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Glucocorticoids modulate human brown adipose tissue thermogenesis *in vivo*

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28 **Abstract**

29 *Introduction.* Brown adipose tissue (BAT) is a thermogenic organ with substantial metabolic capacity
30 and has important roles in the maintenance of body weight and metabolism. Regulation of BAT is
31 primarily mediated through the ß-adrenoceptor (ß-AR) pathway. The in vivo endocrine regulation of
32 this pathway in humans is unkown. The objective of our study was to assess the in vivo BAT
33 temperature responses to acute glucocorticoid administration.

34 *Methods.* We studied 8 healthy male volunteers, not pre-selected for BAT presence or activity and
35 without prior BAT cold-activation, on two occasions, following an infusion with hydrocortisone (0.2
36 $mg.kg^{-1}.min^{-1}$ for 14 hours) and saline, respectively. Infusions were given in a randomized double-blind
37 order. They underwent assessment of supraclavicular BAT temperature using infrared thermography
38 following a mixed meal, and during ß-AR stimulation with isoprenaline (25 ng.kg fat-free $mass^{-1}.min^{-1}$
39 for 60 min) in the fasting state.

40 *Results.* During hydrocortisone infusion, BAT temperature increased both under fasting basal
41 conditions and during ß-AR stimulation. We observed a BAT temperature threshold, which was not
42 exceeded despite maximal ß-AR activation. We conclude that BAT thermogenesis is present in humans
43 under near-normal conditions. Glucocorticoids modulate BAT function, representing important
44 physiological endocrine regulation of body temperature at times of acute stress.

45

46 *Keywords:* brown adipose tissue, glucocorticoids, humans, infrared thermography, beta adrenoceptor

47

48

Abbreviations: ^{18}FDG-PET/CT, 18F-fluorodeoxyglucose-positron emission tomography/computed tomography; ATP, adenosine triphosphate; AUC, area under the curve; BAT, brown adipose tissue; CRF, clinical research facility; DXA, dual energy x-ray absorptiometry; HC, hydrocortisone; HOMA, homeostatic model assessment; ISO, isoprenaline; IT, infrared thermography; NEFA, non-esterified fatty acids; NIHR, National Institute for Health Research; NRES, National Research Ethics Service; REC, research ethics committee; tAUC, time-averaged area under the curve; T_{REF}, reference point temperature; T_{SCR}, supraclavicular temperature; UCP1, uncoupling protein 1; β-AR, beta adrenoceptor

49 **1. Introduction**

50 There is increasing evidence that brown adipose tissue (BAT) has important physiological roles beyond
51 thermoregulation in newborn infants and rodents [1]. Adult humans have significant amounts of BAT
52 [2] and, as a highly metabolic tissue with the capacity to oxidize both glucose and lipid, attention has
53 turned to its involvement in the pathogenesis of obesity and the metabolic syndrome [3]. BAT is
54 characterized by the presence of uncoupling protein (UCP) 1 which uncouples adenosine triphosphate
55 (ATP) production by the mitochrondrial respiratory chain, allowing the dissipation of excess chemical
56 energy as heat [4]. The principal factors regulating BAT function in healthy adults have yet to be fully
57 established due, in part, to the technical limitations of assessing BAT function *in vivo*. The majority of
58 studies in humans have used 18F-fluorodeoxyglucose-positron emission tomography/computed
59 tomography (^{18}FDG-PET/CT) as the gold standard to assess BAT activity, but this is constrained by
60 exposure to ionising radiation, the scanning protocols involved [5] and its unsuitability for live tracking
61 of BAT activation especially after feeding. Systemic β-adrenoceptor (β-AR) activation promotes BAT
62 activity in humans [6], but the role of other endocrine factors remains largerly unknown. The pre-partum
63 elevation of cortisol is pivotal in the initiation of nonshivering BAT thermogenesis at birth [7], and
64 glucocorticoids have recently been proposed as regulators of BAT activity in healthy adult females [8]
65 and in individuals pre-selected for the presence of active BAT [9]. BAT has also been considered to
66 contribute to dietary-induced thermogenesis [10, 11], although this concept remains controversial [12].
67 We, therefore, studied whether BAT is activated by feeding, or by an acute increase in cortisol under
68 basal and β-AR stimulated conditions.

69 **2. Materials and Methods**

70

71 **2.1** ***Subjects***

72 Eight healthy male volunteers participated in this randomized, double-blind, placebo controlled study,
73 conducted between January and March 2015. Individuals were recruited using print and electronic
74 advertising and none was selected or screened on the basis of presence of any active BAT. All subjects
75 underwent a medical evaluation during a screening visit to ensure they were healthy. No subject had
76 any significant past medical history, smoked tobacco or took any regular medications that could affect
77 the study's outcome measures.

78 **2.2** ***Study approval***

79 The study was approved by the Edgbaston NRES Committee, UK (REC reference 14/WM/1085). All
80 participants provided written informed consent.

81 **2.3** ***Study design***

82 *2.3.1. Clinical Research Facility*

83 All parts of this study were conducted in a temperature controlled room at the National Institute for
84 Health Research (NIHR)/Wellcome Trust Clinical Research Facility (CRF) of the University of
85 Birmingham at the Queen Elizabeth Hospital Birmingham, UK. Room temperature was held constant
86 at 23-26°C and was monitored using an ambient temperature probe.

87 *2.3.2 Anthropometric measurements*

88 Measurements were taken during the screening visit. Waist circumference was measured midway
89 between the lower margin of the last palpable rib and the top of the iliac crest, and hip circumference
90 at the level of the greater trochanters. Total and regional fat masses were measured by dual-energy x-
91 ray absorptiometry (DXA). Visceral fat mass was estimated by DXA using a proprietary algorithm
92 provided by the manufacturer [13]. Core temperature was measured with a tympanic thermometer.

