

1 **Title:**

2 Females exposed to 24 hours of sleep deprivation do not experience greater physiological
3 strain, but do perceive heat illness symptoms more severely, during exercise-heat stress

4 **Running title:**

5 Sleep deprivation and heat illness in females

6 **Key words:**

7 Metabolic heat production, thermoregulation, sleep loss, heat injury, females.

8 **Authors:**

9 Relf, Rebecca¹; Willmott, Ashley¹; Mee, Jessica²; Gibson, Oliver³; Saunders, Arron¹; Hayes,
10 Mark¹; Maxwell, Neil¹.

11 ¹Centre for Sport and Exercise Science and Medicine (SESAME), Environmental Extremes
12 Laboratory, University of Brighton, Eastbourne, UK BN20 7SR

13 ²School of Sport, Health and Exercise Sciences (SSHES), Bangor University, North Wales,
14 UK, LL57 2PZ.

15 ³Centre for Human Performance, Exercise and Rehabilitation (CHPER), Brunel University
16 London, Uxbridge, UK UB8 3PH.

17

18 **Corresponding author**

19 Rebecca L. Relf R.Relf@brighton.ac.uk

20 Centre for Sport and Exercise Science and Medicine (SESAME), Environmental Extremes
21 Laboratory, University of Brighton, Eastbourne, UK BN20 7SR

22 Tel +44(0)1273 643754

23

24 **Conflict of interest:** None

25 **Abstract Word Count:** 200

26 **Text-Only Word count:** 4297

27 **Number of Figures:** Three

28 **Number of Tables:** Two

29

30

31

32

33

34 **Abstract**

35

36 **Purpose:** There is limited and inconclusive evidence surrounding the physiological and
37 perceptual responses to heat stress while sleep deprived, especially for females. This study
38 aimed to quantify the effect of 24-hrs sleep deprivation on physiological strain and perceptual
39 markers of heat-related illness in females.

40 **Method:** Nine females completed two 30 min heat stress tests (HST) separated by 48 hrs in
41 39°C, 41% relative humidity at a metabolic heat production of 10 W.kg⁻¹. The non-sleep
42 deprived HST was followed by the sleep deprivation (SDHST) trial for all participants, during
43 the follicular phase of the menstrual cycle. Physiological and perceptual measures were
44 recorded at 5 min intervals during the HSTs. On the cessation of the HSTs, heat illness
45 symptom index (HISI) was completed.

46 **Results:** HISI scores increased after sleep deprivation by 28±16 vs. 20±16 (P=0.01). Peak
47 (39.40±0.35°C vs. 39.35±0.33°C) and change in rectal temperature (1.91±0.21 vs.
48 1.93±0.34°C), and whole body sweat rate (1.08±0.31 vs. 1.15±0.36 L.h⁻¹) did not differ
49 (P>0.05) between tests. No difference was observed in peak, nor rise in; heart rate, mean skin
50 temperature, perceived exertion or thermal sensation during the HSTs.

51 **Conclusion:** 24 hrs sleep deprivation increased perceptual symptoms associated with heat-
52 related illness, however, no thermoregulatory alterations were observed.

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69 **Introduction**

70

71 Physically stressful occupational and athletic activities performed in hot conditions increase
72 physiological strain and impair endurance performance (Galloway & Maughan, 1997).
73 Uncompensable heat stress may increase the risk of developing a heat-related illness (HRI),
74 through increased core temperature, cardiovascular strain and a substantial loss of fluids and
75 electrolytes (Coris et al., 2004). HRIs are categorised by severity and occur along a continuum;
76 where relatively minor symptoms (e.g. heat rash or cramps) can rapidly progress into serious
77 and life-threatening events (e.g. cognitive dysfunction, loss of consciousness) (Heled et al.,
78 2004). HRI onset can be caused and exacerbated by a combination of risk factors including;
79 anthropometric characteristics, age, sex, acclimation state and sleep deprivation, with random
80 or sporadic onsets (Moran et al., 2004).

