The acute and chronic effects of hot water immersion on inflammation and metabolism in sedentary, overweight adults

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Abstract

Regular exercise-induced acute inflammatory responses are suggested to improve the inflammatory profile and insulin sensitivity. As body temperature elevations partly mediate this response, passive heating might be a viable tool to improve the inflammatory profile. This study investigated the acute, and chronic effects of hot water immersion on inflammatory and metabolic markers. Ten sedentary, overweight males (BMI: 31.0±4.2 kg/m^2) were immersed in water set at 39°C for 1 h (HWI) or rested for 1 h at ambient temperature (AMB). Venous blood was obtained prior to, immediately post and 2 h post-session for assessment of monocyte intracellular heat shock protein 72 (iHsp72) and plasma concentrations of extracelullar heat shock protein 72 (eHsp72), interleukin-6 (IL-6), fasting glucose, insulin and nitrite. Thereafter, participants underwent a 2-week intervention period, consisting of 10 hot water immersion sessions (INT). Eight BMI-matched participants (BMI: 30.0±2.5 kg/m^2) were included as control (CON). Plasma IL-6 and nitrite concentrations were higher immediately following HWI compared to AMB (IL-6 $p<0.001$, HWI: 1.37±0.94 to 2.51±1.49 pg/ml; nitrite $p=0.04$, HWI: 271±52 to 391±72 nM), while iHsp72 expression was unchanged ($p=0.57$). In contrast to resting iHsp72 expression ($p=0.59$), fasting glucose ($p=0.04$, INT: 4.44±0.93 to 3.98±0.98 mmol/l), insulin ($p=0.04$, INT: 68.1±44.6 to 55.0±29.9 pmol/l) and eHsp72 ($p=0.03$, INT: 17±41% reduction) concentrations were lowered after INT compared to CON. HWI induced an acute inflammatory response and increased nitric oxide bioavailability. The reductions in fasting glucose and insulin concentrations following the chronic intervention suggest that hot water immersion may serve as a tool to improve glucose metabolism.

Passive heating; chronic low-grade inflammation; heat shock protein; interleukin-6; glucose metabolism

New and noteworthy

A single hot water immersion (HWI) session induces an acute increase in plasma interleukin-6 and nitrite concentrations, but does not acutely elevate heat shock protein 72 expression in monocytes (iHsp72). A chronic HWI intervention reduces fasting glucose and insulin concentrations in the absence of changes in resting iHsp72. Therefore, HWI shows potential as a strategy to combat chronic low-grade inflammation and improve glucose metabolism in individuals without the physical capacity to do so using exercise.

Introduction

Passive heating interventions have been linked to several positive health outcomes, such as improved vascular function (4), mental health (11), weight loss (33) and enhanced insulin sensitivity (42). Although observations of a lowering in fasting glycosylated haemoglobin and blood glucose concentrations following hot water immersion (HWI) in individuals with type 2 diabetes supports the notion of improved insulin sensitivity following HWI (33), the mechanisms that underlie this beneficial effect are currently unclear. Chronic low-grade inflammation has been implicated in the aetiology of insulin resistance (9), as evidenced by the positive association between pro-inflammatory proteins and insulin resistance (9, 39), while the body of evidence for a causal relationship of these proteins with insulin resistance is growing (35). Moreover, it is well documented that exercise training can counteract chronic low-grade inflammation (57) and improve insulin sensitivity (29). However, since it is not feasible for all populations to adhere to the recommended exercise guidelines due to a low physical capacity or health conditions that hinder exercise participation, the development of alternative strategies that can reduce chronic low-grade inflammation in populations without the capacity to engage in sufficient volumes of exercise is warranted to mitigate risk factors for insulin resistance and non-communicable diseases.

The acute inflammatory response provoked by a physical stressor, such as exercise, can induce a subsequent protracted anti-inflammatory response. For instance, elevations in circulating interleukin (IL)-6 concentrations immediately following exercise activate the release of anti-inflammatory cytokines such as IL-1ra and IL-10, typically 1 to 4 h following the exercise bout (57). In addition, recent studies have identified an enhanced acute inflammatory response following exercise when body temperature is augmented (43). Increasing body temperature therefore likely serves as an independent stressor able to induce the acute inflammatory responses needed to reduce chronic low-grade inflammation in the

long term. This is supported by Welc et al. (66), showing that passive heating for 1 h at 42.4°C can activate heat shock factor 1, which in turn upregulates the production of IL-6 and intracellular heat shock protein 72 (iHsp72) in mice skeletal muscle.

In humans, 1-2 h of hot water immersion (HWI), at a temperature 2-3°C higher than resting core temperature, has been reported to acutely elevate IL-6, IL-1ra (45), extracellular Hsp72 (eHsp72) (16) and monocyte intracellular Hsp72 (iHsp72) (54). Elevations in iHsp72 can block the inflammatory actions of c-jun amino terminal kinase (JNK) and nuclear factor κB (NF-κB), resulting in enhanced insulin sensitivity (31). In contrast to the beneficial functions of iHsp72, Hsp72 found in plasma (i.e. eHsp72) can activate circulating monocytes, resulting in an increase in pro-inflammatory cytokine release (1). Although the transient increase in eHsp72 following an acute bout of exercise is suggested to be part of the beneficial inflammatory response to exercise (67), a reduction in resting eHsp72 is suggestive of an improved inflammatory profile and may improve insulin sensitivity (41).

In addition to modulating inflammation, an increase in body temperature has been linked to increased nitric oxide (NO) production through enhanced NO synthase (NOS) (4, 36), possibly mediated by an increased expression of Hsp90 (70). It is well documented that NO impacts a myriad of biological processes, including tissue glucose uptake (19, 20, 58, 60). Therefore, an increase in NO synthesis following HWI might contribute to changes in insulin sensitivity resulting from this intervention. Moreover, an acute increase in NO bioavailability exerts an anti-inflammatory effect on human leukocytes (58) and increases the iHsp72 expression in peripheral mononuclear blood cells (63), indicating cross-talk between NO and the immune system. However, the extent to which acute and chronic HWI influences NO synthesis and its role in chronic low-grade inflammation and insulin sensitivity is presently unclear.

Although there is now evidence for the potential of HWI to induce an *acute* inflammatory response (16, 45, 54), *chronic* intervention studies in humans are scarce. Notwithstanding, the reduction in fasting blood glucose concentrations in patients with diabetes (33) and resting plasma IL-6 concentrations in patients with chronic heart failure (55) are promising initial results. These studies, however, focussed on clinical populations, did not address the mechanistic link between inflammatory and metabolic markers and provided little detail on the acute (thermo-)physiological responses to HWI. For instance, while animal studies have provided compelling evidence for the potential of HWI to chronically elevate basal iHsp72 levels (26, 6, 61), it is not known whether this holds true in humans. The smaller acute core temperature increases reported in human compared to animal studies might make HWI less effective as a strategy to elevate resting iHsp72 levels in humans (27).

Therefore, the present study investigated the acute inflammatory response to a single HWI session as well as the potential of a chronic HWI intervention to improve the inflammatory and metabolic profile at rest. It is hypothesised that an HWI session induces acute increases in plasma IL-6 concentrations, NO bioavailability as well as iHsp72 expression in monocytes. Chronically, the 2-week HWI intervention is hypothesised to increase resting levels of iHsp72, while reducing IL-6 and eHsp72 concentrations. Finally, in line with Hooper et al. (33), the intervention period is expected to result in reductions in fasting glucose and insulin concentrations.

