013). On arrival, nude
162 body mass (kg) was obtained (Seca, Hamburg, Germany). A rectal probe was self-
163 inserted and a heart rate (HR) monitor was positioned across the chest. Participants
164 then rested supine for 30 min for instrumentation and to stabilize physiological
165 status, wearing training shorts. Following baseline measurements (10 min),
166 participants completed 4 sets of 10 RM squats interspersed with a 2 min rest period
167 between sets. Participants then returned to the supine position for 10 min for post-
168 exercise/pre-immersion measurements. Participants were then raised from the bed in
169 a semi-recline position using an electronic hoist (Bianca, Arjo Ltd, Gloucester,
170 United Kingdom) and either lowered into the water tank (ECB, Gloucester, U.K.) to
171 the iliac crest for 10 min, or remained suspended above the bed (Control). At the

172 end of immersion, participants were returned to the bed using the electronic hoist
173 and remained supine for 30 min. The use of the hoist to raise and lower the
174 participants was important to avoid the effect of muscle activation on blood flow

175 Rectal and skin temperatures, HR and thigh and calf cutaneous blood flow
176 were continuously monitored. Muscle temperature, superficial femoral artery blood
177 flow and mean arterial blood pressure (MAP) were measured at baseline, pre-
178 immersion and during post immersion. At the same time points, both perceived
179 thermal comfort, rated using a 9-point scale (0 = unbearably cold to 9 = very hot)
180 (Young, Sawka, Epstein, Decristofano & Pandolf, 1987) and shivering, rated using a
181 4-point scale (1 = no shivering to 4 = heavy shivering) (Wakabayashi, Hanai,
182 Yokoyama & Nomura, 2006) were recorded.

183

184 **Measurements**

185 *Rectal, Thigh, Skin, and Muscle Temperatures*

186 A rectal probe (Rectal probe (adult), Ellab UK, Norwich, England) was
187 inserted 15 cm beyond the anal sphincter for the assessment of rectal temperature.
188 Skin thermistors (Surface temperature probe (stationary), Ellab UK, Norwich,
189 England) were attached to the chest, forearm, upper thigh, and calf for the
190 assessment of local and mean skin temperature (Ramanathan, 1964). Muscle
191 temperature was assessed using a needle thermistor inserted into the vastus lateralis
192 (Multi-purpose needle probe, Ellab UK, Norwich, England). Thigh skinfold
193 thickness was measured using Harpenden skinfold calipers (HSK BI, Baty
194 International, West Sussex, United Kingdom) and divided by 2 to determine the
195 thickness of the thigh subcutaneous fat layer over the vastus lateralis (Enwemeka, et
196 al., 2002). The needle thermistor was inserted at a depth of 3 cm plus one-half the

197 skinfold measurement for determination of deep muscle temperature (3 cm). The
198 thermistor was then withdrawn at 1 cm increments for determination of muscle
199 temperature at 2 cm and 1 cm below the subcutaneous layer. Rectal, skin and
200 muscle temperatures were recorded using an electronic measuring system (E-Val
201 Flex, TMN9616, Ellab UK, Norwich, England).

202

203 *Heart Rate and Arterial Blood Pressure*

204 HR was continuously measured using short-range telemetry (S610; Polar
205 Electro Oy, Kempele, Finland). Arterial blood pressure was measured via
206 automated brachial auscultation (Dinamap, GE Pro 300V2, Tampa, Florida, USA),
207 and MAP was calculated as $[\text{Diastolic} + (0.333 \times (\text{Systolic} - \text{Diastolic}))]$.

208

209 *Femoral Artery Blood Flow*

210 A 15 MHz multi-frequency linear array transducer attached to a high-
211 resolution ultrasound machine (Acuson P50, Siemens, Germany) was used to
212 measure femoral artery diameter and velocity. Images were taken at the superficial
213 femoral artery in the proximal third of the left leg approximately 3 cm distal to the
214 bifurcation. This position was marked on the skin for ultrasound head repositioning
215 during repeated measures. Ultrasound parameters were set to optimize longitudinal
216 B-mode images of the lumen/arterial wall interface. Continuous and synchronized
217 pulsed wave Doppler velocities were also obtained. Data were collected using an
218 insonation angle of 60° and each measurement was recorded for 2 min. Analysis of
219 blood flow velocity and diameter was performed using custom designed edge-
220 detection and wall-tracking software (Green, Cheetham, Reed, Dembo &
221 O'Driscoll, 2002; Thijssen et al., 2011; Woodman et al., 2001). Blood flow was

222 calculated as the product of cross-sectional area and blood flow velocity. Resting
223 diameter, blood flow velocity and blood flow were sampled as the mean of a 20 s
224 period of each 2 min image. Femoral vascular conductance was calculated as the
225 ratio of blood flow/MAP.