81 Sleep deprivation has been reported to contribute to exertional heat illnesses in a multitude of
82 occupational literature (McDermott et al., 2007). Furthermore, 83% of HRI cases were related
83 to a prior episode of sleep deprivation (3-4 hrs per night) (Rav-Acha et al., 2004). Contributing
84 factors to HRIs while sleep deprived include the larger (+0.7°C) exercising core temperature
85 (T_{re}) (Sawka et al., 1984), impaired sudomotor function [reduced ability to dissipate heat
86 through evaporation] (Fujita et al., 2003; Sawka et al., 1984) and increments in ratings of
87 perceived exertion (RPE) and thermal sensation (TS) (Muginshtein-Simkovitch et al., 2015).
88 While sleep is a naturally recurring state, characterized by circadian periodicity (Garcia-
89 Garcia et al., 2014), sleep loss (<6.5 hrs recommended per night) and, or deprivation (e.g.
90 partial or full) disrupts the circadian rhythm, and is highly prevalent among healthy adults and
91 adolescents (Fullagar et al., 2015). Moreover, sleep deprivation is associated with health risks
92 (e.g. increase diurnal blood pressure and cortisol levels) and cognitive impairments (e.g.
93 decision making, memory) (Short & Banks, 2014). Acute 24 hrs sleep deprivation observed
94 during operational duties such as; nursing, mining, aviation and trucking, negatively
95 influences cognitive function, which may influence, and potentially cause several catastrophic
96 incidents and accidents (Horne & Reyner, 1995).

97 Aside from occupations, the multitude of athletes regularly travelling to environmentally
98 challenging conditions (i.e. heat stress), across many time zones to train and compete are
99 exposed to short-term or chronic **sleep loss/deprivation** on a regular basis (Oliver et al.,
100 2009). Whilst experiencing symptoms of HRI may not indicate a medically reportable case, it
101 does suggest an increased susceptibility due to an increased physiological strain and emphasis
102 that the body is unable to meet the demands of thermoregulation (Heled et al., 2004). In an
103 attempt to assess and quantify milder forms of HRI, a heat illness symptom index (HISI) was
104 developed (Coris et al., 2006). This was formed from an in-depth literature review analysing
105 the most common symptoms associated with HRI, to which thirteen were chosen (see Figure
106 2). The HISI was developed to allow a better understanding of the potential pathophysiologic
107 and symptomatic progression of HRI, presenting good reliability and validity in American
108 football players' training (Coris et al., 2006). However, correlation with core temperature was
109 advised for further validation in relation to HRI.

110 A paucity of evidence exists surrounding the physiological and perceptual responses while
111 sleep deprived, especially for females when acknowledging the differences in
112 thermoregulatory function between sexes (Fujita et al., 2003; Oliver et al., 2009). Moreover,
113 controlling for metabolic heat production (\dot{H}_{prod}) during sleep deprivation exercise protocols
114 reduces the systematic differences in T_{re} despite differences in body mass and aerobic capacity
115 (Cramer & Jay, 2014). Therefore, the aim of this study was to quantify the effect of acute sleep
116 deprivation (24 hrs) on perceptual markers related to HRI and physiological strain in females
117 when menstrual cycle is controlled for. It was hypothesised that sleep deprivation would
118 increase the perception of symptoms of HRI, determined by an increased HISI score.
119 Secondly, sleep deprivation would significantly increase the rate of T_{re} rise during exercise.