Methods

Participants

Participants were sedentary (<2 hours exercise/week), overweight (body mass index >27 kg/m^2), otherwise healthy males (Table 1). Exclusion criteria were the usage of anti-inflammatory medication and contra-indications to engage in HWI. The latter was assessed

with a medical health questionnaire according to the American College for Sport and Exercise Medicine guidelines for exercise testing and prescription (32). Engagement in structured exercise was reported prior to and following the chronic intervention period, using the International Physical Activity Questionnaire (8). Participants gave informed consent after being instructed about the procedures of the study, which were approved by the Local Ethical Committee of Loughborough University, in accordance with the declaration of Helsinki.

Procedures

An outline of the procedures for the intervention group is given in Fig. 1. Participants visited the laboratory for a HWI (HWI_{pre}) and control trial (AMB) in a counterbalanced order, with a minimum of 72 h between the visits. Participants refrained from exercise, alcohol and caffeine and standardised their diet using a food diary in the 24 hours prior to the visits. All visits started between 8-10 am, with the starting consistently applied for each individual to account for a possible circadian rhythm in any of the outcome measures. After an overnight fast, nude body mass, height, hip and waist circumference were measured and skinfold thickness was assessed at four sites (biceps, triceps, subscapular and supra iliac) (14) for the estimation of body fat percentage.

***** Insert Figure 1 around here *****

Thereafter, participants underwent 15 min of seated rest in an environmental chamber (27°C, 40% humidity) for baseline measurements (21). Following the "pre" blood sample, participants entered the water tank for the HWI_{pre} or remained seated for another hour in the same conditions as AMB. This control condition (instead of immersion in thermoneutral water) was chosen because this study was designed to evaluate the effects of HWI as a stand-alone health intervention rather than to investigate the effects of an increase in body

temperature per se. Evidence suggests that the effects of hydrostatic pressure on inflammatory markers are negligible (43).

During HWI_{pre}, participants were immersed up to the neck for 1 hour in water set at 39°C. Participants sat in an upright position and were allowed to drink water *ad libitum*. During both HWI_{pre} and AMB, measurements were taken every 15 min. Blood pressure (Microlife BP3AC1-1, Cambridge, UK) was measured in duplicate at the level of the heart, while thermal sensation, thermal comfort (21) and basic affect using the Feeling Scale (68) were reported. Expired air was collected for 3 min into Douglas bags for the determination of oxygen uptake ($\dot{V}O_2$) using a Servomex 1440 gas analyser (Servomex Ltd, Crowborough, UK). Tympanic temperature was measured with a tympanic temperature probe (Squirrel, Grant Instruments, Shepreth, UK), using cotton wool to cover the external canal of the ear. Rectal temperature (T_{rec}) was recorded every 5 min throughout the trials, using a rectal probe (YSI 400 series, Ohio, USA) that was inserted 10 cm beyond the anal sphincter. Heart rate (HR) (Polar RS400, Kempele, Finland) was continuously measured throughout.

Immediately on completion of the session, a "post" blood sample was taken and participants rested seated in the environmental chamber for 30 min. Thereafter, nude body weight was measured and a breakfast snack was provided (Sainsbury breakfast biscuits; 212 kcal, 5.8 g fat, 34.3 g carbohydrates, 4.0 g protein). The change in nude body weight and water consumed was used to estimate sweat loss. Participants were then allowed to rest and perform light work such as reading. Two hours after completion of the session, the "post 2 h" blood sample was taken following 15 min of seated rest.

Following the first two visits, participants enrolled in an intervention period consisting of ten HWI sessions, all executed within fourteen days. The first five sessions of this period lasted 45 minutes, while the last five lasted 60 minutes. As pilot work suggested that the HWI sessions can be experienced as uncomfortable, this progression was chosen to avoid drop-out

during the intervention period. In all sessions the temperature of the water was set at 39°C and participants were immersed up to their neck. During the ten sessions, HR, tympanic temperature, thermal sensation, thermal comfort and basic affect were assessed every 15 min. Three days after completion of the last session of the intervention period, an acute trial (HWI_{post}) was conducted to study the effects of the intervention period on the acute inflammatory response to HWI. The procedures during this session were identical to HWI_{pre}. The "pre" blood sample of the first session (either HWI_{pre} or AMB) and HWI_{post} were used to study the chronic effects of the intervention period. Eight individuals matched for body composition, age and physical activity levels were included as control for the chronic arm of the study (CON). These participants visited the laboratory for two resting blood samples only, with the time between both samples held equal to the intervention group. In the intervention group, an additional resting blood sample was taken one week following HWI_{post} to investigate whether any adaptations detected following the intervention period would remain after one week.

Biochemical analyses

Blood was collected in K_3EDTA (plasma markers) and sodium heparin (flow cytometry) monovettes. The K_3EDTA tubes were spun down immediately for 5 min at 1500 g and 4°C, and plasma was stored at -80°C until batch analysis. Flow cytometry was used to assess changes in iHsp72 in monocytes and the distribution in monocyte subsets. In addition, changes in the expression of iHsp72 in the respective monocyte subsets were assessed. Sixty µL of whole blood was incubated together with 5 µL of PerCP-conjugated cluster of differentiation (CD)14 and 2.5 µL of PE-conjugated CD16 antibodies in the dark at room temperature for 15 min. Thereafter, samples were lysed (750 µL; Facs lysing solution (BD biosciences, San Diego, US), washed (1.5 mL phosphate buffered saline) and fixed using

Leucoperm (60 µL; BD biosciences). Following permeabilisation (60 µL; Leucoperm, BD biosciences) samples were incubated with 4 µL of FITC-conjugated Hsp70 antibody or isotype control for 30 min. Finally, samples were washed and resuspended in phosphate buffered saline prior to running through the Flow Calibur (BD biosciences). All antibodies except CD16 (BD biosciences) were purchased from Miltenyi Biotech (Teterow, Germany). Cell Quest software (BD biosciences) was used for the analysis, collecting 100,000 events per sample. Compensation of the flow cytometer prior to the study was performed manually using a whole blood sample of a male volunteer not participating in the study. Monocytes were selected based on positive CD14 expression, whereafter the percentage of monocyte subsets (CD14++CD16- classical monocytes, CD14+CD16+ intermediate monocytes and CD14-CD16++ non-classical monocytes) was determined using the trapezoid method (68). The iHsp72 expression in monocytes was determined using the geometric mean fluorescence intensity (GMFI) following subtraction of the isotype control GMFI.

All glassware, utensils, and surfaces were rinsed with deionized water to remove residual NO intermediates prior to plasma [nitrite] analysis. Plasma samples were introduced to a gas-tight purge vessel via 200 uL injections into the septum at the top of the vessel. The [nitrite] of the undiluted (non-deproteinized) plasma was determined by its reduction to NO in the presence of glacial acetic acid and aqueous sodium iodide (4% w/v). The spectral emission of electronically excited nitrogen dioxide, derived from the reaction of NO with ozone, was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence nitric oxide analyzer (Sievers NOA 280i, Analytix Ltd, Durham, UK). The [nitrite] was determined by plotting signal (mV) area against a calibration plot of sodium nitrite standards. Interleukin-6 (High-sensitivity, RnD systems, Abington, UK), eHsp72 (Amp`d HSP70 high-sensitivity, Enzo life sciences, Farmingdale, US) and insulin (Mercodia AB, Uppsala, Sweden) were measured in plasma, in duplicate, using

enzyme linked immunosorbent assays (ELISA). For the determination of eHsp72 concentrations, plasma samples were diluted 1:4 prior to running the ELISA. The intra-assay coefficients of variation were 7.0%, 6.2% and 2.5% for IL-6, eHsp72 and insulin, respectively. A Biosen C-line (Biosen, Barleben, Germany) was used to determine blood glucose concentrations in whole blood (52). A whole blood count was obtained using a Yumizen H500 cell counter (Horiba Medical, Montpellier, France) for the determination of leukocyte subsets, haematocrit and haemoglobin. The latter two were used to correct the post and post+2h plasma IL-6 and eHsp72 concentrations for changes in plasma volume (10).