93 *2.3.3. Study visits*

94 Study visits were identical, except for the nature of overnight infusion, and were at least 2 weeks apart
95 (**Fig. 1A**). Subjects were admitted to the CRF in the afternoon, and a cannula for infusion purposes was
96 inserted into a right antecubital fossa vein. At 1800 hours, they were served a standardized calorie-
97 controlled meal (vegetable lasagne; total energy 2634 kJ; typical nutritional values per 100g of product:
98 1.9g fat, 12.2g carbohydrates, 3.3g protein, 1.5g fibre), and then fasted until study completion the next
99 day. BAT thermogenesis assessment was performed immediately before and after the meal, which was
100 ingested within 20 min and was acompanied by tap water at room temperature. At 1900 hours, a constant
101 infusion of either hydrocortisone (HC, 0.2 $mg.kg^{-1}.h^{-1}$) or normal saline (control study visit) was started
102 and given until study completion the following day. Infusions were administered in a double-blind,
103 randomized fashion. At 2200 hours, lights were switched off for night rest. In the morning, cannulations
104 for blood sampling purposes were performed and, at 0900 hours, the isoprenaline infusion protocol
105 commenced. After baseline measurements for 45 min, a one-step infusion of isoprenaline (ISO, 25
106 ng.kg fat-free $mass^{-1}.min^{-1}$) was given for 60 minutes. BAT thermogenic activity was measured at
107 baseline and throughout the infusion.

108 ***2.4. BAT thermogenesis assessment***

109 An infrared thermography (IT) camera (FLIR E60 2.3 Megapixel Infrared Camera; FLIR Systems AB,
110 Danderyd, Sweden) was used to acquire images of the anterior neck and upper chest region, which were
111 sequentially analyzed and processed by an automated analysis program, as described previously [14].
112 Areas of interest for temperature analysis were the supraclavicular region (T_{SCR}) representing BAT, and
113 a non-adipose tissue reference point (T_{REF}) on the chest, close to the xiphoid. In addition, during the
114 periods of IT, two skin contact temperature sensors (iButton DS1922L, Maxim Integrated, Winnersh,
115 UK) recording skin temperature every minute were taped within the supraclavicular fossa (main BAT

116 site) and lateral to the umbilicus (white adipose tissue). For skin contact temperature measurements,
117 data were collected every minute, and analysis was performed using 5-minute averages. For meal
118 meaurements, the mean of both study days was calculated. Fasting and pre-ISO baseline were defined
119 as the average of time points -15 to 0 min. Postprandial and peak post-ISO infusion periods were defined
120 as time points 0 to 15 min and 40 to 50 min, respectively. For the duration of the study, participants
121 were wearing a hospital gown, with their torso exposed for the duration of all measurements.

122 ### *2.5. Analytical methods*

123 Blood samples were drawn into heparinized syringes, and plasma was prepared rapidly at 4°C and
124 immediately frozen at -80°C before analysis. Plasma glucose and NEFA concentrations were measured
125 enzymatically using commercially available kits on an ILAB600 or ILAB650 clinical analyser
126 (Instrumentation Laboratory UK, Warrington, UK). Insulin and C-peptide were measured by ELISA
127 (Invitron, Monmouth, UK) at a reference laboratory (Diabetes Research Unit Cymru, Swansea
128 University, UK). Cortisol was measured by a colorimetric assay (R&D Systems, Abingdon, UK).

129 ### *2.6 Calculations and statistics*

130 Indexes of β-cell function and insulin resistance were calculated according to the homeostatic model
131 assessment (HOMA) method, whereby the mean of three consecutive plasma glucose and insulin
132 postabsorptive measurements were used. Energy expenditure was calculated based on heart rate, age
133 and weight as previously described [15]. Area under the curve (AUC) was calculated using the trapezoid
134 rule and is presented as a time-averaged value (tAUC; AUC divided by the relevant time period).
135 Comparisons between groups were analyzed using *t* test or non-parametric tests for data that were not
136 normally distributed. A $p<0.05$ was considered statistically significant. Based on previous studies using
137 a similar integrative physiology design [16], the sample size was designed to have 85% power to detect
138 a difference of 0.75 standard deviations at the 5% significance level for metabolic parameters. Data
139 were analysed using IBM Statistics for Windows v21 and GraphPad Prism for Windows v6.05. All data
140 are presented as mean ± SEM, unless otherwise stated.

141 ## 3. Results

142 ### *3.1 Meal ingestion leads to increased BAT thermogenic activity*

143 Baseline anthropometric and metabolic characteristics of participants are shown in **Table 1** and
144 environmetnal temperature data for each individual study day are shown in **Supplemental Table 1**.
145 There was no difference in outside or room temperature between study days. Following the mixed meal,
146 postprandial T_{SCR} increased, whereas T_{REF} remained stable (**Fig. 1B**). All participants responded with
147 an increase in BAT thermogenic activity (**Fig. 1C**), while core temperature did not change (**Fig. 1D**).
148 Skin contact measurements showed a similar postprandial temperature increase of 0.39±0.10°C over

149 supraclavicular BAT ($p<0.05$ compared to fasting), whereas skin temperature over white adipose tissue
150 remained the same.

151 ***3.2 Acute hypercortisolemia induces peripheral insulin resistance and increases basal BAT***
152 ***thermogenic activity***

153 Overnight HC infusion resulted in significantly increased plasma cortisol concentrations (**Fig. 2A**).
154 From a metabolic perspective, basal plasma non-esterified fatty acids (NEFA) were high due to fasting
155 (**Fig. 2B**). HC increased basal NEFA and glucose (**Fig. 2C**), as well as insulin (basal insulin 30.4±6.0
156 pmol/L *vs.* 55.2±7.4 pmol/L, $p=0.025$ control compared to hypercortisolemia) and C-peptide
157 concentrations (basal C-peptide 0.25±0.03 pmol/mL *vs.* 0.38±0.04 pmol/mL, $p=0.001$ control compared
158 to hypercortisolemia). In line with this, HOMA indices of peripheral insulin resistance increased
159 (HOMA IR index control 0.62±0.11 *vs.* hypercortisolemia 1.11±0.16, $p=0.016$) (**Table 2**).