120

121 **Method**

122 **Participant characteristics and requirements**

123 Nine recreationally active females (mean \pm standard deviation [SD]; aged: 22 ± 3 yrs, stature:
124 1.66 ± 0.10 m, body mass: 63.8 ± 10.6 kg, body surface area [BSA]: 1.7 ± 0.2 m², peak oxygen
125 uptake ($\dot{V}O_{2peak}$) in $40.1 \pm 0.4^{\circ}C$, 42 ± 1 % relative humidity: 44.1 ± 3.4 mL.kg⁻¹.min⁻¹)
126 volunteered and provided prior written informed consent. Participants had regular sleeping
127 patterns confirmed by sleep diaries (average of >6.5 hrs per night) and had not been exposed
128 to heat stress in the month prior to testing, nor had previously incurred a HRI. The study was
129 approved by the University of Brighton's ethics committee and conformed to the revised
130 Declaration of Helsinki (World Medical Association, 2013). Participants abstained from
131 caffeine (Muginshtein-Simkovitch et al., 2015), strenuous exercise and alcohol in the 24 hrs
132 prior to testing. Moreover, no food was consumed within the 2 hrs prior to each trial and
133 participants were instructed to consume 3-5 mL.kg⁻¹ of water during this period (Sawka et al.,
134 2007). All testing occurred in the morning (08:00-10:00) to control for circadian rhythm. Self-
135 reported menstrual cycle questionnaires were completed in order to schedule testing, which
136 occurred in the early follicular stage of their menstrual cycle (day 0-7), as higher resting T_{re}
137 (0.3 - $0.6^{\circ}C$) and a delayed onset of sweating and cutaneous vasodilation have been reported to
138 occur in the luteal phase (Pivarnik et al, 1992). Participants taking oral contraceptive pills
139 undertook testing during the no pill, placebo phase; these timings were selected to control for
140 hormonal fluctuations in line with previous literature (Stachenfeld & Taylor, 2014).

141 **Experimental design**

142 Participants undertook a repeated measures design, requiring three visits to the laboratory; a
143 lactate threshold and $\dot{V}O_{2peak}$ test, a heat stress test (HST) and finally a sleep deprived HST
144 (SDHST), all separated by 48 hrs. Due to the time restriction of completing tests during the
145 follicular phase of the menstrual cycle, the sleep deprivation test was completed last as the
146 recovery period is still unclear within the literature (Belenky et al., 2003). These logistical
147 constraints necessitated the order of trials and non-randomised approach.

148

149 **Preliminary testing**

150 **Lactate threshold and $\dot{V}O_2$ peak**

151 The pre-programmed lactate threshold protocol was standardised for all participants,
152 beginning at 5 km.hr⁻¹ on a motorised treadmill (Woodway, Germany) within a purpose built
153 environmental chamber (TISS, UK) set to 39.9 ± 0.8°C and 41 ± 3% RH. Participants
154 performed five submaximal (Jay et al., 2011), 3 min incremental stages of 0.8 km.hr⁻¹
155 (Spurway & Jones, 1997) at 1% gradient (Jones & Doust, 1996). Expired air was collected
156 using open-circuit spirometry for 45-s in the last minute of each stage to estimate metabolic
157 heat production for prescription of workload for the subsequent HSTs. Each Douglas bag was
158 analysed using a gas analyser (Servomex International Ltd., UK) to give oxygen (O₂) and
159 carbon dioxide (CO₂) percentages. The temperatures and volumes of the gases were acquired
160 using a dry gas flow meter (Harvard Apparatus Ltd., UK), and a fixed flow pump model
161 Dymax 30 (Charles Austin Pumps Ltd., UK). A two-point calibration was undertaken using a
162 mixture of gases and pre-determined O₂ and CO₂ percentages [15 and 5%, respectively] (BOC,
163 UK) prior to every trial. T_{re}, heart rate (HR), TS (Toner et al., 1986) and RPE (Borg, 1982)
164 were recorded at the end of each 3-min stage. Following a 15 min rest, participants began
165 running at 8.0 km.hr⁻¹, with 1 min stages and increments of 1.0 km.hr⁻¹ (James et al., 2014)
166 until volitional exhaustion. Expired air was collected in a Douglas bag for 45s during each
167 stage, HR and T_{re} were recorded at the end of each stage. Due to the physiological strain,
168 $\dot{V}O_2$ peak was obtained, not maximal as not all criteria were met (e.g. plateau in $\dot{V}O_2$)
169 (Spurway & Jones, 1997).

