Whole body hyperthermia, but not skin hyperthermia, accelerates brain and locomotor limb circulatory strain and impairs exercise capacity in humans

Steven J. Trangmar[1], Scott T. Chiesa[1], Kameljit K. Kalsi[1], Niels H. Secher[1,2] and José González-Alonso[1]

[1]Centre for Human Performance, Exercise and Rehabilitation, Brunel University London, Uxbridge, UK
[2]The Copenhagen Muscle Research Centre, Department of Anaesthesia, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Running title: Hyperthermia and circulatory strain during maximal exercise

Key words: Hyperthermia, maximal exercise, regional blood flow and metabolism

Corresponding Author:
José González-Alonso, [1]Centre for Human Performance, Exercise and Rehabilitation, Brunel University London, UB8 3PH, UK; email: j.gonzalez-alonso@brunel.ac.uk: telephone: +44 (0) 1895 267324; fax: +44 (0) 1895 269769

Table of contents category: Integrative physiology

Key points

- Whole-body hyperthermia impairs cardiovascular function and aerobic exercise capacity, but the contribution of skin hyperthermia to the ensuing regional cardiovascular strain is unclear.
- Body temperature was manipulated with a water-perfused suit to examine how hyperthermia affects brain and active limb circulations and accelerates fatigue during incremental maximal exercise in humans.
- Whole-body hyperthermia, but not skin hyperthermia, accelerated the reductions in brain and active limb perfusion, compromised aerobic metabolism and impaired exercise capacity.
- The attenuation in brain and active limb perfusion was associated with increases in sympathetic vasoconstrictor activity, blunted rise in plasma ATP and a fall in the arterial partial pressure of CO_2.
- These findings challenge the prevailing notion that skin hyperthermia and hyperperfusion *per se* is the dominant factor in the development of cardiovascular strain and fatigue during exercise in hot environments.

Abstract

Cardiovascular strain and hyperthermia are thought to be important factors limiting exercise capacity in heat-stressed humans; however, the contribution of elevations in skin (T_{sk}) *vs.* whole body temperatures on exercise capacity has not been characterised. To ascertain their relationships with exercise capacity, blood temperature (T_B), oxygen uptake ($\dot{V}O_2$), brain perfusion (MCA V_{mean}), locomotor limb haemodynamics, and haematological parameters were assessed during incremental cycling exercise with elevated skin (mild hyperthermia; HYP_{mild}), combined core and skin temperatures (moderate hyperthermia; HYP_{mod}), and under control conditions. Both hyperthermic conditions increased T_{sk} *vs.* control (6.2 ± 0.2 °C; $P < 0.001$), however, only HYP_{mod} increased resting T_B, leg blood flow and cardiac output ($\dot{Q}$), but not MCA V_{mean}. Throughout exercise, T_{sk} remained elevated in both hyperthermic conditions, whereas only T_B was greater in HYP_{mod}. At exhaustion, oxygen uptake and exercise capacity were reduced in HYP_{mod} in association with lower leg blood flow, MCA V_{mean} and MAP, but similar maximal heart rate and T_B. The attenuated brain and leg perfusion with hyperthermia was associated with a plateau in MCA and two-legged vascular conductance (VC). Mechanistically, the falling MCA VC was coupled to reductions in $PaCO_2$ whereas the plateau in leg vascular conductance was related to markedly elevated plasma [NA] and a plateau in plasma ATP. These findings reveal that whole-body hyperthermia, but not skin hyperthermia, compromises exercise capacity in heat-stressed humans through the early attenuation of brain and active muscle blood flow.

Abbreviations. ATP_{fv} femoral venous ATP concentration; $a\text{-}vO_{2diff}$, arteriovenous oxygen content difference; CaO_2, arterial content of oxygen; HR_{max}, maximal heart rate; HYP_{mild}, mild hyperthermia; HYP_{mod}, moderate hyperthermia; MCA V_{mean}, middle cerebral artery blood velocity; [NA], noradrenaline; $PaCO_2$, arterial carbon dioxide pressure; $\dot{Q}$, cardiac output; rSO2%, regional cerebral oxygen saturation; T_a, ambient temperature; T_B, blood temperature; T_i, internal temperature; T_{sk}, mean skin temperature; T_{Oes}, oesophageal temperature; $\dot{V}O_{2max}$, maximal aerobic power; W_{max}, maximal work rate.

Introduction

It is well documented that maximal aerobic exercise capacity ($\dot{V}O_{2max}$) is reduced in hot environments inducing whole body hyperthermia (Rowell *et al.*, 1966; Pirnay *et al.*, 1970; Rowell, 1974; Galloway & Maughan, 1997; González-Alonso *et al.*, 2008; Sawka *et al.*, 2011). The precise mechanisms underpinning the impaired exercise capacity in the heat remain debated, but may result from the interaction of multiple regulatory processes associated with reduced O_2 delivery and modified locomotor muscle and brain metabolism, attainment of high internal and skin temperatures, and altered central nervous system neurotransmitter activity and feedback/reflex mechanisms (González-Alonso *et al.*, 2008; Meeusen & Roelands, 2010; Sawka, 2012; Nybo *et al.*, 2014). The contribution of these factors to early fatigue in the heat appear to be task and exercise-intensity dependent (Nybo *et al.*, 2014). There is, however, surprisingly limited information on the cardiovascular adjustments to incremental exercise with differing extents of skin and whole-body hyperthermia.

Restrictions in active skeletal muscle perfusion may play an important role in the reduced aerobic capacity in hyperthermic conditions. Under control (normothermic) conditions, skeletal muscle O_2 delivery is tightly coupled to the metabolic demand during sub-maximal exercise (Andersen & Saltin, 1985; Delp & Laughlin, 1998; Saltin *et al.*, 1998; González-Alonso *et al.*, 2002; Delp & O'Leary, 2004); regulation that is lost at high intensities as, prior to volitional exhaustion, systemic and active skeletal muscle (in addition to brain and respiratory muscle) blood flow becomes restricted (González-Alonso & Calbet, 2003; Mortensen *et al.*, 2005, 2008; Vogiatzis *et al.*, 2009; Calbet *et al.*, 2015). The attenuated limb blood flow per unit of power when approaching maximal exercise intensities occurs concomitantly with enhanced local vasoconstrictor activity and reductions in stroke volume (González-Alonso & Calbet, 2003; Calbet *et al.*, 2007; Mortensen *et al.*, 2008; Stöhr *et al.*, 2011*b*; Munch *et al.*, 2014). As a consequence, and in contrast to other important regions of the body such as the brain (Nybo *et al*., 2002; González-Alonso *et al*., 2004; Trangmar *et al*., 2014), blunted O_2 delivery may compromise local aerobic metabolism, as

maximal skeletal muscle O_2 extraction is achieved during exhaustive exercise (González-Alonso & Calbet, 2003). It remains unknown whether hyperthermia accelerates cardiovascular strain during incremental exercise with similar non-linear cardiovascular dynamics.

An early restriction in regional blood flow may underpin the reduced $\dot{V}O_{2max}$ with body hyperthermia. The magnitude of the decline in $\dot{V}O_{2max}$ is, however, variable and largely dependent on the extent of the rise in skin and internal temperature (Pirnay *et al.*, 1970; Arngrímsson *et al.*, 2004; Kenefick *et al.*, 2010; Nybo *et al.*, 2014). A critical question is which bodily temperature, or combination of temperatures, is most closely associated with the reduced aerobic capacity in heat stress conditions. On the one hand, brief exposure to heat that does not substantially elevate internal temperature is unlikely to cause a decline in $\dot{V}O_{2max}$ or impair cardiovascular capacity (Arngrímsson *et al.*, 2004). In contrast, a reduced aerobic exercise performance has been associated with the attainment of high skin temperatures without significant elevations in internal (core) temperature (Ely *et al.*, 2009; Kenefick *et al.*, 2010; Lorenzo *et al.*, 2010; Sawka *et al.*, 2012). The hypothesis that elevations in skin temperature, by requiring a large proportion of the cardiovascular capacity, is the primary factor leading to a compromised aerobic performance has generally been substantiated with the classical observation that cardiac output is reduced during the high intensity stages of graded exercise in the heat, compared to a temperate environment, in untrained men (Rowell *et al.*, 1966). High skin temperature has therefore been proposed to be a critical factor underpinning reduced aerobic capacity in the heat (Sawka *et al.*, 2012). However, there is some evidence that $\dot{Q}$ is higher during the early stages of intense constant-load exercise with body hyperthermia in trained individuals (González-Alonso & Calbet, 2003; González-Alonso *et al.*, 2004), suggesting that high skin blood flow requirements *per se* do not compromise systemic perfusion. Hence, whilst it has long been accepted that the extent of the exogenous heat stress is a critical factor for the decline in maximal aerobic power, the precise circulatory

alterations with varying levels of body hyperthermia that partition the roles of skin *vs.* whole-body temperature have yet to be systematically investigated.

The aim of the present study was to investigate the effect of heat stress, inducing two different grades of hyperthermia, on cardiovascular capacity and brain and active limb blood flow and metabolism during incremental cycling exercise to volitional exhaustion. Regional haemodynamics and metabolism during incremental exercise were assessed; 1) after heat exposure sufficient to elevate internal and skin temperature, 2) after a brief heat exposure sufficient to elevate skin temperature and 3) in control conditions. We hypothesised that combined core and skin hyperthermia, but not skin hyperthermia, would compromise $\dot{V}O_{2max}$ and exercise capacity in close association with early restrictions in brain and active-limb perfusion.

Methods

Ethical approval

All procedures in the present study were approved by the Brunel University London Research Ethics Committee (RE54-12) and conformed to the guidelines of the World Medical Association (Declaration of Helsinki). All participants provided their oral and written and informed consent prior to participation.

Participants

Nine healthy experienced cyclists (mean ± SD; age 26 ± 6 yrs, stature 181 ± 6 cm, mass 76 ± 9 kg and $\dot{V}O_{2max}$ 4.5 ± 0.1 $l \cdot min^{-1}$) participated in the study. Participants arrived at the laboratory postprandial with a normal hydration status and were required to abstain from strenuous exercise and alcohol intake for 24 h and caffeine consumption for 12 h.

Experimental design

Participants visited the laboratory on 3 occasions, comprising of a preliminary trial, a hyperthermia trial and a control trial, each separated by one week. The preliminary trial familiarised participants with the testing methodology, prior to

194 performing an incremental exercise test on a cycle ergometer (Lode Excalibur,
195 Groningen, Netherlands) to establish maximal work rate (W_{max}), maximal heart
196 rate (HR_{max}) and $\dot{V}O_{2max}$. The initial work rate was equivalent to 50% of
197 predicted $\dot{V}O_{2max}$, for 2.5 min, followed by increments of 10% predicted every
198 2.5 min until the limit of tolerance. Participants were instructed to maintain a
199 cadence between 70-90 r.p.m. and the test was terminated when cycling speed
200 dropped below 60 r.p.m. for more than 3 s, despite strong verbal
201 encouragement to continue. After a 1 h recovery period, participants were
202 dressed in a water-perfused suit (covering the arms, legs and torso), and laid in
203 a supine position whilst hot water (50 °C) was circulated through, by a
204 temperature controlled water circulator (Julabo F-34, Seelbach, Germany). A
205 foil blanket, gloves and hat were worn to minimise heat loss to the environment.
206 After target increases in skin and core temperature (+6 and +1 ^{o}C, respectively),
207 participants repeated the incremental test to establish HYP_{mod} W_{max}.
208
209 On the hyperthermia trial, participants completed three incremental cycling
210 ergometer exercise tests in the upright position with; 1) HYP_{mod} (with moderate
211 T_c and high T_{sk}, after 52 $\pm$ 3 min of heat exposure), 2) HYP_{mild} (with a high T_{sk}
212 but normal T_c, after 13 $\pm$ 1 min of heat exposure) and, 3) control conditions (T_a
213 18 °C; 36% RH; with fan cooling). On the control trial, the participants
214 completed three incremental cycling ergometer exercise tests in a thermo-
215 neutral environment (20 °C; ≤ 50% RH; with fan cooling). Each of the
216 incremental cycling tests consisted of 5 x ~2.5 min stages at 20, 40, 60, 80 and
217 100% W_{max}, and cycling pedal cadence was stable between 70-90 r.p.m. On
218 both the hyperthermia and control trials, each incremental test was separated
219 by 1 h of passive recovery while hydration was maintained through the regular
220 consumption of water.
221
222 On the hyperthermia trial, brain, active limb and systemic haemodynamics and
223 blood samples from the brachial artery and femoral vein were obtained
224 simultaneously at rest and in the final minute of each exercise stage. Skin and
225 femoral venous temperatures and arterial and femoral venous pressures were

recorded continuously. The same measures were collected in the control trial, except for the arterio-venous blood sampling, leg blood flow (LBF) and blood pressure measurements, and with the addition of oesophageal temperature (T_{Oes}). Full depiction of the experimental protocol of the study is presented in figure 1.