Statistical analyses

All values are given as mean ± standard deviation. Normality of the data was checked using the Shapiro-Wilk test and a log transformation was performed when non-normality was detected. Log transformation was performed on the eHsp72 data. Analysis of variance (ANOVA) with repeated measures where appropriate was used to detect differences in the acute responses between AMB and HWI_{pre} , HWI_{pre} and HWI_{post} as well as the effects of the intervention period on baseline measures compared to CON. Due to a difference in baseline plasma nitrite concentrations between HWI_{pre} and AMB, a one-way ANCOVA was employed to detect differences between HWI and AMB at “post” and “post+2h” using nitrite concentrations at “pre” as a covariate. *R*, the fold change in the eHsp72/iHsp72 ratio, was determined for the acute as well as chronic arm of the study (41). The homeostasis model assessment for insulin resistance (HOMA-IR) was determined using fasting glucose and insulin concentrations (47). For all analyses, a Bonferroni corrected post-hoc test was used for exploration of the differences at every time point when significance was detected. Effect sizes (ES) (Cohen`s *d*) and their 95% confidence intervals were calculated where appropriate, whereby an ES of 0.20, 0.50 and 0.80 refers to a small, moderate or large effect, respectively

(7). The effect sizes for a Time x Group (T x G) or Time x Condition (T x C) interaction were calculated by comparing the pre-post change scores in each group or condition. Correlations were computed using Pearson`s *r*. As the latter was an explorative analysis, the risk for a type II error was not deemed problematic, and no Bonferroni correction was applied (56). The 23rd version of the statistical package SPSS (SPSS inc, Chicago, US) was used for all analyses and statistical significance was set at $p<0.05$.

Results

Participants

Baseline characteristics of the participants in the intervention group (INT) and CON can be seen in Table 1. Apart from a trend towards a larger hip circumference in the control group, there were no differences in anthropometrics and physical activity levels between the groups.

***** Insert Table 1 around here *****

Acute responses to hot water immersion

The physiological and perceptual responses during HWI_{pre} and AMB are given in Table 2. During HWI_{pre}, rectal temperature increased from 37.1±0.6°C to 38.7±0.4°C (Fig 2). Folllowing the intervention period, diastolic blood pressure was lower at the end of HWI_{post} when compared to HWI_{pre} (F: 25.4, $p = 0.001$). Thermal sensation at the end of HWI_{post} was lower than at the end of HWI_{pre} (F: 14.3, $p = 0.01$) and sweat loss during HWI was increased from 1.1±0.6 (HWI_{pre}) to 1.7±0.6 L (HWI_{post}) (F: 26.5, $p = 0.001$).

***** Insert Table 2 and Figure 2 around here *****

Plasma concentrations of IL-6 were higher compared to AMB immediately following $HWI_{pre,}$ (T x C; F: 14.5, $p<0.001$, ES: 1.71 (1.31 – 2.07)). However, this was not accompanied

by a rise in either eHsp72 (T x C; F: 1.9, $p = 0.16$) or iHsp72 in total monocytes (T x C; F: 0.5, $p = 0.57$) directly post or 2 h post-HWI_{pre} (Fig. 3). The same was true for the expression of iHsp72 in classical monocytes (T x C: F: 1.7, $p = 0.22$), intermediate monocytes (T x C; F: 2.3, $p = 0.19$) and non-classical monocytes (T x C; F: 1.5, $p = 0.25$). *R* did not differ between HWI_{pre} and AMB (T x C; pre-post F: 0.6, $p = 0.48$; pre-post+2h F: 0.1, $p = 0.76$).

***** Insert Figure 3 around here *****

The distribution of monocyte subsets changed immediately after HWI_{pre}, with an increase of the intermediate (T x C; F: 9.0, $p = 0.004$, ES: 1.39 (0.36 – 2.03)) and non-classical monocytes (T x C; F: 11.8, $p = 0.001$, ES: 1.34 (0.32 – 1.24)). The proportion of classical monocytes, however, was not reduced (T x C; F: 2.5, $p = 0.10$) (Table 3). Lymphocyte numbers increased to a larger extent directly following HWI_{pre} compared to AMB (T x C; F: 11.0, $p = 0.003$, ES: 1.97 (0.84 – 2.94)). There was no difference between HWI_{pre} and AMB in the acute elevation of total monocyte (T x C; F: 0.8, $p = 0.56$), leukocyte (T x C; F: 2.0, $p = 0.16$) or neutrophil numbers (T x C; F: 2.7, $p = 0.08$). The increase in plasma nitrite concentration directly following HWI_{pre} was larger compared to AMB (F: 11.2, $p = 0.04$, ES:1.82 (0.71 – 2.77); Fig. 2).

***** Insert Table 3 around here *****

The IL-6, eHsp72 and iHsp72 response did not differ following HWI_{post} when compared with HWI_{pre} (T x C; IL-6 F: 0.3, $p = 0.80$, eHsp72 F: 0.9, $p = 0.45$, iHsp72 F: 0.1, $p = 0.71$). The same was true for Hsp72 expression in classical (T x C; F: 1.7, $p = 0.22$), intermediate (T x C; F: 2.2, $p = 0.17$) and non-classical monocytes (T x C; F: 1.5, $p = 0.25$). In contrast to HWI_{pre}, the percentage of intermediate monocytes was not elevated following HWI_{post} (Time; F: 3.4, $p = 0.06$; Table 3). There were no differences in the acute change between HWI_{pre} and HWI_{post} for total leukocyte (T x C; F: 1.3, $p = 0.36$), monocyte (T x C; F: 0.2, $p = 0.92$),

lymphocyte (T x C; F: 1.9, $p = 0.17$) and neutrophil (T x C; F: 0.8, $p = 0.56$) numbers. Finally, the acute change in plasma nitrite concentration was similar between HWI_{pre} and HWI_{post} (T x C; F: 1.3, $p = 0.30$) (Fig. 3).

Chronic effects of the hot water immersion intervention period

Table 4 shows the physiological responses during the HWI sessions of the intervention period. Body mass did not change in INT following the intervention period (92.1±9.2 kg to 92.3±9.5 kg, F: 0.01, $p = 0.92$). Both systolic (T; F: 5.1, $p = 0.05$, ES: 0.60 (0.34 – 1.44)) and diastolic blood pressure (T; F: 14.3, $p = 0.003$, ES: 0.64 (0.32 – 1.47)) were lowered following the intervention period. Resting HR (T; F: 0.3, $p = 0.54$) and Trec (T; F: 0.4, $p = 0.22$) were not affected by the intervention period (Table 2). Physical activity levels were not different from habitual physical activity (as reported at the start of the intervention period) during the intervention period (T; F: 0.2, $p = 0.64$).

