Passive heating, HSP70, IL-6 and Energy Expenditure: A treatment tool for metabolic diseases?

Faulkner, SH; Jackson, S; Fatania, G; and Leicht, CA

National Centre for Sports and Exercise Medicine, School of Sport Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire, LE11 3TU, United Kingdom

Running Head: Passive heating - a treatment tool for metabolic diseases?

Address for correspondence:
Dr Steve H. Faulkner
National Centre for Sports and Exercise Medicine,
School of Sport Exercise and Health Sciences,
Loughborough University,
Loughborough, Leicestershire,
LE11 3TU,
United Kingdom

Email: S.Faulkner2@lboro.ac.uk

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Abstract

Exercise and physical activity remain the gold standard methods of enhancing and maintaining health and wellbeing. However, in populations that benefit most from exercise, adherence is often poor and alternatives to exercise are important to bring about health improvements. Recent work suggests a role for passive heating (PH) and heat shock proteins (HSP) in improving cardio-metabolic health. The aim of this study was to investigate the expression of HSP70 and IL-6 in response to either exercise (EX) or PH and the subsequent effect on glucose control. Fourteen males volunteered and were categorized lean (BMI 23.5 ± 2.2 $Kg \cdot m^{-2}$) or overweight (29.2 ± 2.7 $Kg \cdot m^{-2}$) and completed 60 minutes of either moderate cycling at a fixed rate of metabolic heat production (EX) or warm water immersion in 40°C water (PH). Extracellular HSP70 increased from baseline in both conditions with no differences between PH (0.98 ± 1.1 $ng \cdot mL^{-1}$) or EX (0.84 ± 1.0 $ng \cdot mL^{-1}$, $P=0.814$). IL-6 increased following both conditions with a 2 fold increase after PH and 4 fold after EX. Energy expenditure increased by 61.0 ± 14.4 kcal (79%) after PH. Peak glucose concentration after a meal immediately following PH was reduced when compared with EX (6.3 ± 1.4 $mmol \cdot L^{-1}$ vs. 6.8 ± 1.2 $mmol \cdot L^{-1}$; $P<0.05$). There was no difference in 24-hour glucose area under the curve between conditions. These data indicate the potential for thermal therapy as a novel treatment and management strategy for type 2 diabetes where adherence, or ability to exercise may be compromised.

Keywords

Diabetes, Exercise, HSP70, Immersion, Metabolism, Passive Heating,

Abbreviations

AUC – Area under the curve

BMI – Body mass index

BP – blood pressure

BSA – body surface area

CGM – Continuous glucose monitor

CI – confidence interval

CV – coefficient of variation

eHSP – extracellular heat shock protein

ELISA - sandwich enzyme-linked immunosorbent assays

ES – effect size

EX – Exercise

HDL-C - High density lipoprotein

$\dot{H}_{prod}$ - Metabolic heat production

HSP – Heat shock protein

iHSP – Intracellular heat shock protein

IL-1ra – Interleukin 1 receptor antagonist

IL-6 – Interleukin 6

IL-10 – Interleukin 10

kDa – kilo Dalton

LDL-C – Low density lipoprotein

LEAN – Lean participant group

OW – Overweight participant group

PH – Passive heating

SD – Standard deviation

SEM – Standard error of mean

T2DM – Type 2 diabetes mellitus

T_b - body temperature

T_c – core temperature

T_m – muscle temperature

T_{sk} – skin temperature

TG – Triglyceride

$\dot{V}CO_2$ –carbon dioxide production

$\dot{V}O_2$ – oxygen uptake

$\dot{V}O_{2max}$ – Maximum volume of oxygen uptake

ΔT – change in temperature

Introduction

Overweight, obesity and type 2 diabetes mellitus (T2DM) are characterised by chronic inflammation and impairments to insulin sensitivity and glucose control. Exercise and physical activity remain the gold standard methods of enhancing and maintaining health and wellbeing. However, in populations that benefit most from exercise, adherence is often poor, most likely due to medical conditions and disability,[1] poor motivation and a lack of convenience.[2,3] There are many factors involved in the development and treatment of insulin resistance, with the roles played by both thermal therapy [4] and heat shock proteins (HSPs) receiving increased attention.[5-8] Furthermore, it has been suggested that HSPs may provide an early indicator or the onset of metabolic diseases, such as T2DM, before elevations in plasma glucose become evident.[9,10]

HSPs are synthesised in response to a number of physiological stressors, such as exercise in the heat,[11-13] and are individually characterised by their molecular weight. The most widely studied HSP in humans is the 70 kDa family of HSPs which includes both the constitutive HSP73 and inducible HSP72 forms. HSPs have wide-ranging functions including their roles as thermo-protectants and involvement in protein synthesis.[14] The resting level of extracellular HSP70 (eHSP70) appears to be related to adiposity and inflammation with elevations reported in obesity and T2DM, whereas resting intracellular HSP70 (iHSP70) is decreased in T2DM.[10] A reduction in iHSP70 is an important component in the vicious cycle of chronic inflammation and reduced insulin signalling[15,16] and the development of insulin resistance.[17,18] Indeed, a reduction in iHSP70 has been reported in T2DM and correlates to insulin resistance and glucose disposal rates.[19,20]

eHSP70 is increased following exercise[13] with the magnitude of change dependent on both the duration and intensity.[21,22] A core temperature (T_c) threshold for elevations in eHSP70 has been suggested, with a 0.8-1.5˚C increase in T_c being required in order to elevate eHSP70[23,24] Recently, thermal therapy has been demonstrated to stimulate HSP70 production, although to a lesser extent than following exercise,[5,24] and may offer an alternative way by which the positive effects of HSP may be induced.

The influence of thermal therapy on glycemic regulation and metabolic disease has primarily been investigated using rodent models.[17,18,25-27] Chung et al.,[18] were the first to demonstrate that passive heating of mice resulted in an elevation in

HSP70 which was protective against the deleterious effects of consuming a high fat diet. Therefore, there is a need to investigate these rodent models and how well they translate into human participants.

There is emerging evidence to suggest that thermal therapy may have long-term benefits to metabolic and cardiovascular health in humans.[4,28] However, the mechanisms by which such an effect may occur remain to be elucidated. Notwithstanding, there is evidence to support the view that benefits of thermal therapy are likely to involve heat shock proteins.[10]

Whilst exercise has significant benefits to improving health, individuals with many types of chronic disease often experience low exercise tolerance.[29] Based on the above animal literature, there is evidence to suggest that thermal therapy may replicate some of the health benefits of exercise and alleviate some of the comorbidities often associated with chronic diseases such as T2DM.[30] If successful, the implementation of thermal therapy may help to lessen some of the financial burden of treating chronic by potentially reducing the dependence on pharmacological interventions. Thermal therapy could offer a simple home-based intervention that may appeal to individuals unable or unwilling to participate in regular exercise.