Brain, active limb and resting systemic haemodynamics

Middle cerebral artery velocity (MCA V_{mean}) was measured using a 2 MHz pulsed trans-cranial Doppler ultrasound system (DWL, Sipplingen, Germany). The right MCA was insonated through the temporal ultrasound window, distal to the MCA-anterior cerebral artery bifurcation, at a depth of 45-60 mm (Aaslid *et al.*, 1982). Regional cerebral (frontal lobe) oxygen saturation (rSO_2%) was also assessed using near-infrared spectroscopy (NIRS; INVOS, Somanetics, Troy, MI, USA).

During exercise, LBF was determined using the constant-infusion thermodilution method (Andersen & Saltin, 1985; González-Alonso *et al.*, 2000). Resting blood flow (n=4) was obtained using duplex Doppler ultrasonography (Vivid 7, Dimension, GE Healthcare, UK), or calculated from the directly obtained a-vO_{2diff} and estimated leg $\dot{V}O_2$ (n =5) assuming comparable leg $\dot{V}O_2$ values than those measured in 4 participants in this study and previous reports from this laboratory using similar heating protocols (Pearson *et al.*, 2011; Chiesa *et al.*, 2015). $\dot{Q}$ at rest was estimated using the Modelflow method (Wesseling *et al.*, 1993), from the directly obtained intra-arterial pressure wave forms, corrected for age, height and weight.

Catheter placement and blood sampling

Participants rested with a slight head-down tilt whilst catheters for blood sampling, mean arterial pressure (MAP), femoral venous pressure and blood temperature were inserted after local anaesthesia (1% lidocaine) into the brachial artery of the non-dominant arm and anterograde into the right common femoral vein (Logicath Quad lumen, 18 gauge, 2.3 mm; MXA234X16X85,

Smiths Medical International LTD), the latter using the Seldinger technique. Catheters were inserted by an experienced clinician under ultrasound guidance and were regularly flushed with normal saline (0.9% NaCl) to maintain patency. The time from catheterisation to the commencement of resting measurements was ~1 h to allow time for the restoration of normal haemodynamics.

Blood variables

Arterial and femoral venous blood samples were drawn into pre-heparinised syringes and analysed immediately for blood gas variables (ABL 800 FLEX, Radiometer, Copenhagen, Denmark) corrected to blood temperature in the femoral vein. The analyser was calibrated (one and two-point) at regular intervals in accordance with manufacturer guidelines. Additional arterial blood samples were collected in 2 ml syringes and transferred to EDTA tubes, centrifuged and separated. Plasma noradrenaline was subsequently determined using an enzyme immunoassay kit (DEE6200, Demeditec Diagnostics GmbH, Kiel, Germany).

Heart rate, blood pressure and body temperatures

Heart rate was obtained by telemetry (Polar Electro, Kempele, Finland). Arterial and femoral venous pressure waveforms were recorded using pressure transducers (Pressure Monitoring Kit, TruWave, Edwards Lifesciences, Germany) zeroed at the level of the right atrium in the mid-axillary line (arterial) and at the level of the tip of the catheter (femoral venous). Pressure waveforms were amplified (BP amp, ADIstruments) and sampled at 1000 Hz using a data acquisition unit (Powerlab 16/30, ADInstruments, Oxfordshire, UK) for offline analysis. For measurements of femoral venous blood temperature (T_B), a thermistor (T204a, PhysiTemp, Clifton, New Jersey, USA) was inserted through the femoral venous catheter and connected to a thermocouple meter (TC-2000, Sable Systems, NV: USA) and routed through the data acquisition system. In the control trial, oesophageal temperature (T_{Oes}) was measured using a thermistor (Physitemp, New England, USA), inserted pernasally into the oesophagus at a depth of ¼ standing height. Increases in core temperature

during cycling exercise reflect the rise in femoral venous blood temperature, as T_B and T_{Oes} have been shown to be within ~0.1 °C (González-Alonso *et al.*, 1999). Mean skin temperature (T_{sk}) from four sites (standard weightings of chest, arm, thigh and calf; (Ramanathan, 1964) was obtained using a wireless monitoring system (iButton®, Maxim Integrated, San José, CA, USA).

Calculations

In the hyperthermia trials, brain and active limb vascular conductance (VC) indices were calculated by dividing MCA V_{mean} and LBF (for two-legged) by perfusion pressure (MAP). Direct measurements of $\dot{Q}$ were not possible during exercise; however, to provide some insight into these responses, $\dot{Q}$ was calculated using the Fick principle, by estimation of systemic O_2 extraction from the directly measured limb O_2 extractions (assuming a linear relationship between these variables, reported in similar exercise protocols; Mortensen *et al.*, 2008; Munch *et al.*, 2014, and accounting for the known reduction in systemic O_2 extraction with core hypethermia; González-Alonso et al., 2004). The following equations were used: Y = 1.43X - 44.7; R^2 = 0.99; P > 0.05 for control and HYP_{mild} and Y = 1.7322X -76.126; R^2 = 0.98; P > 0.05 for HYP_{mod}. When leg blood flow measurements were not possible, LBF was calculated from the estimated leg $\dot{V}O_2$ (assuming that the increase in pulmonary $\dot{V}O_2$ from baseline reflected only the increase in leg $\dot{V}O_2$) (Mortensen *et al.*, 2005, 2008; Calbet *et al.*, 2007) and directly measured leg arterial-to-femoral venous O_2 difference.

Statistics

Differences between exercise conditions were assessed using a two-way repeated-measures ANOVA in which condition (Moderate heat stress, mild heat stress and control) and exercise phase (Rest, 20, 40, 60, 80 and 100%) were the main factors. Where a significant main effect was found, pairwise comparisons were made using the Holm-Bonferroni procedure. Statistical significance was set at P < 0.05 and all analyses were made using IBM SPSS Statistics (Version 20, IBM Corporation, Armonk, NY, USA).

Results

Impact of heat stress and repeated incremental exercise on exercise capacity

On the preliminary visit, heat stress exposure sufficient to induce HYP_{mod} resulted in a reduction in W_{max} by ~13 ± 1%, despite a similar HR_{max} compared to control. To ensure a comparable percentage of W_{max} across experimental conditions in the subsequent hyperthermia and control trials, the absolute work rates for the incremental stages in HYP_{mod} were reduced by 13 ± 1% (64 ± 2, 128 ± 4, 193 ± 5, 257 ± 7 and 321 ± 9 W) compared to all other incremental tests (74 ± 2, 148 ± 4, 223 ± 7, 297 ± 9 and 371 ± 11 W; Fig. 1).

During the control trial, where exercise capacity across the three incremental tests was the same, $\dot{V}O_{2max}$ (4.4 ± 0.1, 4.5 ± 0.2 and 4.5 ± 0.1 l min^{-1}), HR_{max} (177 ± 3, 181 ± 3 and 182 ± 3 beats min^{-1}), T_{Oes} (38.2 ± 0.1, 38.6 ± 0.1 and 38.7 ± 0.1 ^{o}C) and end-exercise MCA V_{mean} (68 ± 5, 66 ± 5 and 68 ± 3 cm s^{-1}) were not significantly different (Fig. 2). Moreover, the increase in $\dot{V}O_2$ per unit of power was linear from low to maximal exercise intensities in all 3 tests (9.2 ± 0.3, 9.5 ± 0.3 and 9.1 ± 0.3 ml min^{-1} W^{-1}; R^2 = 0.99; P < 0.001). Given the similar exercise capacity, body temperatures and cardio-respiratory responses to exercise in the control trial, the following sections focus on the effects of temperature manipulation on whole-body haemodynamics in the hyperthermia trial only.

Temperature and cardiorespiratory responses to heat stress

Resting T_B was elevated in HYP_{mod} compared to HYP_{mild} and control exercise conditions (37.5 ± 0.1 *vs.* 36.7 ± 0.1 and 37.0 ± 0.1 °C; P = 0.03), whereas T_{sk} was elevated in both heat stress conditions compared to control (~38.2 ± 0.3 *vs.* 32.3 ± 0.4 °C; P < 0.001: Fig. 3A and B). During incremental exercise in HYP_{mod}, T_B was initially unchanged before increasing to a peak of 39.3 ± 0.1 °C (P < 0.01 *vs.* rest) whereas, in HYP_{mild} and control, T_B increased from rest to W_{max} (39.1 ± 0.1 °C; P < 0.001) and was lower overall compare to HYP_{mod}. T_{sk}

was maintained elevated in both heat stress conditions (~36.9 ± 0.4 *vs.* 32.0 ± 0.4 °C; $P < 0.001$) and was maintained stable throughout exercise.

Cardiorespiratory variables are presented in Table 1. Briefly, systolic and diastolic blood pressures were lower in HYP_{mod} compared to HYP_{mild} and control ($P < 0.001$). Respiratory frequency, CO_2 production ($\dot{V}CO_2$) and minute ventilation ($\dot{V}_E$) increased with exercise intensity and were lower in HYP_{mod} compared to HYP_{mild} and control (both $P < 0.001$). End-tidal PO_2 initially declined before increasing at $W_{max,}$ with the reverse response observed for PCO_2; however, there were no differences between the exercise test conditions ($P = 0.492$).

Brain, active limb and systemic haemodynamics

At baseline, HR was 57 ± 3 beats min^{-1}, two-legged blood flow 0.8 ± 0.1 l min^{-1}, $\dot{Q}$ 5.5 ± 0.4 l min^{-1} and MCA V_{mean} 64 ± 1 cm s^{-1} (Fig. 4). At rest following passive heat stress or control, HR (88 ± 3 *vs.* ~76 ± 5 bpm), two-legged blood flow (1.9 ± 0.1 *vs.* ~1.0 ± 0.1 l min^{-1}) and $\dot{Q}$ (8.9 ± 0.7 *vs.* ~6.9 ± 0.8 l min^{-1}) were elevated in HYP_{mod} compared to HYP_{mild} and control (all $P < 0.05$), whereas MCA V_{mean} was not different (~63 ± 2 cm s^{-1}). From rest to sub-maximal exercise, HR and two-legged blood flow increased with exercise intensity in all conditions ($P < 0.05$ *vs.* rest) and MCA V_{mean} was elevated (Fig. 4C; $P < 0.05$). However, overall, two-legged blood flow was lower (Fig. 4A; $P < 0.05$) and HR higher, in HYP_{mod} exercise compared to control exercise. At exhaustion, HR increased to similar peak values in $HYP_{mod,}$ HYP_{mild} and control respectively (189 ± 4, 187 ± 3 and 184 ± 3 beats/min). In all conditions, the rate of rise in two-legged blood flow was attenuated, and MCA V_{mean} was reduced in all exercise conditions. Final two-legged blood flow (16.2 ± 1.3, 18.4 ± 1.1 and 18.9 ± 1.1 l min^{-1}) and MCA V_{mean} (57 ± 1 *vs.* 66 ± 3 cm s^{-1}) were lower in lower in HYP_{mod} than in HYP_{mild} and control conditions.

On the transition from rest to sub-maximal exercise, estimated $\dot{Q}$ increased at a similar rate among conditions (~0.04 l min^{-1} W). Prior to exhaustion, $\dot{Q}$

paralleled the attenuation in two-legged blood flow, to a greater extent in HYP_{mod} vs. HYP_{mild} and control conditions (Gradient = 0.007 *vs.* 0.017 l min^{-1} W), at a lower absolute work rate, and was similar at end-exercise (26.6 ± 2 l min^{-1}).

Blood pressure, oxygen uptake and brain oxygenation

At rest, MAP and FVP were not different among conditions (Fig. 4D). From rest to maximal exercise, MAP increased in all conditions, but was reduced in HYP_{mod} compared to HYP_{mild} and control, respectively (124 ± 7, 139 ± 7 and 153 ± 7 mmHg; $P < 0.05$). Femoral venous pressure increased with exercise intensity but was not different among exercise conditions.

At rest, leg a-v O_{2diff} was lower in HYP_{mod} compared to HYP_{mild} (24 ± 3 *vs.* ~56 ± 7 ml l^{-1}; $P < 0.05$: Fig. 4B), whereas resting systemic $\dot{V}O_2$ was not different among conditions (0.46 ± 0.03 l min^{-1}; P = 0.47-0.84). During incremental exercise, leg a-vO_{2diff} and systemic $\dot{V}O_2$ increased with intensity in all conditions ($P < 0.05$). At exhaustion, leg a-v O_{2diff} was not different among conditions; however, systemic $\dot{V}O_{2max}$ was reduced in HYP_{mod} compared to HYP_{mild} and control exercise conditions (3.94 ± 0.11 *vs.* 4.23 ± 0.13 and 4.23 ± 0.14 l min^{-1}, respectively; $P < 0.05$). Compared to the 3 maximal incremental tests in the control trial, the rise in systemic $\dot{V}O_2$ per unit of power was identical from 20 to 70-80%W_{max} (9.6 ± 0.3 ml min^{-1} W^{-1}), but became attenuated thereafter (8.2 ± 0.6 ml min^{-1} W^{-1}). At rest, NIRS derived rSO_2% was elevated in HYP_{mod} and HYP_{mild} *vs.* control conditions (77 ± 2 & 75 ± 3 *vs.* 67 ± 3%; $P < 0.05$) and remained unchanged across all conditions during incremental exercise, but declined before exhaustion (rSO_2 ~64%; $P < 0.05$).