**** Insert Table 4 around here ****

The effect of the intervention period on resting IL-6, iHsp72 and eHsp72 levels is presented in Fig. 4. Resting levels of IL-6 and iHsp72 in total monocytes were not altered following the intervention period (T x G; IL-6 F: 0.1, $p = 0.87$, iHsp72 F: 0.2, $p = 0.59$). The same was true for the expression of iHsp72 in the monocyte subsets (T x G; classical monocytes F: 1.8, $p = 0.14$; intermediate monocytes F: 1.2, $p = 0.39$; non-classical monocytes F: 0.3, $p = 0.78$). Extracellular Hsp72 was lowered in INT compared to CON (difference in fold change between groups; F: 6.8; $p = 0.03$, ES: 1.00 (0.73 – 1.26)). This resulted in a lower R in INT as compared to CON (G; F: 6.0, $p = 0.04$, ES: 0.34 (0.21 – 0.51)). The change in the distribution of monocytes subsets in the circulation at rest was not different in INT compared to CON (T x G; classical monocytes F: 0.8, $p = 0.52$, intermediate monocytes F: 1.1, $p = 0.23$, non-classical monocytes F: 1.8, $p = 0.14$) (Fig. 4).

***** Insert Figure 4 around here *****

Fasting blood glucose concentrations were lower in INT compared to CON following the intervention period (T x G; F: 5.0, $p = 0.04$, ES: 0.68 (0.42 – 0.97); Fig. 5). Fasting insulin concentrations did not change in INT compared to CON (T x G; F: 1.3, $p = 0.30$, ES: 0.50 (-0.46 – 1.42)). However, following inspection of the individual data an outlier was detected (Fig. 5, grey line), which was confirmed using the methods for outlier detection postulated by Leys et al. (46). After removing the insulin data of this participant, there was a larger decrease in fasting insulin in INT compared to CON (T x G; F: 4.8, $p = 0.04$, ES: 1.06 (0.02 – 2.00)). HOMA-IR was also reduced to a larger extend in INT compared to CON (T x G; F: 5.5, $p = 0.03$, ES: 1.07 (0.08 – 2.06)). Finally, there was no difference in the change of resting plasma nitrite concentrations between INT and CON (INT 321±69 nM to 234±64 nM; CON 230±57 nM to 262±77 nM; T x G; F: 1.7, $p = 0.17$).

***** Insert Figure 5 around here *****

One week following the post blood sample, resting iHsp72 (pre: 307±53 GMFI, post: 309±69, post+1week: 358±116; T; F: 1.8, $p = 0.22$), IL-6 (pre: 1.22±0.52 pg/ml, post: 1.31±0.53, post+1week: 1.12±0.65; T; F: 0.2, $p = 0.67$), the percentage of classical monocytes (pre: 94.4±1.8%, post: 91.9±4.5%, post+1week: 94.1±1.3%; T; F: 1.7, $p = 0.18$), intermediate monocytes (pre: 1.25±0.38%, post: 1.69±0.73%, post+1week: 1.47±0.51%; T; F: 1.0. $p = 0.27$) and non-classical monocytes (pre: 2.70±0.92%, post: 3.10±1.09%, post+1week: 3.39±1.35%; T; F: 1.0, $p = 0.28$) were not changed compared to either pre or post. Resting concentrations of eHsp72 were elevated compared to post (fold change pre-post: 0.83±0.41, fold change pre-post+1 week: 1.28±0.34, T; F: 5.8, $p = 0.03$, ES: 0.83 (0.20 – 1.84)). The lowering of fasting blood glucose following the intervention period was still present at post+1 week (pre: 4.44±0.93 mmol/L, post: 3.98±0.98 mmol/L, post+1week: 3.89±0.77 mmol/L, T;

F: 25.1, p = 0.001, ES: 0.61 (0.08 – 1.32). However, fasting insulin was elevated at post+1 week compared to post (pre: 68.10±44.65 pmol/l, post: 51.7±27.3 pmol/l, post+1week: 72.6±56.3 pmol/l, T; F: 4.5, p = 0.05, ES: 0.53 (0.05 – 1.08), returning to the insulin concentrations found prior to the intervention (pre- post+1 week, T; F: 1.1, p = 0.21). There was no difference in HOMA-IR between post+1 week compared with post (pre: 13.91±11.09, post: 8.99±7.89, post+1 week: 12.40±10.01, T; F: 4.1, p = 0.06) or pre (T; F: 0.8, p = 0.47). Plasma nitrite concentrations were not changed at post+1 week compared to pre or post (pre: 314±61 nM, post: 247±66 nM, post+1week: 304±91 nM; T; F: 3.9, p = 0.09).

Correlations

During $HWI_{pre,}$ there was no correlation between the peak core temperature attained and the acute change in iHsp72 expression (r = -0.11, p = 0.77), plasma IL-6 (r = 0.23, p = 0.55) or nitrite concentrations (r = 0.04, p = 0.91). Following the chronic intervention, there was a negative correlation between plasma insulin concentration at baseline and its change following the intervention (r = -0.45, p = 0.01). There was no relationship with insulin at baseline and the change in blood glucose concentrations (r = 0.23, p = 0.33). No correlation was observed between baseline blood glucose concentration and the chronic change in insulin (r = -0.28, p = 0.27) or glucose concentrations (r = 0.29, p = 0.25). In addition, there was no correlation between the fold change in eHsp72 following the intervention and the change in insulin (r = 0.61, p = 0.06) or glucose concentrations (r = 0.03, p = 0.94). Finally, there was no correlation between the chronic change in iHsp72 expression and the chronic change in insulin (r = -0.16, p = 0.66) or glucose concentrations (r = 0.21, p = 0.56).

Discussion

This study investigated the acute inflammatory response to HWI as well as the potential of chronic HWI to improve inflammatory and metabolic profiles at rest. Acute HWI evoked elevated plasma IL-6 and nitrite concentrations, and an increase in the percentage of intermediate and non-classical monocytes. This was however not accompanied by an increase in iHsp72 expression. Two weeks of chronic HWI reduced fasting glucose, insulin and eHsp72 concentrations. Together, this indicates that HWI may be a useful strategy to improve aspects of the inflammatory profile and glucose metabolism in individuals without the physical capacity to do so using exercise training.

Acute responses to hot water immersion

Our observation that one hour of HWI in water set at 39°C induced a significant increase in plasma IL-6 concentrations corroborates with the notion that increases in body temperature can serve as an independent stressor to induce an acute inflammatory response. Previous studies employing 1 h of HWI have shown comparable increases in plasma IL-6 concentrations to the current study (16, 45), while 2 h of HWI results in a more marked IL-6 response (43). Consistent with exercise studies (17), this suggests that the IL-6 response to HWI is dose dependent. In line with this, a more intense HWI protocol than used in the present study (i.e. longer duration or warmer water) may be required to induce changes in iHsp72 or eHsp72. Oehler et al. (54) reported an acute increase in iHsp72 following HWI of 2 h in water set at 39.5°C, while a session of 1 h did not result in elevated iHsp72 expression (50). On the other hand, Faulkner et al. (16) reported acute increases in eHsp72 following immersion up to the waistline for 1 h in water set at 40°C, resulting in a ~1°C increase in core temperature. As the acute inflammatory response to HWI seems dose dependent, it is conceivable that there may exist a threshold in core or muscle temperature or time accrued

above this threshold that needs to be reached in order to induce an iHsp72 response. Using exercise as a stressor, Gibson et al. (24) have suggested that at least ~27 min above a core temperature of 38.5°C is needed to induce the upregulation of Hsp72 mRNA. In the current study, participants` rectal temperature exceeded 38.5°C for ~15 min only. This may also explain why an acute increase in iHsp72 following passive heating is a consistent finding in animal studies (28, 64), but not in human studies (50), as the endogenuous heat stress imposed in the former is much higher compared with the present and other studies in humans. Of note, the required heat stress might need to be even higher to induce acute increases in circulating eHsp72 concentrations (23).