The primary aim of this study was to investigate the expression of eHSP70 in response to either exercise (EX) or thermal therapy in the form of passive heating (PH) via warm water immersion. A secondary aim was to investigate the effect of body composition on the eHSP70 response. Finally, we wished to investigate the potential effect of passive heating on glycemic control in response to replicated dietary intake. It was hypothesised that both EX and PH would elevate eHSP70, with a larger magnitude of change following EX. It was further hypothesised that there would be a differential response in eHSP70 between lean and overweight groups.

Methods

Participants. A total of fourteen males volunteered to participate in the study and were split into two groups i) Lean (LEAN, n=7; BMI <25 $kg \cdot m^{-2}$, body fat <15% and fat mass < 12kg) and ii) Overweight (OW, n=7; BMI > 27.0 $kg \cdot m^{-2}$, body fat >20% and fat mass >25kg). Participant characteristics are provided in table 1. All participants were healthy, non-smokers with no history of cardiovascular, haematological or metabolic disorders and had been weight stable for ≥ 3 months. Participants were habitually inactive, performing less than 1.5 hours of structured physical activity per week and had avoided any hot weather exposure in the previous two months, including frequent sauna or spa use.

Ethical approval. Full ethical approval was granted by the Loughborough University Ethical Advisory Committee. All procedures conformed to the principles defined in the Declaration of Helsinki. Participants were fully informed of the experimental protocols and any potential risks were identified before they provided their written consent to participate.

Experimental overview. Participants visited the laboratory on three occasions. Visit one consisted of preliminary measures including a blood profile, body composition and both submaximal and maximal oxygen uptake tests ($\dot{V}O_{2\,max}$ test). Visits two and three comprised the experimental trials which consisted of either 60 minutes of passive heating via warm water immersion (PH), or 60 minutes of exercise at a fixed rate of metabolic heat production (EX) which were completed in a counterbalanced order and were matched for ΔT_c. On arrival, participants were asked to void their bladder and were weighed in minimal clothing and then fully instrumented. Participants then completed either PH or EX as detailed below. On completion of each trial a second blood sample and muscle temperature measure were obtained, with a final blood sample being taken 2 hours after completion (figure 1). Experimental trials were separated by a minimum of 5 days to reduce any acclimation effect[31] and to minimise any effect of prior exercise or heating upon insulin sensitivity.[32] All trials were completed at the same time of day to minimise effects of circadian variation.

Preliminary visit. Participants arrived in a fasted state and a capillary blood sample was drawn for assessment of total cholesterol, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), glucose and triglycerides (TG) (Cardiochek, Polymer Technology Systems, Indianapolis, IN). Participants' height and weight were recorded (Seca 360, Birmingham, UK), and BMI calculated. Body composition was determined with skinfold callipers (Harpenden, Warwickshire, UK) using the 7-site formula.[33] Participants then completed a submaximal exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) with an online gas analyser (Metamax 3B, Cortex Biophysik GmbH, Germany). The test comprised four stages, increasing by 20 W every 4 minutes. $\dot{V}O_2$ and $\dot{V}CO_2$ were determined over a 30 second rolling average from the final minute of the each stage.

On completion of the submaximal test, participants were given a minimum of a 10-minute recovery period prior to commencing the $\dot{V}O_{2\,max}$ test. This test used a continual ramp protocol at a rate of 20 W·min^{-1} until the participant reached volitional exhaustion. $\dot{V}O_{2\,max}$ was determined over a 30 second rolling average from the final minute of the test.

Experimental trials. Seventy-two hours prior to the start of each experimental trail, participants reported to the laboratory where they were fitted with a continuous glucose monitor device (CGM; Freestyle Libre, Abbott Laboratories, Berkshire). The sensor was inserted on the posterior aspect of the upper limb, with interstitial glucose measured every 15 minutes from thereon. CGM data were analysed for 2-h post meal glucose area under the curve and peak glucose concentration.[34] Participants were provided with scales (HoMedics Group Salter, Kent) and detailed instruction on how to measure dietary intake in order to accurately complete a 3-day diet diary during the first trial, which commenced 24 h before and ended 24 h after completion of trial 1. Participants were instructed to consume the first meal after each condition following a standardised 3-hour time period. Participants were then required to replicate their dietary intake for the second trial.

Exercise trial. Participants performed 1 hour cycling at a fixed rate of metabolic heat production ($\dot{H}_{prod}$) equivalent to 7 W/kg in an environmental chamber (25.6 ± 0.7°C; 49.8 ± 3.8% *relative humidity, rh*). The initial workload was determined from the submaximal test and external workload was manipulated throughout the 60 minutes in order to maintain $\dot{H}_{prod}$ at 7 W/kg. Participants completed the exercise at a self-selected cadence. $\dot{V}O_2$ and $\dot{V}CO_2$ were recorded throughout using an online gas analyser. Airflow was provided by three fans stacked vertically, positioned 2.5m in front of the ergometer at an air speed of 1.5 m·s^{-1}.

Passive heating trial. Participants were seated in a water bath (40.2 ± 0.2 °C) and immersed up to the waist for 1 hour. Water was circulated throughout to ensure maintenance of water temperature. $\dot{V}O_2$ and $\dot{V}CO_2$ recorded throughout. Ambient conditions during the trial were 23.9 ± 0.9 °C; 49.9 ± 4.4 % *rh*.

Owing to the effect of temperature and duration on HSP72 and IL-6 expression, we attempted to match the change in core temperature between conditions by eliciting a 1°C increase in core temperature. Furthermore, the duration of both exercise and passive heating was matched to limit the effect of intervention exposure time on key outcome measures.

Instrumentation. Core temperature (T_C) was measured using a rectal thermistor (Grant Instruments Ltd, Cambridge) inserted 10 cm beyond the anal sphincter. Wireless skin sensors (iButtons, DS1922, California, USA) were applied and secured by Medipore tape (3M, Berkshire, UK). Mean skin temperature ($\bar{T}_{sk}$) was calculated according to the formula of Ramanathan.[35] Participants wore a heart rate monitor throughout (RS800, Kempele, Polar, Finland). Estimations of whole body sweat loss were made from changes in body mass, which were corrected for fluid intake and respiratory fluid loss.

Muscle temperature. Muscle temperature (T_m) was measured in the right *vastus lateralis* using a solid needle probe (MKA08050A275TS Ellab, Copenhagen, Denmark) immediately pre and post-trial. Following standard sterile procedure, the needle probe was first inserted to an initial depth of 3 cm beyond the muscle fascia where the temperature was allowed to stabilise before the probe was withdrawn to 2cm and then 1cm depths, with the temperature recorded at each depth.

