

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

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13 **Running title:** Hyperthermia and circulatory strain during maximal exercise

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16 metabolism

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33 **Key points**

- 34 ● Whole-body hyperthermia impairs cardiovascular function and aerobic
35 exercise capacity, but the contribution of skin hyperthermia to the
36 ensuing regional cardiovascular strain is unclear.
- 37 ● Body temperature was manipulated with a water-perfused suit to
38 examine how hyperthermia affects brain and active limb circulations and
39 accelerates fatigue during incremental maximal exercise in humans.
- 40 ● Whole-body hyperthermia, but not skin hyperthermia, accelerated the
41 reductions in brain and active limb perfusion, compromised aerobic
42 metabolism and impaired exercise capacity.
- 43 ● The attenuation in brain and active limb perfusion was associated with
44 increases in sympathetic vasoconstrictor activity, blunted rise in plasma
45 ATP and a fall in the arterial partial pressure of CO₂.
- 46 ● These findings challenge the prevailing notion that skin hyperthermia and
47 hyperperfusion *per se* is the dominant factor in the development of
48 cardiovascular strain and fatigue during exercise in hot environments.

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65 **Abstract**

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

88

89

90 **Abbreviations.** ATP_{fv} femoral venous ATP concentration; $a-vO_{2diff}$,
91 arteriovenous oxygen content difference; CaO_2 , arterial content of oxygen;
92 HR_{max} , maximal heart rate; HYP_{mild} , mild hyperthermia; HYP_{mod} , moderate
93 hyperthermia; $MCA V_{mean}$, middle cerebral artery blood velocity; [NA],
94 noradrenaline; $PaCO_2$, arterial carbon dioxide pressure; \dot{Q} , cardiac output;
95 $rSO_2\%$, regional cerebral oxygen saturation; T_a , ambient temperature; T_B , blood
96 temperature; T_i , internal temperature; T_{sk} , mean skin temperature; T_{Oes} ,
97 oesophageal temperature; $\dot{V}O_{2max}$, maximal aerobic power; W_{max} , maximal work
98 rate.

99 **Introduction**

100 It is well documented that maximal aerobic exercise capacity ($\dot{V}O_{2max}$) is
101 reduced in hot environments inducing whole body hyperthermia (Rowell *et al.*,
102 1966; Pirnay *et al.*, 1970; Rowell, 1974; Galloway & Maughan, 1997; González-
103 Alonso *et al.*, 2008; Sawka *et al.*, 2011). The precise mechanisms underpinning
104 the impaired exercise capacity in the heat remain debated, but may result from
105 the interaction of multiple regulatory processes associated with reduced O₂
106 delivery and modified locomotor muscle and brain metabolism, attainment of
107 high internal and skin temperatures, and altered central nervous system
108 neurotransmitter activity and feedback/reflex mechanisms (González-Alonso *et*
109 *al.*, 2008; Meeusen & Roelands, 2010; Sawka, 2012; Nybo *et al.*, 2014). The
110 contribution of these factors to early fatigue in the heat appear to be task and
111 exercise-intensity dependent (Nybo *et al.*, 2014). There is, however, surprisingly
112 limited information on the cardiovascular adjustments to incremental exercise
113 with differing extents of skin and whole-body hyperthermia.

114

115 Restrictions in active skeletal muscle perfusion may play an important role in
116 the reduced aerobic capacity in hyperthermic conditions. Under control
117 (normothermic) conditions, skeletal muscle O₂ delivery is tightly coupled to the
118 metabolic demand during sub-maximal exercise (Andersen & Saltin, 1985; Delp
119 & Laughlin, 1998; Saltin *et al.*, 1998; González-Alonso *et al.*, 2002; Delp &
120 O'Leary, 2004); regulation that is lost at high intensities as, prior to volitional
121 exhaustion, systemic and active skeletal muscle (in addition to brain and
122 respiratory muscle) blood flow becomes restricted (González-Alonso & Calbet,
123 2003; Mortensen *et al.*, 2005, 2008; Vogiatzis *et al.*, 2009; Calbet *et al.*, 2015).
124 The attenuated limb blood flow per unit of power when approaching maximal
125 exercise intensities occurs concomitantly with enhanced local vasoconstrictor
126 activity and reductions in stroke volume (González-Alonso & Calbet, 2003;
127 Calbet *et al.*, 2007; Mortensen *et al.*, 2008; Stöhr *et al.*, 2011*b*; Munch *et al.*,
128 2014). As a consequence, and in contrast to other important regions of the body
129 such as the brain (Nybo *et al.*, 2002; González-Alonso *et al.*, 2004; Trangmar *et*
130 *al.*, 2014), blunted O₂ delivery may compromise local aerobic metabolism, as

131 maximal skeletal muscle O₂ extraction is achieved during exhaustive exercise
132 (González-Alonso & Calbet, 2003). It remains unknown whether hyperthermia
133 accelerates cardiovascular strain during incremental exercise with similar non-
134 linear cardiovascular dynamics.