Brain and active limb conductance, blood gases, plasma catecholamines and ATP

Arterial and venous haemoglobin [Hb] and arterial oxygen content increased with incremental exercise in all conditions, despite a reduction in arterial oxygen saturation (all $P < 0.05$: Table 2 and 3). Arterial oxygen content was elevated in

418 both heat stress conditions and was higher in HYP_{mod} compared to HYP_{mild} and
419 control up to 60% W_{max} ($P < 0.05$). Blood lactate increased exponentially and
420 reached similar values at exhaustion in all experimental conditions (Table 3).
421 However, arterial and venous glucose concentrations were elevated at exercise
422 intensities ≥ 60% W_{max} in the HYP_{mod} compared to control and HYP_{mild}.
423
424 At rest, MCA vascular conductance was not different among conditions (Fig.
425 5A). The elevations in limb and systemic perfusion in HYP_{mod} were coupled to
426 an enhanced limb vascular conductance (Fig. 5B; $P < 0.05$). MCA vascular
427 conductance declined with exercise intensity. Contrastingly, limb vascular
428 conductance increased with exercise intensity ($P < 0.05$), but was not different
429 among conditions.
430
431 At rest, arterial [NA] (Fig. 5C) was augmented in HYP_{mod} *vs.* HYP_{mild} and control
432 conditions (3.7 $\pm$ 0.8 vs. ~1.9 $\pm$ 0.5 nmol l^{-1}; $P < 0.05$), whereas venous [NA]
433 was not different (data not shown). Thereafter, both arterial and venous [NA]
434 increased with exercise intensity to a similar peak value (~48 $\pm$ 5 nmol l^{-1}). The
435 rise in arterial [NA] was coupled to a blunted two-legged vascular conductance
436 at maximal exercise intensities (R^2 = 0.64; $P < 0.01$). At rest and during
437 submaximal exercise, P_aCO_2 was maintained stable (Fig. 5D. However, beyond
438 sub-maximal intensities, and in association with a marked increase in $\dot{V}_E$ (Table
439 1), P_aCO_2 declined to a similar end-exercise value across conditions ($P < 0.05$).
440 The decline in P_aCO_2 was moderately related to the fall in MCA vascular
441 conductance (R^2 = 0.29; $P < 0.01$). Lastly, femoral venous (ATP_{fv}) and arterial
442 (ATP_a) ATP concentrations were not different at rest, but increased up to sub-
443 maximal exercise intensities in all conditions (Fig. 5E and F). Beyond 80%
444 W_{max}, both ATP_{fv}, in association with an attenuation in two-legged vascular
445 conductance (R^2 = 0.46; $P < 0.01$), and ATP_a plateaued.
446
447 **Discussion**
448 To our knowledge this is the first study to separate the effects of skin
449 hyperthermia from the combined effects of skin and internal hyperthermia on

brain and locomotor limb perfusion, aerobic metabolism and exercise capacity. The major novel finding was that marked skin hyperthermia was insufficient to compromise $\dot{V}O_{2max}$ and incremental exercise capacity. A second novel finding was that superimposed internal and skin hyperthermia led to a faster decline, attenuation or plateau in brain and locomotor limb perfusion, which was mechanistically coupled to a plateau or decline in regional vascular conductance and a reduced arterial pressure. Finally, acceleration of the attenuation in brain and exercising limb flow appears to be an important mechanism by which combined skin and internal hyperthermia reduces aerobic metabolism and exercise capacity. Together, these findings demonstrate that the combination of skin and internal body hyperthermia is a critical factor in whether or not brain and active muscle perfusion and aerobic metabolism is compromised during incremental exercise to volitional exhaustion in hot environments.

Skin hyperthermia does not independently compromise cardiovascular capacity or aerobic exercise performance

In the present study we clamped skin temperature at a high level (i.e., ~37 ^{o}C vs. ~32 ^{o}C), without increasing internal temperature, prior to and during incremental exercise (HYP_{mild}). To achieve this, the participants were first exposed to passive whole body heat stress for ~13 min and then combined whole body heat stress and exercise for an additional ~12.5 min. An important finding under these conditions was that $\dot{V}O_{2max}$ and exercise capacity was the same compared to control exercise. Narrow core-to-skin temperature gradients, as seen in both HYP conditions in the present study (range; 0-2.6 ^{o}C), are purported to place a significant burden on cardiovascular capacity owing to the increased demand for skin blood flow (Rowell, 1986; Sawka *et al.*, 2012). This theory has been taken to mean that high skin temperatures play a dominant role in reduced exercise capacity in the heat, by promoting the displacement of blood volume and flow to the skin thereby compromising active muscle perfusion (Tatterson *et al.*, 2000; Ely *et al.*, 2009; Kenefick *et al.*, 2010; Lorenzo *et al.*, 2010; Cheuvront *et al.*, 2010; Sawka *et al.*, 2012). However, we show that

brain, active limb and systemic blood flow during HYP_{mild} is not reduced compared to that observed during control conditions. Moreover, the results on the experimental trial are supported by those on the control trial (Fig. 2) where, despite some differences in exercise internal temperature (~0.5 ^{o}C at exhaustion), exercise capacity was not different during repeated incremental exercise with normal skin temperature (~32 ^{o}C). Our findings collectively suggest that skin hyperthermia or small elevations in internal temperature alone do not compromise aerobic power or exercise capacity in trained individuals.

In contrast, when combined internal and skin hyperthermia was present (i.e., achieved by extending the exposure to passive whole body heat stress to ~52 min, while the exercise duration was not different), $\dot{V}O_{2max}$ was reduced by ~8%; a decline similar to that previously reported (Rowell *et al.*, 1966; Klausen *et al.*, 1967; Pirnay *et al.*, 1970; Sawka *et al.*, 1985; Nybo *et al.*, 2001; Arngrímsson *et al.*, 2004). The reduced aerobic power and work capacity were associated with a diminished arterial pressure, an early attenuation in active limb (and systemic perfusion), an advanced fall in brain blood flow and high internal and skin temperatures (39.3 and 37 ^{o}C, respectively) (Fig. 4). Restricted LBF, via a plateau in local vascular conductance, precedes fatigue during incremental (Mortensen *et al.*, 2008) and constant load maximal exercise (González-Alonso & Calbet, 2003); whole body hyperthermia advances this cardiovascular instability and may explain the reduced maximal aerobic power (González-Alonso & Calbet, 2003). Our data demonstrate that the duration of heat exposure is critical to whether or not cardiovascular function is impaired during strenuous exercise in the heat stressed human.

Impact of hyperthermia on blood flow and pressure at rest and during incremental exercise

To understand the responses to regional hyperthermia during exercise and the potential underlying mechanisms, we need to first scrutinise the resting responses. At rest, combined internal and skin hyperthermia led to elevations in LBF and $\dot{Q}$, accompanying a fall in limb a-vO_{2diff} and a lower MAP, in close

agreement with the responses to passive heat stress (Barcroft *et al.*, 1947; Rowell *et al.*, 1969; Rowell, 1974; Minson *et al.*, 1998; Crandall *et al.*, 2008; Stöhr *et al.*, 2011*a*; Pearson *et al.*, 2011; Heinonen *et al.*, 2011; Chiesa *et al.*, 2016). Interestingly, brief heat exposure, sufficient to raise T_{sk} to that experienced during combined internal and skin hyperthermia (HYP_{mild}), but without elevations in T_c, led to a small increase in systemic (+ 1.3 l min^{-1}) and limb blood flow (+ 0.25 l min^{-1}) compared to baseline values. During passive whole body heat stress, interspersed by single leg exercise, elevations in whole-body perfusion (e.g. $\dot{Q}$; 1.1 – 1.8 ± 0.3 l min^{-1}, LBF; 0.5 ± 0.1 l min^{-1}) and small but significant reductions in MAP have been observed with skin hyperthermia at rest without increases in T_c (Pearson *et al.*, 2011; Stöhr *et al.*, 2011*a*). In a recent study from this laboratory, mild heat stress was also shown to induce small but significant increases in systemic and leg perfusion and HR (e.g. $\dot{Q}$; 0.9 l min^{-1}, LBF; 0.2 l min^{-1}; 12 beats min^{-1}), although these alterations occurred concomitant to small increases in T_c (~0.4ºC) (Chiesa *et al.*, 2016). It is therefore likely that any increased demand for skin and deep limb tissue blood flow, during passive mild hyperthermia, is met by blood flow redistribution from splanchnic vascular beds and a small increase in $\dot{Q}$ and small reduction in MAP (Rowell *et al.*, 1968; Crandall *et al.*, 2008).

A key question is whether passive hyperthermia-induced hyperperfusion and hypotension alters cardiovascular dynamics during incremental exercise. A novel finding in the present study was that the rise in LBF, limb a-vO_{2diff} and the early increase in MCA V_{mean} from rest to sub-maximal exercise was similar among temperature manipulations. However, between 20-80% of W_{max}, blood pressure and MCA V_{mean} were reduced with combined skin and internal hyperthermia compared to control exercise (Fig. 4), despite an estimated ~1.8 l min^{-1} elevation in $\dot{Q}$. The present blood flow responses during sub-maximal exercise are consistent with a similar LBF and limb a-vO_{2diff} but elevated $\dot{Q}$ in trained individuals (Savard *et al.*, 1988; Nielsen *et al.*, 1993, 1997), but are in contrast to the classical observations of a suppressed $\dot{Q}$ (from an exercise intensity equivalent to ~60% $\dot{V}O_{2max}$) in the study of Rowell *et al.* (1966). Prior to

546 exhaustion, brain perfusion was markedly reduced, whilst there was evidence of
547 a plateau in two-legged (and systemic) blood flow. In all cases, combined
548 internal and skin hyperthermia advanced this restriction in regional blood flow,
549 despite a similar HR_{max} rate and limb O_2 extraction.
550
551 The attenuation in blood flow and vascular conductance at maximal exercise
552 may involve the interaction of various reflex, chemical and thermal mechanisms,
553 in different tissues of the body, responsible for regulating local vascular tone
554 (Rowell, 1974; González-Alonso *et al.*, 2004; González-Alonso, 2008;
555 Mortensen *et al.*, 2008; Mortensen & Saltin, 2014). To provide mechanistic
556 insight into these circulatory alterations during incremental exercise, with
557 differing combinations of body temperatures, we assessed a number of
558 vasoactive substances implicated in the regulation of brain and muscle blood
559 flow. Irrespective of the temperature manipulation, the fall (brain) or plateau in
560 (two-legged) blood flow at maximal exercise intensities was coupled to a similar
561 fall or plateau in regional vascular conductance (Fig. 5), indicative of
562 vasoconstriction in the active brain and muscle vascular beds. Mechanistically,
563 the brain blood flow velocity decline towards baseline values was associated
564 with a hyperventilation-induced fall in P_aCO_2 (r = 0.54; P < 0.05); a potent
565 vasoactive substance affecting cerebrovascular tone (Willie *et al.*, 2012). These
566 dynamics during graded exercise are supported by the literature (Hellstrom *et*
567 *al.*, 1996; Sato *et al.*, 2011; Trangmar *et al.*, 2014). On the other hand, the
568 restriction in two-legged conductance, prior to exhaustion in all conditions, was
569 related to a plateau in plasma ATP and an exponential rise in sympathetic
570 vasoconstrictor activity even when leg vascular conductance and plasma ATP
571 and [NA] were or tended to be higher in the hyperthermic trials (Fig. 5). It has
572 previously been postulated that the influence of sympathetic vasoconstriction on
573 vascular conductance can be “overridden” by metabolic vasodilation
574 (Remensnyder *et al.*, 1962; Rosenmeier *et al.*, 2004). This theory can explain
575 the regulation of muscle perfusion when exercising limb blood flow, and the
576 intravascular vasodilator milieu including ATP, increase progressively during
577 exercise against a background of relatively low sympathetic drive (González-

Alonso *et al.*, 2002; Rosenmeier *et al.*, 2004; Mortensen *et al.*, 2011). However, the present findings together with those during maximal and supra-maximal exercise (Mortensen *et al.*, 2008), indicate that local vasoconstriction prevails during whole body, intense exercise in association with marked increases in sympathetic nerve activity (Saito *et al.*, 1993; Ichinose *et al.*, 2008) and a blunted rise in plasma ATP concentration. Thus, functional sympatholysis does not prevail at the maximal and supramaximal exercise domain with normal or elevated levels of local hyperthermia.