226

227 *Cutaneous Blood Flow*

228 Red blood cell flux was used as an index of skin blood flow via laser
229 Doppler flowmetry (Periflux System 5001, Perimed Instruments, Jarfalla, Sweden).
230 An integrated laser Doppler probe (Probe 413, Perimed, Suffolk, United Kingdom)
231 was attached to the mid-anterior thigh halfway between the inguinal line and the
232 patella, and on the calf in the region of the largest circumference. Once affixed, the
233 probes were not removed until the completion of each trial. Cutaneous vascular
234 conductance was calculated as the ratio of laser Doppler flux to MAP (cutaneous
235 vascular conductance = laser Doppler flux/MAP x 100) and expressed as a
236 percentage change from pre immersion values. Thigh and calf skin conductance are
237 expressed as percentage change from pre immersion (zero)

238

239 **Statistical Analysis**

240 It was estimated that a sample size of at least 6 participants would have 90%
241 power to detect a $175 \text{ ml}\cdot\text{min}^{-1}$ reduction in femoral artery blood flow following 10
242 min of cool (22°C) water immersion, using a standard deviation of the differences
243 of $99 \text{ ml}\cdot\text{min}^{-1}$ (Mawhinney et al., 2013). A two-factor (condition x time) general
244 linear model (GLM) was used to evaluate treatment differences between the 8°C ,
245 22°C and control conditions. A three-way GLM (condition x depth x time) was
246 employed to analyse muscle temperature. Significant main effects and interactions

247 were followed up using multiple comparisons (Student-Newman-Keuls). The α
248 level for evaluation of statistical significance was set at $P < 0.05$ and were analysed
249 using Statistical Package for the Social Sciences (Chicago, IL). All data are
250 presented as mean \pm s.

251

252 **RESULTS**

253 *Thermoregulatory responses*

254 Exercise elicited an increase in rectal temperature (8°C; $\Delta 0.3\pm 0.2^\circ\text{C}$; 22°C;
255 $\Delta 0.2\pm 0.1^\circ\text{C}$; control; $0.3\pm 0.1^\circ\text{C}$; $P < 0.001$) but rectal temperature was not different
256 between conditions ($P > 0.05$; Figure 1a). Rectal temperature decreased over the
257 post immersion recovery period ($P < 0.001$) with no difference observed between
258 conditions ($P = 0.19$; Figure. 1a).

259 Exercise elicited an increase in thigh (8°C; $\Delta 0.4\pm 0.6^\circ\text{C}$; 22°C; $\Delta 0.8\pm 0.6^\circ\text{C}$;
260 control; $\Delta 0.6\pm 0.8^\circ\text{C}$; $P = 0.002$) and mean skin temperature (8°C; $\Delta 0.3\pm 0.2^\circ\text{C}$;
261 22°C; $\Delta 0.2\pm 0.1^\circ\text{C}$; control; $0.3\pm 0.1^\circ\text{C}$; $P < 0.001$) but skin temperatures were not
262 different between conditions ($P > 0.05$; Figure. 1). The colder water reduced local
263 thigh and mean skin temperatures to a greater extent compared to 22°C throughout
264 post-immersion ($P < 0.001$; Figure 1); both skin temperatures were lower in both
265 cooling conditions compared with the control condition. Both temperatures
266 gradually increased during the 30 min recovery period in both cooling conditions
267 whilst values remained relatively stable in the control condition. Local thigh and
268 mean skin temperature remained below baseline at the end of the recovery period in
269 the 8°C and 22°C conditions ($P < 0.001$) and were unchanged in the control
270 condition ($P > 0.05$; Figure. 1).

271 Exercise induced increases in muscle temperature at 3 cm (8°C; Δ
272 0.8±0.3°C; 22°C; Δ 1.4±0.5°C; control; Δ 1.0±0.4°C), 2 cm (8°C; Δ 0.9±0.4°C;
273 22°C; Δ 1.3±0.7°C; control; Δ 1.1±0.6°C), and 1 cm (8°C; Δ 1.0±0.6°C; 22°C; Δ
274 1.2±0.9°C; control; Δ 1.1±0.7°C) depths ($P < 0.001$), which were similar between
275 conditions ($P > 0.05$; Figure. 2). During the post immersion recovery period, a
276 greater reduction in muscle temperature was observed in both cooling conditions
277 compared with the control condition at all 3 probe depths and at each time point (P
278 < 0.001 ; Figure 2). There was also a greater reduction in muscle temperature at each
279 depth in 8°C cooling compared with 22°C at each time point ($P < 0.001$; Figure 2).