170

171 **Metabolic heat production (\dot{H}_{prod})**

172 In conformity with the recommendations from Jay et al. (2011) and Cramer and Jay (2014);
173 \dot{H}_{prod} was prescribed from metabolic energy expenditure and velocity during the running
174 submaximal lactate threshold. Metabolic energy expenditure (Nishi, 1981) was calculated
175 from each stage for oxygen consumption ($\dot{V}O_2$) and the respiratory exchange ratio (RER) (Jay
176 et al., 2011), using the equation below:

$$M = \dot{V}_{O_2} \frac{\left(\frac{RER - 0.7}{0.3} e_c \right) + \left(\frac{1 - RER}{0.3} e_f \right)}{60} \times 1000 \text{ Watts}$$

177

178 where: e_c is the caloric equivalent per litre of O_2 for the oxidation of carbohydrates (21.13 kJ),
 179 and e_f is the caloric equivalent per litre of oxygen for the oxidation of fat (19.62 kJ). \dot{H}_{prod} was
 180 determined as the difference between metabolic energy expenditure (M) and external
 181 mechanical power output (W), divided by body mass (BM) to obtain relative \dot{H}_{prod} ($W \cdot \text{kg}^{-1}$):
 182 $\dot{H}_{\text{prod}} = (M - W) / \text{BM}$.

183

184 **Main experimental tests**

185 The HST consisted of 30 min running at a \dot{H}_{prod} of $10 \text{ W} \cdot \text{kg}^{-1}$ (pre-determined by pilot work)
 186 at 1% gradient (Jones & Doust, 1996) on a motorised treadmill. The treadmill velocity did not
 187 differ between HSTs for each participant ($8\text{-}10 \text{ km} \cdot \text{hr}^{-1}$, $77 \pm 5\% \dot{V}O_{2\text{peak}}$). The test occurred
 188 within hot conditions $39.8 \pm 0.7^\circ\text{C}$ and $41 \pm 2\% \text{ RH}$, which were controlled using automated
 189 computer feedback (WatFlow control system, TISS, UK).

190

191 **Pre- trial preparation**

192 On arrival to the laboratories, participants provided a fresh mid-flow urine sample.
 193 Euhydration was confirmed by the following criteria (Sawka et al., 2007); urine osmolality
 194 ($U_{\text{osm}} \leq 700 \text{ mOsm} \cdot \text{kg}^{-1} \text{ H}_2\text{O}$ (Advanced Micro Osmometer 3300, Vitech Scientific Ltd.,
 195 UK) and specific gravity ($U_{\text{sg}} \leq 1.020$ (URC-Ne handheld refractometer, ATAGO CO Ltd.,
 196 Japan). Following this, nude body mass (NBM) was recorded to the nearest gram (GFK 150,
 197 Adam Equipment Inc., USA). Differences between pre and post exercise NBM determined
 198 non-urine fluid loss (whole body sweat rate, $\text{L} \cdot \text{hr}^{-1}$). After a 15 min rest period, in a controlled
 199 laboratory ($21.9 \pm 1.7^\circ\text{C}$, $50 \pm 10\% \text{ RH}$), baseline measures were recorded.