Does cardiovascular strain contribute to hyperthermia-induced fatigue?

An important question from the present study is which cardiovascular process underpins the reduced exercise capacity under physiologically stressful environments. Prevailing theory suggests that reduced aerobic capacity during exercise in the heat is due to reductions in active muscle blood flow, secondary to a substantial increase in skin perfusion, and despite active redistribution of blood flow from non-active tissues (Rowell, 1974, 1986). This theory was based on observations that body hyperthermia suppressed $\dot{Q}$ during treadmill running, in un-trained and un-acclimatised individuals, compared to control conditions (Rowell *et al.*, 1966); thus giving rise to the premise that the limited cardiovascular capacity is insufficient to meet the combined demands of heat dissipation (skin perfusion) and active muscle perfusion. Our findings demonstrate that the attenuated rise in systemic $\dot{V}O_2$ (from 9.6 to 8.2 ml min^{-1} W^{-1}) and reduced exercise capacity with combined internal and skin hyperthermia were coupled to an advanced fall in brain blood flow, and an early attenuation in LBF (that is, occurring at a lower absolute work rate); temporal responses that could feasibly result in a compromised local tissue aerobic metabolism when oxygen extraction reaches its upper limits (~ 90% in the 3 conditions of this study) (González-Alonso & Calbet, 2003; Mortensen *et al.*, 2005, 2008; Calbet *et al.*, 2007). In addition, our estimates of $\dot{Q}$ suggest that systemic blood flow is similar at exhaustion among temperature manipulations; a conclusion supported by findings in trained participants, during constant-load cycling to volitional exhaustion, with combined internal and skin hyperthermia

610 (González-Alonso & Calbet, 2003). It is therefore unlikely that the absolute
611 values of $\dot{Q}$ and high skin blood flow explain early fatigue during incremental
612 exercise.
613
614 Reductions in cerebral O_2 delivery (and oxygenation) might contribute to fatigue
615 processes when hyperthermic (Nielsen & Nybo, 2003; Nybo & Secher, 2004;
616 Todd *et al.*, 2005; Rasmussen *et al.*, 2010; Ross *et al.*, 2012). However, it is
617 unlikely that the moderate reductions in cerebral perfusion, seen here, and in
618 previous studies (González-Alonso *et al*., 2004; Trangmar *et al*., 2014, 2015),
619 can compromise cerebral metabolism to the extent that can explain the reduced
620 aerobic power with a moderate hyperthermia. Rather, the advanced fall in
621 cerebral perfusion, at lower absolute exercise intensities, is likely a
622 consequence of the overall cardiovascular strain induced by strenuous exercise
623 in the heat and the concomitant respiratory alkalosis. This is supported by
624 similar findings in hypoxia where cerebral O_2 delivery is markedly attenuated,
625 despite elevated systemic blood flow and perfusion pressure (Subudhi *et al.*,
626 2009; Vogiatzis *et al.*, 2011). Restoring reductions in cerebral O_2 delivery,
627 during exercise in hypoxia and with body hyperthermia, does not improve
628 maximal aerobic power (Subudhi *et al.*, 2011; Keiser *et al.*, 2015), indicating
629 that processes other than a suppressed cerebral O_2 metabolism explain the
630 early fatigue under physiological stressful environments.
631
632 Our present findings highlight that combined skin and internal hyperthermia
633 accelerates the attenuation in regional and systemic perfusion and reduces
634 aerobic capacity during strenuous exercise. Blunted skeletal muscle and
635 systemic blood flow and O_2 delivery, with and without body hyperthermia,
636 appear to be an important factor limiting aerobic capacity (González-Alonso &
637 Calbet, 2003; Mortensen *et al.*, 2005, 2008). We recognise that many
638 interrelating factors likely contribute to the development of fatigue during
639 exercise. In this context, exhaustion in the present experimental conditions may
640 have resulted from the interaction of multiple inhibitory and excitatory regulatory
641 processes in response to reduced O_2 delivery, modified locomotor muscle and

brain metabolism, hyperthermia, altered central motor output, changed central nervous system neurotransmitter activity, and stimulation of muscle feedback mechanisms sensing local metabolic milieu (González-Alonso *et al.*, 2008; Amann & Calbet, 2008; Meeusen & Roelands, 2010; Amann *et al.*, 2011; Noakes, 2012; Sawka, 2012; Nybo *et al.*, 2014; Morales-Alamo *et al.*, 2015; Blain *et al.*, 2016). Supporting the idea that the aetiology of fatigue during exercise is multifactorial and typified by cardiovascular strain and disturbed physiological homeostasis, we found that the single stressor skin hyperthermia was apparently met by compensatory physiological adjustments such that muscle and whole body aerobic energy provision was not compromised compared to control. The combination of multiple stressors triggered by whole body hyperthermia, however, resulted in a compromised aerobic capacity, associated with a blunted rise in active muscle and systemic perfusion.

Methodological considerations

Resting blood flow measurements were made using Doppler ultrasonography, rather than thermodilution, as less blood flow variability is seen with ultrasonography in resting conditions. We were unable to obtain direct measures of $\dot{Q}$ during exercise; on this basis our conclusions based on estimated $\dot{Q}$ are purposefully tempered. On the other hand, it is established that systemic O_2 difference shares a strong linear relationship with leg O_2 extraction during incremental exercise (Mortensen *et al.*, 2008; Munch *et al.*, 2014). Moreover, the adjustment to this relationship in HYP_{mod} is in accordance with previous literature demonstrating a reduced systemic O_2 extraction, per unit of leg O_2 extraction with body hyperthermia (González-Alonso *et al.*, 2004). Finally, our estimations on $\dot{Q}$ dynamics during exercise were supported by those obtained with the Modelflow method. Nevertheless, future studies measuring central haemodynamics with different manipulations of internal and skin temperature are required to confirm the present observations.

Conclusion

The present findings show that skin hyperthermia, in the absence of high internal temperatures, does not compromise cardiovascular capacity, maximal oxygen uptake or exercise performance during strenuous whole-body dynamic exercise. The fall in maximal aerobic power with combined internal and skin hyperthermia was associated with compromised active muscle metabolism due to reduced oxygen delivery. Taken together, these observations explain why aerobic exercise performance in hot environments is not universally impaired across all exercise modalities, as the deleterious effects of environmental heat stress are directly dependent upon heat exposure inducing whole-body hyperthermia and uncompensable physiological strain.

References

Aaslid R, Markwalder TM & Nornes H (1982). Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* **57,** 769–774.

Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF & Dempsey JA (2011). Implications of group {III} and {IV} muscle afferents for high-intensity endurance exercise performance in humans. *J Physiol* **589,** 5299–5309.

Amann M & Calbet JAL (2008). Convective oxygen transport and fatigue. *J Appl Physiol* **104,** 861–870.

Andersen P & Saltin B (1985). Maximal perfusion of skeletal muscle in man. *J Physiol* **366,** 233–249.

Arngrímsson SA, Petitt DS, Borrani F, Skinner KA & Cureton KJ (2004). Hyperthermia and maximal oxygen uptake in men and women. *Eur J Appl Physiol* **92,** 524–532.

Barcroft H, Bonnar WM & Edholm OG (1947). Reflex vasodilatation in human skeletal muscle in response to heating the body. *J Physiol* **106,** 271–278.

Blain GM, Mangum TS, Sidhu SK, Weavil JC, Hureau TJ, Jessop JE, Bledsoe AD, Richardson RS & Amann M (2016). Group III/IV muscle afferents limit the intramuscular metabolic perturbation during whole body exercise in humans. *J Physiol*; DOI: 10.1113/JP272283.

Calbet JAL, Gonzalez-Alonso J, Helge JW, S\ondergaard H, Munch-Andersen T, Boushel R & Saltin B (2007). Cardiac output and leg and arm blood flow during incremental exercise to exhaustion on the cycle ergometer. *J Appl Physiol* **103,** 969–978.

Calbet JAL, Losa-Reyna J, Torres-Peralta R, Rasmussen P, Ponce-González JG, Sheel AW, de la Calle-Herrero J, Guadalupe-Grau A, Morales-Alamo D, Fuentes T, Rodríguez-García L, Siebenmann C, Boushel R & Lundby C (2015). Limitations to oxygen transport and utilization during sprint exercise in humans: evidence for a functional reserve in muscle O_2 diffusing capacity. *J Physiol* **593,** 4649–4664.

Cheuvront SN, Kenefick RW, Montain SJ & Sawka MN (2010). Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol* **109,** 1989–1995.

Chiesa ST, Trangmar SJ & González-Alonso J (2016). Temperature and blood flow distribution in the human leg during passive heat stress. *J Appl Physiol* **120,** 1047–1058.

Chiesa ST, Trangmar SJ, Kalsi KK, Rakobowchuk M, Banker DS, Lotlikar MD, Ali L & González-Alonso J (2015). Local temperature-sensitive mechanisms are important mediators of limb tissue hyperemia in the heat-stressed human at rest and during small muscle mass exercise. *Am J Physiol Hear Circ Physiol* **309,** H369–H380.

Crandall CG, Wilson TE, Marving J, Vogelsang TW, Kjaer A, Hesse B & Secher NH (2008). Effects of passive heating on central blood volume and ventricular dimensions in humans. *J Physiol* **586,** 293–301.

Delp MD & Laughlin MH (1998). Regulation of skeletal muscle perfusion during exercise. *Acta Physiol Scand* **162,** 411–419.

Delp MD & O'Leary DS (2004). Integrative control of the skeletal muscle microcirculation in the maintenance of arterial pressure during exercise. *J Appl Physiol* **97,** 1112–1118.

Ely BR, Ely MR, Cheuvront SN, Kenefick RW, Degroot DW & Montain SJ (2009). Evidence against a 40 degrees {C} core temperature threshold for fatigue in humans. *J Appl Physiol* **107,** 1519–1525.

737 Galloway SD & Maughan RJ (1997). Effects of ambient temperature on the
738 capacity to perform prolonged cycle exercise in man. *Med Sci Sport Exerc*
739 **29,** 1240–1249.
740 González-Alonso J (2008). {ATP}: a double-edged signalling molecule
741 regulating the flow of oxygen. *J Physiol* **586,** 4033–4034.
742 González-Alonso J, Calbet JA & Nielsen B (1999). Metabolic and
743 thermodynamic responses to dehydration-induced reductions in muscle
744 blood flow in exercising humans. *J Physiol* **520 Pt 2,** 577–589.
745 González-Alonso J & Calbet JAL (2003). Reductions in systemic and skeletal
746 muscle blood flow and oxygen delivery limit maximal aerobic capacity in
747 humans. *Circulation* **107,** 824–830.
748 González-Alonso J, Crandall CG & Johnson JM (2008). The cardiovascular
749 challenge of exercising in the heat. *J Physiol* **586,** 45–53.
750 González-Alonso J, Dalsgaard MK, Osada T, Volianitis S, Dawson EA, Yoshiga
751 CC & Secher NH (2004). Brain and central haemodynamics and
752 oxygenation during maximal exercise in humans. *J Physiol* **557,** 331–342.
753 González-Alonso J, Olsen DB & Saltin B (2002). Erythrocyte and the regulation
754 of human skeletal muscle blood flow and oxygen delivery: role of circulating
755 {ATP}. *Circ Res* **91,** 1046–1055.
756 González-Alonso J, Quistorff B, Krustrup P, Bangsbo J & Saltin B (2000). Heat
757 production in human skeletal muscle at the onset of intense dynamic
758 exercise. *J Physiol* **524 Pt 2,** 603–615.
759 Heinonen I, Brothers RM, Kemppainen J, Knuuti J, Kalliokoski KK & Crandall
760 CG (2011). Local heating, but not indirect whole body heating, increases
761 human skeletal muscle blood flow. *J Appl Physiol* **111,** 818–824.
762 Hellstrom G, Fischer-Colbrie W, Wahlgren NG & Jogestrand T (1996). Carotid
763 artery blood flow and middle cerebral artery blood flow velocity during
764 physical exercise. *J Appl Physiol* **81,** 413–418.
765 Ichinose M, Saito M, Fujii N, Ogawa T, Hayashi K, Kondo N & Nishiyasu T
766 (2008). Modulation of the control of muscle sympathetic nerve activity
767 during incremental leg cycling. *J Physiol* **586,** 2753–2766.
768 Keiser S, Flück D, Stravs A, Hüppin F & Lundby C (2015). Restoring heat