280 Thermal comfort was lower after cooling; both immediately (8°C, 2±1 AU;
281 22°C, 3±1 AU; control, 5±1 AU, $P < 0.001$) and 10 min post immersion (8°C, 3±1
282 AU; 22°C, 4±1 AU; control, 5±1 AU, $P < 0.01$) compared with the control
283 condition. A lower thermal comfort rating also occurred in the 8°C condition, 20
284 min after immersion, compared with the control condition ($P < 0.001$). Thermal
285 comfort was also lower in the colder water compared with 22°C for up to 10 min
286 after immersion ($P < 0.001$). There was no difference in thermal comfort between
287 conditions at the end of the 30 min recovery period ($P > 0.05$) with similar ratings
288 to baseline. Slight to moderate shivering was observed during immersion in both
289 cooling conditions compared with no shivering in control (8°C, 2±1 AU; 22°C, 2±1
290 AU; control, 1±0 AU). There was no shivering observed throughout the post
291 immersion period in any experimental condition.

292

293 *Heart rate, mean arterial pressure and ratings of perceived exertion (RPE)*

294 Each set of 10 repetitions of squat exercise increased HR ($P < 0.01$), which
295 remained elevated prior to immersion (8°C; 77±11 beats·min⁻¹; 22°C; 73±11

296 beats·min⁻¹; control; 73±10 beats·min⁻¹; $P < 0.001$). HR was increased during colder
297 water immersion (8°C, 80±14 beats·min⁻¹; 22°C, 69±9 beats·min⁻¹; control; 71±7
298 beats·min⁻¹; $P < 0.001$), but remained similar between all conditions during the post
299 immersion recovery period ($P > 0.05$).

300 MAP was not different between conditions immediately prior to immersion
301 (8°C; 89±5 mmHg; 22°C; 88±5 mmHg; control; 88±6 mmHg; $P > 0.05$). MAP was
302 higher during the 10 min immersion period and immediately post immersion in 8°C
303 water (95±7 mmHg) compared to 22°C, (88±7 mmHg) and control (87±4 mmHg)
304 conditions ($P < 0.01$). MAP was similar between all conditions throughout the
305 remaining period of the post immersion phase ($P > 0.05$). MAP returned towards
306 baseline values at the end of the 30 min recovery period in the 22°C and control
307 conditions ($P > 0.05$), but still remained elevated in the 8°C condition (8°C, 90±6;
308 22°C, 90±5; control, 89±7 mm Hg; $P = 0.02$).

309 RPE was similar between trials in the first set of exercise (8°C; 13±2 AU;
310 22°C; 13±1 AU; control; 13±1 AU; $P > 0.05$). There was a higher rating with each
311 subsequent set of squat exercise ($P < 0.001$) with RPE remaining similar between
312 conditions until the end of exercise (8°C; 15±2 AU; 22°C; 15±2 AU; control; 15±2
313 AU; $P > 0.05$).

314

315 *Femoral artery and cutaneous blood flow responses.*

316 Exercise increased femoral blood flow and conductance by ~75% and ~80%
317 respectively ($P < 0.001$) which was not different between conditions ($P > 0.05$;
318 Figure 3). A lower femoral artery blood flow and conductance (~50%) was
319 observed during post-immersion recovery period in both cooling conditions
320 compared with control (8°C, 22°C, $P < 0.01$; Figure 3). Cooling reduced femoral

321 artery blood flow and conductance by ~60% and ~75% relative to baseline and pre-
322 immersion values, respectively, at the end of the 30 min recovery period.

323 Pre-immersion thigh (8°C, 0.23±0.15 AU; 22°C, 0.28±0.21 AU; control,
324 0.31±0.15 AU; $P = 0.31$) and calf (8°C, 0.22±0.20 AU; 22°C, 0.16±0.10 AU;
325 control, 0.17±0.08 AU; $P = 0.45$) cutaneous vascular conductance were not
326 different between conditions. A greater skin vasoconstriction was observed in both
327 cooling conditions at the thigh ($P < 0.01$) and calf ($P < 0.01$) relative to the control
328 throughout the post-immersion recovery period (~50-60%; $P > 0.05$). No
329 differences were observed between cooling conditions (Figure 4).

330

331 **DISCUSSION**

332 The purpose of this study was to investigate the effects of CWI of various
333 water temperatures on lower limb blood flow following resistance exercise. We
334 found no differences in the blood flow responses to CWI at 8°C and 22°C following
335 resistance exercise despite greater reductions in muscle and skin temperatures after
336 CWI of 8°C. Moreover, these responses were similar in time course and magnitude
337 to our previous findings following endurance cycling exercise (Mawhinney et al.,
338 2013). Taken together, these findings suggest that the application of CWI is
339 similarly effective with regards to vascular responses following different modes of
340 moderate intensity exercise.