200 **Experimental Measurements**

201 Rectal probes (Henley, UK) were self-inserted 10 cm past the anal sphincter provided
202 continuous T_{re} measurement throughout tests. Participants were familiarised to the HISI (0-
203 130), TS (0 unbearably cold to +8 unbearably hot) and RPE (6 = very, very light to 20 =
204 exhaustion) scales, and then affixed a HR monitor to the chest (Polar FT1, Polar Electro,
205 Finland). Skin temperature (T_{skin}) was recorded using skin thermistors (Eltek Ltd, Cambridge,
206 UK) attached to four sites; the midpoint of the right pectoralis major (T_{chest}), midpoint of the
207 right triceps brachii lateral head (T_{arm}), right rectus femoris ($T_{upper\ leg}$) and right gastrocnemius
208 lateral head ($T_{lower\ leg}$), and connected to a temperature logger (Squirrel 1000 series, Eltek Ltd.,
209 UK). This device has been found to have a typical error of measurement (TEM) of 0.18°C
210 (James et al., 2014). T_{skin} was calculated using the equation by Ramanathan (1964); Mean T_{skin}
211 = $(0.3 \times [T_{chest} + T_{arm}]) + (0.2 \times [T_{upper\ leg} + T_{lower\ leg}])$. Both physiological and perceptual
212 measurements were taken at 5 min intervals throughout the 30 min running HST. Expired air
213 was collected at three time points during the run (minutes 4-5, 14-15 and 24-25) to assess the
214 accuracy of the \dot{H}_{prod} prescription. The HISI scale (Coris et al., 2006) is a 10 point index of 13
215 symptoms including that of thirst, dizziness etc, which are rated on a scale of 0 (no symptoms)
216 to 10 (had to stop exercise). Guidelines were given to participants prior to tests and during
217 familiarisation / pilot work, to make the differentiation between symptoms easier, HISI was
218 recorded during the last minute of the HSTs.

219

220 **Sleep deprivation protocol**

221 A 7 day sleep diary was self-reported by the participants in the week prior to testing to assess
222 average sleep (hrs) and to ensure participants were not banking sleep. Participants were asked
223 to complete the diaries in the morning after first waking and reported; time they went to bed,
224 total hours slept and quality of sleep. Participants reported to the laboratories at 22:00, having
225 been awake 14 hrs, to remain awake for the entirety of the night prior to testing at 08:00 (awake
226 24 hrs). Participants were continuously monitored and allowed to consume snacks and non-
227 caffeinated beverages, each of which was recorded (Hom et al., 2012). This sleep deprivation

228 protocol ensured participants remained in an energy balanced state. The calorie content of
229 food consumed was equal to average female calories ($1348 \pm 125 \text{ kcal}\cdot\text{day}^{-1}$) expended in the
230 10 hrs overnight due to sleep deprivation, $\sim 562 \text{ kcal}$ (Arciero et al., 1993).

231

232 **Blood sampling and analysis**

233 Prior to both HSTs (follicular phase) and on day 20-22 (luteal phase) of the participants' self-
234 reported menses, a resting 6 mL venous blood sample was drawn from the median cubical
235 vein, and centrifuged in duplicate at 4400 rpm and 4°C for 10-min (5702R centrifuge,
236 Eppendorf UK Ltd.). Plasma was then pipetted into 1.5 mL microtubes (Western laboratory
237 science, UK) and stored at -86°C (VIP series, Sanyo Electric Biomedical Co Ltd., Japan) for
238 later analysis. Following the manufacturer's guidelines, analysis involved the use of
239 commercially available 17β -estradiol (ab108667) and progesterone (ab108670)
240 immunoenzymatic assay kits (Abcam plc, UK). Incubation, including the required quality
241 control standards was performed on an orbital platform shaker (Titramax 1000, Heidolph UK)
242 at 1.5 mm vibration and read by a microplate reader using absorption at 450 nm (elx800,
243 BioTek UK). As described by the manufacturer, the intra-assay and inter-assay variability was
244 9% and 10% for 17β -estradiol and 4% and 9.3% for progesterone, respectively. Moreover, the
245 lowest detectable concentration of 17β -estradiol and progesterone was 20.26 and 0.24 ng.mL-
246 1, respectively.