Blood sampling. Venous blood samples were collected by venepuncture from an antecubital vein in the right arm into a 10 mL Vacutainer that had been pre-cooled and pre-treated with K^3EDTA (BD Biosciences, San Diego, USA). Samples were obtained before and on completion of each heating protocol with a final sample taken 120 minutes later (figure 1). Samples were stored on ice until they were centrifuged at 3,500 rpm for 10 min at 4°C and the plasma stored at -80°C until subsequent analysis.

Enzyme-linked immunosorbent assays. Plasma HSP70 (ENZ-101, AMP'D® hs HSP70, Enzo Life Sciences, Exeter, UK) and IL-6 (HS600B, Quantikine® HS IL-6, R&D Systems, Abingdon, UK) concentrations were analysed using sandwich enzyme-linked immunosorbent assays (ELISAs) according to the manufacturers' instructions. Plasma HSP70 and IL-6 concentrations were determined in relation to a four-parameter standard curve (version 6.0 GraphPad Software, La Jolla, CA, USA). All samples were analysed in duplicate with a mean intra plate coefficient of variation (CV) of 4.5% for HSP70 and 5.8% for IL-6. The inter plate CV was 6.3% for HSP70 and 6.1% for IL-6.

Calculations. Energy expenditure during PH and EX was calculated via indirect calorimetery. Heat balance parameters were estimated via partitional calorimetery and are presented as the mean value for each condition. All parameters were calculated in W/m^2 but presented as W/kg where appropriate. The rate of metabolic energy expenditure was estimated as:

$$M\ (W/m^2) = \dot{V}O_2 \cdot \frac{\left[\left(\frac{RER-0.7}{0.3}\right)\cdot e_c\right] + \left[\left(\frac{1.0-RER}{0.3}\right)\cdot e_f\right]}{60 \cdot BSA} \cdot 1000 \tag{1}$$

where RER is the respiratory exchange ratio and e_c and e_f represent the energy equivalent of carbohydrate (21.13 kJ) and fat (19.69 kJ) respectively per litre of O_2 consumed per minute ($L \cdot min^{-1}$). $\dot{H}_{prod}$ was determined as the difference between M and the external work rate (W):

$$\dot{H}_{prod}\ (W/m^2) = M - W \tag{2}$$

Mean body temperature ($\Delta\overline{T}_b$) was estimated using the three-compartment model as follows:

$$\Delta\overline{T}_b = (0.63\ \Delta\ T_c) + (0.24\ \Delta\overline{T}_{sk}) + (0.13\ \Delta\ T_m) \tag{3}$$

where ΔT_c represents the change in core temperature, $\Delta\overline{T}_{sk}$ the change in mean skin temperature and ΔT_m the change in muscle temperature at a depth of 3cm.[36] Area under the curve (AUC) was calculated using the trapezoid method.

Statistical analysis. The normality and distribution of data was assessed using the Shapiro-Wilk normality test. Where data failed to meet the criteria of normal distribution the data were log transformed. Mean participant characteristics were compared using independent samples *t*-tests. T_c, $\overline{T}_{sk}$, T_m, HSP70, IL-6, were analysed using repeated measures ANOVA. Where significance was obtained *post hoc* tests were completed using Sidak's test for multiple comparisons. $\dot{H}_{prod}$, $\Delta\overline{T}_b$ and energy expenditure were analysed using one-way ANOVA with Sidak's test for multiple comparisons. Correlational analysis was done using Pearson's correlation coefficient. Linear regression was used to analyse the relative contributions of ΔT_c , ΔT_{sk}, ΔT_m and $\Delta\overline{T}_b$ to ΔHSP70 and ΔIL-6. The R^2 value was employed to determine the variance explained by each predictor variable. Where reported, the adjusted R^2 value is provided for multiple linear regression in order to account for the number of predictor variables in the model. All statistical analyses were performed using GraphPad Prism (version 6.0 GraphPad Software, La Jolla, CA, USA). All data are presented as mean ± SD unless otherwise stated. P values ≤ 0.05 were considered statistically significant. Effect sizes (ES) corrected for bias using Hedge's g were calculated as the ratio of the mean difference to the pooled standard deviation of the difference, with 95% confidence intervals (95% CI) for differences also presented. The magnitude of the ES was classed as trivial (<0.2), small (0.2– 0.6), moderate (0.6– 1.2), large (1.2– 2.0) and very large (≥ 2.0).[37]

Results

$\dot{H}_{prod}$. There were differences in $\dot{H}_{prod}$ between PH (1.9 ± 0.2 W/kg) and EX (7.3 ± 0.5 W/kg, P<0.001). $\dot{H}_{prod}$ was successfully matched between groups for both PH (LEAN 2.0 ± 0.2 W/kg, OW = 1.8 ± 0.2 W/kg, P=0.433, CI = -0.03 to 0.43, ES=0.9) and EX (LEAN 7.3 ± 0.4, OW 7.2 ± 0.5 W/kg; P=0.763, CI = -0.63 to 0.43, ES = 0.21). However, when lean body mass is accounted for, differences in $\dot{H}_{prod}$ were evident (LEAN 8.4 ± 0.4 W/kg/LBM^{-1}; OW 9.8 ± 1.6 W/kg/LBM^{-1}, P<0.05, CI = 0.04 to 2.76, ES=1.1).

e*HSP70.* There was a main effect of time (P<0.005) but not condition (P = 0.887) on the eHSP70 response (figure 2A). The increase in eHSP70 following PH and EX was 23% and 24% respectively (figure 2B). There was a large effect of group on the change in eHSP70 following PH (40.2% vs. 13.2%, P = 0.273, CI = 2.9 to 51.1, ES = 1.4) and a small effect following EX (37% vs. 14%, P = 0.340, CI = -48.1 to 94.9, ES = 0.4), although this did not reach statistical significance.. Across conditions ΔeHSP70 correlated with mean T_m (r=0.485, P<0.05). ΔeHSP70 was negatively correlated to body mass (r = -0.400, P<0.05). Of the thermal variables, ΔT_m provided the best predictor of ΔHSP70, with the ΔT_m explaining 24.7% of the total variance in ΔHSP70 (r=0.498, P<0.01). Body mass also predicted ΔHSP70, explaining 16.4% of the total variance (r = -0.405, P<0.05). When combined, ΔT_m and body mass provided the strongest predictor, accounting for 28% of the variance in ΔHSP70 (Adjusted R^2 = 0.280, r = 0.585, P<0.01).