135

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

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

164

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

175

176 **Methods**

177 ***Ethical approval***

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

182

183 ***Participants***

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

189

190 ***Experimental design***

191 Participants visited the laboratory on 3 occasions, comprising of a preliminary
192 trial, a hyperthermia trial and a control trial, each separated by one week. The
193 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 °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 ± 3 min of heat exposure), 2) HYP_{mild} (with a high T_{sk}
212 but normal T_c , after 13 ± 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; \leq 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

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

231

232 ***Brain, active limb and resting systemic haemodynamics***

233 Middle cerebral artery velocity (MCA V_{mean}) was measured using a 2 MHz
234 pulsed trans-cranial Doppler ultrasound system (DWL, Sippligen, Germany).
235 The right MCA was insonated through the temporal ultrasound window, distal to
236 the MCA-anterior cerebral artery bifurcation, at a depth of 45-60 mm (Aaslid *et al.*,
237 1982). Regional cerebral (frontal lobe) oxygen saturation (rSO₂%) was also
238 assessed using near-infrared spectroscopy (NIRS; INVOS, Somanetics, Troy,
239 MI, USA).

240

241 During exercise, LBF was determined using the constant-infusion thermodilution
242 method (Andersen & Saltin, 1985; González-Alonso *et al.*, 2000). Resting blood
243 flow (n=4) was obtained using duplex Doppler ultrasonography (Vivid 7,
244 Dimension, GE Healthcare, UK), or calculated from the directly obtained a-
245 vO_{2diff} and estimated leg $\dot{V}O_2$ (n =5) assuming comparable leg $\dot{V}O_2$ values than
246 those measured in 4 participants in this study and previous reports from this
247 laboratory using similar heating protocols (Pearson *et al.*, 2011; Chiesa *et al.*,
248 2015). \dot{Q} at rest was estimated using the Modelflow method (Wesseling *et al.*,
249 1993), from the directly obtained intra-arterial pressure wave forms, corrected
250 for age, height and weight.

251

252 ***Catheter placement and blood sampling***

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

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

263

264 ***Blood variables***

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

274

275 ***Heart rate, blood pressure and body temperatures***

276 Heart rate was obtained by telemetry (Polar Electro, Kempele, Finland). Arterial
277 and femoral venous pressure waveforms were recorded using pressure
278 transducers (Pressure Monitoring Kit, TruWave, Edwards Lifesciences,
279 Germany) zeroed at the level of the right atrium in the mid-axillary line (arterial)
280 and at the level of the tip of the catheter (femoral venous). Pressure waveforms
281 were amplified (BP amp, ADInstruments) and sampled at 1000 Hz using a data
282 acquisition unit (Powerlab 16/30, ADInstruments, Oxfordshire, UK) for offline
283 analysis. For measurements of femoral venous blood temperature (T_B), a
284 thermistor (T204a, PhysiTemp, Clifton, New Jersey, USA) was inserted through
285 the femoral venous catheter and connected to a thermocouple meter (TC-2000,
286 Sable Systems, NV: USA) and routed through the data acquisition system. In
287 the control trial, oesophageal temperature (T_{Oes}) was measured using a
288 thermistor (Physitemp, New England, USA), inserted pernasally into the
289 oesophagus at a depth of $\frac{1}{4}$ standing height. Increases in core temperature

290 during cycling exercise reflect the rise in femoral venous blood temperature, as
291 T_B and T_{Oes} have been shown to be within ~ 0.1 °C (González-Alonso *et al.*,
292 1999). Mean skin temperature (T_{sk}) from four sites (standard weightings of
293 chest, arm, thigh and calf; (Ramanathan, 1964) was obtained using a wireless
294 monitoring system (iButton[®], Maxim Integrated, San José, CA, USA).

295

296 **Calculations**

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

313

314 **Statistics**

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

322

323 **Results**

324 ***Impact of heat stress and repeated incremental exercise on exercise*** 325 ***capacity***

326 On the preliminary visit, heat stress exposure sufficient to induce HYP_{mod}
327 resulted in a reduction in W_{\max} by $\sim 13 \pm 1\%$, despite a similar HR_{max} compared
328 to control. To ensure a comparable percentage of W_{\max} across experimental
329 conditions in the subsequent hyperthermia and control trials, the absolute work
330 rates for the incremental stages in HYP_{mod} were reduced by $13 \pm 1\%$ (64 ± 2 ,
331 128 ± 4 , 193 ± 5 , 257 ± 7 and 321 ± 9 W) compared to all other incremental
332 tests (74 ± 2 , 148 ± 4 , 223 ± 7 , 297 ± 9 and 371 ± 11 W; Fig. 1).

333

334 During the control trial, where exercise capacity across the three incremental
335 tests was the same, $\dot{V}O_{2\max}$ (4.4 ± 0.1 , 4.5 ± 0.2 and 4.5 ± 0.1 l min⁻¹), HR_{max}
336 (177 ± 3 , 181 ± 3 and 182 ± 3 beats min⁻¹), T_{Oes} (38.2 ± 0.1 , 38.6 ± 0.1 and 38.7
337 ± 0.1 °C) and end-exercise MCA V_{mean} (68 ± 5 , 66 ± 5 and 68 ± 3 cm s⁻¹) were
338 not significantly different (Fig. 2). Moreover, the increase in $\dot{V}O_2$ per unit of
339 power was linear from low to maximal exercise intensities in all 3 tests ($9.2 \pm$
340 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
341 similar exercise capacity, body temperatures and cardio-respiratory responses
342 to exercise in the control trial, the following sections focus on the effects of
343 temperature manipulation on whole-body haemodynamics in the hyperthermia
344 trial only.