160 Acute hypercortisolemia increased T_{SCR} in the basal state (**Fig. 2D**). This was accompanied by an
161 increase in basal core temperature (**Fig. 2E**), but we did not observe any effect on blood pressure or
162 heart rate (**Fig. 2F**).

163 ***3.3 Acute β-AR stimulation increases BAT thermogenic activity during control and***
164 ***hypercortisolemia conditions***

165 From a metabolic perspective, ISO infusion significantly increased systemic NEFA concentrations,
166 both under control and hypercortisolemia conditions (**Fig. 2B**). Despite the augmentation of basal
167 systemic lipolysis by HC, the β-AR dependent rise in plasma NEFA was of similar magnitude compared
168 to control conditions (Δ AUC 953±155 *vs* 979±175 µmol/L; $p=0.926$ compared to control). Following
169 the initial peak, there was a sharp decline in NEFA concentrations despite continuing ISO infusion.
170 Control plasma glucose concentrations were unaffected by ISO, while the observed increase in
171 concentrations during HC infusion is due to glucocorticoid-induced peripheral tissue insulin resistance
172 (**Fig. 2C**). This is supported by the concomitant changes in insulin, C-peptide and HOMA indexes
173 showing a decrease in glucose sensitivity despite a significant increase in insulin and C-peptide
174 concentrations during ISO infusion (**Table 1**). Expectedly, non-selective β-AR stimulation with ISO
175 increased heart rate and systolic blood pressure, responses not significantly affected by HC **(Fig. 2F**
176 and **Supplemental Fig. 1**). Basal and ISO-induced BAT thermogenic activity measures did not show
177 any significant correlation with BMI or measures of adipose tissue distribution (data not shown).

178 Adrenergic stimulation resulted in a highly localized increase in temperature within the supraclavicular
179 region, representative of BAT thermogenic activity, both under control and hypercortisolemic
180 conditions (**Fig. 3**). All study participants responded to ISO with an increase in BAT temperature
181 (**Supplemental Fig. 2**). Under control conditions, ISO increased T_{SCR} by 0.7°C, plateaued and then
182 returned to baseline after the infusion, implying cessation of β-adrenergic-mediated BAT thermogenesis

183 (**Fig. 3A**). These responses to ISO were similar during hypercortisolemia (**Fig. 3B**), whereby peak T_{SCR}
184 was slightly higher (**Fig. 3C**). The ISO-induced T_{SCR} increase was greater than the physiological
185 stimulus of diet-induced thermogenesis (**Fig. 3D**). Skin temperature showed similar results
186 (**Supplemental Fig. 3**). Energy expenditure increased significantly during ISO (**Supplemental Fig. 4**).
187 During hypercortisolemia, ISO-induced energy expenditure was closely correlated with basal T_{SCR}
188 during control conditions (Pearson r=0.742, p=0.035) and peak T_{SCR} during HC (r=0.870, p=0.005). In
189 response to ISO, peak core temperature was similar between control and hypercortisolemia conditions
190 (**Supplemental Fig. 5**).

191 **4. Discussion**

192 Human supraclavicular BAT is characterised by the presence of thermogenically functional UCP1, with
193 a respiratory capacity that substantially exceeds that of white fat [17]. Understanding the endocrine
194 factors regulating BAT function is an important prerequisite before being able to utilise the metabolic
195 capabilities of this tissue. In this study we sought to study BAT *in vivo* following exposure to a
196 combination of physiological stimuli in order to determine the relative importance of diet and edocrine
197 mediated effects.

198 BAT glucose uptake has been reported to be increased following a single carbohydrate-rich meal [18],
199 although overfeeding for 24h did not have any effect [19]. This has led to some controversy regarding
200 the contribution of BAT to dietary-induced thermogenesis in humans. We sought to investigate this
201 using a single standardized mixed meal, serving as a physiological stimulus. While we did not measure
202 whole body energy expenditure, we observed selective temperature changes over the supraclavidular
203 region only, immediately after the meal, suggesting direct BAT activation and not a thermic effect of
204 food. Interestingly, from a mechanistic perspective, postprandial BAT activation would be
205 characterized by both systemic cortisol secretion [20] and sympathetic β-AR stimulation [21],
206 suggesting an acute maximal response following feeding.

207 Cortisol promotes important physiological maturation effects around the time of birth, including raised
208 UCP1 abundance in adipose tissue [7, 22]. However, in adult rodents, glucocorticoids inhibit BAT [23]
209 by interfering with adrenergic signalling [24, 25]. Human data are scarce with one study reporting
210 dexamethasone-induced inhibition of UCP1 expression and metabolic rate in human brown adipocytes
211 in vitro [26], and another reporting BAT activation following administration of the synthetic
212 glucocorticoid prednisolone *in vivo* [9]. In our study, we chose hydrocortisone to model a physiological
213 acute surge of cortisol, as seen during the perinatal period and at times of acute stress. We observed an
214 increase of basal T_{SCR} during hypercortisolemia, supporting a physiological role for cortisol in BAT
215 activation, as we did not observe any additive effects on blood pressure or heart rate. The duration of
216 the infusion was chosen to allow for glucocorticoid-mediated genomic effects to take place [27]. While

217 the achieved plasma cortisol concentrations were in excess of those typical for acute stress [28], it is
218 important to note that tissue-responsiveness can be determined by tissue-specific glucocorticoid
219 metabolism rather than absolute plasma concentrations [29]. Taken together our data indicate the
220 positive relationship between cortisol and BAT temperature as previously indicated from a small study
221 on healthy adult females [8].