341 Previous studies, which have examined the influence of CWI on limb blood
342 flow responses after exercise, have used an endurance exercise stimulus (Ihsan et
343 al., 2013; Mawhinney et al., 2013; Vaile et al., 2011). These endurance type
344 protocols typically produce a greater level of systemic (e.g., core temperature)
345 hyperthermia and different metabolic perturbations, compared with resistance

346 exercise (Deschenes et al., 1998; Mortensen et al., 2008). A relative decrease in
347 blood volume in the leg muscle microcirculation after CWI of 10°C has been
348 reported after knee extensor resistance exercise using near-infrared spectroscopy
349 (Roberts et al., 2015a), however, this method is associated with several limitations
350 (Davis, Fadel, Cui, Thomas & Crandall, 2006; Ferrari, Mottola & Quaresima, 2004)
351 compared with absolute measures of femoral and skin blood flow. In the present
352 study, 10-min of lower body immersion in either 8°C or 22°C water reduced femoral
353 artery blood flow by ~75% and ~50%, respectively, compared with the control
354 condition. The magnitude of change in femoral artery conductance after CWI was
355 similar to our previous observations (~55%) after cycling exercise (Mawhinney et
356 al., 2013) and other studies, which assessed limb blood flow with other methods
357 (Ihsan et al., 2013; Vaile et al., 2011). The lack of difference in the femoral artery
358 conductance response to cold (8°C) and cool (22°C) water in the current study,
359 despite greater decreases in muscle temperature in cold water, are in agreement with
360 our previous work (Gregson et al., 2011; Mawhinney et al., 2013) and are likely due
361 to an insufficiently large enough difference in deep muscle temperature between
362 cooling conditions (~1°C) to directly modify femoral artery blood flow.

363

364 It has previously been observed that heat stress from cycling exercise
365 (Mawhinney et al, 2013) can cause a different cutaneous blood flow response to
366 CWI compared with resting conditions (Gregson et al, 2011), e.g., a lack of
367 difference in cutaneous vasoconstriction after immersion in cold and cool water
368 temperatures following cycling exercise. However, it remains to be elucidated
369 whether a smaller level of thermal strain after a bout of resistance exercise could
370 influence the cutaneous blood flow response to CWI. This is important to establish

371 because a greater cutaneous blood flow during cooling may infer less muscle blood
372 flow (Gregson et al, 2011). In the present study, rises in core ($\sim 0.3^{\circ}\text{C}$) and local
373 limb temperatures (muscle 3 cm, $\sim 1^{\circ}\text{C}$; skin, $\sim 0.6^{\circ}\text{C}$) after resistance exercise led to
374 increases in thigh and calf cutaneous vascular conductance. Despite differences in
375 lower limb skin temperature after immersion in 8°C and 22°C water, reductions in
376 lower limb cutaneous vascular conductance were similar between cooling
377 conditions and in agreement with our previous work (Mawhinney et al, 2013) that
378 elicited a higher thermoregulatory strain (core 0.9°C , muscle 3 cm; 1.6°C and skin
379 1.7°C). It is therefore conceivable that only a small hyperthermic load (systemic or
380 local limb) is required to blunt cutaneous vasoconstrictor responsiveness (Wilson,
381 Cui & Crandall, 2002). In addition, cold-induced vasodilation can occur in 8°C
382 water, albeit under resting conditions with no change in body temperature, which
383 may contribute to a similar skin blood flow after 8°C CWI relative to 22°C CWI
384 (Gregson et al, 2011). In combination, similar changes in femoral artery and
385 cutaneous blood flow after CWI in 8°C and 22°C water suggest that both cooling
386 conditions will be equally effective in reducing blood flow when applied after
387 resistance exercise and that the 22°C water may be more tolerable based on the
388 increased thermal comfort ratings in this condition.

389

390 It is difficult to directly measure muscle blood flow in humans, particularly
391 across a broad area of muscle. Our approach, measuring total limb and cutaneous
392 blood flow simultaneously, allows some inferences to be drawn regarding
393 generalized changes in blood flow to muscle. In response to cooling in the present
394 experiment, changes in both total limb and cutaneous flow were similar. This
395 suggests that despite distinct impacts of 8°C and 22°C cooling on skin and muscle

396 temperatures (especially deeper muscle temperatures), the impact on muscle blood
397 flow was qualitatively similar. Collectively, these data infer that, if different degrees
398 of post-exercise cooling have an impact upon recovery following resistance
399 training, they are independent of blood flow to muscle.

400

401 Muscle temperature-induced reductions in microvascular blood flow may
402 reduce inflammation, edema, swelling and pain after tissue injury and limit
403 secondary injury (Lee et al, 2005). The proposal that cooling induced reductions in
404 limb blood flow are beneficial in limiting the inflammatory response after muscle
405 damaging exercise is largely based on animal research, which has shown muscle
406 cooling to reduce markers of inflammation in damaged muscle (Lee et al, 2005;
407 Ramos et al, 2016; Schaser et al, 2007). A recent novel study using humans has
408 recently challenged this view by showing that CWI (10 min at 10°C), applied after
409 lower body resistance exercise, has no impact on the muscle inflammatory or
410 cellular stress response compared with active recovery (Peake et al, 2016).
411 Additionally, the chronic application of CWI (2 d·w⁻¹ over 12 weeks) applied after
412 resistance-training exercise also blunts the cellular adaptation responses and long-
413 term gains in muscle mass and strength (Roberts et al, 2015b). Nevertheless, a
414 reduction in muscle blood flow may still provide benefits to the acute recovery of
415 muscle function after resistance exercise (Roberts et al., 2015a) by attenuating
416 edema and swelling *per se* (Dolan, Thornton, Fish & Mendel, 1997; Yanagisawa, et
417 al, 2003) and associated pain (e.g. soreness) upon movement (Diong & Kamper,
418 2014). These findings have implications for the use of CWI in the periodization of
419 training. For example, CWI may be better utilized in situations where repeated
420 bouts of intense resistance exercise are required in short periods of time rather than

421 as a regular adjunct to resistance training.