345

346 ***Temperature and cardiorespiratory responses to heat stress***

347 Resting T_B was elevated in HYP_{mod} compared to HYP_{mild} and control exercise
348 conditions (37.5 ± 0.1 vs. 36.7 ± 0.1 and 37.0 ± 0.1 °C; $P = 0.03$), whereas T_{sk}
349 was elevated in both heat stress conditions compared to control ($\sim 38.2 \pm 0.3$ vs.
350 32.3 ± 0.4 °C; $P < 0.001$: Fig. 3A and B). During incremental exercise in
351 HYP_{mod}, T_B was initially unchanged before increasing to a peak of 39.3 ± 0.1 °C
352 ($P < 0.01$ vs. rest) whereas, in HYP_{mild} and control, T_B increased from rest to
353 W_{\max} (39.1 ± 0.1 °C; $P < 0.001$) and was lower overall compare to HYP_{mod}. T_{sk}

354 was maintained elevated in both heat stress conditions ($\sim 36.9 \pm 0.4$ vs. $32.0 \pm$
355 0.4 °C; $P < 0.001$) and was maintained stable throughout exercise.

356

357 Cardiorespiratory variables are presented in Table 1. Briefly, systolic and
358 diastolic blood pressures were lower in HYP_{mod} compared to HYP_{mild} and control
359 ($P < 0.001$). Respiratory frequency, CO₂ production ($\dot{V}CO_2$) and minute
360 ventilation (\dot{V}_E) increased with exercise intensity and were lower in HYP_{mod}
361 compared to HYP_{mild} and control (both $P < 0.001$). End-tidal PO₂ initially
362 declined before increasing at W_{max} , with the reverse response observed for
363 PCO₂; however, there were no differences between the exercise test conditions
364 ($P = 0.492$).

365

366 ***Brain, active limb and systemic haemodynamics***

367 At baseline, HR was 57 ± 3 beats min⁻¹, two-legged blood flow 0.8 ± 0.1 l min⁻¹,
368 \dot{Q} 5.5 ± 0.4 l min⁻¹ and MCA V_{mean} 64 ± 1 cm s⁻¹ (Fig. 4). At rest following
369 passive heat stress or control, HR (88 ± 3 vs. $\sim 76 \pm 5$ bpm), two-legged blood
370 flow (1.9 ± 0.1 vs. $\sim 1.0 \pm 0.1$ l min⁻¹) and \dot{Q} (8.9 ± 0.7 vs. $\sim 6.9 \pm 0.8$ l min⁻¹)
371 were elevated in HYP_{mod} compared to HYP_{mild} and control (all $P < 0.05$),
372 whereas MCA V_{mean} was not different ($\sim 63 \pm 2$ cm s⁻¹). From rest to sub-
373 maximal exercise, HR and two-legged blood flow increased with exercise
374 intensity in all conditions ($P < 0.05$ vs. rest) and MCA V_{mean} was elevated (Fig.
375 4C; $P < 0.05$). However, overall, two-legged blood flow was lower (Fig. 4A; $P <$
376 0.05) and HR higher, in HYP_{mod} exercise compared to control exercise. At
377 exhaustion, HR increased to similar peak values in HYP_{mod}, HYP_{mild} and control
378 respectively (189 ± 4 , 187 ± 3 and 184 ± 3 beats/min). In all conditions, the rate
379 of rise in two-legged blood flow was attenuated, and MCA V_{mean} was reduced in
380 all exercise conditions. Final two-legged blood flow (16.2 ± 1.3 , 18.4 ± 1.1 and
381 18.9 ± 1.1 l min⁻¹) and MCA V_{mean} (57 ± 1 vs. 66 ± 3 cm s⁻¹) were lower in lower
382 in HYP_{mod} than in HYP_{mild} and control conditions.

383

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

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

390

391 ***Blood pressure, oxygen uptake and brain oxygenation***

392 At rest, MAP and FVP were not different among conditions (Fig. 4D). From rest
393 to maximal exercise, MAP increased in all conditions, but was reduced in
394 HYP_{mod} compared to HYP_{mild} and control, respectively (124 ± 7, 139 ± 7 and 153
395 ± 7 mmHg; *P* < 0.05). Femoral venous pressure increased with exercise
396 intensity but was not different among exercise conditions.

397

398 At rest, leg a-v O_{2diff} was lower in HYP_{mod} compared to HYP_{mild} (24 ± 3 vs. ~56 ±
399 7 ml l⁻¹; *P* < 0.05; Fig. 4B), whereas resting systemic $\dot{V}O_2$ was not different
400 among conditions (0.46 ± 0.03 l min⁻¹; *P* = 0.47-0.84). During incremental
401 exercise, leg a-vO_{2diff} and systemic $\dot{V}O_2$ increased with intensity in all conditions
402 (*P* < 0.05). At exhaustion, leg a-v O_{2diff} was not different among conditions;
403 however, systemic $\dot{V}O_{2max}$ was reduced in HYP_{mod} compared to HYP_{mild} and
404 control exercise conditions (3.94 ± 0.11 vs. 4.23 ± 0.13 and 4.23 ± 0.14 l min⁻¹,
405 respectively; *P* < 0.05). Compared to the 3 maximal incremental tests in the
406 control trial, the rise in systemic $\dot{V}O_2$ per unit of power was identical from 20 to
407 70-80%W_{max} (9.6 ± 0.3 ml min⁻¹ W⁻¹), but became attenuated thereafter (8.2 ±
408 0.6 ml min⁻¹ W⁻¹). At rest, NIRS derived rSO₂% was elevated in HYP_{mod} and
409 HYP_{mild} vs. control conditions (77 ± 2 & 75 ± 3 vs. 67 ± 3%; *P* < 0.05) and
410 remained unchanged across all conditions during incremental exercise, but
411 declined before exhaustion (rSO₂ ~64%; *P* < 0.05).