Although the HWI protocol used in this study did not elevate iHsp72 expression, the acute increase in IL-6 concentrations indicates that in analogy to exercise, passive heating can also induce an acute inflammatory response, possibly leading to the circulating anti-inflammatory milleau postulated by Petersen and Pedersen as one of the benefits of exercise (57). While it is now widely acknowledged that contracting skeletal muscle is the main source of IL-6 during acute exercise (17), it is not clear whether this is also the case for HWI. However, skeletal muscle is suggested to secrete IL-6 in response to increases in local temperature (66). HWI for 1 h in water set at 40°C leads indeed to a muscle temperature increase of ~2.5°C (16). Suggested mechanisms for the acute inflammatory response following passive heating are the influx of calcium via the opening of the thermosensitive transient receptor potential 1 (53) and the activation of heat shock factor 1, which can both result in the production of IL-6 and Hsp72 (66). In addition, circulating monocytes are potent producers of cytokines and might be a source of IL-6 found in the circulation following HWI (1). The acute recruitment of intermediate and non-classical monocytes seen following HWI in this study could indeed have led to increased IL-6 secretion into the circulation as these subsets are known to release more IL-6 in response to an in-vitro stimulant such as

lipopolysacharide (30). However, since monocytes only represent a small percentage of leukocytes, it is not known what the impact of acute changes in circulating monocyte subsets on circulating cytokines is (65). Nevertheless, since the proportion of relatively inflammatory monocytes (i.e. intermediate and non-classical monocytes) at rest are positively associated with the risk for a range of chronic diseases (69), the acute shift following HWI found in this study provides rationale for further research in the potential of HWI interventions to chronically alter the distribution of monocyte subsets in the circulation.

While the interest in HWI to reduce chronic low-grade inflammation is a relatively recent phenomenon, its potential to increase blood flow and enhance vascular function is more established (13). Nevertheless, we show for the first time an acute increase in the bioavailability of the vasodilator NO in response to HWI in humans, possibly mediated by the enhanced activation of eNOS in response to the increase in shear stress and/or local temperature (19). Additionally, as Hsp90 acts as an agonist for NO production by eNOS, the acute increase in NO bioavailability may have been mediated by an increased expression of Hsp90 (22). Future studies are therefore needed to identify the potential of HWI to increase Hsp90 expression. Since the acute increase in NO following HWI has the potential to aid tissue blood flow and is implicated in the translocation of GLUT4 to the plasma membrane of skeletal muscle cells during exercise (59), HWI has the potential to facilitate glucose disposal in skeletal muscle and other tissues (2, 20). In support, animal studies suggest GLUT4 translocation (25) and enhanced insulin sensitivity in skeletal muscle (27) following an acute HWI session. Of note, in the current study the acute effects of HWI on glucose disposal were not assessed and the implications of an acute increase in NO bioavailability on glucose disposal are therefore only speculative. Indeed, the chronic reduction in fasting glucose and insulin found in the current study occured independently of changes in resting plasma nitrite concentrations.

If passive heating is to be successfully introduced as a health promoting intervention in practice, it is important to assess perceptual responses to provide insight into its potential to influence adherence rates to the intervention (68). In the current study, the perceptual responses during 1 h of HWI of indicated profound feelings of discomfort similar to those reported during high-intensity interval training (32, 38). This implies that further increases in water temperature or session duration would result in an activity that is difficult to adhere to (15). Therefore, although more intense HWI sessions than the one used in the current study seems to be needed to induce an acute Hsp72 response, the practical application of HWI sessions such as the one applied in the study of Oehler et al. (54; 2 h at 39.5°C) in the general population is questionable. Moreover, the absence of more positive affective responses during HWI_{post} as compared to HWI_{pre} suggests that no short-term improvements in the perceptual responses can be expected as a result of regular engagement in HWI. Therefore, future studies could test different HWI protocols in an attempt to optimise the balance between delivering a HWI stimulus that evokes the neccessary inflammatory and metabolic benefits without eliciting negative affective responses that have the potential to limit adherence to the intervention. Finally, although HWI did not induce acute changes in Hsp72, we did observe acute elevations of nitrite and IL-6 in addition to chronic improvements in fasting glucose, insulin and eHsp72. This suggests that there may be no need to further increase the thermal load of the HWI sessions to improve metabolic health and that the focus could be directed towards the improvement of the perceptual responses during HWI. A titration study in which the thermal load is gradually reduced may be useful to gain insight in the minimal passive heat stress needed to induce acute changes in factors such as plasma IL-6 concentrations and NO bioavailability and its impact on the perceptual responses during HWI.

As suggested by several authors (34, 42, 48), HWI interventions could serve as a strategy to improve insulin sensitivity, possibly via the elevation of iHsp72 expression and/or reduced chronic low-grade inflammation. In line with this suggestion that emanated from the pilot study by Hooper et al. (33), fasting glucose and insulin concentrations were reduced following the 2-week HWI intervention period applied in the current study. This was accompanied by a reduction in eHsp72 concentrations. However, no changes in resting iHsp72 expression, or plasma IL-6 and nitrite concentrations were found.

Animal studies suggest increased basal iHsp72 levels as a mechanism behind the beneficial changes in insulin sensitivity reported following hot water immersion (6, 26). Moreover, Hsp72 knock-out mice are highly insulin resistant and do not experience similar benefits from passive heating strategies compared to mice expressing Hsp72 (12). However, in the current study reductions in fasting glucose and insulin were found in the absence of changes in iHsp72. The reason for this discrepancy might lie in the tissue in which iHsp72 was assessed. While most animal studies have investigated iHsp72 in skeletal muscle, in the current study iHsp72 expression was assessed in monocytes. Although the acute iHsp72 responses in leukcoytes follow the same pattern as those found in skeletal muscle (64) and in-vitro heat shock upregulates iHsp72 expression in monocytes (62), the chronic adaptations to heat therapy and health interventions in monocytes are less clear. While heat acclimation using exercise can induce increases in monocyte iHsp72 expression (44), trained runners actually express lower levels of iHsp72 in leukocytes compared to their sedentary counterparts (18). More studies that simulatiously measure iHsp72 expression in both tissues following health interventions are therefore needed to resolve the mechanisms for enhanced glucose metabolism after HWI. It should be acknowledged that the chronic intervention may

have impacted on other factors implicated in glucose metabolism, as for instance passive lower-limb heating can chronically elevate peroxisome proliferator-activated receptor-gamma coactivator 1-α (PGC-1α) expression (28).

Despite no changes in resting iHsp72, eHsp72 concentrations were significantly lowered following the intervention period. When present in the circulation, eHsp72 can activate monocytes via the Toll-like receptor 4/CD14 complex, resulting in the secretion of pro-inflammatory cytokines such as IL-6, tumour necrosis factor-α (TNF-α) and IL-β (1). As the latter cytokines can directly interfere with insulin sensitivity (35), it is suggested that the deletirious effects of eHsp72 on health are exhibited via this mechanism (37). Additionally, the positive change in *R* in INT might be indicative of an improved inflammatory profile following the intervention period, as suggested by Krause et al. (41). However, the influence of eHsp72 and changes in *R* on glucose metabolism needs to be studied in more detail.