422

423 In line with our previous observations (Gregson et al, 2011; Mawhinney et
424 al, 2013), the increases in MAP and HR during 8°C immersion are characteristic of
425 the well-established cold pressor response (Victor, Leimbach, Seals & Wallin,
426 1987). The changes in these cardiovascular indices are initiated by the activation of
427 noxious skin thermoreceptors that cause a reflex increase in sympathetic nervous
428 activity leading to peripheral vasoconstriction and reductions in arterial blood flow
429 (Gregson et al, 2011). In the 22°C condition, there was no observed increase in HR
430 or MAP despite a reduction in limb blood flow. These findings are consistent with
431 the activation of non-noxious thermoreceptors operable at similar temperatures
432 (Gregson et al, 2011). The stimulation of these particular thermoreceptors are
433 related to the difference in skin temperatures and ratings of thermal sensation during
434 immersion in the different cooling conditions.

435

436 In the present study, seated rest in ambient air was selected as the control;
437 consequently, the effect of hydrostatic pressure on limb blood flow *per se*,
438 independent of the water temperature effect, was not assessed. The pressure effect
439 of water has previously been shown to increase femoral artery blood flow by ~250-
440 300 ml·min⁻¹ in thermoneutral immersion under non-exercise conditions (Ménétrier
441 et al, 2015). Therefore, in our study, it is possible that the hydrostatic effect of water
442 *per se* may have prevented a greater magnitude of decrease in arterial blood flow
443 being observed after cooling.

444

445

446 **CONCLUSION**

447 The application of lower limb immersion in 8°C and 22°C water after a bout
448 of resistance exercise decreases femoral artery and cutaneous blood flows compared
449 with rest and to a similar extent between cold and cool water temperatures.
450 Individuals who may not tolerate colder water temperatures may therefore use less
451 noxious water temperatures after resistance exercise. These findings have practical
452 implications for the acute use of cold-water immersion for recovery in clinical and
453 athletic settings.

454

455 **ACKNOWLEDGEMENT**

456 We would like to thank all the participants, ECB Cold Spa for providing the water
457 tanks and UK Sport for funding the present investigation.

458

459 **DISCLOSURE STATEMENT**

460 WG has received funding from ECB Cold Spas Ltd for the CWI facility and from
461 UK Sport for part funding of a PhD degree programme. All other authors have no
462 conflicts of interest.

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638 Figure captions

639

640 **Figure 1.** Rectal temperature (A), mean skin temperature (B) and thigh skin
641 temperature (C) pre and post immersion in 8°C, 22°C and control (n = 12, mean ±
642 SD). Main effects for condition ($P<0.001$) and time ($P<0.001$), alongside a
643 significant interaction between condition and time ($P<0.001$), were found for thigh
644 and mean skin temperature. Main effects for time ($P<0.001$) were found for rectal
645 temperature. Significant difference from baseline in the 8°C condition (*), 22°C
646 condition (**) and control conditions (***) ($P<0.01$). Significant difference
647 between cooling conditions vs control (+) ($P<0.001$). Significant difference
648 between cooling conditions (#) ($P<0.05$).

649

650 **Figure 2.** Muscle temperature pre and post immersion, at temperature probe depths
651 of 3 cm (A), 2 cm (B), and 1cm (C) (n =12, mean ± SD). Main effects for condition
652 ($P<0.001$) and time ($P<0.001$) were found along with a significant interaction
653 between condition, time and probe depth ($P<0.001$). Significant difference from
654 baseline in the 8°C (*), 22°C (**), and control conditions (***) ($P<0.001$).
655 Significant difference between cooling conditions vs control (+) ($P<0.001$).
656 Significant difference between cooling conditions (#) ($P<0.05$).