412

413 ***Brain and active limb conductance, blood gases, plasma catecholamines*** 414 ***and ATP***

415 Arterial and venous haemoglobin [Hb] and arterial oxygen content increased
416 with incremental exercise in all conditions, despite a reduction in arterial oxygen
417 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 $\geq 60\%$ 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 ± 0.8 vs. $\sim 1.9 \pm 0.5$ 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 ($\sim 48 \pm 5$ 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

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

464

465 **Skin hyperthermia does not independently compromise cardiovascular** 466 **capacity or aerobic exercise performance**

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

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

490

491 In contrast, when combined internal and skin hyperthermia was present (i.e.,
492 achieved by extending the exposure to passive whole body heat stress to ~52
493 min, while the exercise duration was not different), $\dot{V}O_{2max}$ was reduced by
494 ~8%; a decline similar to that previously reported (Rowell *et al.*, 1966; Klausen
495 *et al.*, 1967; Pirnay *et al.*, 1970; Sawka *et al.*, 1985; Nybo *et al.*, 2001;
496 Arngrímsson *et al.*, 2004). The reduced aerobic power and work capacity were
497 associated with a diminished arterial pressure, an early attenuation in active
498 limb (and systemic perfusion), an advanced fall in brain blood flow and high
499 internal and skin temperatures (39.3 and 37 °C, respectively) (Fig. 4). Restricted
500 LBF, via a plateau in local vascular conductance, precedes fatigue during
501 incremental (Mortensen *et al.*, 2008) and constant load maximal exercise
502 (González-Alonso & Calbet, 2003); whole body hyperthermia advances this
503 cardiovascular instability and may explain the reduced maximal aerobic power
504 (González-Alonso & Calbet, 2003). Our data demonstrate that the duration of
505 heat exposure is critical to whether or not cardiovascular function is impaired
506 during strenuous exercise in the heat stressed human.

507

508 **Impact of hyperthermia on blood flow and pressure at rest and during** 509 **incremental exercise**

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

514 agreement with the responses to passive heat stress (Barcroft *et al.*, 1947;
515 Rowell *et al.*, 1969; Rowell, 1974; Minson *et al.*, 1998; Crandall *et al.*, 2008;
516 Stöhr *et al.*, 2011a; Pearson *et al.*, 2011; Heinonen *et al.*, 2011; Chiesa *et al.*,
517 2016). Interestingly, brief heat exposure, sufficient to raise T_{sk} to that
518 experienced during combined internal and skin hyperthermia (HYP_{mild}), but
519 without elevations in T_c , led to a small increase in systemic (+ 1.3 l min⁻¹) and
520 limb blood flow (+ 0.25 l min⁻¹) compared to baseline values. During passive
521 whole body heat stress, interspersed by single leg exercise, elevations in
522 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
523 small but significant reductions in MAP have been observed with skin
524 hyperthermia at rest without increases in T_c (Pearson *et al.*, 2011; Stöhr *et al.*,
525 2011a). In a recent study from this laboratory, mild heat stress was also shown
526 to induce small but significant increases in systemic and leg perfusion and HR
527 (e.g. \dot{Q} ; 0.9 l min⁻¹, LBF; 0.2 l min⁻¹; 12 beats min⁻¹), although these alterations
528 occurred concomitant to small increases in T_c (~0.4°C) (Chiesa *et al.*, 2016). It
529 is therefore likely that any increased demand for skin and deep limb tissue
530 blood flow, during passive mild hyperthermia, is met by blood flow redistribution
531 from splanchnic vascular beds and a small increase in \dot{Q} and small reduction in
532 MAP (Rowell *et al.*, 1968; Crandall *et al.*, 2008).