While previous studies have found changes in the inflammatory profile following short-term health interventions, the relatively short duration of the HWI intervention period might have been the reason for the absence of changes in resting levels of iHsp72, IL-6, monocyte subset distrubution and NO bioavailbility. On the other hand, it is striking that only 10 HWI sessions resulted in reductions in fasting glucose, insulin and blood pressure in males that were sedentary and overweight, but did not show signs of pre-diabetes or strongly elevated inflammatory markers at baseline. The positive correlation between baseline fasting insulin concentrations and the reduction in fasting insulin following the intervention suggests that those with more impaired metabolic health might benefit most from HWI. The lowered blood pressure following the intervention period supports recent findings by Brunt et al. (4), suggesting that HWI may also be a potent strategy to improve vascular health. While iHsp72- and NO-mediated mechanisms are suggested to play a role in this effect (5), the

improvements in blood pressure in the present study were independent of changes in resting levels of both measures.

Together, the current study provides a strong rationale to pursue further research on the potential of passive heating strategies to enhance (cardio)metabolic health. For instance, future studies should consider using more robust measures of insulin sensitivity (e.g. oral glucose tolerance testing), implementing longer-term interventions and explore its effectiveness and feasibility in populations that could benefit most from this alternative health intervention (e.g. individuals with a spinal cord injury, frail elderly or those with other conditions that interfere with exercise participation). Additionally, future studies in humans are needed to clarify the role of inflammatory markers in glucose metabolism. In this regard, the relatively modest heat stress imposed in the present study may be considered a limitation. Although here an applicable model of passive heating is presented, future mechanistic studies may consider increasing body temperature to a larger extent and for longer durations. For instance, a passive heating model that is more likely to elevate iHsp72 expression may aid our understanding on the importance of this marker for glucose metabolism in humans. Finally, although there was no acute iHsp72 response following HWI and resting iHsp72 expression in monocytes was not changed following the intervention, an elevated iHsp72 expression in skeletal muscle for up to 7 days has been reported following exercise (51). Therefore, the resting and post-immersion inflammatory and metabolic markers may have been influenced by the potentially elevated iHsp72 expression in skeletal muscle.

In summary, a single HWI session induces an acute inflammatory response, indicated by acute elevations in IL-6, changes in the monocyte subset distribution, and increase in NO synthesis, indicated by increased plamsa nitrite concentrations. However, these responses were not accompanied by acute increases in iHsp72 or eHsp72. The 2-week HWI intervention period reduced fasting glucose and insulin, concomitant with lower resting eHsp72

concentrations, but independent of iHsp72 expression, plasma IL-6 and nitrite concentrations at rest, as the latter markers did not change following the chronic intervention. Therefore, this study provides support for the use of HWI to improve aspects of the inflammatory profile and enhance glucose metabolism in sedentary, overweight males, and might have implications for improving metabolic health in populations unable to meet the current physical activity recommendations.

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Fig. 1 Outline of the study procedures for the intervention group (INT). An acute HWI (HWI_{pre}) and control trial (AMB) were followed by ten HWI sessions within two weeks. A second acute HWI trial (HWI_{post}) was conducted three days after completion of the intervention period and a resting blood sample was taken seven days following HWI_{post} (Post). For the control group (CON), a resting blood sample was taken at the time-points corresponding to visit 1 and 13 of the intervention group.

Fig. 2 Rectal temperature during and following AMB, HWI_{pre} and HWI_{post} (n = 10). * Significantly different from AMB.

Fig. 3 The acute changes in plasma IL-6, eHsp72, iHsp72 and nitrite concentrations following AMB, HWI_{pre} and HWI_{post}. Black lines represent individual data points, while the bars represent the group mean (n = 10). *Significant time x trial interaction when compared with AMB.

Fig. 4 Resting levels of the inflammatory outcome measures before and after the HWI intervention period. INT: intervention group (n = 10), CON: control group (n = 8). The black lines represent individual data points, while the bars represent the group mean. ^ Significant difference between groups.

Fig. 5 Fasting blood glucose and plasma insulin concentrations for the intervention and control group. INT: intervention group (n = 10), CON: control group (n = 8). The black lines represent individual data points, while the bars represent the group mean. ^ Significant time x group interaction. Participant with grey line does not contribute to the bar representing the group mean.

814 Table 1. Participant characteristics at baseline. Data are presented as mean ± SD.

815

816 Table 2. Physiological responses to the hot water immersion and control trial. Data are presented as
817 mean ± SD.

818

819 Table 3. The distribution of monocyte subsets in whole blood following hot water immersion and the
820 control trial. Data are presented as mean ± SD.

821

822 Table 4. Physiological responses during the sessions of the 2-week intervention period. Data are
823 presented as mean ± SD.