769 stress-associated reduction in middle cerebral artery velocity does not
770 reduce fatigue in the heat. *Scand J Med Sci Sport* **25 Suppl 1,** 145–153.
771 Kenefick RW, Cheuvront SN, Palombo LJ, Ely BR & Sawka MN (2010). Skin
772 temperature modifies the impact of hypohydration on aerobic performance.
773 *J Appl Physiol* **109,** 79–86.
774 Klausen K, Dill DB, Phillips Jr EE & McGregor D (1967). Metabolic reactions to
775 work in the desert. *J Appl Physiol* **22,** 292–296.
776 Lorenzo S, Halliwill JR, Sawka MN & Minson CT (2010). Heat acclimation
777 improves exercise performance. *J Appl Physiol* **109,** 1140–1147.
778 Meeusen R & Roelands B (2010). Central fatigue and neurotransmitters, can
779 thermoregulation be manipulated? *Scand J Med Sci Sport* **20 Suppl 3,** 19–
780 28.
781 Minson CT, Wladkowski SL, Cardell AF, Pawelczyk JA & Kenney WL (1998).
782 Age alters the cardiovascular response to direct passive heating. *J Appl*
783 *Physiol* **84,** 1323–1332.
784 Morales-Alamo D, Losa-Reyna J, Torres-Peralta R, Martin-Rincon M, Perez-
785 Valera M, Curtelin D, Ponce-González JG, Santana A & Calbet JAL (2015).
786 What limits performance during whole body incremental exercise to
787 exhaustion in humans? *J Physiol*.
788 Mortensen SP, Damsgaard R, Dawson EA, Secher NH & González-Alonso J
789 (2008). Restrictions in systemic and locomotor skeletal muscle perfusion,
790 oxygen supply and {VO2} during high-intensity whole-body exercise in
791 humans. *J Physiol* **586,** 2621–2635.
792 Mortensen SP, Dawson EA, Yoshiga CC, Dalsgaard MK, Damsgaard R, Secher
793 NH & González-Alonso J (2005). Limitations to systemic and locomotor
794 limb muscle oxygen delivery and uptake during maximal exercise in
795 humans. *J Physiol* **566,** 273–285.
796 Mortensen SP & Saltin B (2014). Regulation of the skeletal muscle blood flow in
797 humans. *Exp Physiol* **99,** 1552–1558.
798 Mortensen SP, Thaning P, Nyberg M, Saltin B & Hellsten Y (2011). Local
799 release of {ATP} into the arterial inflow and venous drainage of human
800 skeletal muscle: insight from {ATP} determination with the intravascular

microdialysis technique. *J Physiol* **589,** 1847–1857.

Munch GDW, Svendsen JH, Damsgaard R, Secher NH, González-Alonso J & Mortensen SP (2014). Maximal heart rate does not limit cardiovascular capacity in healthy humans: insight from right atrial pacing during maximal exercise. *J Physiol* **592,** 377–390.

Nielsen B, Hales JR, Strange S, Christensen NJ, Warberg J & Saltin B (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *J Physiol* **460,** 467–485.

Nielsen B & Nybo L (2003). Cerebral changes during exercise in the heat. *Sport Med* **33,** 1–11.

Nielsen B, Strange S, Christensen NJ, Warberg J & Saltin B (1997). Acute and adaptive responses in humans to exercise in a warm, humid environment. *Pflugers Arch* **434,** 49–56.

Noakes TD (2012). Fatigue is a {Brain-Derived} Emotion that Regulates the Exercise Behavior to Ensure the Protection of Whole Body Homeostasis. *Front Physiol* **3,** 82.

Nybo L, Jensen T, Nielsen B & González-Alonso J (2001). Effects of marked hyperthermia with and without dehydration on {VO(2}) kinetics during intense exercise. *J Appl Physiol* **90,** 1057–1064.

Nybo L, M\oller K, Volianitis S, Nielsen B & Secher NH (2002). Effects of hyperthermia on cerebral blood flow and metabolism during prolonged exercise in humans. *J Appl Physiol* **93,** 58–64.

Nybo L, Rasmussen P & Sawka MN (2014). Performance in the heat-physiological factors of importance for hyperthermia-induced fatigue. *Compr Physiol* **4,** 657–689.

Nybo L & Secher NH (2004). Cerebral perturbations provoked by prolonged exercise. *Prog Neurobiol* **72,** 223–261.

Pearson J, Low DA, Stöhr E, Kalsi K, Ali L, Barker H & González-Alonso J (2011). Hemodynamic responses to heat stress in the resting and exercising human leg: insight into the effect of temperature on skeletal muscle blood flow. *Am J Physiol Regul Integr Comp Physiol* **300,** R663–R673.

833 Pirnay F, Deroanne R & Petit JM (1970). Maximal oxygen consumption in a hot
834 environment. *J Appl Physiol* **28,** 642–645.
835 Ramanathan NL (1964). A new weighting system for mean surface temperature
836 of the human body. *J Appl Physiol* **19,** 531–533.
837 Rasmussen P, Nybo L, Volianitis S, M\oller K, Secher NH & Gjedde A (2010).
838 Cerebral oxygenation is reduced during hyperthermic exercise in humans.
839 *Acta Physiol* **199,** 63–70.
840 Remensnyder JP, Mitchell JH & Sarnoff SJ (1962). Functional sympatholysis
841 during muscular activity. Observations on influence of carotid sinus on
842 oxygen uptake. *Circ Res* **11,** 370–380.
843 Rosenmeier JB, Hansen J & González-Alonso J (2004). Circulating {ATP-
844 induced} vasodilatation overrides sympathetic vasoconstrictor activity in
845 human skeletal muscle. *J Physiol* **558,** 351–365.
846 Ross EZ, Cotter JD, Wilson L, Fan J-L, Lucas SJE & Ainslie PN (2012).
847 Cerebrovascular and corticomotor function during progressive passive
848 hyperthermia in humans. *J Appl Physiol* **112,** 748–758.
849 Rowell LB (1974). Human cardiovascular adjustments to exercise and thermal
850 stress. *Physiol Rev* **54,** 75–159.
851 Rowell LB (1986). Circulatory adjustments to dynamic exercise and heat stress:
852 competing controls. In *Human Circulation: Regulation during Physical*
853 *Stress*, pp. 363–406. Oxford University Press, New York.
854 Rowell LB, Brengelmann GL, Blackmon JR, Twiss RD & Kusumi F (1968).
855 Splanchnic blood flow and metabolism in heat-stressed man. *J Appl*
856 *Physiol* **24,** 475–484.
857 Rowell LB, Marx HJ, Bruce RA, Conn RD & Kusumi F (1966). Reductions in
858 cardiac output, central blood volume, and stroke volume with thermal stress
859 in normal men during exercise. *J Clin Invest* **45,** 1801–1816.
860 Rowell LB, Murray JA, Brengelmann GL & Kraning 2nd KK (1969). Human
861 cardiovascular adjustments to rapid changes in skin temperature during
862 exercise. *Circ Res* **24,** 711–724.
863 Saito M, Tsukanaka A, Yanagihara D & Mano T (1993). Muscle sympathetic
864 nerve responses to graded leg cycling. *J Appl Physiol* **75,** 663–667.

865 Saltin B, R\aadegran G, Koskolou MD & Roach RC (1998). Skeletal muscle
866 blood flow in humans and its regulation during exercise. *Acta Physiol*
867 *Scand* **162,** 421–436.
868 Sato K, Ogoh S, Hirasawa A, Oue A & Sadamoto T (2011). The distribution of
869 blood flow in the carotid and vertebral arteries during dynamic exercise in
870 humans. *J Physiol* **589,** 2847–2856.
871 Savard GK, Nielsen B, Laszczynska J, Larsen BE & Saltin B (1988). Muscle
872 blood flow is not reduced in humans during moderate exercise and heat
873 stress. *J Appl Physiol* **64,** 649–657.
874 Sawka MN (2012). Career perspectives of Michael N. Sawka. *Extrem Physiol*
875 *Med* **1,** 10.
876 Sawka MN, Cheuvront SN & Kenefick RW (2012). High skin temperature and
877 hypohydration impair aerobic performance. *Exp Physiol* **97,** 327–332.
878 Sawka MN, Leon LR, Montain SJ & Sonna LA (2011). Integrated physiological
879 mechanisms of exercise performance, adaptation, and maladaptation to
880 heat stress. *Compr Physiol* **1,** 1883–1928.
881 Sawka MN, Young AJ, Cadarette BS, Levine L & Pandolf KB (1985). Influence
882 of heat stress and acclimation on maximal aerobic power. *Eur J Appl*
883 *Physiol Occup Physiol* **53,** 294–298.
884 Stöhr EJ, González-Alonso J, Pearson J, Low DA, Ali L, Barker H & Shave R
885 (2011*a*). Effects of graded heat stress on global left ventricular function and
886 twist mechanics at rest and during exercise in healthy humans. *Exp Physiol*
887 **96,** 114–124.
888 Stöhr EJ, González-Alonso J & Shave R (2011*b*). Left ventricular mechanical
889 limitations to stroke volume in healthy humans during incremental exercise.
890 *Am J Physiol Hear Circ Physiol* **301,** H478–H487.
891 Subudhi AW, Miramon BR, Granger ME & Roach RC (2009). Frontal and motor
892 cortex oxygenation during maximal exercise in normoxia and hypoxia. *J*
893 *Appl Physiol* **106,** 1153–1158.
894 Subudhi AW, Olin JT, Dimmen AC, Polaner DM, Kayser B & Roach RC (2011).
895 Does cerebral oxygen delivery limit incremental exercise performance? *J*
896 *Appl Physiol* **111,** 1727–1734.

Tatterson AJ, Hahn AG, Martini DT & Febbraio MA (2000). Effects of heat stress on physiological responses and exercise performance in elite cyclists. *J Sci Med Sport* **3,** 186–193.

Todd G, Butler JE, Taylor JL & Gandevia SC (2005). Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol* **563,** 621–631.

Trangmar SJ, Chiesa ST, Llodio I, Garcia B, Kalsi KK, Secher NH & González-Alonso J (2015). Dehydration accelerates reductions in cerebral blood flow during prolonged exercise in the heat without compromising brain metabolism. *Am J Physiol Hear Circ Physiol* **309,** H1598–H1607.

Trangmar SJ, Chiesa ST, Stock CG, Kalsi KK, Secher NH & González-Alonso J (2014). Dehydration affects cerebral blood flow but not its metabolic rate for oxygen during maximal exercise in trained humans. *J Physiol* **592,** 3143–3160.

Vogiatzis I, Athanasopoulos D, Habazettl H, Kuebler WM, Wagner H, Roussos C, Wagner PD & Zakynthinos S (2009). Intercostal muscle blood flow limitation in athletes during maximal exercise. *J Physiol* **587,** 3665–3677.

Vogiatzis I, Louvaris Z, Habazettl H, Athanasopoulos D, Andrianopoulos V, Cherouveim E, Wagner H, Roussos C, Wagner PD & Zakynthinos S (2011). Frontal cerebral cortex blood flow, oxygen delivery and oxygenation during normoxic and hypoxic exercise in athletes. *J Physiol* **589,** 4027–4039.

Wesseling KH, Jansen JR, Settels JJ & Schreuder JJ (1993). Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol* **74,** 2566–2573.

Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA & Ainslie PN (2012). Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol* **590,** 3261–3275.

Additional information

Competing interests

All authors ascertain no conflict of interests associated with this work.

Author contributions

Experiments were performed at the Centre for Human Performance, Exercise and Rehabilitation, Brunel University London. S.J.T & J.G.A. were involved in the conception and design of the experiment. All authors were involved in data collection, analysis and interpretation of data. S.J.T drafted the article and it was critically revised for important intellectual content by S.T.C., K.K.K., C.G.S., M. R., N.H.S. & J.G.A. All authors qualify as authors, are accountable for the integrity of the data collected and approved the final version of the manuscript.

Acknowledgements

The authors would like to thank all participants for their time, effort and commitment, without which this study would not have been possible. Additional thanks are reserved to Eurico Wilhelm, Christopher Stock, Thomas Topham, Omar Labarta and Drs. Andrew Simpson and Raúl López-Grueso for their technical assistance throughout the project.

Funding

This study was supported by a grant from the Gatorade Sports Science Institute, PepsiCo Inc, USA. The views contained within this document are those of the authors and do not necessarily reflect those of PepsiCo Inc.

Present addresses

Steven J Trangmar, Department of Life Sciences, University of Roehampton, Whitelands College, Holybourne Avenue, London, SW15 4JD; **Scott T Chiesa,** Institute of Cardiovascular Science, University College London, Gower Street, London, WC1E 6BT; **Kameljit K Kalsi,** St George's University London, Cranmer Terrace, Tooting, London, SW17 0RE.