533

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

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

586

587 **Does cardiovascular strain contribute to hyperthermia-induced fatigue?**

588 An important question from the present study is which cardiovascular process
589 underpins the reduced exercise capacity under physiologically stressful
590 environments. Prevailing theory suggests that reduced aerobic capacity during
591 exercise in the heat is due to reductions in active muscle blood flow, secondary
592 to a substantial increase in skin perfusion, and despite active redistribution of
593 blood flow from non-active tissues (Rowell, 1974, 1986). This theory was based
594 on observations that body hyperthermia suppressed \dot{Q} during treadmill running,
595 in un-trained and un-acclimatised individuals, compared to control conditions
596 (Rowell *et al.*, 1966); thus giving rise to the premise that the limited
597 cardiovascular capacity is insufficient to meet the combined demands of heat
598 dissipation (skin perfusion) and active muscle perfusion. Our findings
599 demonstrate that the attenuated rise in systemic $\dot{V}O_2$ (from 9.6 to 8.2 ml min⁻¹
600 W⁻¹) and reduced exercise capacity with combined internal and skin
601 hyperthermia were coupled to an advanced fall in brain blood flow, and an early
602 attenuation in LBF (that is, occurring at a lower absolute work rate); temporal
603 responses that could feasibly result in a compromised local tissue aerobic
604 metabolism when oxygen extraction reaches its upper limits (~ 90% in the 3
605 conditions of this study) (González-Alonso & Calbet, 2003; Mortensen *et al.*,
606 2005, 2008; Calbet *et al.*, 2007). In addition, our estimates of \dot{Q} suggest that
607 systemic blood flow is similar at exhaustion among temperature manipulations;
608 a conclusion supported by findings in trained participants, during constant-load
609 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 O₂ 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 O₂ 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 O₂ 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 O₂ 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 O₂ 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 O₂ delivery, modified locomotor muscle and

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

655

656 **Methodological considerations**

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

671

672 **Conclusion**

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

683

684 **References**

- 685 Aaslid R, Markwalder TM & Nornes H (1982). Noninvasive transcranial Doppler
686 ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg*
687 **57**, 769–774.
- 688 Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF & Dempsey JA
689 (2011). Implications of group {III} and {IV} muscle afferents for high-
690 intensity endurance exercise performance in humans. *J Physiol* **589**, 5299–
691 5309.
- 692 Amann M & Calbet JAL (2008). Convective oxygen transport and fatigue. *J Appl*
693 *Physiol* **104**, 861–870.
- 694 Andersen P & Saltin B (1985). Maximal perfusion of skeletal muscle in man. *J*
695 *Physiol* **366**, 233–249.
- 696 Arngrímsson SA, Petitt DS, Borrani F, Skinner KA & Cureton KJ (2004).
697 Hyperthermia and maximal oxygen uptake in men and women. *Eur J Appl*
698 *Physiol* **92**, 524–532.
- 699 Barcroft H, Bonnar WM & Edholm OG (1947). Reflex vasodilatation in human
700 skeletal muscle in response to heating the body. *J Physiol* **106**, 271–278.
- 701 Blain GM, Mangum TS, Sidhu SK, Weavil JC, Hureau TJ, Jessop JE, Bledsoe
702 AD, Richardson RS & Amann M (2016). Group III/IV muscle afferents limit
703 the intramuscular metabolic perturbation during whole body exercise in
704 humans. *J Physiol*; DOI: 10.1113/JP272283.

705 Calbet JAL, Gonzalez-Alonso J, Helge JW, S'olndergaard H, Munch-Andersen
706 T, Boushel R & Saltin B (2007). Cardiac output and leg and arm blood flow
707 during incremental exercise to exhaustion on the cycle ergometer. *J Appl*
708 *Physiol* **103**, 969–978.

709 Calbet JAL, Losa-Reyna J, Torres-Peralta R, Rasmussen P, Ponce-González
710 JG, Sheel AW, de la Calle-Herrero J, Guadalupe-Grau A, Morales-Alamo
711 D, Fuentes T, Rodríguez-García L, Siebenmann C, Boushel R & Lundby C
712 (2015). Limitations to oxygen transport and utilization during sprint exercise
713 in humans: evidence for a functional reserve in muscle O₂ diffusing
714 capacity. *J Physiol* **593**, 4649–4664.

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

718 Chiesa ST, Trangmar SJ & González-Alonso J (2016). Temperature and blood
719 flow distribution in the human leg during passive heat stress. *J Appl Physiol*
720 **120**, 1047–1058.

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

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

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

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

734 Ely BR, Ely MR, Cheuvront SN, Kenefick RW, Degroot DW & Montain SJ
735 (2009). Evidence against a 40 degrees {C} core temperature threshold for
736 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, Krstrup 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

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

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

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

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

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

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

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

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

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

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

828 Pearson J, Low DA, Stöhr E, Kalsi K, Ali L, Barker H & González-Alonso J
829 (2011). Hemodynamic responses to heat stress in the resting and
830 exercising human leg: insight into the effect of temperature on skeletal
831 muscle blood flow. *Am J Physiol Regul Integr Comp Physiol* **300**, R663–
832 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, Møller 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, Raadegran 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 & Sanna 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 (2011a). 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 (2011b). 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.

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

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

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

906 Trangmar SJ, Chiesa ST, Stock CG, Kalsi KK, Secher NH & González-Alonso J
907 (2014). Dehydration affects cerebral blood flow but not its metabolic rate for
908 oxygen during maximal exercise in trained humans. *J Physiol* **592**, 3143–
909 3160.

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

913 Vogiatzis I, Louvaris Z, Habazettl H, Athanasopoulos D, Andrianopoulos V,
914 Cherouveim E, Wagner H, Roussos C, Wagner PD & Zakynthinos S
915 (2011). Frontal cerebral cortex blood flow, oxygen delivery and oxygenation
916 during normoxic and hypoxic exercise in athletes. *J Physiol* **589**, 4027–
917 4039.

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

921 Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K,
922 Graham J, Lewis NC, Day TA & Ainslie PN (2012). Regional brain blood
923 flow in man during acute changes in arterial blood gases. *J Physiol* **590**,
924 3261–3275.

