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

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ABSTRACT

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

Keywords: blood flow; cooling; muscle damage; inflammation

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

Participants

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

Experimental Design

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

146 The experimental trials were performed in a randomized counterbalanced
147 order, at least 7-days following the second familiarization session and at least 7-days
148 apart. For each trial, participants arrived at the laboratory at least 3 h postprandial,
149 having refrained from exercise, alcohol, tobacco and caffeine during the previous 24
150 h and having consumed 5 ml·kg^{-1} of water 2 h before arrival. All participants recorded
151 their nutritional and fluid intake for 24 h prior to their first experimental trial. This
152 record was photocopied and returned to them to repeat for their remaining trials. All
153 trials were conducted under an ambient temperature of 22-24°C to control variability
154 in cutaneous blood flow (Cracowski, Minson, Salvat-Melis & Halliwill, 2006) and at
155 the same time of day in order to avoid the circadian variation in internal body
156 temperature.
157 Each participant was required to complete 4 sets of 10 RM squats followed
158 by a 10 min period of immersion in either 8°C or 22°C water or seated rest (Control).
159 The water temperatures and immersion protocol was based on our previous studies
160 (Gregson et al., 2011; Mawhinney et al., 2013). On arrival, nude body mass (kg) was
161 obtained (Seca, Hamburg, Germany). A rectal probe was self-inserted and a heart rate
162 (HR) monitor was positioned across the chest. Participants then rested supine for 30
163 min for instrumentation and to stabilize physiological status, wearing training shorts.
164 Following baseline measurements (10 min), participants completed 4 sets of 10 RM
165 squats interspersed with a 2 min rest period between sets. Participants then returned
166 to the supine position for 10 min for post-exercise/pre-immersion measurements.
167 Participants were then raised from the bed in a semi-recline position using an
168 electronic hoist (Bianca, Arjo Ltd, Gloucester, United Kingdom) and either lowered
169 into the water tank (ECB, Gloucester, U.K.) to the iliac crest for 10 min, or remained
170 suspended above the bed (Control). At the end of immersion, participants were

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

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

Measurements

Rectal, Thigh, Skin, and Muscle Temperatures

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

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

Heart Rate and Arterial Blood Pressure

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

Femoral Artery Blood Flow

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

velocity and blood flow were sampled as the mean of a 20 s period of each 2 min image. Femoral vascular conductance was calculated as the ratio of blood flow/MAP.

Cutaneous Blood Flow

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

Statistical Analysis

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

of statistical significance was set at $P < 0.05$ and were analysed using Statistical Package for the Social Sciences (Chicago, IL). All data are presented as mean±*s*.

RESULTS

Thermoregulatory responses

Exercise elicited an increase in rectal temperature (8°C; Δ 0.3±0.2°C; 22°C; Δ 0.2±0.1°C; control; 0.3±0.1°C; $P < 0.001$) but rectal temperature was not different between conditions ($P > 0.05$; Figure 1a). Rectal temperature decreased over the post immersion recovery period ($P < 0.001$) with no difference observed between conditions ($P = 0.19$; Figure. 1a).

Exercise elicited an increase in thigh (8°C; Δ 0.4±0.6°C; 22°C; Δ 0.8±0.6°C; control; Δ 0.6±0.8°C; $P = 0.002$) and mean skin temperature (8°C; Δ 0.3±0.2°C; 22°C; Δ 0.2±0.1°C; control; 0.3±0.1°C; $P < 0.001$) but skin temperatures were not different between conditions ($P > 0.05$; Figure. 1). The colder water reduced local thigh and mean skin temperatures to a greater extent compared to 22°C throughout post-immersion ($P < 0.001$; Figure 1); both skin temperatures were lower in both cooling conditions compared with the control condition. Both temperatures gradually increased during the 30 min recovery period in both cooling conditions whilst values remained relatively stable in the control condition. Local thigh and mean skin temperature remained below baseline at the end of the recovery period in the 8°C and 22°C conditions ($P < 0.001$) and were unchanged in the control condition ($P > 0.05$; Figure. 1).

Exercise induced increases in muscle temperature at 3 cm (8°C; Δ 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; 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 1.2±0.9°C;

control; Δ 1.1±0.7°C) depths ($P < 0.001$), which were similar between conditions ($P > 0.05$; Figure. 2). During the post immersion recovery period, a greater reduction in muscle temperature was observed in both cooling conditions compared with the control condition at all 3 probe depths and at each time point ($P < 0.001$; Figure 2). There was also a greater reduction in muscle temperature at each depth in 8°C cooling compared with 22°C at each time point ($P < 0.001$; Figure 2).

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

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

Each set of 10 repetitions of squat exercise increased HR ($P < 0.01$), which remained elevated prior to immersion (8°C; 77±11 beats·min^{-1}; 22°C; 73±11 beats·min^{-1}; control; 73±10 beats·min^{-1}; $P < 0.001$). HR was increased during colder water immersion (8°C, 80±14 beats·min^{-1}; 22°C, 69±9 beats·min^{-1}; control; 71±7

294 beats·min^{-1}; $P < 0.001$), but remained similar between all conditions during the post
295 immersion recovery period ($P > 0.05$).