IL-6. There was a main effect of time (P<0.001), condition (P<0.05) and an interaction effect (P<0.01, figure 2C) on IL-6 concentration. IL-6 was higher immediately following EX (1.74 ± 1.79 pg·mL^{-1}) compared to PH (0.97 ± 0.86 pg·mL^{-1}, P<0.0001, ES=0.5) and equated to an increase of 346% after EX and 118% following PH (figure 2D) from baseline values. IL-6 had returned to baseline at 2h post for PH (0.58 ± 0.47 pg·mL^{-1}) and EX (0.73 ± 0.79 pg·mL^{-1}). When considering differences between LEAN and OW, there was a main effect of time (P<0.05) but not group (P = 0.533) on the IL-6 response. None of the thermal variables, nor body mass, provided an effective model for predicting the change in IL-6.

Energy expenditure. There were no differences in resting metabolic rate between LEAN (1811.2 ± 82.3 kcal·day^{-1}) and OW (1873.9 ± 140.7 kcal·day^{-1}; P = 0.330, ES = 0.5). There was an overall effect of condition on energy expenditure during heating ($P<0.0001$) and an interaction effect ($P<0.05$). PH increased energy expenditure by 61.0 ± 14.4 kcal compared to rest ($P<0.0001$, ES = 4.8), which equates to a 79.5% increase. EX resulted in an additional energy expenditure of 556.3 ± 92.0 kcal compared to rest ($P<0.0001$, ES = 7.1), equating to a 721% increase. EX energy expenditure was greater for OW compared to LEAN (11.3 ± 1.9 $kcal \cdot min^{-1}$ vs. 9.8 ± 0.9 $kcal \cdot min^{-1}$ respectively, $P<0.05$, ES = 1.0), resulting in an additional caloric expenditure from rest of 597.7 ± 107.9 kcal for OW (748%), and 515 ± 52.1 kcal for LEAN (681%). During PH, there was no difference in the increase in energy expenditure between LEAN and OW (2.3 ± 0.3 $kcal \cdot min^{-1}$ vs. 2.3 ± 0.3 $kcal \cdot min^{-1}$ respectively, P = 0.997, ES = 0.0). Caloric expenditure increased from rest by 63.4 ± 16.6 kcal (84%) and 58.5 ± 12.7 kcal (74%) kcal for LEAN and OW respectively although there was no difference between groups (P=0.623, ES = 0.3).

Substrate oxidation. Total EX carbohydrate oxidation was higher for OW (125.3 ± 20.8 g) compared to LEAN (92.5 ± 28.2 g; $P<0.05$, ES=1.3). There was a moderate effect of body mass on total carbohydrate oxidation in PH between OW (7.5 ± 4.2 g) and LEAN (13.2 ± 8.3 g, ES=0.83) although this did not reach significance (P=0.13). There was no difference between OW and LEAN in total fat oxidation during EX (P=0.46). There was a tendency for OW to display a greater total fat oxidation than LEAN in PH, although this did not reach statistical significance (10.6±1.54g vs. 9.0±3.1g; ES 0.7; P=0.13).

Continuous glucose monitoring. Owing to instances of poor compliance to diet replication and sensor malfunction and drop out, CGM analysis was only completed on 8 participants, with an unequal split between LEAN and OW. Therefore, it was only possible to conduct analysis on PH compared to EX. Peak glucose concentration in response to the meal following PH (6.3 ± 1.4 $mmol \cdot L^{-1}$) was lower compared to the same meal after EX (6.8 ± 1.2 $mmol \cdot L^{-1}$; $P<0.05$, CI = -0.9 to 1.9, ES = 0.4, figure 4a). There was no difference in glucose AUC for PH compared to EX following this meal (P=0.875, CI = -11.2 to 12.4, ES = 0.1 figure 4b). There was no difference in 24h AUC between PH and EX.

Core temperature. There was a main effect of time ($P<0.0001$) and an interaction effect ($P<0.0001$), but no main effect of condition on ΔT_c (P = 0.112, figure 4a). PH ΔTc was lower in OW compared to LEAN from 35 minutes ($P<0.05$), although by 60 minutes there was no difference in ΔT_c (1.0 ± 0.2˚C vs. 0.9 ± 0.2˚C, LEAN vs. OW respectively P = 0.089, CI = -0.13 to 0.33, ES = 0.5). There were no differences in ΔT_c between OW and LEAN during EX at any time point, with ΔT_c at 60 minutes being 0.8 ± 0.1˚C vs. 0.8 ± 0.2˚C for LEAN and OW respectively (P=0.999).

Skin temperature. There were main effects of time (P<0.0001), condition (P<0.0001) and an interaction effect (P<0.0001) on $\Delta\overline{T}_{sk}$ (figure 4B). There were no differences in $\Delta\overline{T}_{sk}$ between OW and LEAN following EX (LEAN 1.7 ± 0.7˚C vs OW 1.7 ± 1.6˚C, P=0.999, ES=0.0) or PH (LEAN 4.2 ± 0.4˚C vs OW 4.7 ± 0.6˚C, P=0.095, ES=0.9).

Muscle temperature. There was an effect of depth on ΔT_m (P<0.001), but no effect of condition (P=0.285) or interaction (P=0.111, figure 4C). Following PH, the ΔT_m was greater for LEAN compared to OW at a depth of 1cm (LEAN, 2.5 ± 0.8˚C; OW 1.9 ± 0.6˚C, P<0.0005, CI = -0.2 to 1.4, ES=0.8) and 3cm (LEAN, 2.3±0.6˚C; OW 1.9± 0.7, P<0.005, CI = -0.4 to 1.2, ES=0.6). After EX, ΔT_m was only different at 1cm (LEAN, 2.3±0.6˚C; OW 2.0 ± 0.7, P<0.005, CI = -0.5 to 1.1, ES = 0.4).

Mean body temperature. There was a difference in $\Delta\overline{T}_b$ between conditions (P<0.0001, figure 4D). There were differences between LEAN PH and LEAN EX (1.7 ± 0.3˚C vs. 1.1 ± 0.3 ˚C, P<0.005, CI = 0.3 to 1.0, ES = 1.9); OW PH and OW EX (1.8 ± 0.2˚C vs. 1.0 ± 0.4˚C; P<0.001, CI = 0.4 to 1.2, ES = 2.4). There were no differences between LEAN and OW within the same condition (both P>0.9).

Heart rate. There were significant effects of time, condition and interaction effect of heart rate (all P<0.0001). Mean percentage HR_{max} EX was 78±8% compared to 54±7% in PH (P<0.0001). There were no differences in the mean percentage of HR_{max} between groups for either PH (LEAN = 54.0 ± 6.0% vs. OW = 55.2 ± 6.2%, P=0.719) or EX (LEAN = 78.5 ± 7.3%; OW = 81.7 ± 7.4%, P=0.428).