222 ß-AR stimulation induces BAT thermogenesis in humans [6, 30], although findings are inconsistent
223 depending on the ß-AR employed [31, 32]. We found a localised increase in supraclavicular temperature
224 during ISO infusion both under control and hypercortisolemia conditions. This temperature change was
225 temporally limited for the duration of the infusion, suggesting underlying BAT activation. T_{SCR}
226 responses for all subjects increased within the first 5 minutes which is in accordance with acute cold
227 exposure on BAT [14]. The observed T_{SCR} plateau is suggestive of a limit to BAT thermogenesis *in*
228 *vivo*. The concomitant sharp decline in NEFA concentrations during the later stages of the infusion is
229 consistent with β-AR desensitization due to maximal receptor stimulation [16]. The finding of a slightly
230 higher peak T_{SCR} during hypercortisolemia suggests a minor synergistic effect between cortisol and ß-
231 AR stimulation. Interestingly, the two pathways are intrinsically connected as catecholamine synthesis
232 is under glucocorticoid control [33].

233 Previous studies have shown that active BAT decreases with age and obesity, and its activation varies
234 between sexes [14, 34]. We studied BAT activity in healthy males using IT to assess temperature
235 changes in the supraclavicular region and a non-adipose tissue reference point. Supraclavicular skin
236 temperature increases upon BAT activation [35, 36] and IT has been shown to measure changes in skin
237 temperature overlying the main BAT depot in humans [14]. It has been confirmed as a reliable
238 alternative for *in vivo* BAT activity assessment, correlating with ^{18}FDG-PET/CT [9, 37], with the
239 additional benefit of enabling real-time tracking of temperature changes. IT-derived BAT temperature
240 measurements might be influenced by subcutaneous adipose tissue thickness [38], however, in our study
241 participants were lean and we monitored dynamic temperature changes over time, as opposed to a
242 single, static measurement. Adrenoceptor-induced vasodilation, both as a result of HC and ISO
243 infusions, could increase skin blood flow and interfere with IT measurements. However, compared to
244 T_{SCR}, there were clear temporal differences in the change in T_{REF} which showed a later initial increase
245 and a sustained increase post-infusion. Overall, we demonstrate a BAT-specific thermogenic and
246 vasodilation response to both HC and ISO, clearly differentiated from non-BAT reference areas,
247 confirming that the temperature responses we measured are confined to BAT.

248 Our findings support previous studies showing β-AR stimulation as a means of activating BAT in
249 humans [6, 30], confirming IT as a sensitive, non-invasive method for the *in vivo* assessment of BAT
250 function in humans under near-normal conditions [9, 14, 37]. This is particularly important when

comparing our results with those studies using glucose tracer uptake as an index of BAT activity. Similar ISO doses did not show any significant BAT glucose tracer uptake, likely due to competition between the tracer and fatty acids from ISO-induced lipolysis combined with increased insulin resistance [32]. Given that BAT primarily utilizes fatty acids for heat generation [1], it is possible that ^{18}FDG-PET/CT underestimates the amount of active BAT in humans. This limitation has led to the development of alternative BAT assessment methods, in addition to IT, either using different PET/CT tracers [39], or based on magnetic resonance imaging techniques [40]. We demonstrate that temperature changes in the supraclavicular area upon β-AR stimulation are indicative of localised BAT activity in a cohort of unselected young individuals, maintained at room temperature. This supports the prospect of harnessing BAT activity and the associated increase in energy expenditure as a potential treatment for metabolic diseases. We provide further evidence that in humans, in contrast to rodents, acute hypercortisolemia does not inhibit BAT function, but results in BAT activation [9]. Despite this, there is a threshold of activity that cannot be overcome even during maximal short-term β-AR stimulation.

Our study has some limitations by design, including the small size of our sample, although it is standard for a healthy volunteer study of this type. The acute infusion of hydrocortisone limits the conclusions we can draw in relation to states of chronic glucocorticoid excess that are associated with profound metabolic changes, i.e. Cushing's Syndrome. In addition, the concomitant induction of relative insulin resistance during hydrocortisone infusion might have obscured glucocorticoid-specific effects on BAT function, especially since there is a complex relationship between insulin-mediated glucose uptake and BAT perfusion and activity *in vivo* [41]. The strenghts of our study are the randomized double-blind design of the infusion protocol and that all measurements were carried out within a short period of time, reducing the confounding effect of variations seasonal temperature, and thus endogenous BAT activity. In addition, by using IT for the assessment of BAT thermogenic activity, we were able to perform live tracking of BAT function in response to experimental stimuli.

In conclusion, glucocorticoids modulate BAT thermogenesis and may represent an important physiological mechanism for maintaining human body temperature at times of acute stress. Our study suggests that transient stress could act to promote BAT function. This suggests that depending on the type and magnitude of stress BAT could be utilised to improve body weight regulation and metabolic homeostasis.

Authors contributions

KNM and MES designed the study; HS and KNM conducted the experiments and analyzed the data with JL, HS, MES, JL, HB, DS and KNM wrote the manuscript.

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Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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400

401 **Figure captions**

402 **Figure 1**

403 Study design and temperature responses to a meal. Each participant underwent the study twice, whereby
404 BAT thermogenic activity was studied with infrared thermography before and after a standardized meal,
405 followed by either a 14 h overnight constant hydrocortisone or normal saline (control) infusion.
406 Infusions were given in a randomized, double-blind order and continued during and after β-
407 adrenoceptor stimulation with isoprenaline (**A**). Mean changes in supraclavicular region (T_{SCR}, grey
408 circles) and non-adipose tissue reference (T_{REF}, open squares) temperatures (dotted line indicates time
409 of meal) (**B**), individual responses (fasting, open squares; postprandial, black squares) (**C**), and changes
410 in core temperature (fasting, open circles; postprandial, black squares) (**D**) following the meal. *p<0.05
411 compared to fasting baseline, n=8

412

413 **Figure 2**

414 Metabolic and cardiovascular responses following saline infusion (S) or hydrocortisone infusion (HC)
415 at baseline and during an isoprenaline infusion (ISO, dotted lines indicate infusion period). Plasma
416 cortisol concentrations (saline, open circles; hydrocortisone, black squares) (**A**), non-esterified fatty
417 acids (NEFA) (saline, open circles; hydrocortisone, black squares) (**B**), glucose (saline, open circles;
418 hydrocortisone, black squares) (**C**), supraclavicular temperature (T_{SCR}) (saline, open circles;
419 hydrocortisone, black circles) (**D**) and core temperature at baseline (saline, open squares;
420 hydrocortisone, black squares) (**E**). Systolic (sys) and diastolic (dia) blood pressure (BP) and heart rate
421 (HR) at baseline (saline, open bars; hydrocortisone, black bars) (**F**). *p<0.05 vs. control, n=8