Tables

Table 1. Cardiorespiratory responses to incremental exercise with different grades of hyperthermia. Values are means±SEM for 9 subjects. Data presented are from the dehydration trial only. * different from rest $P < 0.05$, # different from moderate hyperthermia, ‡ mild hyperthermia, † different from control. Presented symbols denote differences between conditions at the same relative percentage of $W_{max.}$

Table 2. Haematological responses to incremental exercise with different grades of hyperthermia. Values are means±SEM for 9 subjects. Dehydration and rehydration exercise trials are represented. * different from rest $P < 0.05$, ‡ mild hyperthermia, † different from control. Presented symbols denote differences between conditions at the same relative percentage of $W_{max.}$

Table 3. Haematological responses to incremental exercise with different grades of hyperthermia. Values are means±SEM for 9 subjects. Dehydration and rehydration exercise trials are represented. * different from rest $P < 0.05$, ‡ mild hyperthermia, † different from control. Presented symbols denote differences between conditions at the same relative percentage of $W_{max.}$

Table 1. Cardiorespiratory responses to incremental exercise with different grades of hyperthermia

% of W_{max}	SBP (mmHg)	DBP (mmHg)	r*f* (breaths/min)	$\dot{V}CO_2$ (l min^{-1})	$\dot{V}E$ (l min^{-1})	$PetO_2$ (mmHg)	$PetCO_2$ (mmHg)
HYP_{mod}							
Rest	138 ± 5†	73 ± 2	18 ± 1	464 ± 46	17 ± 2	111 ± 3	33 ± 2
20	157 ± 7*	72 ± 3‡†	25 ± 2*	1353 ± 74*	39 ± 2*	103 ± 2*	38 ± 2*
40	165 ± 10*‡†	73 ± 3‡†	29 ± 2*	1990 ± 81*	55 ± 2*	102 ± 1*	40 ± 1*
60	179 ± 12*	74 ± 3†	32 ± 2*	2779 ± 72*‡	77 ± 3*‡	106 ± 1	40 ± 1*
80	192 ± 11*‡†	79 ± 3‡†	41 ± 2*	3684±110*‡†	110 ± 5*‡†	111 ± 2	38 ± 1*
100	211 ± 9*‡†	84 ± 3‡†	51 ± 3*	4422 ± 83*‡†	148 ± 7*‡†	115 ± 1*	34 ± 1
HYP_{mild}							
Rest	135 ± 5*†	72 ± 4†	16 ± 2	392 ± 19	14 ± 1	108 ± 2	34 ± 1
20	162 ± 6*†	76 ± 4*†	25 ± 2*	1138 ±744*	39 ± 2*	100 ± 2*	39 ± 1*
40	182 ± 8*	80 ± 3*	29 ± 2*	2124 ± 73*	58 ± 2*	102 ± 1*	40 ± 1*
60	196 ± 12*	81 ± 5*†	34 ± 2*	3052 ± 92*†	86 ± 3*†	106 ± 1	40 ± 1*
80	211 ± 11*	86 ± 5*	41 ± 2*†	4102 ± 103*†	126 ± 4*†	113 ± 1	36 ± 1*
100	229 ± 11*	96 ± 6*	52 ± 3*	4733 ± 158*	161 ± 7*	116 ± 1*	34 ± 1
Control							
Rest	155 ± 6‡	83 ± 4	17 ± 2	404 ± 27	14 ± 1	108 ± 3	33 ± 1
20	180 ± 4*‡	85 ± 3	26 ± 2*	1332 ± 75*	39 ± 2*	99 ± 1*	38 ± 1*
40	200 ± 7*	87 ± 3	29 ± 2*	2058 ± 80*	57 ± 2*	102 ± 2*	40 ± 1*
60	217 ± 7*	91 ± 3*	32 ± 2*	2878 ± 84*	79 ± 3*	104 ± 2	40 ± 1*
80	227 ± 7*	92 ± 4*	39 ± 2*	3882 ± 114*	116 ± 6*	110 ± 2	38 ± 1*
100	245 ± 8*	100 ± 5*	52 ± 3*	4729 ± 124*	165 ± 7*	117 ± 1*	33 ± 1

Values are mean±SEM for 9 participants. Heart rates (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), respiratory frequency (r*f*), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), end-tidal oxygen ($PetO_2$) and carbon dioxide tension ($PetCO_2$). * different *vs.* rest $P < 0.05$**,** ‡ different *vs.* mild hyperthermia, † different *vs.* control. Presented symbols denote differences between conditions at the same relative percentage of $W_{max.}$

Table 2. Blood gases and metabolite responses to incremental exercise with different grades of hyperthermia

	pH		Hb ($g\ l^{-1}$)		SO_2 (%)		PO_2 (mmHg)		PCO_2 (mmHg)	
% of W_{max}	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous
HYP_{mod}										
Rest	7.46 ± 0.01‡†	7.44 ± 0.01‡†	148 ± 3‡†	151 ± 3‡†	97.5 ± 0.3	85.1 ± 1.3‡†	94.5 ± 2.9	51.8 ± 1.3‡†	38.2 ± 1.3	42.1 ± 1.8
20	7.47 ± 0.01‡†	7.39 ± 0.01*‡†	154 ± 3*‡†	157 ± 3*‡†	98.0 ± 0.2	36.3 ± 1.2*	100.3 ± 2.8	24.1 ± 0.6*‡†	36.2 ± 1.8*	53.7 ± 2.8*
40	7.45 ± 0.01*‡†	7.35 ± 0.01*	154 ± 3*‡†	157 ± 3*‡†	97.8 ± 0.2	25.9 ± 2.0*	99.4 ± 3.1	21.1 ± 0.8*	37.0 ± 1.5‡	60.3 ± 2.7*
60	7.42 ± 0.01*†	7.31 ± 0.01*	155 ± 3*‡†	158 ± 3*‡†	97.5 ± 0.2*	21.0 ± 2.3*	99.3 ± 2.2	20.2 ± 1.1*	38.1 ± 1.2	65.9 ± 2.1*
80	7.40 ± 0.01*‡†	7.26 ± 0.01*‡	156 ± 3*	159 ± 4*‡†	97.2 ± 0.2*	16.8 ± 1.9*‡	98.2 ± 2.9	18.9 ± 1.1*	36.2 ± 0.9	72.1 ± 1.9*
100	7.36 ± 0.01*‡†	7.19 ± 0.01*‡	157 ± 3*	161 ± 3*‡†	96.7 ± 0.2*	11.6 ± 1.3*	100.3 ± 2.4	17.8 ± 1.1*	33.7 ± 1.0*	78.1 ± 2.1*
HYP_{mild}										
Rest	7.44 ± 0.01	7.41 ± 0.01	141 ± 2†	143 ± 3	97.9 ± 0.1	71.5 ± 2.1	95.7 ± 2.2	38.2 ± 1.2	38.2 ± 1.0	44.3 ± 1.2
20	7.44 ± 0.01	7.38 ± 0.01*	147 ± 3*†	149 ± 3*†	97.8 ± 0.1	32.4 ± 1.6*	95.1 ± 2.0	21.8 ± 0.5*	37.7 ± 1.1	52.5 ± 1.6*
40	7.42 ± 0.01*	7.33 ± 0.01*	148 ± 3*†	150 ± 3*†	97.6 ± 0.2	23.4 ± 1.2*	95.9 ± 1.8	20.0 ± 0.5*	39.3 ± 0.9	61.4 ± 1.5*
60	7.41 ± 0.00*	7.29 ± 0.00*	150 ± 3*†	153 ± 3*†	97.3 ± 0.1*	18.0 ± 1.2*	96.0 ± 1.7	18.6 ± 0.6*	38.6 ± 1.0	67.6 ± 1.2*
80	7.38 ± 0.01*	7.23 ± 0.01*†	153 ± 3*	155 ± 3*	97.1 ± 0.2*	13.9 ± 1.4*†	97.3 ± 2.0	17.8 ± 1.0*	36.6 ± 1.2†	74.0 ± 1.5*
100	7.32 ± 0.01*†	7.15 ± 0.01*†	156 ± 3*†	152 ± 3*	96.2 ± 0.3*	11.1 ± 1.2*	99.2 ± 2.5	17.9 ± 1.2*	33.3 ± 1.1*	79.0 ± 2.7*
Control										
Rest	7.44 ± 0.01	7.41 ± 0.01	138 ± 3	140 ± 3	97.9 ± 0.1	66.6 ± 3.3	95.8 ± 1.6	36.4 ± 1.9	37.4 ± 1.0	43.9 ± 1.6
20	7.44 ± 0.01	7.39 ± 0.01*	145 ± 3*	146 ± 3*	97.7 ± 0.2	32.3 ± 1.2*	93.5 ± 2.1	21.7 ± 0.3*	37.2 ± 1.0	50.2 ± 1.5*
40	7.42 ± 0.00*	7.34 ± 0.01*	146 ± 3*	147 ± 3*	97.7 ± 0.2	23.0 ± 1.6*	97.5 ± 2.4	19.6 ± 0.6*	38.7 ± 1.0	59.5 ± 1.5*
60	7.40 ± 0.00*	7.29 ± 0.00*	148 ± 3*	151 ± 3*	97.2 ± 0.2*	19.7 ± 1.6*	95.2 ± 1.8	19.2 ± 0.8*	39.4 ± 1.0*	66.0 ± 1.4*
80	7.38 ± 0.01*	7.24 ± 0.01*	150 ± 3*	151 ± 3*	96.8 ± 0.2*	15.5 ± 1.5*	95.6 ± 2.5	18.6 ± 1.1*	38.3 ± 1.4	71.4 ± 1.6*
100	7.33 ± 0.01*	7.17 ± 0.01*	153 ± 3*	153 ± 3*	96.3 ± 0.3*	12.0 ± 1.2*	98.8 ± 2.6	17.9 ± 1.1*	34.3 ± 1.3*	76.7 ± 2.2*

Values are mean±SEM for 9 participants. pH, Haemoglobin (Hb), oxygen saturation (SO_2%), partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2) for arterial and femoral venous blood. * different *vs.* rest, ‡ different *vs.* mild hyperthermia, † different *vs.* control (all $P < 0.05$). Presented symbols denote differences between conditions at the same relative percentage of W_{max}.

Table 3. Blood gases and metabolite responses to incremental exercise with different grades of hyperthermia

	CtO_2 (ml l^{-1})		[Lac] (mmol l^{-1})		[Glu] (mmol l^{-1})		$[HCO_3^-]$ (mmHg)		ABE (mmol l^{-1})	
% of W_{max}	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous
HYP_{mod}										
Rest	199 ± 4‡†	176 ± 3‡†	1.0 ± 0.1	1.1 ± 0.1	5.8 ± 0.1	5.8 ± 0.2	27.3 ± 0.3‡†	27.7 ± 0.3‡†	3.0 ± 0.4‡†	4.1 ± 0.5†
20	209 ± 4*‡†	79 ± 2*‡†	1.7 ± 0.2*	2.1 ± 0.3*	5.9 ± 0.2	5.8 ± 0.3	26.6 ± 0.3*†	27.5 ± 0.4†	2.0 ± 0.5*†	6.5 ± 0.5*†
40	208 ± 4*‡†	56 ± 4*	2.2 ± 0.3*	2.4 ± 0.4*	6.0 ± 0.2	6.0 ± 0.3	26.1 ± 0.5*†	27.0 ± 0.5*	1.5 ± 0.6*	6.8 ± 0.6*†
60	209 ± 4*‡†	46 ± 5*	3.0 ± 0.4*	3.3 ± 0.5*	6.1 ± 0.2‡†	6.0 ± 0.3‡†	25.2 ± 0.5*†	26.0 ± 0.6*†	0.5 ± 0.7*	6.1 ± 0.8*†
80	210 ± 4*	37 ± 4*	4.8 ± 0.5*	5.4 ± 0.5*	6.1 ± 0.3‡†	6.0 ± 0.3‡†	23.3 ± 0.5*‡†	23.8 ± 0.6*‡†	-1.9 ± 0.7*‡	4.2 ± 0.8‡†
100	210 ± 4*	26 ± 3*	8.6 ± 0.6*	9.7 ± 0.5*	6.3 ± 0.3*‡†	6.3 ± 0.3*‡†	20.1 ± 0.6*‡†	20.3 ± 0.5*‡†	-6.1 ± 0.8*‡†	0.4 ± 0.7*
HYP_{mild}										
Rest	191 ± 3†	140 ± 6	1.3 ± 0.2	1.5 ± 0.1	5.9 ± 0.2	5.7 ± 0.3	26.0 ± 0.3†	26.4 ± 0.3	1.7 ± 0.4†	3.4 ± 0.4
20	198 ± 4*†	67 ± 4*	1.5 ± 0.2*	1.6 ± 0.2	5.9 ± 0.3	5.8 ± 0.3	26.0 ± 0.3	26.7 ± 0.3	1.6 ± 0.4	5.6 ± 0.4*
40	199 ± 4*†	49 ± 2*	1.7 ± 0.2*	2.0 ± 0.3*	5.7 ± 0.3*	5.6 ± 0.3	25.7 ± 0.3*	26.3 ± 0.3	1.3 ± 0.4*	6.0 ± 0.5*
60	202 ± 4*†	38 ± 3*	2.8 ± 0.3*	3.3 ± 0.4*	5.4 ± 0.2	5.3 ± 0.2	24.7 ± 0.3*	25.2 ± 0.4*	-0.1 ± 0.5*	5.3 ± 0.5*
80	205 ± 4*	30 ± 3*	5.7 ± 0.6*	6.3 ± 0.7*	5.3 ± 0.2*	5.2 ± 0.3	22.2 ± 0.5*†	22.5 ± 0.5*	-3.1 ± 0.7*†	2.7 ± 0.7
100	208 ± 4*	23 ± 2*	10.5 ± 0.8*	11.0 ± 0.8*	5.3 ± 0.3	5.2 ± 0.3	18.3 ± 0.5*†	18.8 ± 0.5*	-8.3 ± 0.7*†	-1.8 ± 0.8*
Control										
Rest	187 ± 4	129 ± 9	1.4 ± 0.2	1.7 ± 0.2	6.1 ± 0.2	5.9 ± 0.1	25.5 ± 0.2	26.0 ± 0.3	1.0 ± 0.3	3.0 ± 0.5
20	195 ± 4*	65 ± 3*	1.6 ± 0.2	1.7 ± 0.2	6.0 ± 0.1*	6.1 ± 0.1	25.5 ± 0.3	26.4 ± 0.3	1.0 ± 0.4	4.9 ± 0.5*
40	197 ± 4*	47 ± 4*	1.7 ± 0.3	2.0 ± 0.3	5.9 ± 0.1	5.9 ± 0.2	25.4 ± 0.3	26.0 ± 0.4	0.9 ± 0.5	5.4 ± 0.6*
60	198 ± 4*	41 ± 4*	2.6 ± 0.3*	3.2 ± 0.4*	5.6 ± 0.2*	5.5 ± 0.2*	24.5 ± 0.4*	24.9 ± 0.5*	-0.1 ± 0.5*	4.8 ± 0.6*
80	201 ± 4*	33 ± 3*	4.8 ± 0.5*	5.5 ± 0.6*	5.3 ± 0.2*	5.2 ± 0.3*	22.7 ± 0.5*	22.8 ± 0.6*	-2.4± 0.8*	2.7 ± 0.8
100	203 ± 4*	26 ± 3*	9.3 ± 0.6*	10.3 ± 0.9*	5.2 ± 0.3*	5.1 ± 0.3*	19.1 ± 0.5*	19.4 ± 0.6*	-7.2± 0.8*	-0.8 ± 0.8*