Discussion

The primary outcome of the present study was that the concentration of eHSP70 in the plasma was similar between EX and PH when both conditions were matched for ΔT_c, with ΔT_m being the best individual predictor of ΔeHSP70, explaining ~25% of the total variance. This suggests that changes to T_m are an important determinant of eHSP70 appearance and that the HSP70 response may be hindered by excess body mass. An important secondary outcome is the increase in energy expenditure evident as a consequence of PH. This has important implications for the use of PH as an intervention that may help to reduce body fat, particularly for individuals unable or unwilling to complete regular physical activity. Finally, the reduction in peak glucose in the meal following PH compared to EX suggests that PH may have a beneficial effect on glycaemic regulation and provide an alternative or augmentative intervention by which individuals with metabolic diseases may be able to improve their glycemic control. However, additional research needs to be conducted to confirm these data in larger cohorts over a prolonged period of time.

A greater degree of variability in the eHSP70 response to heating in the OW group was evident. Regression analysis demonstrated a link between body mass and the ΔeHSP70. A greater body mass was associated with an impaired eHSP70 response to heating, via either EX or PH, explaining 16% of the total variance. Such variation may be indicative of an impaired HSP response in overweight individuals in the face of physiological stress and would likely be compounded by low aerobic capacity.[38] The implications for such a deficiency are wide ranging and may increase susceptibility to metabolic disease[38,39] and impaired vascular function.[40]

Chronic exercise training causes diminishing elevations in eHSP70 with each bout of subsequent exercise, whilst iHSP70 concentration increases. Such changes in the compartmentalised concentration of HSP70 will lead to a shift in the ratio of iHSP70 to eHSP70. This ratio is postulated to be important in determining the systemic inflammatory profile and associated insulin resistance,[9] with a shift in favour of iHSP70 lowering inflammation and improving insulin sensitivity. However, given the variability in the HSP70 response in OW, any such changes to iHSP70:eHSP70 will likely require long term PH in order to achieve a positive balance in favour of iHSP70. Given the suggested mechanism through which HSP70 may influence insulin signalling,[41] the increase in iHSP70 represents a critical adaptation through which passive heating may elicit positive metabolic effects.

An increase in iHSP70 has been reported previously following 12 weeks of heat therapy and was associated with reductions in HbA1c, fasting glucose and insulin.[42] The beneficial effects of iHSP70 relating to insulin resistance and metabolism are suggested to occur by increasing mitochondrial number and oxidative capacity.[18,43] Given the link between reduced HSP70 and T2DM[18] it appears that an intervention capable of inducing HSP70 in humans would be effective at alleviating some of the symptoms of metabolic diseases.[25] The present data demonstrates comparable increases in eHSP70 following either PH or EX, therefore it is possible that PH may offer a mechanism by which individuals who are unwilling or unable to exercise regularly, could augment similar beneficial effects by incorporating passive heating into their daily routine.

Whilst there were no clear differences in the HSP70 response between either PH or EX, there were clear differences in the IL-6 response. Previous work has shown that temperature increases independently elevate IL-6.[44] However, the present data does not support the view that skeletal muscle may act as a "heat stress sensor" triggering a subsequent cytokine response, as none of the thermal measures taken during the trials were effective predictors of ΔIL-6 and could not account for a significant proportion of the variation in IL-6 following heating. Our data also indicate that the difference in IL-6 between PH and EX is not due to HSP70 induction of IL-6[45,46] as ΔHSP70 was similar following PH and EX. This suggests the IL-6 response to exercise occurs independently of HSP70 expression and is more dependent on intensity and duration of exercise,[47] or exposure to heat stress. IL-6 combined with its receptor (IL-6R), may also be a pathway by which insulin independent glucose uptake occurs at rest.[48] Should chronic PH enhance IL-6R expression as is evident following exercise training,[49] interventions capable of increasing both IL-6 and IL-6R, may provide a therapeutic basis for improving glycemic control. Moreover, it has recently been shown that acute passive heating elevates the anti-inflammatory cytokines IL-1ra and IL-10,[50] suggesting that chronic passive heating may benefit the inflammatory profile in a similar way to exercise, albeit to a lesser extent, although long term investigation of PH is required to corroborate this hypothesis.

IL-6 appears to play a central role in the metabolic activity of brown adipose tissue (BAT). In a rodent model, BAT transplanted from IL-6 knockout mice did not display its characteristically profound metabolic effect on glucose homoeostasis and insulin sensitivity.[51] Coupled with the present data, this suggests that PH induced elevations in energy expenditure may occur as a consequence of IL-6 derived elevation in BAT activity. Although this was not determined in the present study, it presents an intriguing line for future research.

In addition to benefits to metabolic regulation, PH has been shown to benefit cardiovascular health. In a recent meta-analysis Laukkanen et al., report that lifelong sauna use reduced cardiovascular and all cause mortality, with the largest benefits associated with more frequent sauna use.[52] Although this relationship does not establish causality [28,53] it is nonetheless and interesting link that is supported by recent investigations.[54,55] PH appears to improve vascular function, with improvements to flow-mediated dilatation, arterial stiffness and blood pressure reported after 8 weeks of PH.[54] Moreover, Thomas et al., have shown that 30 minutes of PH can induce a greater shear stress response and reduction in mean arterial pressure compared to matched duration exercise, in patients with peripheral arterial disease.[55] Based on heart rate responses, the cardiovascular strain in the present study was comparable to that of Thomas et al., which elicited ~50% HR_{max} during their immersion protocol. These recent findings indicate that PH has the potential to induce significant systemic benefits, which in some instances may be comparable to or even greater than the effects evident following exercise.

In summary, the data presented here demonstrate that both acute PH and EX result in comparable increases in HSP70. It is suggested that ΔHSP70 is sensitive to the ΔT_m. The elevations in both HSP70 and IL-6 may promote an anti-inflammatory milieu and help combat the chronic inflammation associated with many disease states. The increase in energy expenditure following PH demonstrates a systemic effect of PH, which has the ability to reduce adipose tissue and further contribute to reducing inflammation. Finally, the present data, in agreement with that of other authors, indicates that PH might provide an alternative to exercise in some individuals who are too physically impaired to undertake prolonged aerobic activity to improve their cardio-metabolic health.

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Table 1. Participant characteristics.