422

423 **Figure 3**

424 BAT thermogenic responses. Supraclavicular temperature (T_{SCR}) and non-adipose tissue reference point
425 (T_{REF}) following saline (S) infusion (T_{SCR}, grey circles; T_{REF} open squares) (**A**) or hydrocortisone (HC)
426 infusion (T_{SCR}, grey circles; T_{REF} open squares) (**B**) during and after isoprenaline stimulation (ISO,
427 dotted lines indicate infusion period). Individual peak BAT temperatures during ISO (S, open circles;
428 HC, black circles) (**C**). Change in temperature during ISO or following a standardized meal (T_{SCR}, grey
429 bars; T_{REF} open bars) (**D**). *p<0.001 vs. basal, †p<0.05 vs. saline, #p<0.001 vs. saline, ##p<0.001 vs.
430 T_{REF}, n=8

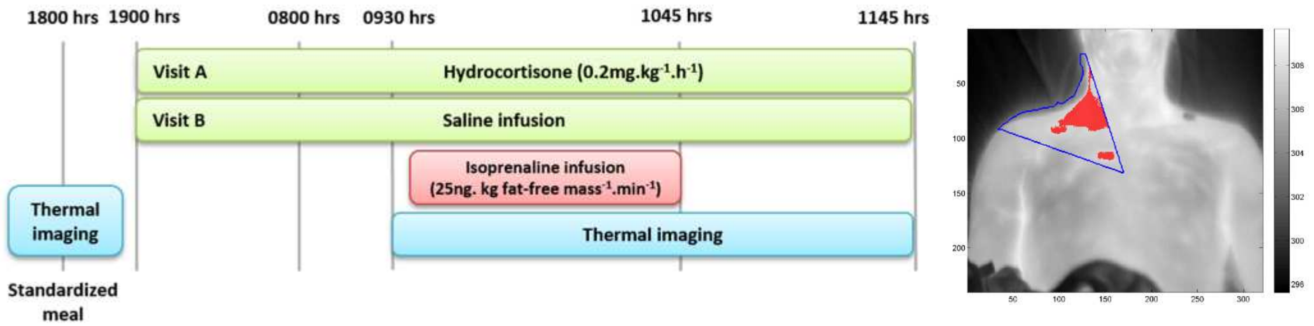
431

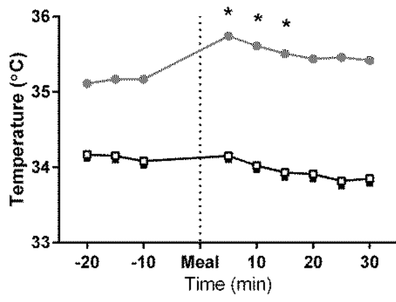
432

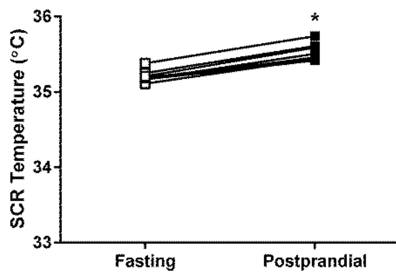
433 **Figures**

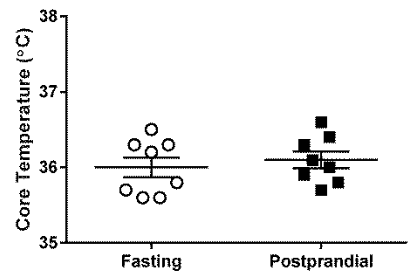
434

435 **Figure 1**

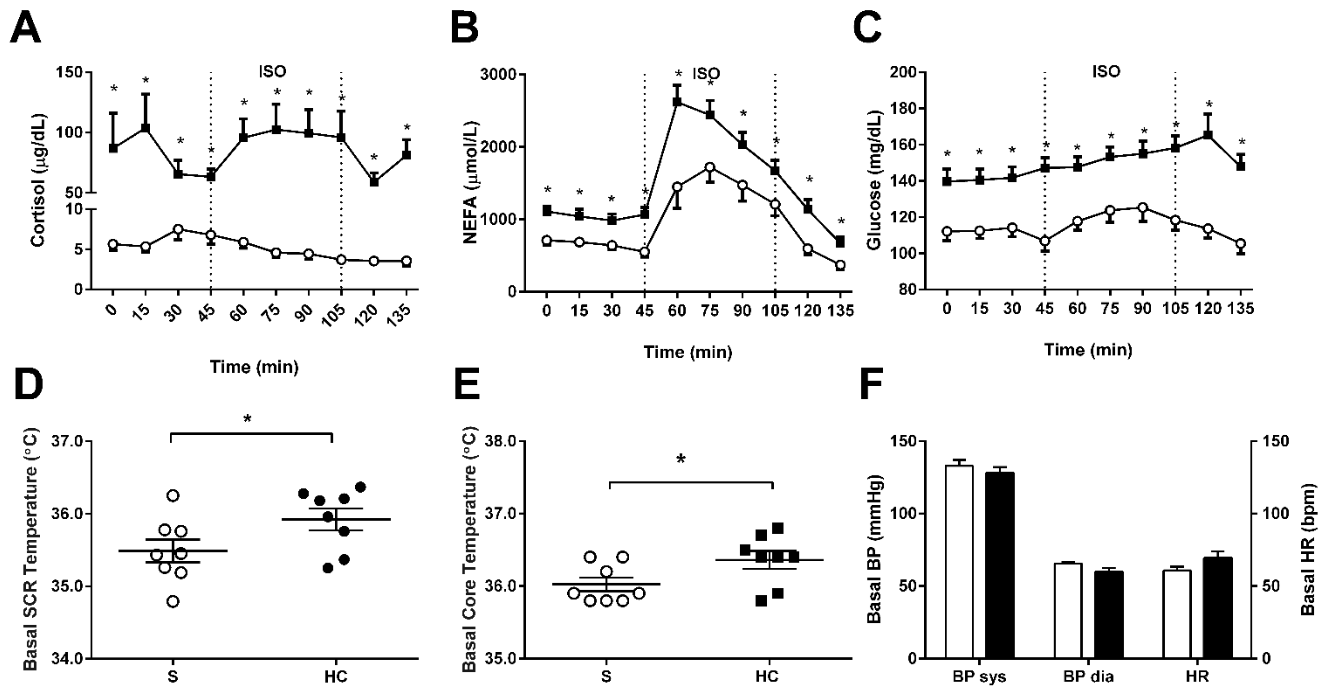








436 **Figure 2**



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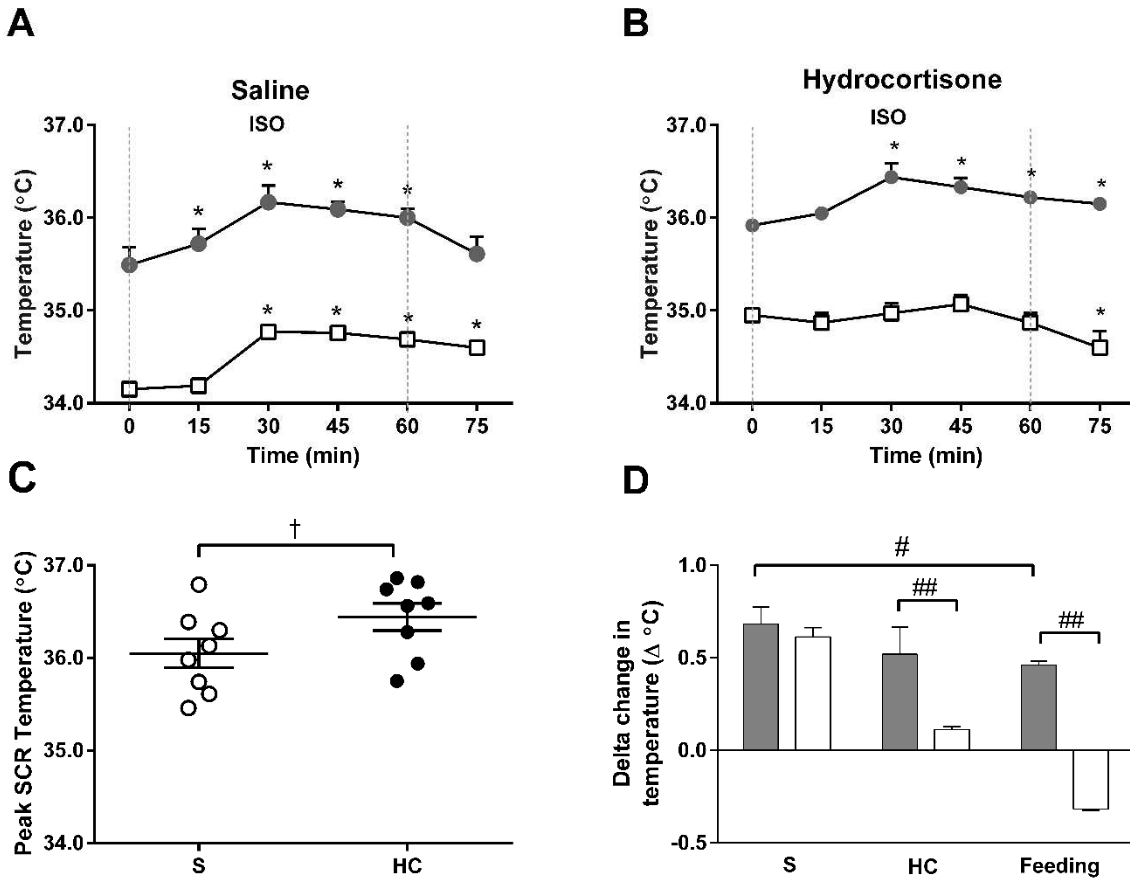
438 **Figure 3**



439

440 **Table 1:** Baseline anthropometric and metabolic characteristics of participants, n=8

441–458

Characteristic	
Age (years)	20 (18-34)
Weight (kg)	75.0 (61.5-81.7)
BMI (kg/m^2)	23.0 (20.6-24.2)
WHR	0.84 (0.78-0.9)
Trunk fat (kg)	7.7 (5.1-9.2)
Leg fat (kg)	5.5 (4.3-7.5)
Visceral fat (kg)	0.2 (0.1-0.4)
Systolic BP (mmHg)	124 (105-145)