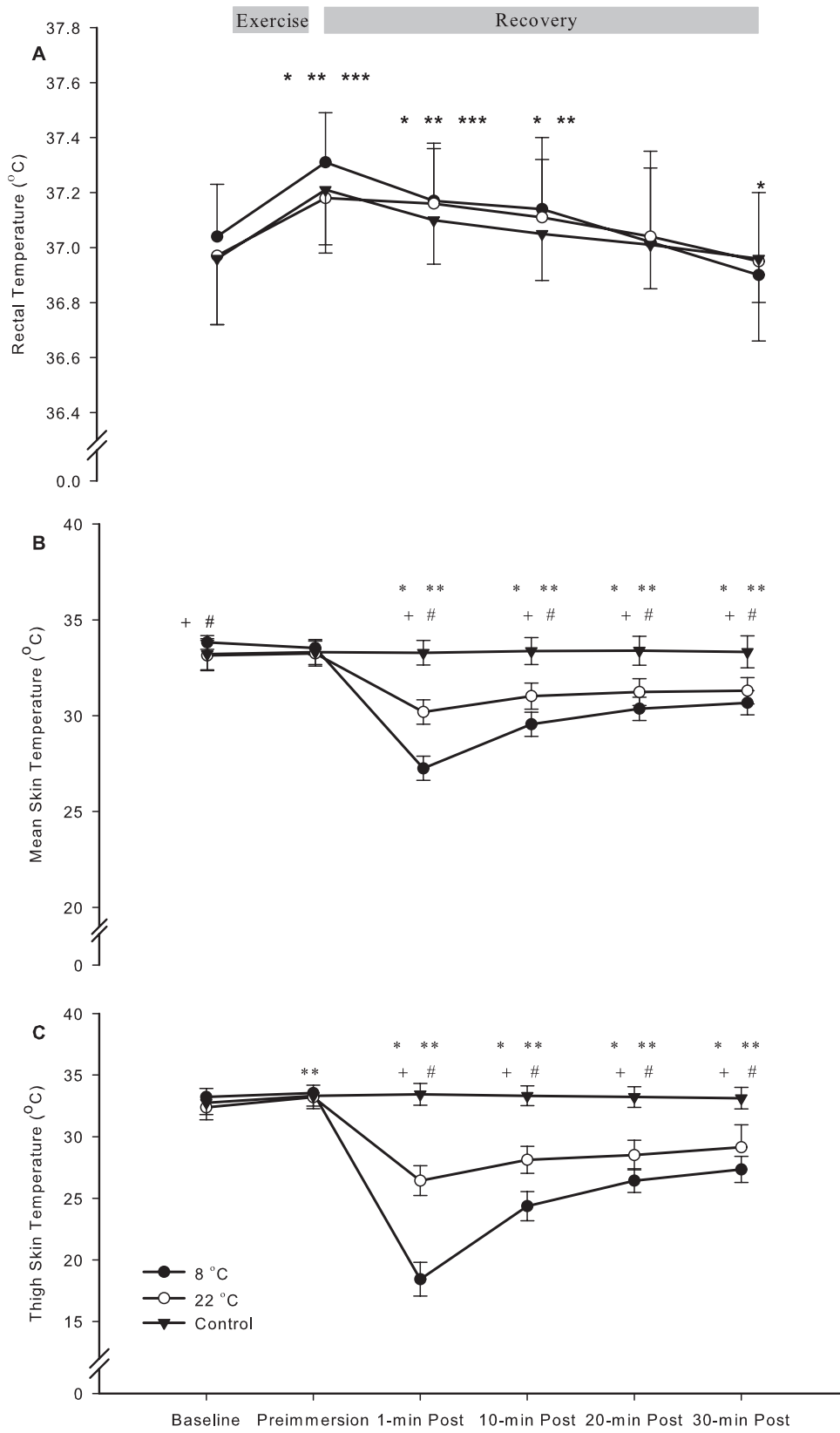
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658 **Figure 3.** Femoral artery blood flow (A) and conductance (B) pre and post
659 immersion in 8°C, 22°C and control (n = 12, mean ± SD). A main effect for
660 condition ($P<0.001$) and time ($P<0.001$) was found for both artery flow and
661 conductance. There was also a significant interaction between condition and time
662 for both artery flow ($P<0.01$) and conductance ($P<0.01$). Significant difference
663 from baseline in the 8°C (*), 22°C (**), and control conditions (***) ($P<0.05$).
664 Significant difference between cooling conditions vs control (+) ($P<0.01$).