	Lean	Overweight
Age (years)	23.9 ± 3.5	30.7 ± 12.0
Height (m)	1.83 ± 0.05	1.78 ± 0.05*
Weight (kg)	78.9 ± 7.01	92.3 ± 12.3*
BMI ($kg^{.}m^2$)	23.5 ± 2.2	29.2 ± 2.7**
Waist (cm)	79.6 ± 4.8	92.9 ± 8.2**
Hip (cm)	99.6 ± 4.4	105.1 ± 6.8
Waist:Hip	0.80 ± 0.03	0.88 ± 0.04**
BP Systolic	130 ± 14	137 ± 10
BP Diastolic	77 ± 12	87 ± 0
Cholesterol ($mmol^{.}L^{-1}$)	3.27 ± 0.54	3.71 ± 1.04
LDL-C ($mmol^{.}L^{-1}$)	1.5 ± 0.5	1.8 ± 0.6
HDL-C ($mmol^{.}L^{-1}$)	1.3 ± 0.3	1.2 ± 0.2
Glucose ($mmol^{.}L^{-1}$)	4.6 ± 0.6	4.1 ± 0.8
Triglyceride ($mmol^{.}L^{-1}$)	1.1 ± 0.2	1.6 ± 0.8
Body fat (%)	12.5 ± 5.5	20.3 ± 1.6***
Fat Mass (kg)	10.1 ± 5.1	28.1 ± 9.9**
Lean mass (kg)	68.8 ± 4.2	64.2 ± 6.9
$\dot{V}O_{2\,max}$ ($L^{.}min^{-1}$)	3.86 ± 0.35	3.35 ± 0.81
$\dot{V}O_{2\,max}$ ($mL^{.}kg^{.}min^{-1}$)	49.03 ± 5.74	36.60 ± 9.66*
Percent $\dot{V}O_{2\,max}$ – EX (%)	53.9 ± 7.5	69.2 ± 10.2**
Percent $\dot{V}O_{2\,max}$ – PH (%)	18.3 ± 6.7	25.5 ± 6.7
Basal Metabolic Rate ($kcal^{.}day^{-1}$)	1811.2 ± 82.3	1873.9 ± 140.7

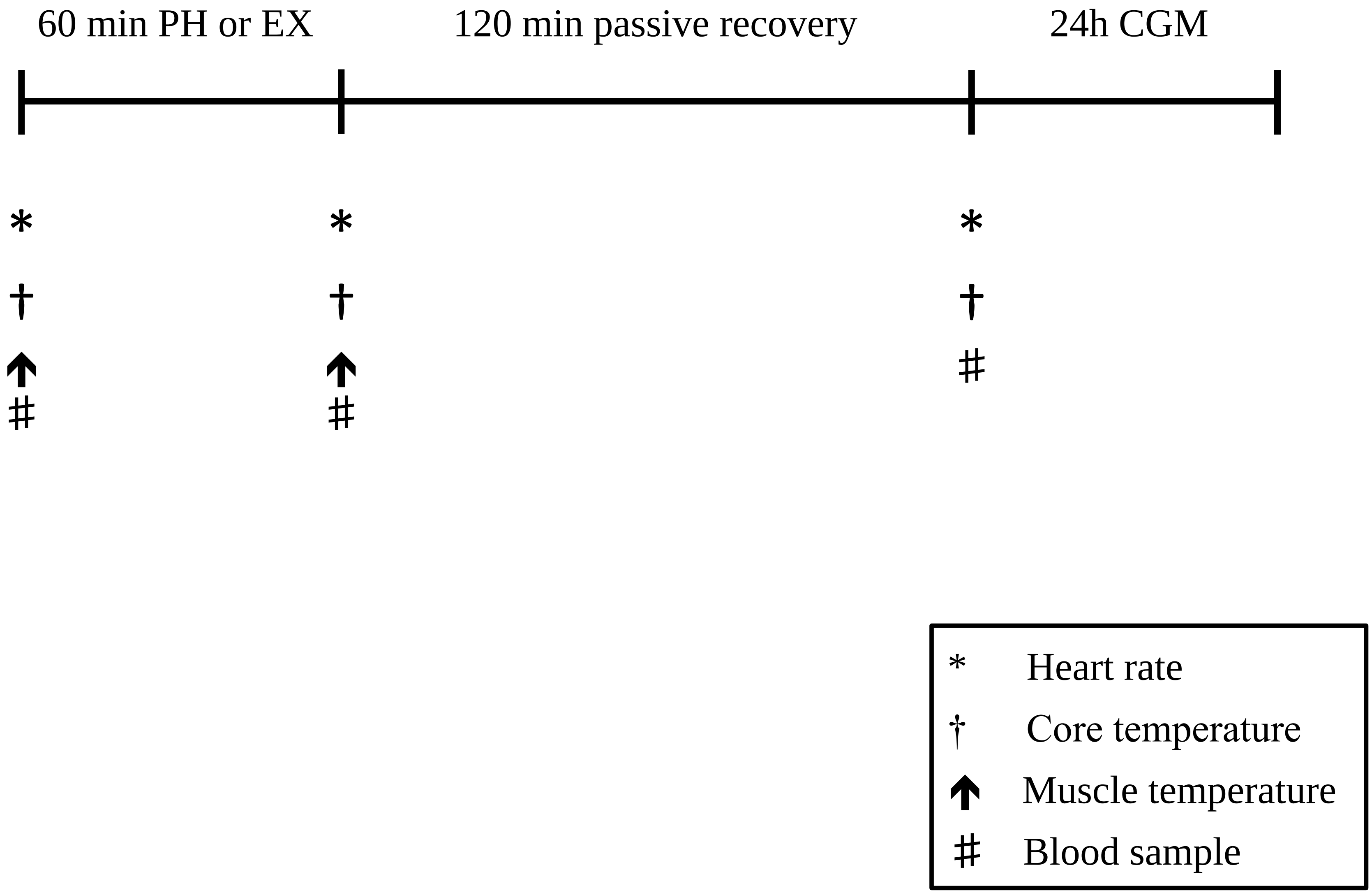
Data presented as mean ± SD. Asterix denotes * ($P<0.05$); ** ($P < 0.01$); *** ($P<0.005$). Abbreviations: BMI – Body mass index, BP – blood pressure, EX – Exercise trial, HDL – High density lipoprotein, LDL – Low density lipoprotein, PH – Passive heating trial, TG – Triglyceride