Diastolic BP (mmHg)	72 (59-79)
Heart rate (bpm)	58 (53-62)
Fasting glucose (mg/dL)	113 (89-122)
Fasting insulin (pmol/L)	30.6 (14.4-68.5)
Fasting NEFA (µmol/L)	707 (450-873)
TSH (mIU/L)	1.30 (0.93-4.83)
FT4 (pmol/L)	17.7 (15.0-18.8)

459

460 Median and range shown. BMI, body mass index; WHR, waist-to-hip ratio; BP, blood pressure; TSH,
461 thyroid stimulating hormone; FT4, free thyroxine

462

463

464

465 **Table 2:** Comparison of insulin and C-peptide AUC, and homeostatic model assessment (HOMA)
466 indexes during basal and isoprenaline-stimulated conditions, n=8

	Control		Cortisol		*P* value control *vs* cortisol		*P* value basal *vs* isoprenaline	
	Basal	ISO	Basal	ISO	Basal	ISO	Control	Cortisol
Insulin (pmol/L)	30.4±6.0	100.4±15.7	55.2±7.4	206.7±30.5	0.025	0.012	0.012	0.012
C-peptide (pmol/mL)	0.25±0.03	0.47±0.05	0.38±0.04	0.90±0.09	0.001	0.001	<0.0001	<0.0001

	Control	Cortisol	*P* value
Δ insulin basal to ISO (pmol/L)	70.0±10.1	151.5±28.9	0.012
Δ C-peptide basal to ISO (pmol/mL)	0.22±0.03	0.52±0.07	0.012
HOMA %B	42.2±5.9	40.5±4.5	0.731
HOMA %S	192.2±29.5	105.9±19.2	0.004
HOMA IR	0.62±0.11	1.11±0.16	0.016

467 Mean and SEM shown

468

469 **Supplemental data**

470 **Supplemental Table 1:** Environmental temperatures in relation to the time of year throughout which
471 each study was conducted. Environmental outside temperature data obtained from the Meteorological
472 office. Note overnight duration of infusion as outlined in main text.