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927 **Additional information**

928 ***Competing interests***

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

930

931 ***Author contributions***

932 Experiments were performed at the Centre for Human Performance, Exercise
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934 the conception and design of the experiment. All authors were involved in data
935 collection, analysis and interpretation of data. S.J.T drafted the article and it was
936 critically revised for important intellectual content by S.T.C., K.K.K., C.G.S., M.
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958

959 **Tables**

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

966

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

972

973 **Table 3. Haematological responses to incremental exercise with different**
974 **grades of hyperthermia.** Values are means±SEM for 9 subjects. Dehydration
975 and rehydration exercise trials are represented. * different from rest $P < 0.05$, ‡
976 mild hyperthermia, † different from control. Presented symbols denote
977 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)	rf (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 (rf), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$), end-tidal oxygen (PetO₂) and carbon dioxide tension (PetCO₂). * 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

% of W_{max}	pH		Hb (g l ⁻¹)		SO ₂ (%)		PO ₂ (mmHg)		PCO ₂ (mmHg)	
	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 (SO₂%), partial pressures of oxygen (PO₂) and carbon dioxide (PCO₂) 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

% of W_{max}	CtO_2 (ml l ⁻¹)		[Lac] (mmol l ⁻¹)		[Glu] (mmol l ⁻¹)		[HCO ₃] (mmHg)		ABE (mmol l ⁻¹)	
	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 $\dot{V}O_{2\max}$. As HYP_{mod} reduced $\dot{V}O_{2\max}$ (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 $\dot{V}O_{2\max}$, in either HYP_{mod} or HYP_{mild/control}, where the latter two conditions did not reduce $\dot{V}O_{2\max}$. 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 \pm 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 \pm 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 \pm 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 $\sim 80\%$ 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 \pm 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