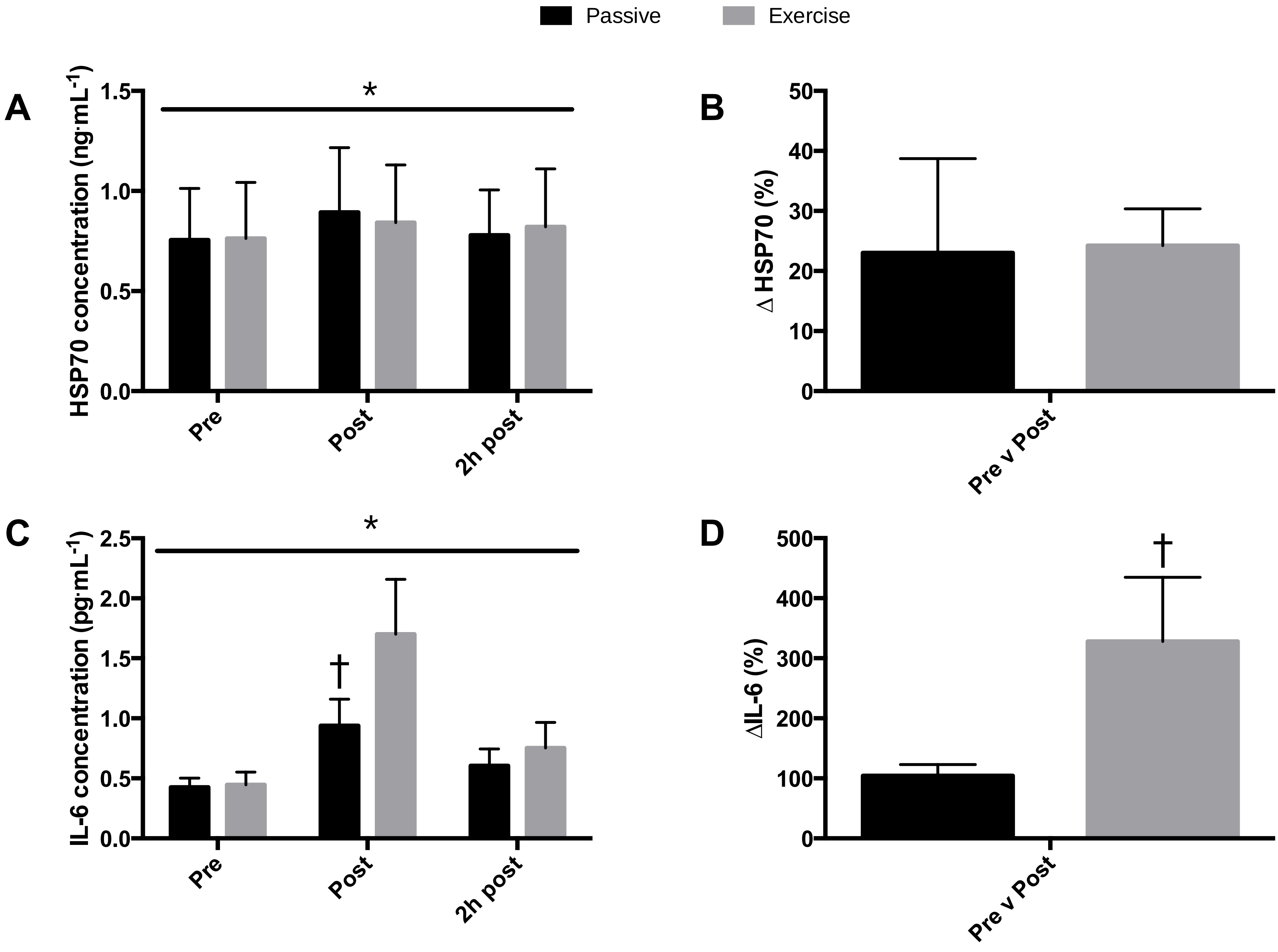
Figure 1: Schematic representation of the experimental trials. PH, passive heating; EX, exercise; CGM, continuous glucose monitoring.

Figure 2: The effect of 60 minutes passive heating at 40˚C, exercise at 7 W/kg and body composition on IL-6 and HSP70. A) the effect of passive heat and exercise on HSP70; B) the change in IL-6 in response to heating. C) The effect of passive heat and exercise on IL-6; D) the change in IL-6 in response to heating. *above the line denotes main effect of time. †denotes difference between passive heating and exercise. Data presented as mean ± SEM

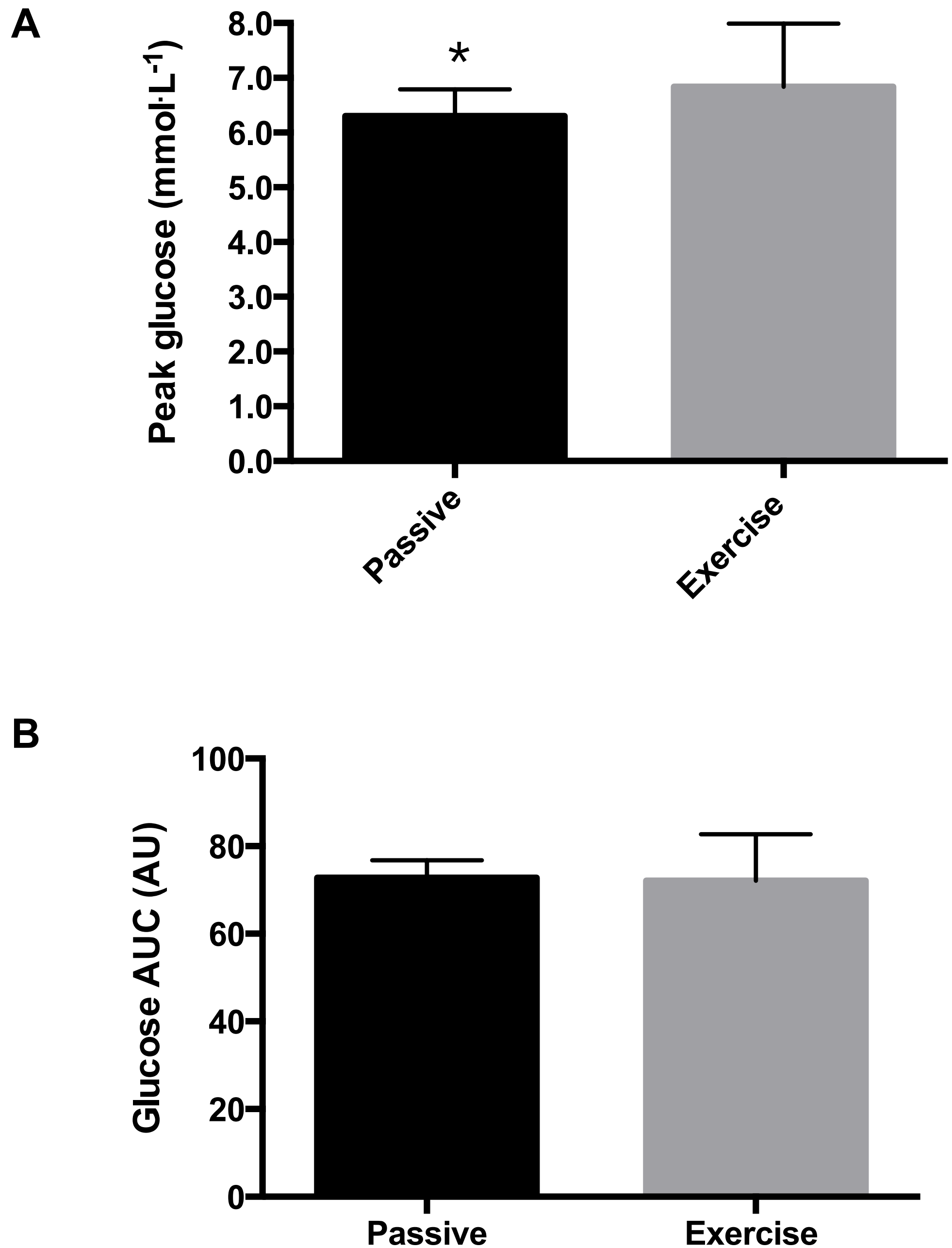
Figure 3: Passive heating resulted in a reduction in peak interstitial glucose concentration compared to exercise in the meal immediately following the intervention. *denotes passive different to exercise ($P<0.05$). Data presented as mean ± SEM. n=8