247 **Statistical analyses**

248 All data was analysed using a standard statistical package (SPSS version 20.0), and reported
249 as mean \pm SD. All data were analysed for normality using Shapiro-Wilk and sphericity using
250 the Greenhouse-Geisser method. As a measure of retest correlation, relative measures of intra
251 class correlation (ICC) with 95% confidence intervals (CI) were calculated for the HISI scale
252 at rest and during exercise, alongside Spearman's correlation (non-parametric data). Absolute
253 measures of reliability were calculated using Bland-Altman limits of agreement (LOA)
254 showing the mean bias and 95% CI; at rest LOA = 0.38 (-0.64, 1.39), ICC = 0.918, and during

255 exercise LOA = 0.13 (-1.82, 2.07), ICC = 0.986. Non-parametric datasets; average and peak
256 RPE, TS and HISI, were analysed using a Wilcoxon signed-rank test with Bonferroni
257 correction applied. Paired samples T-Tests were used for resting and end-test results. A 2-way
258 (trial x time) repeated measures analysis of variance (ANOVA) was completed for
259 physiological measures. Effect size (*d*) was categorised as small (0.2), medium (0.5) and large
260 (0.8) (Cohens, 1988). Statistical significance was accepted at the level of $P \leq 0.05$.

261 **Results**

262 **Participant characteristics**

263 Participants arrived to the laboratories for both main tests in a similar physiological resting
264 state ($P > 0.05$) (Table 1) and completed the HST for both trials. Participants had a weekly
265 average sleep of 7.50 ± 0.45 hrs per day and 7.20 ± 0.39 hrs prior to the first HST. No sleep
266 occurred in the 24 hrs prior to SDHST with 375 ± 50 kcals consumed overnight to balance
267 energy expenditure. Plasma concentrations of 17β -estradiol ($P = 0.48$) and progesterone
268 ($P = 0.72$) were not different across the two main HSTs and higher on day 20-22 of the self-
269 reported menstrual cycle questionnaire (Table 1). None of the experimental sessions had to be
270 withdrawn or repeated based on blood sample results.

271 **** INSERT TABLE 1 APPROXIMATELY HERE ****

272 **Perception of HRI symptoms**

273 The HISI score was significantly higher after sleep deprivation (HST 20 ± 16 vs. 28 ± 16
274 SDHST, $Z = -2.675$, $P = 0.01$) (Figure 1). The symptoms; heat sensations on the head or neck,
275 chills, stopping sweating and vomiting were not reported in either of the main trials by any of
276 the participants. Percentage increases in the SDHST vs. HST for the other nine symptoms
277 varied from 15 to 50%. The largest increases following sleep deprivation occurred in; nausea
278 (50%), lightheaded (47%) and confusion (45%). The most commonly reported two symptoms
279 for all participants reported were; feeling tired and thirst, highlighted in Figure 2.

280 **** INSERT FIGURE 1 APPROXIMATELY HERE ****

281

**** INSERT FIGURE 2 APPROXIMATELY HERE ****

282 **Physiological responses**

283 Peak T_{re} was not different ($P = 0.22$, $d = 0.05$) between SDHST ($39.35 \pm 0.33^{\circ}\text{C}$) and HST
284 ($39.40 \pm 0.35^{\circ}\text{C}$). No difference ($P=0.81$, $d = 0.1$) was found in the ΔT_{re} as displayed in Figure
285 3. There was no difference between the two HSTs for any physiological variable, except
286 average HR (HST 182 ± 7 vs. SDHST 180 ± 7 beats.min⁻¹, $d = 0.44$, $P= 0.01$) (Table 2).

287

**** INSERT TABLE 2 APPROXIMATELY HERE ****

288

**** INSERT FIGURE 3 APPROXIMATELY HERE ****

289 **Correlational analysis**

290 Spearman's correlation coefficient indicated a non-significant medium-positive trend,
291 between change in T_{re} and end HISI score ($r=0.58$, $P=0.11$). This was also the case for peak
292 T_{re} and end HISI score ($r=0.44$, $P=0.24$).