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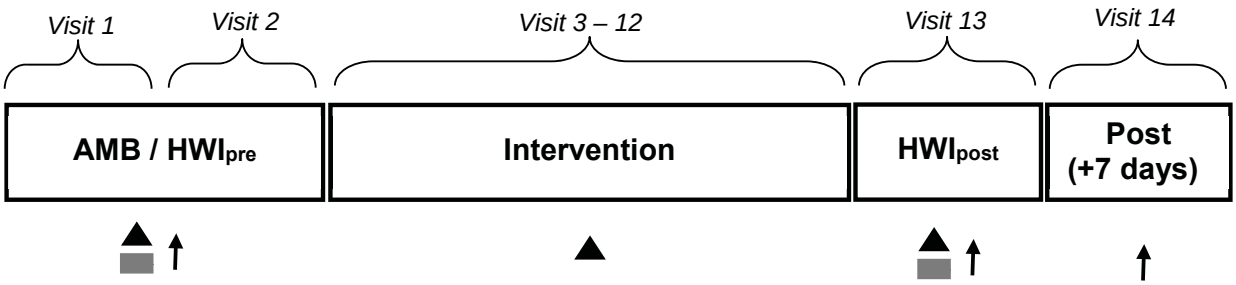
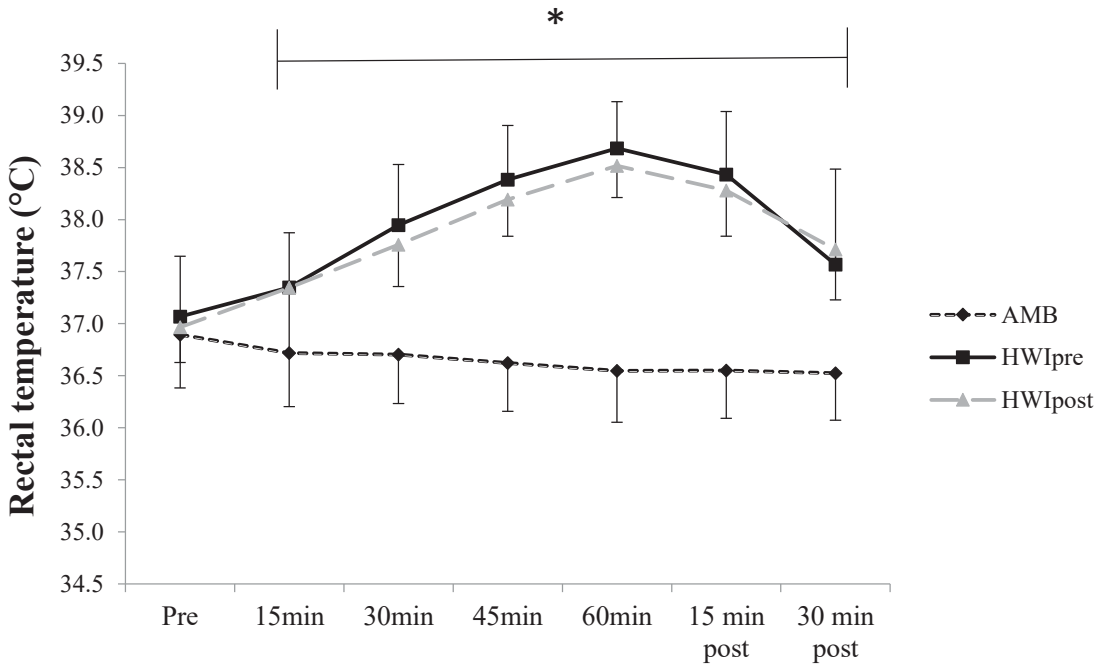
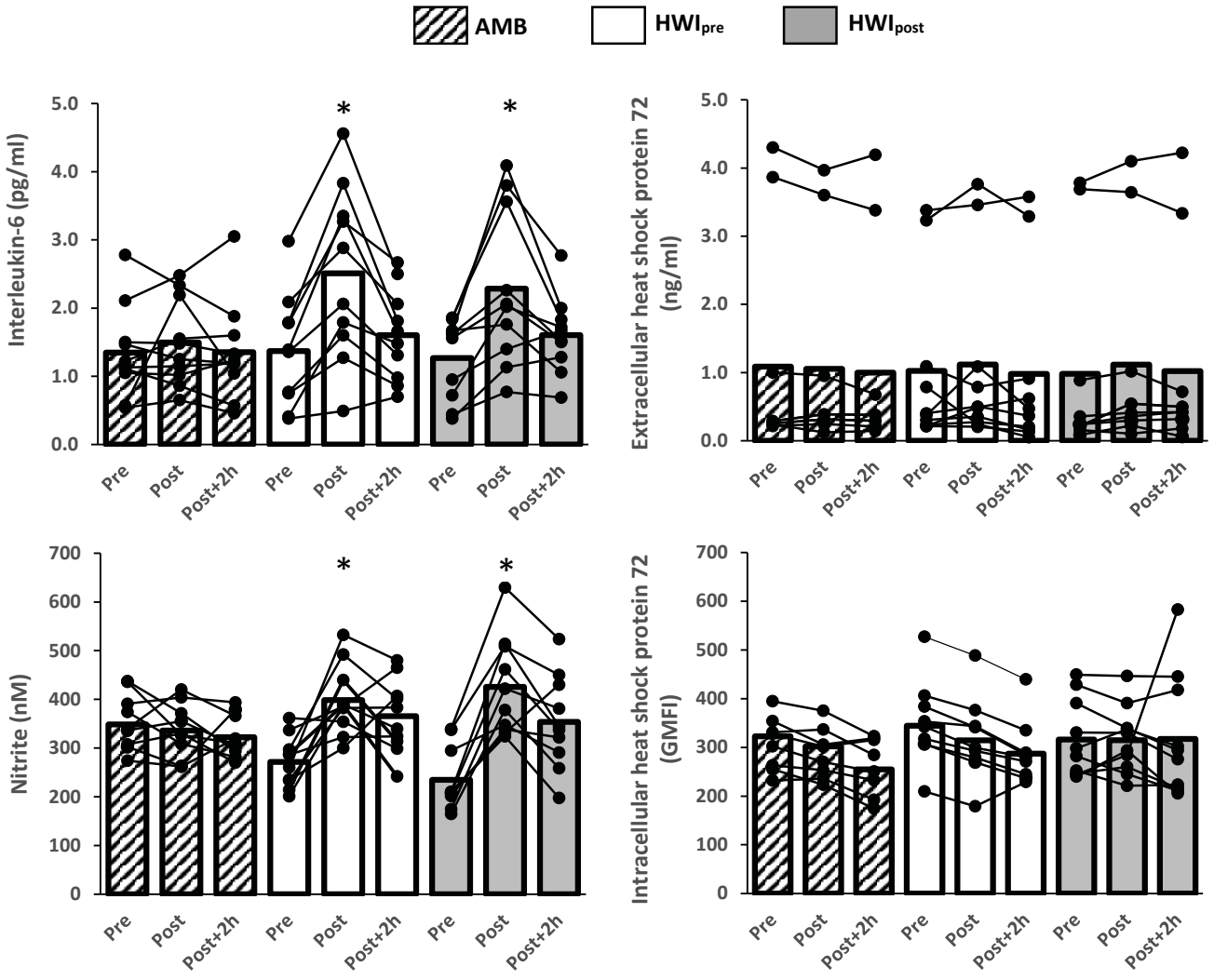


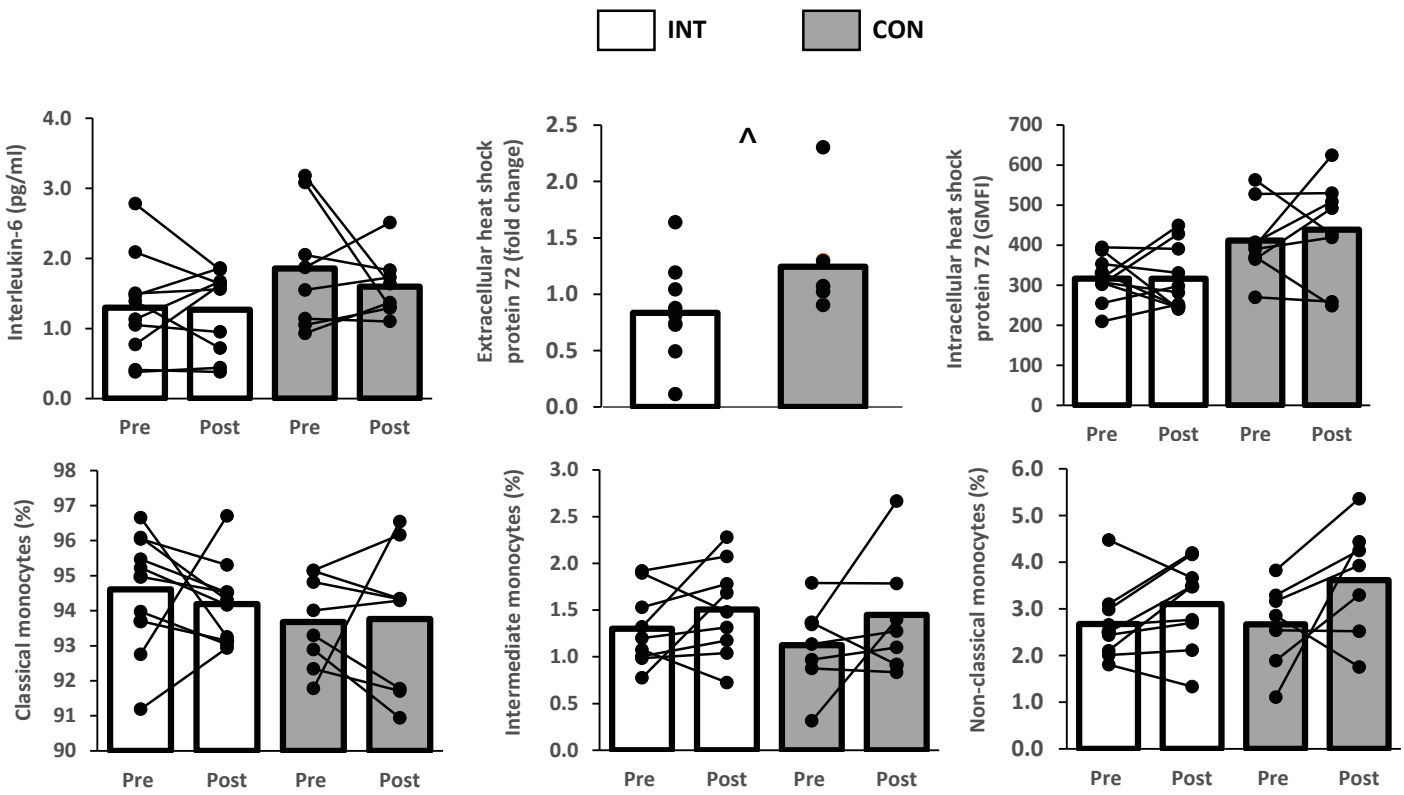
Blood sample(s)