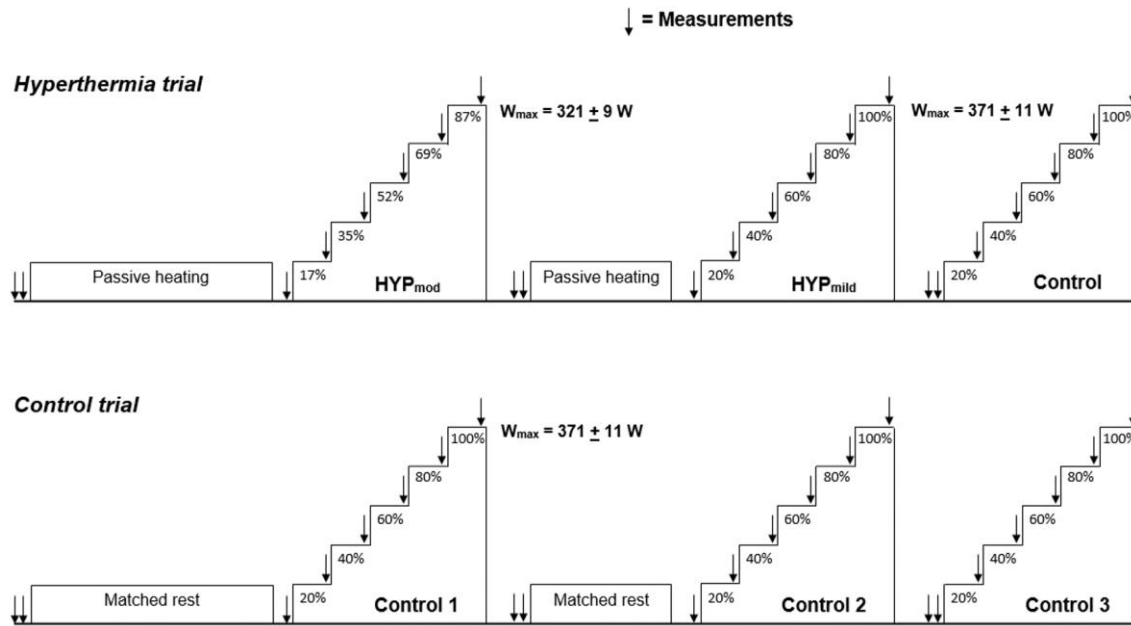


Figure 2

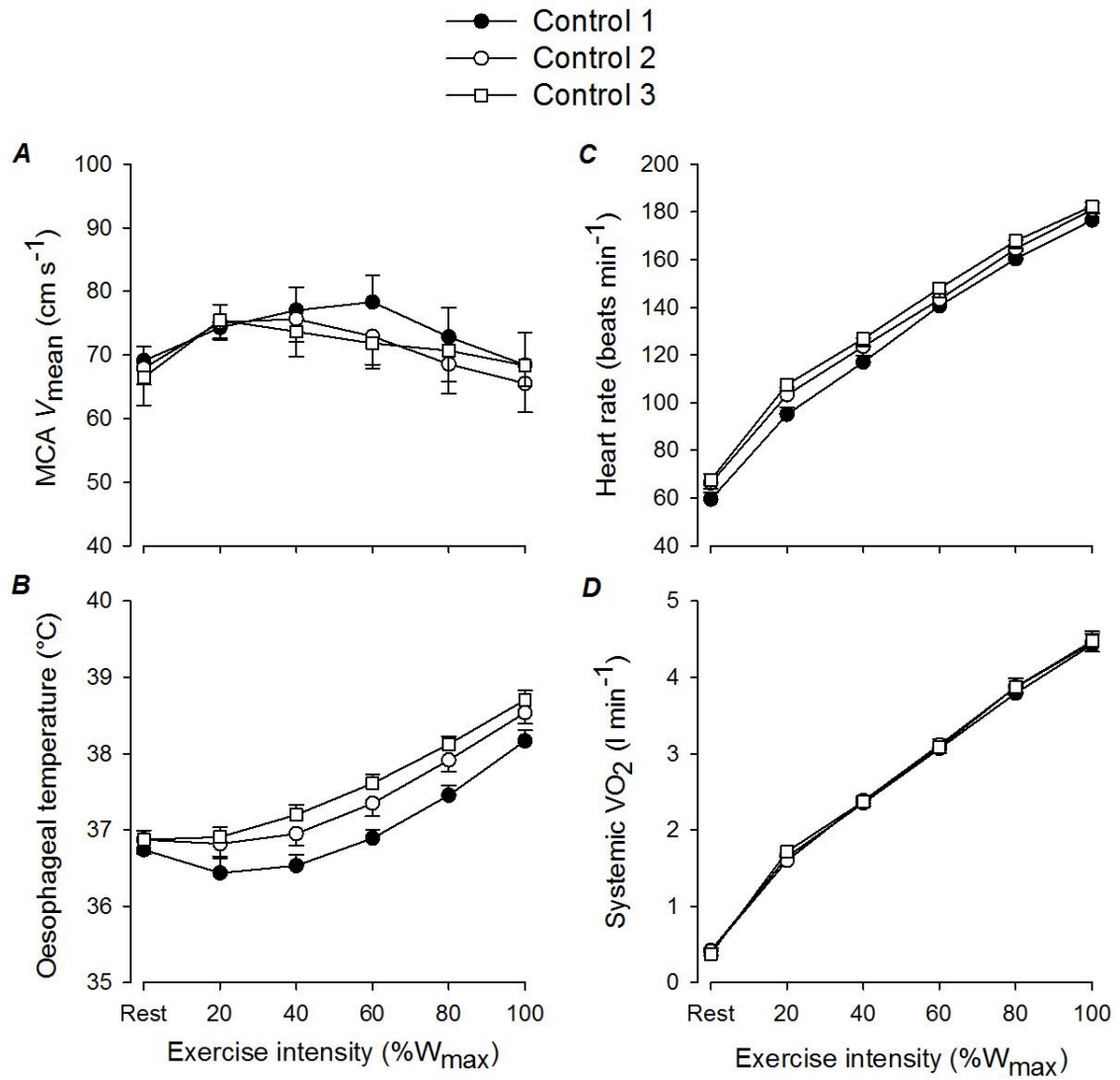


Figure 3