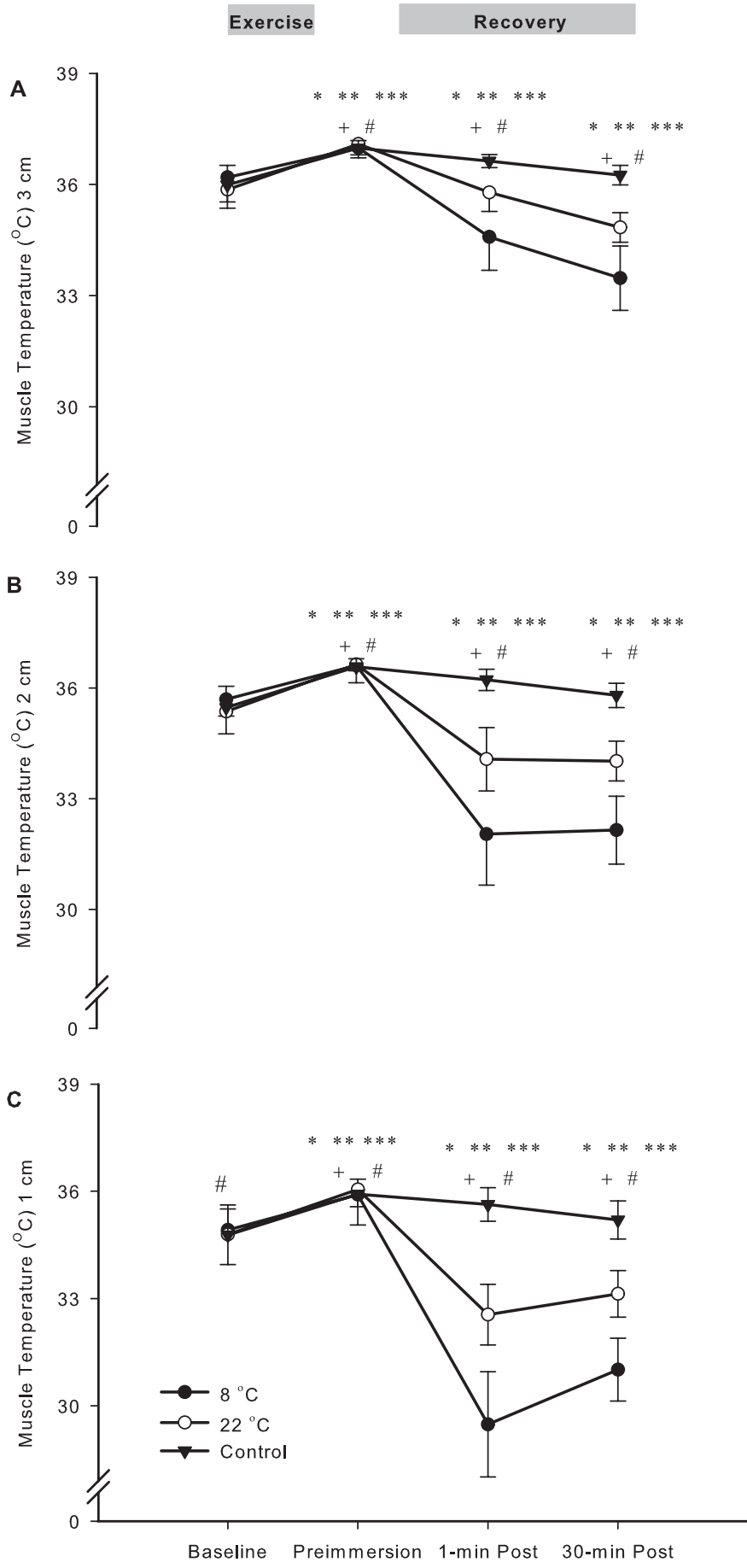
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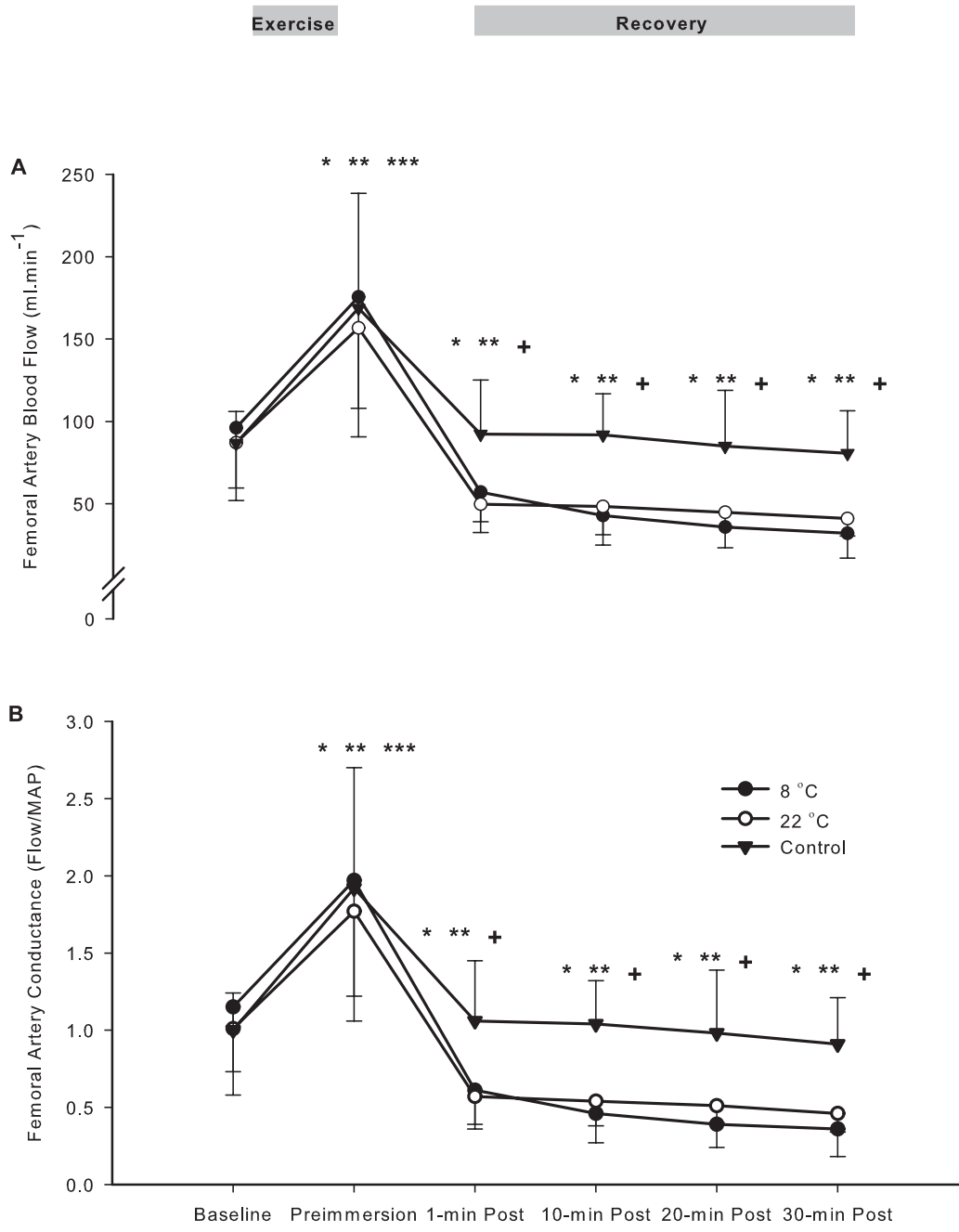
666 **Figure 4.** Percentage change in thigh cutaneous vascular conductance (A) and calf
667 vascular conductance (B) from pre immersion in 8°C, 22°C and control (n =12,
668 mean ± SD). Main effects for condition ($P<0.01$) were found for both thigh and calf
669 cutaneous vascular conductance. A main effect for time ($P<0.05$) was also found
670 for thigh conductance. There were no interactions between condition and time in
671 thigh ($P=0.78$) or calf vascular conductance ($P=0.42$). Significant difference from
672 baseline in the 8°C (*), 22°C (**), and control conditions (***) ($P<0.05$).
673 Significant difference between cooling conditions vs control (+) ($P<0.01$).