Heart rate, perceptual responses, core temperature

Oxygen uptake, blood pressure







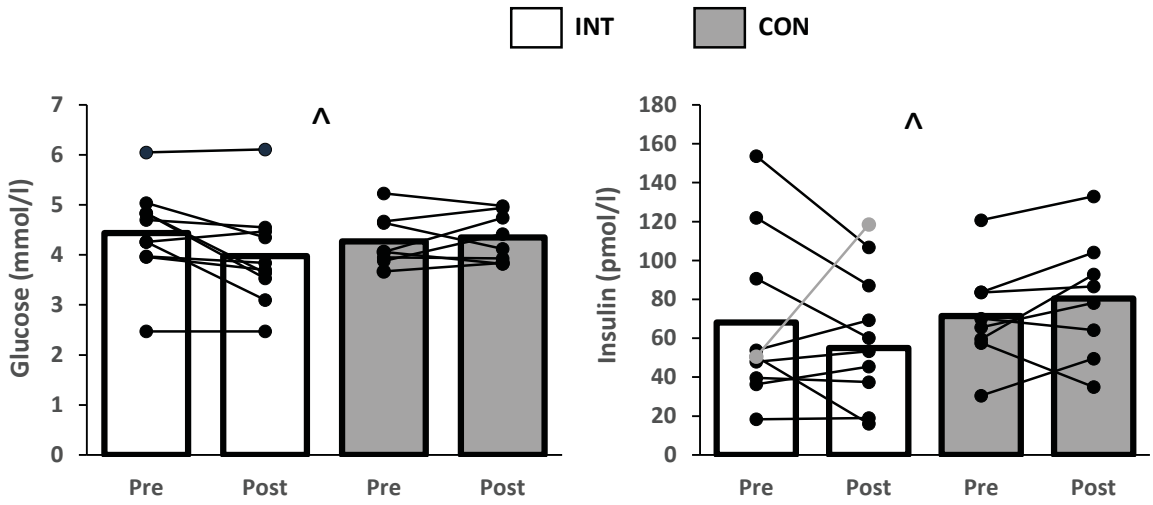


Table 1. Participant characteristics at baseline. Values are given in mean ± SD

Parameter	INT (N=10)	CON (N=8)	*p* (ES)
Age (yrs)	33.2±10.1	32.0±7.5	0.39(0.14)
Height (cm)	173.2±6.9	176.9±4.2	0.17(0.67)
Body mass (kg)	92.1±9.2	93.9±9.4	0.36(0.21)
BMI (kg/m^2)	31.0±4.2	30.0±2.5	0.37(0.30)
Fat percentage (%)	25.1±3.5	25.7±2.3	0.23(0.18)
Waist circumference (cm)	96.9±4.7	100.1±8.4	0.14(0.52)
Hip circumference (cm)	103.9±4.1	109.4±5.1	0.08(1.28)
Structured exercise (min/week)	38±54	29±44	0.49(0.19)

Abbreviations: BMI = body mass index; ES = Cohen`s d effect size, INT = intervention group, CON = control group

Table 2. Physiological responses to the hot water immersion and control trial. Data are presented as mean ± SD.

Parameter	AMB Pre	AMB 60 min	HWI_{pre} Pre	HWI_{pre} 60 min	HWI_{post} Pre	HWI_{post} 60 min
T_{rec} (°C)	36.9±0.5	36.6±0.5	37.1±0.6	38.7±0.4*	37.0±0.3	38.5±0.3*
T_{tymp} (°C)	35.5±0.4	35.2±0.4	35.4±0.7	37.5±0.7*	35.2±0.3	37.6±0.3*
HR (bpm)	69±17	64±15	67±14	105±13*	68±12	104±10*
VO_2 (L/min)	0.20±0.04	0.23±0.04	0.21±0.04	0.42±0.10*	0.19±0.05	0.37±0.03**
SBP (mmHg)	127±10	123±12	126±13	138±15*	118±15^	126±13^
DBP (mmHg)	86±9	85±10	83±9	78±9	79±11^	71±12*^
Basic affect (-5 to +5)	0.9±1.4	0.7±1.3	1.3±2.0	-1.1±2.2*	1.1±1.9	-1.2±1.9*
TS (1 to 9)	4.9±0.6	4.8±0.8	5.1±0.9	7.4±1.0*	4.7±0.5	6.7±0.8*^
TC (-5 to +5)	0.0±0.0	0.0±0.5	0.2±0.4	2.2±1.0*	-0.1±0.3	2.3±1.3*
Sweat loss (L)	N/A	0.17±0.19	N/A	1.12±0.56*	N/A	1.65±0.57*^
PV change (%)	N/A	98±3	N/A	95±8	N/A	95±4

Abbreviations: AMB = control trial; HWIpre = hot water immersion session prior to HWI intervention period; HWIpost = hot water immersion session following HWI intervention period; T_{rec} = rectal temperature; T_{tymp}: tympanic temperature; HR = heart rate; VO_2 = oxygen uptake; SBP = systolic blood pressure; DBP = diastolic blood pressure; TS = thermal sensation; TC = thermal comfort (higher TC scores reflect reduced feelings of thermal comfort), PV = plasma volume

* Significantly different from AMB; ^ Significant difference between HWI_{pre} and HWI_{post}

Table 3. The distribution of monocyte subsets in whole blood following hot water immersion and the control trial.

	Classical monocytes (%)	Intermediate monocytes (%)	Non-classical monocytes (%)
AMB pre	94.6±2.3	1.3±0.4	2.6±0.7
AMB post	94.0±4.8	1.3±0.3	2.4±0.5
AMB p2h	94.2±1.9	1.4±0.6	2.6±1.1
HWI_{pre} pre	94.50±4.0	1.3±0.6	2.2±0.9
HWI_{pre} post	91.2±5.8	1.7±0.7*	3.4±1.9*
HWI_{pre} p2h	94.4±2.7	1.3±0.4	2.0±0.4
HWI_{post} pre	94.2±1.2	1.5±0.6	3.1±1.0
HWI_{post} post	93.9±1.7	1.4±0.4^	3.5±0.4*
HWI_{post} p2h	94.7±1.5	1.2±0.5	2.7±1.3

Date are mean±SD. Abbreviations: AMB = control trial; HWIpre = hot water immersion session prior to HWI intervention period; HWIpost = hot water immersion session following HWI intervention period, p2h = 2 hours post hot water immersion

*Significantly different from AMB; ^ Significant difference between HWI_{pre} and HWI_{post}

Table 4. Physiological responses during the sessions of the 2-week intervention period.

Parameter	Pre session 1-5	End session 1-5	Pre session 6-10	End session 6-10
Tympanic temperature (°C)	35.3±0.4	37.5±0.2*	35.1±0.3	37.5±0.3*
TS (1 to 9)	4.8±0.5	6.6±0.2*	4.9±0.4	6.7±0.2*
Basic affect (-5 to +5)	1.0±1.0	0.0±2.0	1.2±1.6	-0.7±1.8
HR (bpm)	67±13	105±2*	68±14	105±3*

Abbreviations: TS = thermal sensation; HR = heart rate. Data are means of five sessions during INT. End = measurement taken in the final 30 s of the session. Session 1-5 lasted 45 min, while session 6-10 lasted 60 min.
* Significantly different from Pre session.