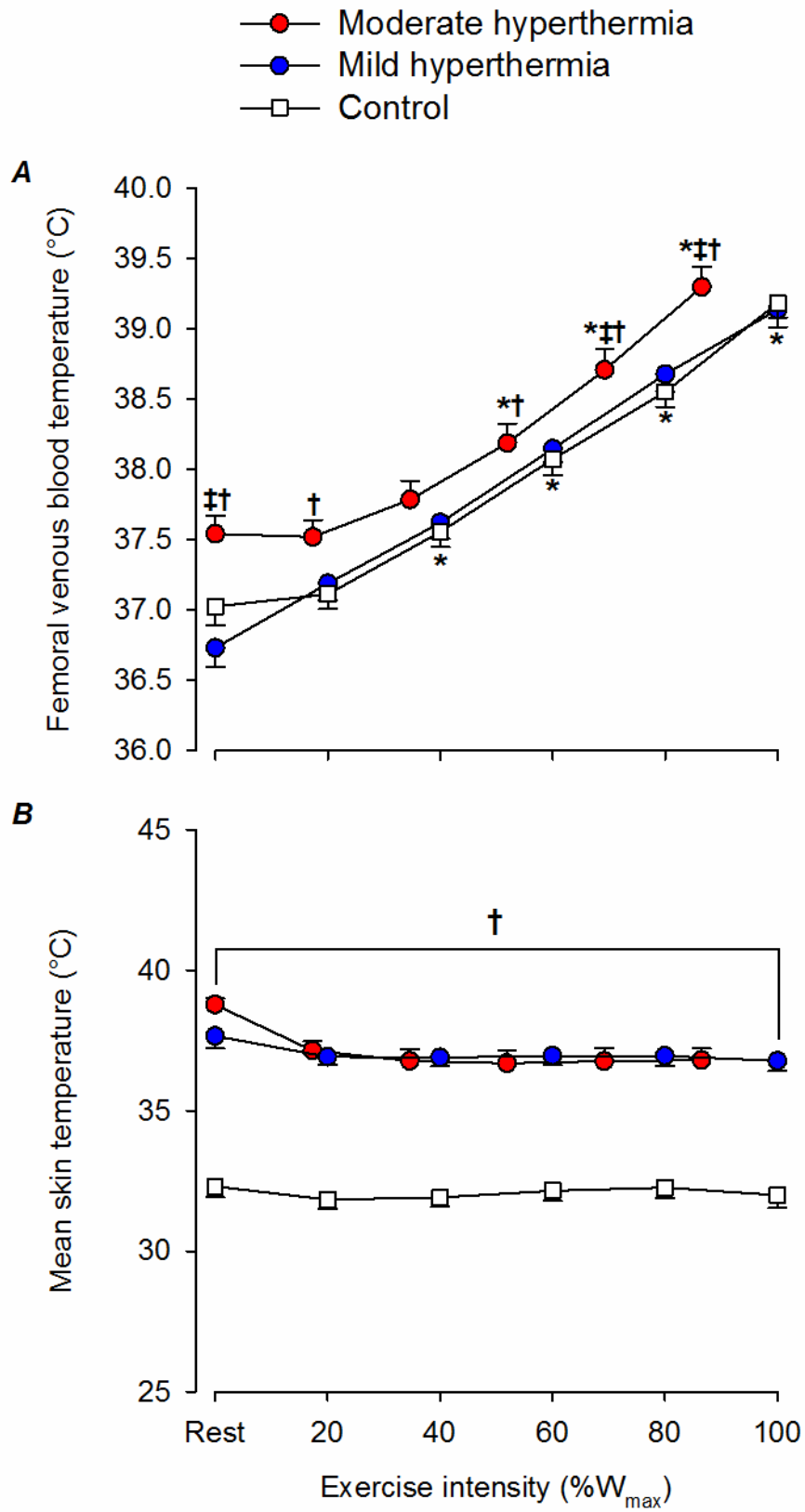


Figure 4

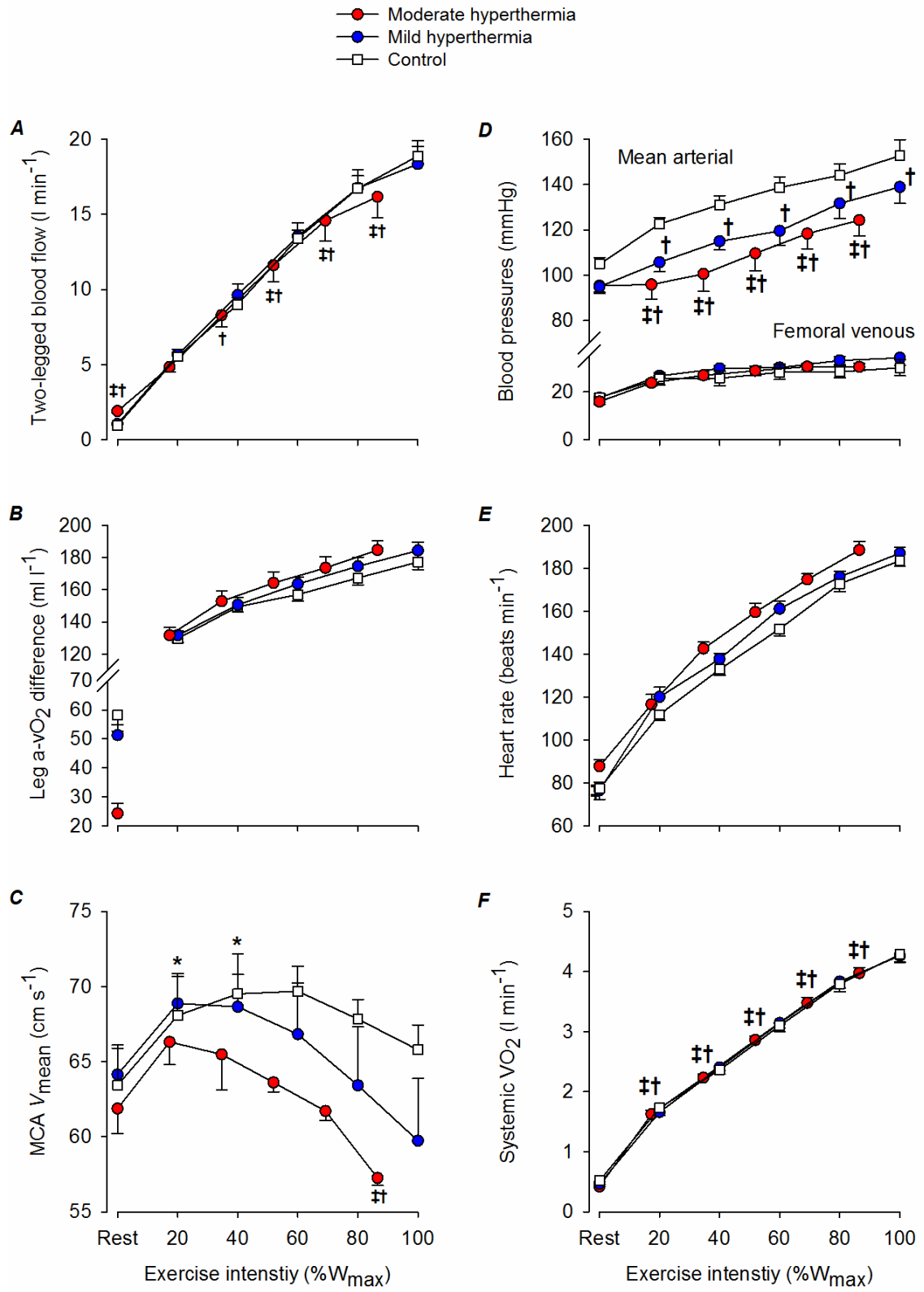


Figure 5