473

Saline infusion					
Participant	Date of study	Environmental room temperature (°C)		Environmental outside temperature (°C)	
		Infusion start	Infusion end	Infusion start	Infusion end
1	13/01/2015	23.7	25.2	6	4
2	24/02/2015	22.8	25.8	6	5
3	11/02/2015	24.4	24.0	5	5
4	21/01/2015	24.2	24.6	4	4
5	22/01/2015	24.1	23.9	4	4
6	26/02/2015	24.2	23.2	5	5
7	26/03/2015	25.2	25.2	7	7
8	24/03/2015	24.1	24.6	7	7
Mean ± SEM		24.1 ± 0.2	24.6 ± 0.3	5.5 ± 0.4	5.1 ± 0.4

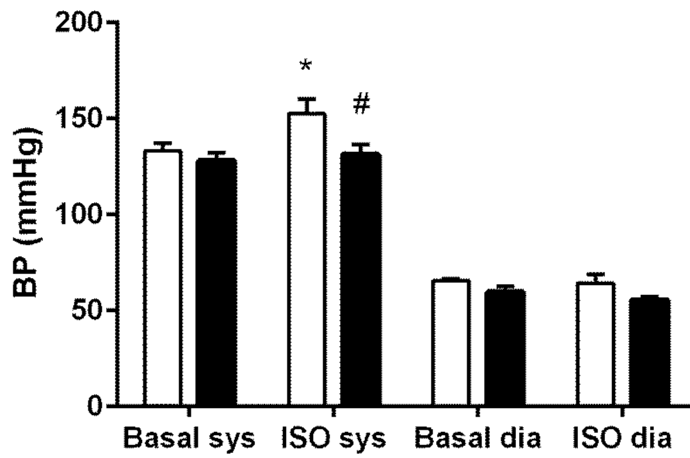
474

Hydrocortisone infusion					
Participant	Date of study	Environmental room temperature (°C)		Environmental outside temperature (°C)	
		Infusion start	Infusion end	Infusion start	Infusion end
1	10/02/2015	23.7	23.9	4	5
2	14/01/2015	24.0	25.3	4	5
3	15/01/2015	22.2	23.5	5	4
4	25/02/2015	25.5	26.2	5	5
5	12/02/2015	24.0	24.8	5	4
6	25/03/2015	25.2	23.6	7	7
7	11/03/2015	25.1	25.2	6	6
8	12/03/2015	25.3	23.6	6	5
Mean ± SEM		24.4 ± 0.4	24.5 ± 0.4	5.3 ± 0.4	5.1 ± 1.0

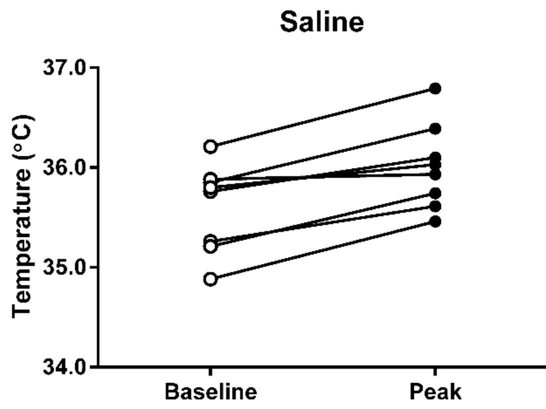
475

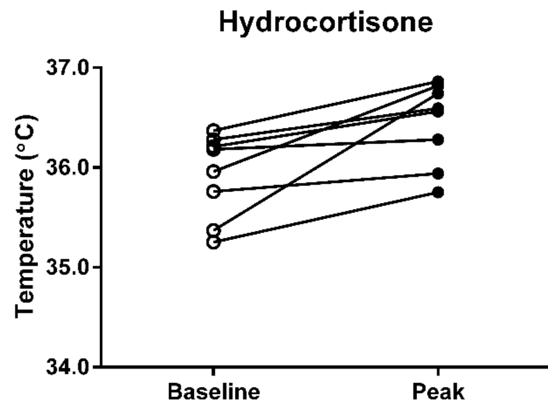
476

477 **Supplemental Figure 1**



478 Systolic (sys) and diastolic (dia) blood pressure before (basal) and after 50 min of isoprenaline
479 infusion (ISO), under control conditions (open bars) and following an overnight hydrocortisone
480 infusion (black bars). * $p<0.05$ compared to basal control, # p=<0.05 compared to basal
481 hydrocortisone, n=8





482

483 **Supplemental Figure 2**

484 Individual BAT temperature responses from baseline (open circles) to peak (black circles) following
485 saline infusion **(A)** or hydrocortisone infusion **(B)**, n=8