Values are mean±SEM for 9 participants. Oxygen content (CtO_2), Lactate concentration ([Lac]), Glucose concentration ([Glu]), sodium bicarbonate concentration ([HCO3$^-$])) and acid-base excess (ABE) for arterial and femoral venous blood. * different *vs.* rest $P < 0.05$, ‡ different *vs.* mild hyperthermia, † different *vs.* control. Presented symbols denote differences between conditions at the same relative percentage of $W_{max.}$

Figure legends

Figure 1. Sequence of the exercise protocols. Participants visited the laboratory on two occasions, with each trial consisting of 3 incremental cycling exercise tests at intensities relative to $\dot{V}O_{2ma}$. As HYP_{mod} reduced $\dot{V}O_{2max}$ (obtained on the preliminary trial), the absolute work rates of each stage were lower than all other incremental tests (321 ± 9 W *vs.* 371 ± 11 W). This adjustment allowed for comparisons between incremental tests, relative to $\dot{V}O_{2max}$, in either HYP_{mod} or HYP_{mild}/control, where the latter two conditions did not reduce $\dot{V}O_{2max}$. Passive heating/matched rest durations prior to exercise in HYP_{mod} and HYP_{mild} were 52 ± 3 and 13 ± 1 min respectively. A minimum of 1 h passive rest separated each incremental exercise bout.

Figure 2. Brain and systemic haemodynamics, and systemic oxygen uptake in response to three incremental exercise bouts on the control trial. Values are means±SEM for 7 participants. Variables in figure 2B, C and D increased with exercise intensity ($P < 0.01$).

Figure 3. Temperature responses to incremental exercise with different grades of hyperthermia. Femoral venous blood (*A*) and mean skin (*B*) temperatures are reported. Values are means±SEM for 9 participants. Moderate (internal and skin), mild (skin only) hyperthermia and control exercise are represented. * different *vs.* rest $P < 0.05$, ‡ different *vs.* mild hyperthermia, † different *vs.* control. Presented symbols denote differences between conditions at the same relative percentage of $W_{max.}$

Figure 4. Two-legged and brain haemodynamics, blood pressures and limb and systemic oxygen uptake in response to exercise with different grades of hyperthermia. Values are means±SEM for 9 participants. Variables in all figures (except Fig. 4C) increased with exercise intensity. Limb blood flow (Fig. 4A) increased with exercise intensity to ~80% W_{max} ($P < 0.05$), but plateaued prior to exhaustion. ‡ different *vs.* mild hyperthermia, † different *vs.*

control. Presented symbols denote differences between conditions at the same relative percentage of $W_{max.}$

Figure 5. Brain and two-legged vascular conductances, arterial [NA], P_aCO_2 and femoral venous and arterial plasma ATP in response to incremental exercise with different grades of hyperthermia. Values are means±SEM for 7 participants. ‡ different *vs.* mild hyperthermia, † different *vs.* control. Presented symbols denote differences between conditions at the same relative percentage of W_{max}.

Figure 1

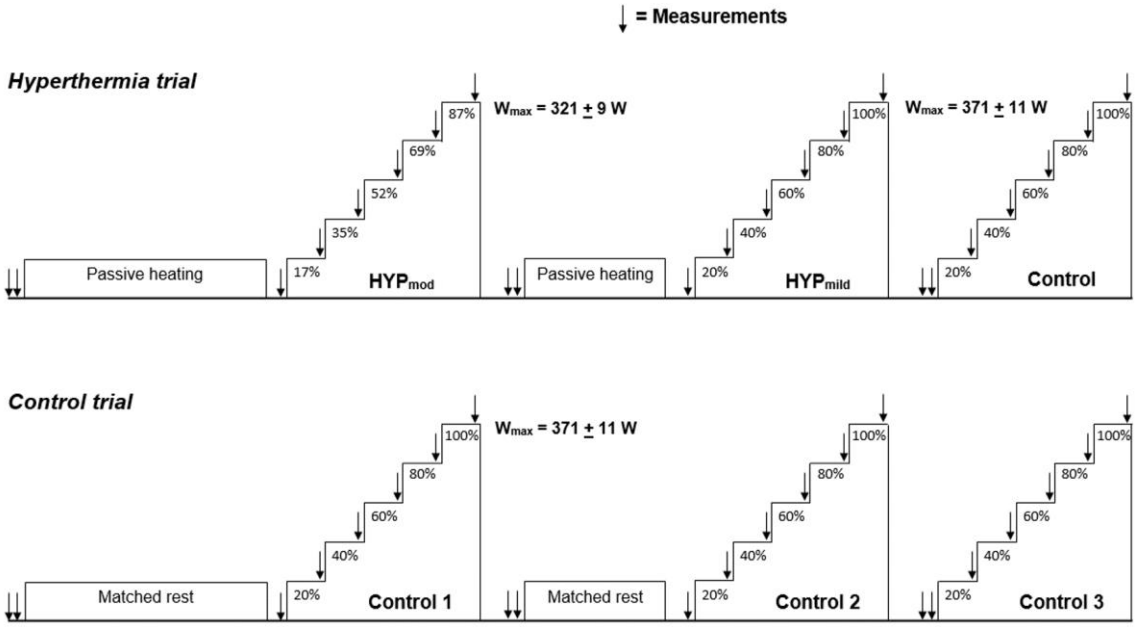

↓ = Measurements
Hyperthermia trial
Passive heating
17%
35%
52%
69%
87%
HYPmod
Wmax = 321 ± 9 W
Passive heating
20%
40%
60%
80%
100%
HYPmild
Wmax = 371 ± 11 W
20%
40%
60%
80%
100%
Control
Control trial
Matched rest
20%
40%
60%
80%
100%
Control 1
Wmax = 371 ± 11 W
Matched rest
20%
40%
60%
80%
100%
Control 2
20%
40%
60%
80%
100%
Control 3

Figure 2

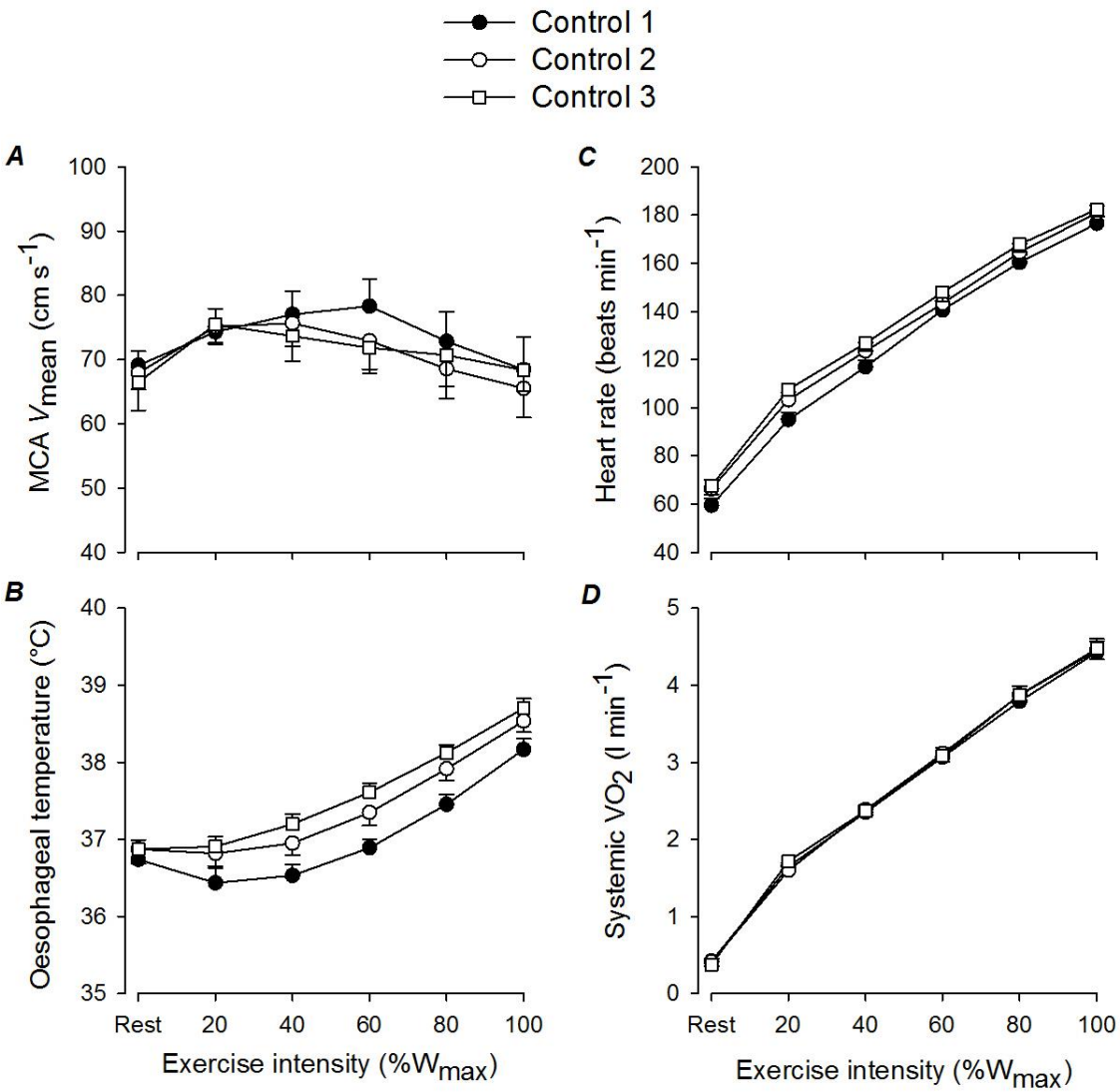

Control 1
Control 2
Control 3
A
MCA V_{mean} (cm s^{-1})
100
90
80
70
60
50
40
C
Heart rate (beats min^{-1})
200
180
160
140
120
100
80
60
40
B
Oesophageal temperature (°C)
40
39
38
37
36
35
D
Systemic VO_2 (l min^{-1})
5
4
3
2
1
0
Rest
20
40
60
80
100
Exercise intensity (%W_{max})
Rest
20
40
60
80
100
Exercise intensity (%W_{max})