296 MAP was not different between conditions immediately prior to immersion
297 (8°C; 89±5 mmHg; 22°C; 88±5 mmHg; control; 88±6 mmHg; $P > 0.05$). MAP was
298 higher during the 10 min immersion period and immediately post immersion in 8°C
299 water (95±7 mmHg) compared to 22°C, (88±7 mmHg) and control (87±4 mmHg)
300 conditions ($P < 0.01$). MAP was similar between all conditions throughout the
301 remaining period of the post immersion phase ($P > 0.05$). MAP returned towards
302 baseline values at the end of the 30 min recovery period in the 22°C and control
303 conditions ($P > 0.05$), but still remained elevated in the 8°C condition (8°C, 90±6;
304 22°C, 90±5; control, 89±7 mm Hg; $P = 0.02$).

305 RPE was similar between trials in the first set of exercise (8°C; 13±2 AU;
306 22°C; 13±1 AU; control; 13±1 AU; $P > 0.05$). There was a higher rating with each
307 subsequent set of squat exercise ($P < 0.001$) with RPE remaining similar between
308 conditions until the end of exercise (8°C; 15±2 AU; 22°C; 15±2 AU; control; 15±2
309 AU; $P > 0.05$).

310

311 *Femoral artery and cutaneous blood flow responses.*

312 Exercise increased femoral blood flow and conductance by ~75% and ~80%
313 respectively ($P < 0.001$) which was not different between conditions ($P > 0.05$; Figure
314 3). A lower femoral artery blood flow and conductance (~50%) was observed during
315 post-immersion recovery period in both cooling conditions compared with control
316 (8°C, 22°C, $P < 0.01$; Figure 3). Cooling reduced femoral artery blood flow and
317 conductance by ~60% and ~75% relative to baseline and pre-immersion values,
318 respectively, at the end of the 30 min recovery period.

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

DISCUSSION

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

Previous studies, which have examined the influence of CWI on limb blood flow responses after exercise, have used an endurance exercise stimulus (Ihsan et al., 2013; Mawhinney et al., 2013; Vaile et al., 2011). These endurance type protocols typically produce a greater level of systemic (e.g., core temperature) hyperthermia and different metabolic perturbations, compared with resistance exercise (Deschenes et al., 1998; Mortensen et al., 2008). A relative decrease in blood volume in the leg muscle microcirculation after CWI of 10°C has been reported after knee extensor

resistance exercise using near-infrared spectroscopy (Roberts et al., 2015a), however, this method is associated with several limitations (Davis, Fadel, Cui, Thomas & Crandall, 2006; Ferrari, Mottola & Quaresima, 2004) compared with absolute measures of femoral and skin blood flow. In the present study, 10-min of lower body immersion in either 8ºC or 22ºC water reduced femoral artery blood flow by ~75% and ~50%, respectively, compared with the control condition. The magnitude of change in femoral artery conductance after CWI was similar to our previous observations (~55%) after cycling exercise (Mawhinney et al., 2013) and other studies, which assessed limb blood flow with other methods (Ihsan et al., 2013; Vaile et al., 2011). The lack of difference in the femoral artery conductance response to cold (8ºC) and cool (22ºC) water in the current study, despite greater decreases in muscle temperature in cold water, are in agreement with our previous work (Gregson et al., 2011; Mawhinney et al., 2013) and are likely due to an insufficiently large enough difference in deep muscle temperature between cooling conditions (~1°C) to directly modify femoral artery blood flow.

It has previously been observed that heat stress from cycling exercise (Mawhinney et al, 2013) can cause a different cutaneous blood flow response to CWI compared with resting conditions (Gregson et al, 2011), e.g., a lack of difference in cutaneous vasoconstriction after immersion in cold and cool water temperatures following cycling exercise. However, it remains to be elucidated whether a smaller level of thermal strain after a bout of resistance exercise could influence the cutaneous blood flow response to CWI. This is important to establish because a greater cutaneous blood flow during cooling may infer less muscle blood flow (Gregson et al, 2011). In the present study, rises in core (~0.3ºC) and local limb temperatures

(muscle 3 cm, ~1ºC; skin, ~0.6ºC) after resistance exercise led to increases in thigh and calf cutaneous vascular conductance. Despite differences in lower limb skin temperature after immersion in 8ºC and 22ºC water, reductions in lower limb cutaneous vascular conductance were similar between cooling conditions and in agreement with our previous work (Mawhinney et al, 2013) that elicited a higher thermoregulatory strain (core 0.9ºC, muscle 3 cm; 1.6ºC and skin 1.7ºC). It is therefore conceivable that only a small hyperthermic load (systemic or local limb) is required to blunt cutaneous vasoconstrictor responsiveness (Wilson, Cui & Crandall, 2002). In addition, cold-induced vasodilation can occur in 8ºC water, albeit under resting conditions with no change in body temperature, which may contribute to a similar skin blood flow after 8ºC CWI relative to 22ºC CWI (Gregson et al, 2011). In combination, similar changes in femoral artery and cutaneous blood flow after CWI in 8ºC and 22ºC water suggest that both cooling conditions will be equally effective in reducing blood flow when applied after resistance exercise and that the 22ºC water may be more tolerable based on the increased thermal comfort ratings in this condition.