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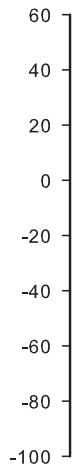
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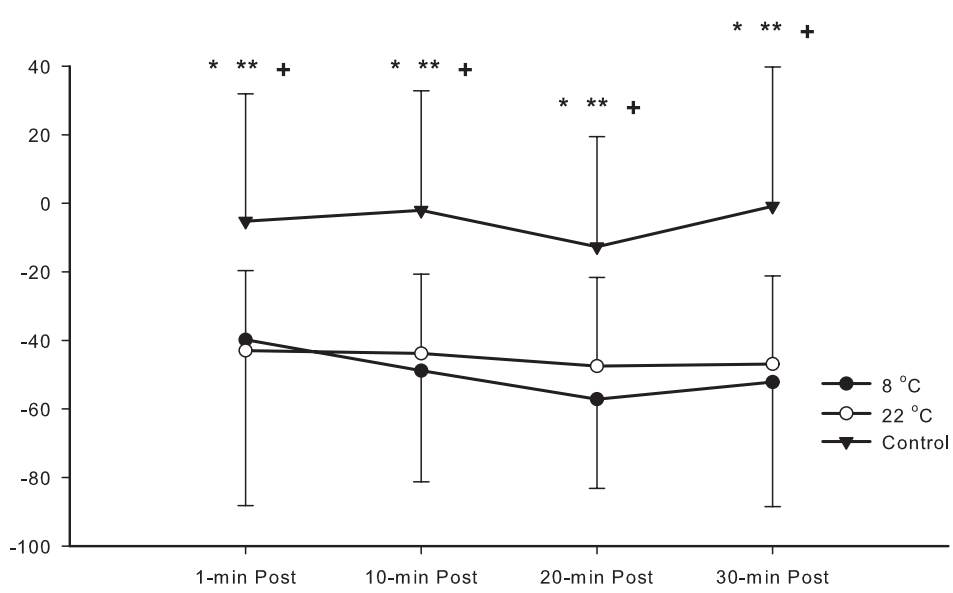


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A
Change in Cutaneous Vascular Conductance (% Pre-cooling)



B
Change in Cutaneous Vascular Conductance (% Pre-cooling)



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