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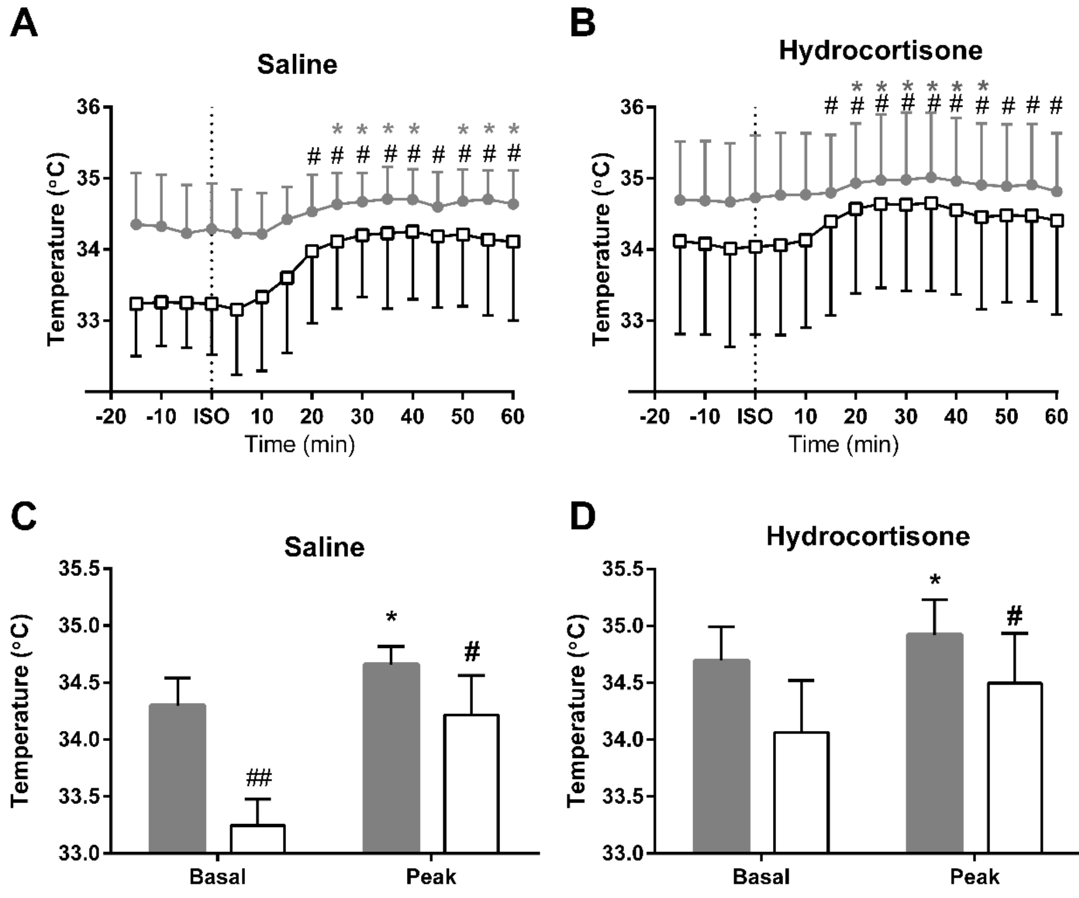

487

488 **Supplemental Figure 3**

489 Brown (BAT) and white adipose tissue (WAT) thermogenic responses to isoprenaline (ISO) using skin
490 contact temperature sensors placed over the supraclavicular BAT region (grey circles and bars) or over
491 an area of abdominal WAT (open squares and bars) under control conditions (**A**) and following an
492 overnight hydrocortisone infusion (**B**). Dotted line indicates start of ISO infusion. Mean temperatures
493 at baseline and at peak during ISO (**C**, control and **D**, hydrocortisone). * $p<0.05$ peak/post-ISO BAT
494 temperaure compared to basal BAT, # $p<0.05$ peak/post-ISO WAT temperature compared to basal
495 WAT, ## $p<0.05$ compared to basal BAT, n=8

496

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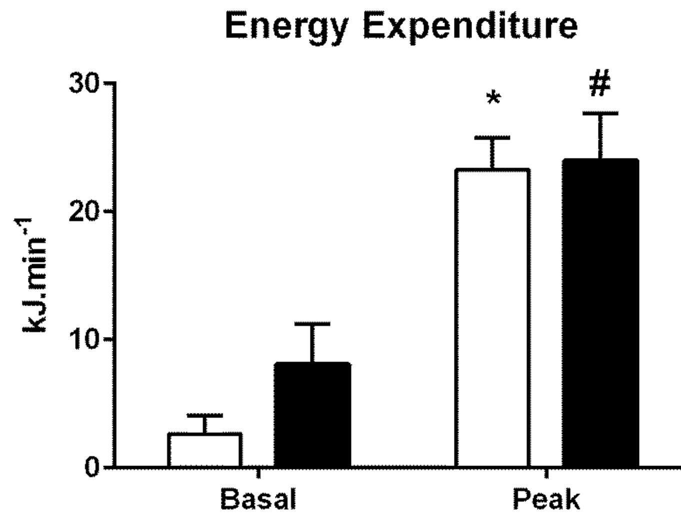

Supplemental Figure 4

Whole body energy expenditure under control conditions (open bars) and following an overnight hydrocortisone infusion (black bars) during basal conditions and after 50 min of isoprenaline infusion (peak). * $p<0.05$ compared to basal control, # $p=<0.05$ compared to basal hydrocortisone, $n=8$

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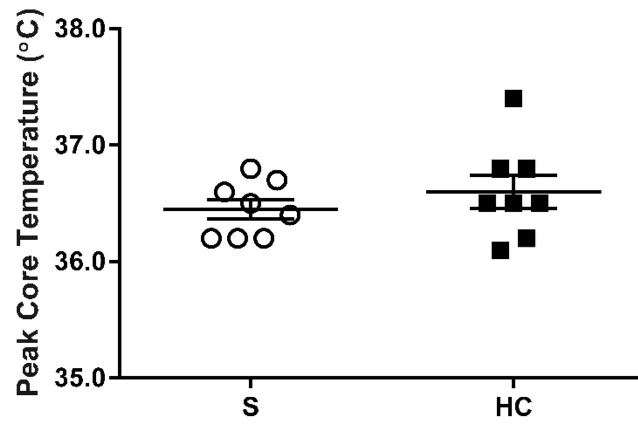

512

513 **Supplemental Figure 5**

514 Individual peak core temperature responses to isoprenaline infusion following saline infusion (S, open
515 circles) or hydrocortisone infusion (HC, black squares), n=8

516