It is difficult to directly measure muscle blood flow in humans, particularly across a broad area of muscle. Our approach, measuring total limb and cutaneous blood flow simultaneously, allows some inferences to be drawn regarding generalized changes in blood flow to muscle. In response to cooling in the present experiment, changes in both total limb and cutaneous flow were similar. This suggests that despite distinct impacts of 8ºC and 22ºC cooling on skin and muscle temperatures (especially deeper muscle temperatures), the impact on muscle blood flow was qualitatively similar. Collectively, these data infer that, if different degrees of post-exercise cooling

394 have an impact upon recovery following resistance training, they are independent of
395 blood flow to muscle.
396
397 Muscle temperature-induced reductions in microvascular blood flow may
398 reduce inflammation, edema, swelling and pain after tissue injury and limit secondary
399 injury (Lee et al, 2005). The proposal that cooling induced reductions in limb blood
400 flow are beneficial in limiting the inflammatory response after muscle damaging
401 exercise is largely based on animal research, which has shown muscle cooling to
402 reduce markers of inflammation in damaged muscle (Lee et al, 2005; Ramos et al,
403 2016; Schaser et al, 2007). A recent novel study using humans has recently challenged
404 this view by showing that CWI (10 min at 10ºC), applied after lower body resistance
405 exercise, has no impact on the muscle inflammatory or cellular stress response
406 compared with active recovery (Peake et al, 2016). Additionally, the chronic
407 application of CWI (2 $d \cdot w^{-1}$ over 12 weeks) applied after resistance-training exercise
408 also blunts the cellular adaptation responses and long-term gains in muscle mass and
409 strength (Roberts et al, 2015b). Nevertheless, a reduction in muscle blood flow may
410 still provide benefits to the acute recovery of muscle function after resistance exercise
411 (Roberts et al., 2015a) by attenuating edema and swelling *per se* (Dolan, Thornton,
412 Fish & Mendel, 1997; Yanagisawa, et al, 2003) and associated pain (e.g. soreness)
413 upon movement (Diong & Kamper, 2014). These findings have implications for the
414 use of CWI in the periodization of training. For example, CWI may be better utilized
415 in situations where repeated bouts of intense resistance exercise are required in short
416 periods of time rather than as a regular adjunct to resistance training.
417
418 In line with our previous observations (Gregson et al, 2011; Mawhinney et al,

2013), the increases in MAP and HR during 8ºC immersion are characteristic of the well-established cold pressor response (Victor, Leimbach, Seals & Wallin, 1987). The changes in these cardiovascular indices are initiated by the activation of noxious skin thermonociceptors that cause a reflex increase in sympathetic nervous activity leading to peripheral vasoconstriction and reductions in arterial blood flow (Gregson et al, 2011). In the 22ºC condition, there was no observed increase in HR or MAP despite a reduction in limb blood flow. These findings are consistent with the activation of non-noxious thermonociceptors operable at similar temperatures (Gregson et al, 2011). The stimulation of these particular thermonociceptors are related to the difference in skin temperatures and ratings of thermal sensation during immersion in the different cooling conditions.

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

CONCLUSION

The application of lower limb immersion in 8ºC and 22ºC water after a bout of resistance exercise decreases femoral artery and cutaneous blood flows compared

with rest and to a similar extent between cold and cool water temperatures. Individuals who may not tolerate colder water temperatures may therefore use less noxious water temperatures after resistance exercise. These findings have practical implications for the acute use of cold-water immersion for recovery in clinical and athletic settings.

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Figure captions

Figure 1. Rectal temperature (A), mean skin temperature (B) and thigh skin temperature (C) pre and post immersion in 8°C, 22°C and control (n = 12, 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 and mean skin temperature. Main effects for time ($P<0.001$) were found for rectal temperature. Significant difference from baseline in the 8°C condition (*), 22°C condition (**) and control conditions (***) ($P<0.01$). Significant difference between cooling conditions vs control (+) ($P<0.001$). Significant difference between cooling conditions (#) ($P<0.05$).

Figure 2. Muscle temperature pre and post immersion, at temperature probe depths of 3 cm (A), 2 cm (B), and 1cm (C) (n =12, 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 ($P<0.001$). Significant difference from baseline in the 8°C (*), 22°C (**) and control conditions (***) ($P<0.001$). Significant difference between cooling conditions vs control (+) ($P<0.001$). Significant difference between cooling conditions (#) ($P<0.05$).

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

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