Figure 3

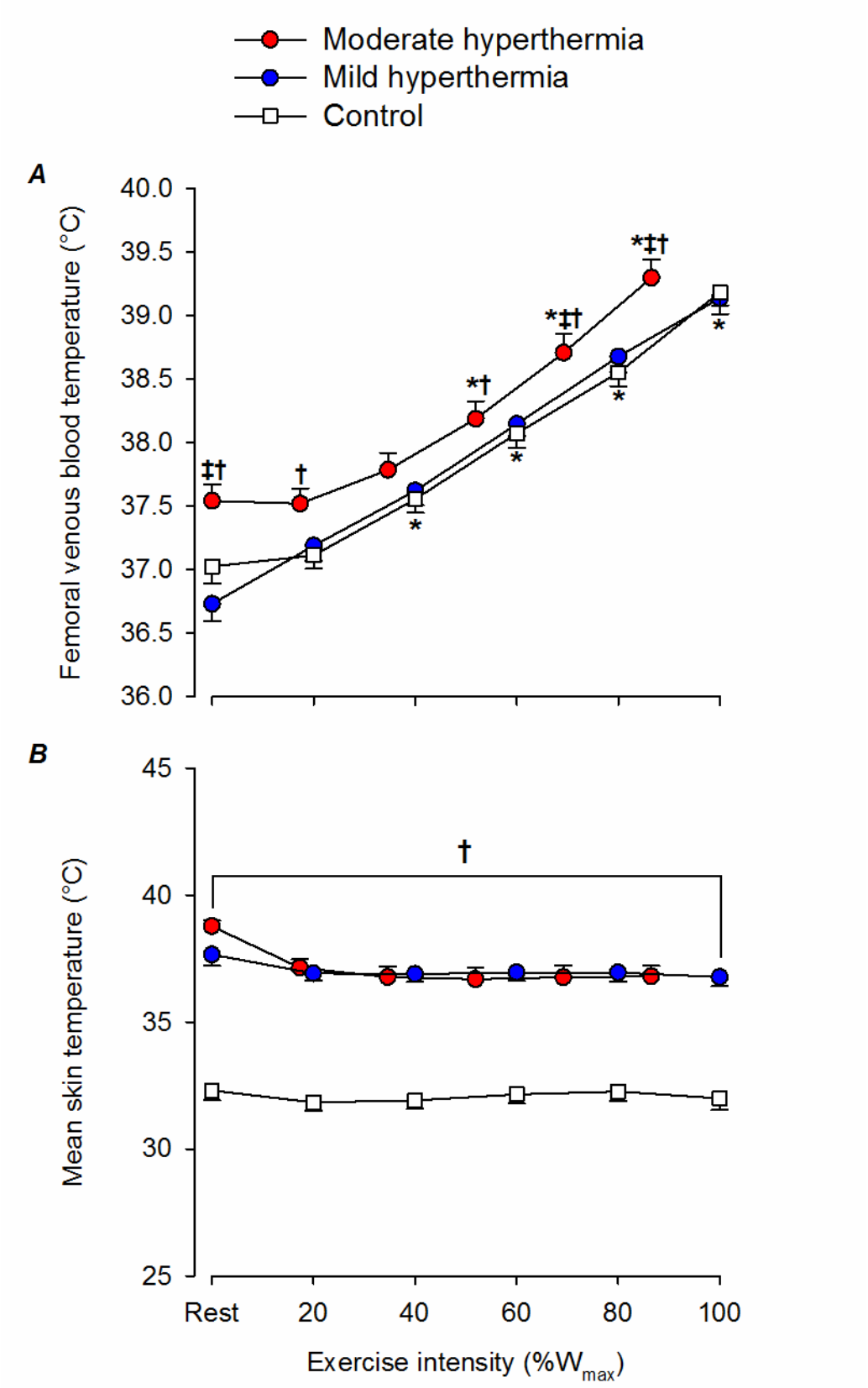

Moderate hyperthermia
Mild hyperthermia
Control
A
Femoral venous blood temperature (°C)
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
‡†
†
*†
*‡†
*‡†
*
*
*
*
B
Mean skin temperature (°C)
45
40
35
30
25
†
Rest
20
40
60
80
100
Exercise intensity (%W$_{max}$)

Figure 4

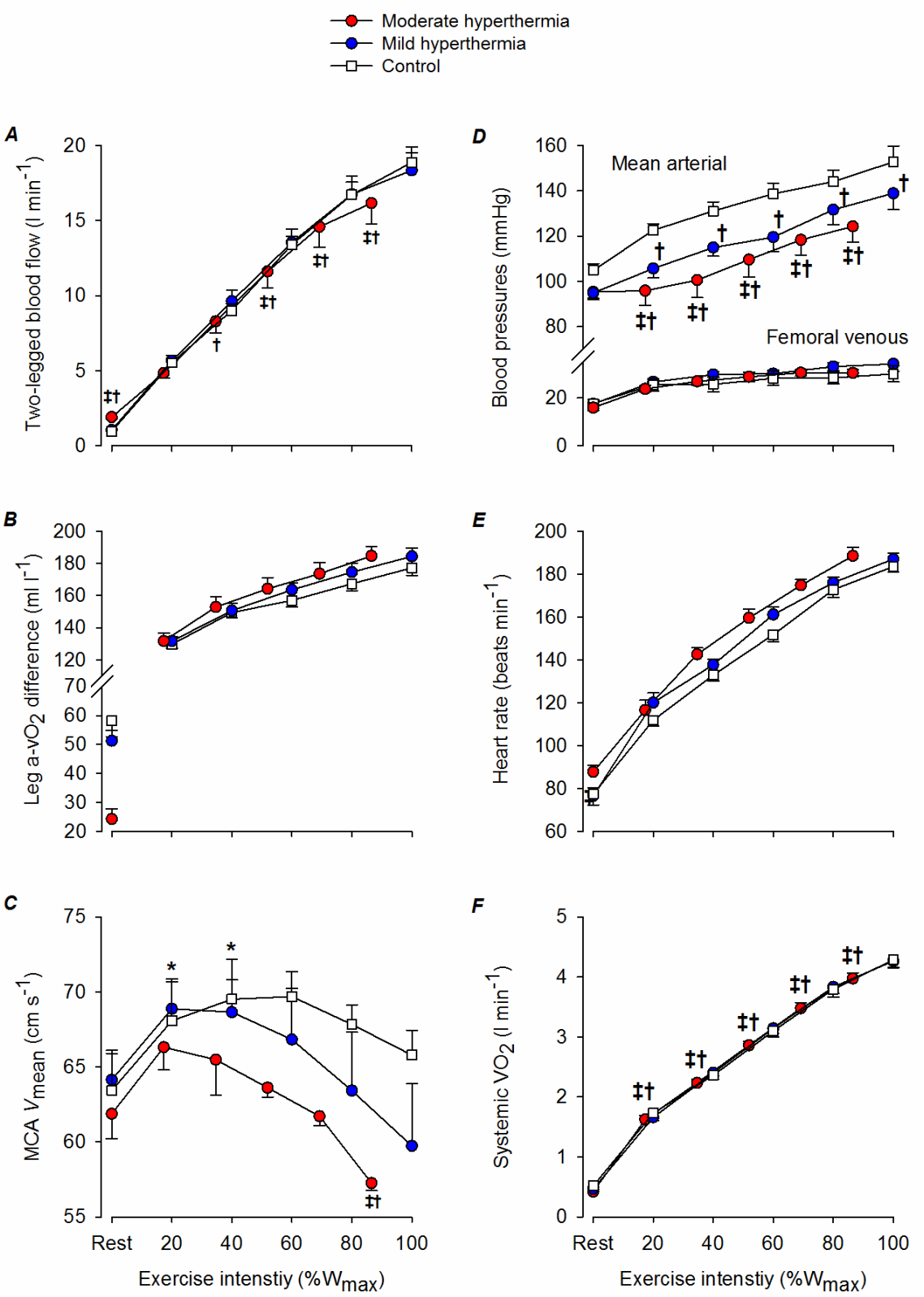
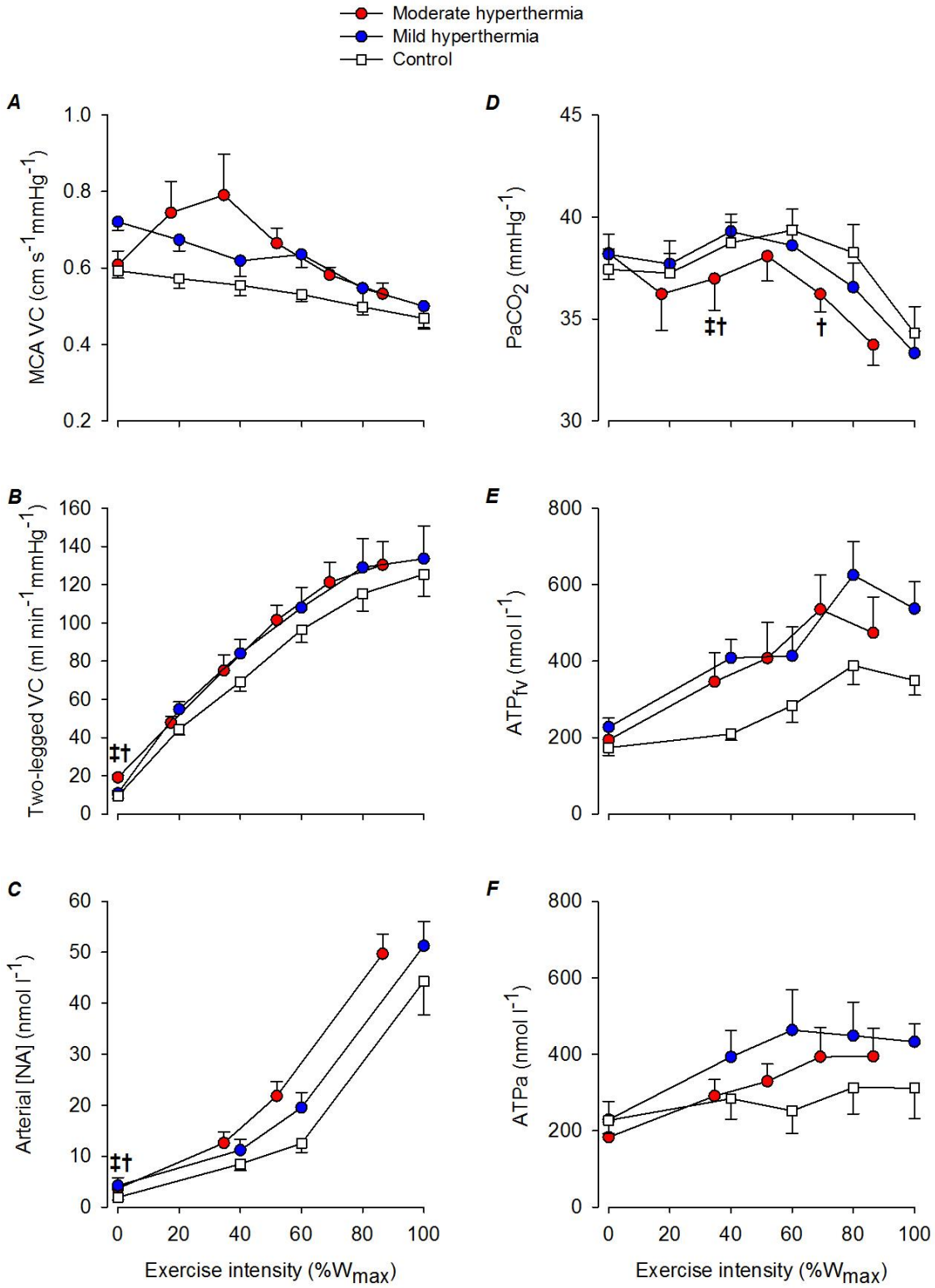

Moderate hyperthermia
Mild hyperthermia
Control
A
Two-legged blood flow (l min-1)
D
Blood pressures (mmHg)
Mean arterial
Femoral venous
B
Leg a-vO2 difference (ml l-1)
E
Heart rate (beats min-1)
C
MCA Vmean (cm s-1)
F
Systemic VO2 (l min-1)
Rest
Exercise intenstiy (%Wmax)

Figure 5


Moderate hyperthermia
Mild hyperthermia
Control
A
MCA VC (cm s-1mmHg-1)
1.0
0.8
0.6
0.4
0.2
D
PaCO2 (mmHg-1)
45
40
35
30
‡†
†
B
Two-legged VC (ml min-1 mmHg-1)
160
140
120
100
80
60
40
20
0
‡†
E
ATPfv (nmol l-1)
800
600
400
200
0
C
Arterial [NA] (nmol l-1)
60
50
40
30
20
10
0
‡†
0
20
40
60
80
100
Exercise intensity (%Wmax)
F
ATPa (nmol l-1)
800
600
400
200
0
0
20
40
60
80
100
Exercise intensity (%Wmax)