Figure 4: The effect of 60 minutes passive heating at 40˚C, exercise at 7 W/kg and body composition on determinants of mean body temperature. A) the change in core temperature, B) change in mean skin temperature, C) muscle temperature at depths of 1cm, 2cm and 3cm, D) change in mean body temperature. # above line denotes main effect of time, * denotes difference between LEAN and OW in passive heating condition ($P<0.05$). †denotes difference between LEAN and OW in exercise condition ($P<0.05$) **denotes difference between PH and EX within each condition Data presented as mean ± SEM

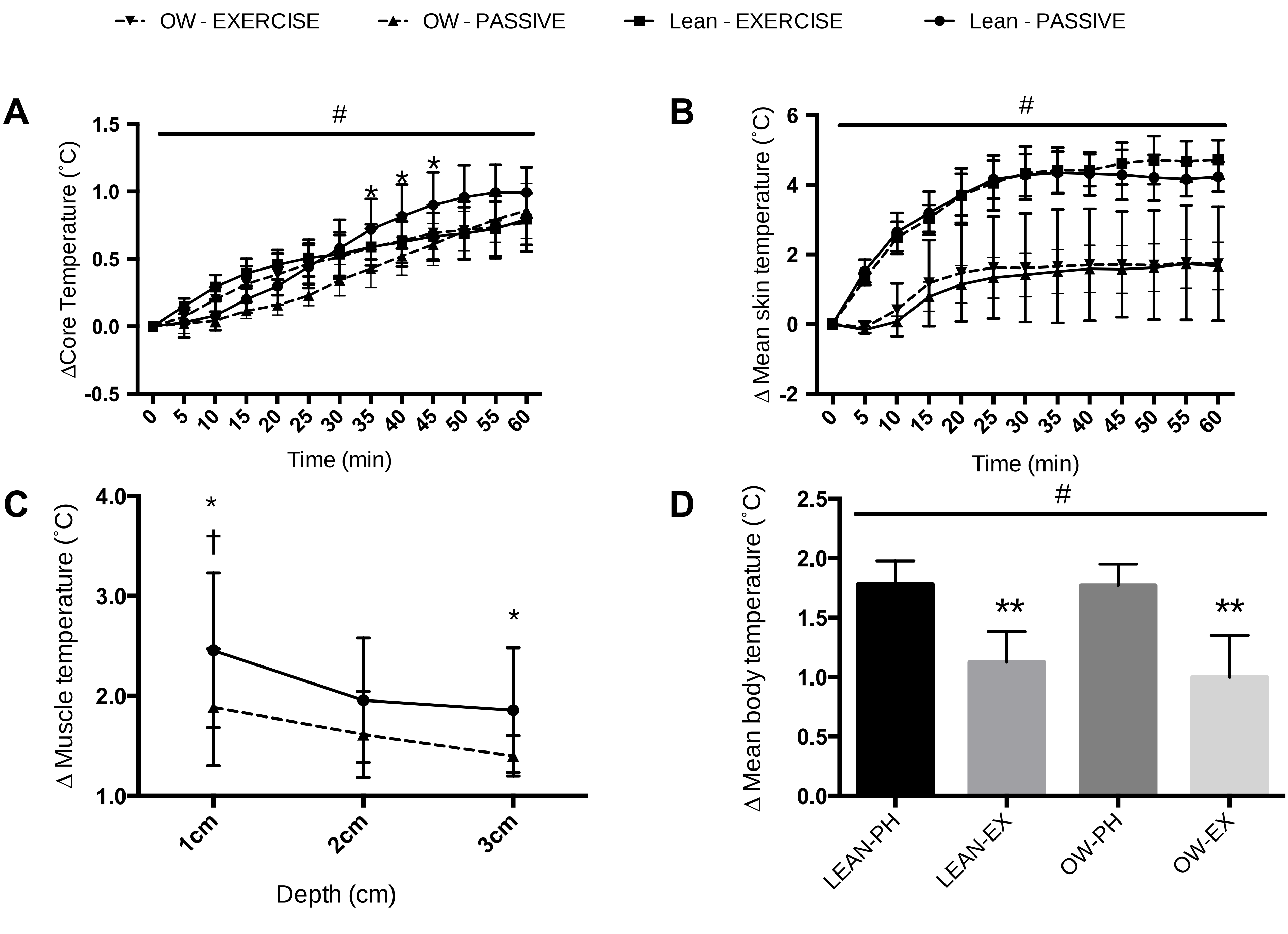

60 min PH or EX
120 min passive recovery
24h CGM
* Heart rate
† Core temperature
Muscle temperature
Blood sample



Passive
Exercise
A
HSP70 concentration (ng·mL-1)
1.5
1.0
0.5
0.0
*
Pre
Post
2h post
B
Δ HSP70 (%)
50
40
30
20
10
0
Pre v Post
C
IL-6 concentration (pg·mL-1)
2.5
2.0
1.5
1.0
0.5
0.0
*
†
Pre
Post
2h post
D
ΔIL-6 (%)
500
400
300
200
100
0
†
Pre v Post



A
Peak glucose (mmol·L-1)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
Passive
Exercise
B
Glucose AUC (AU)
100
80
60
40
20
0
Passive
Exercise


OW - EXERCISE
OW - PASSIVE
Lean - EXERCISE
Lean - PASSIVE
A
ΔCore Temperature (˚C)
Time (min)
B
Δ Mean skin temperature (˚C)
Time (min)
C
Δ Muscle temperature (˚C)
1cm
2cm
3cm
Depth (cm)
D
Δ Mean body temperature (˚C)
LEAN-PH
LEAN-EX
OW-PH
OW-EX