293

294 **Discussion**

295 The aim of this study was to determine if acute sleep deprivation would exacerbate the
296 symptoms associated with HRI in females. The main findings revealed that sleep deprivation
297 increased the perceptual symptoms associated with a HRI as presented by a greater HISI score,
298 in line with the aforementioned hypothesis. Contrary to our second hypothesis, there were no
299 differences in the rate of T_{re} rise following sleep deprivation. The primary variable
300 investigated in this study was the HISI scale; a novel quantitative measurement of heat related
301 illness symptoms (Coris et al., 2006). Mean HISI score increased by 30% following sleep
302 deprivation.

303 There is no existing literature assessing the HISI scale whilst exercising in the heat or sleep
304 deprived, except the original Coris et al. (2006) study, which can offer comparison. They
305 found correlations in HISI score with football training intensity, ambient temperature and fluid

306 loss as a relationship for HRI. However, Coris et al. (2006) did not correlate HISI to T_{re} which
307 might indicate the contribution core temperature has towards HISI symptoms and as a result
308 HRI. In the current study however, found a non-significant, but medium positive correlation
309 between end T_{re} ($r=0.44$) and ΔT_{re} ($r=0.58$), and HISI score; potentially highlighting an
310 association, but not a causal relationship between perceptual symptoms and physiological
311 contributors to HRI. Figure 3 highlights where the differences in symptoms of the HISI
312 occurred for the nine participants over the two HSTs; where the two most commonly reported
313 symptoms were “feeling tired” and “thirst”. It is commonly accepted that the risk of HRI is
314 directly influenced by dehydration (Coris et al., 2006). All participants were hydrated as a
315 control measure prior to the 30 minute run, and so the feeling of thirst is a perceptual indicator
316 of an enhanced risk of potential HRI. No participant reported “stopping sweating”, which is a
317 symptom primarily associated with heat stroke, an uncommon condition not reflective of mild
318 HRI, reflected in the data (Coris et al., 2006). The largest increases following sleep deprivation
319 compared to the HST were found in the symptoms nausea (50%), lightheaded (47%) and
320 confusion (45%), highlighting the presence of some level of cognitive dysfunction, which is
321 associated with heat exhaustion / stroke (Heled et al., 2004).

322 Literature surrounding the influence of sleep deprivation on T_{re} changes is equivocal (Fullagar
323 et al., 2015). The current study concludes no difference in resting or peak T_{re} , in line with
324 other literature (Fujita et al., 2003; Moore et al., 2013; Muginshtein-Simkovitch et al., 2015;
325 Oliver et al., 2009). Conversely, resting T_{re} may be lower following sleep deprivation of
326 greater durations (Sawka et al., 1984); possibly indicating that sleep deprivation of <30 hrs
327 may not be sufficient to induce alterations in thermoregulation. Mechanisms associated with
328 these alterations to thermoregulation have been proposed to be due to an altered central
329 nervous system function or changes in peripheral input (Moore et al., 2013), however findings
330 remain inconclusive.

331 Our study revealed no difference in RPE at any time point between HST and SDHST, in line
332 with other studies (Moore et al., 2013; Oliver et al., 2009). Although, previous literature
333 suggested an increased perception of effort when exercising at fixed exercise intensities

334 (Muginshtein-Simkovitch et al., 2015), a possible explanation for this discrepancy in our
335 findings is interpreted to be exercise intensity-dependent. The methodology of Muginshtein-
336 Simkovitch et al. (2015) consisted of low exercise intensity walking (5 km.hr⁻¹ at 2% gradient),
337 whereas the other two studies (Moore et al., 2013; Oliver et al., 2009) and the current study
338 required participants to run at a considerably higher exercise intensity (70% $\dot{V}O_{2max}$, self-
339 paced treadmill run and at 10 W.kg⁻¹ [77% $\dot{V}O_{2peak}$]). While thermal strain has been proposed
340 to have a direct influence on subjective feelings (Sawka et al., 1984), in the current study TS
341 did not differ between trials. These findings are in line with Moore et al. (2013) following
342 partial sleep deprivation (PSD) (6 hrs over 3 days), although it has been reported that 24 hrs
343 sleep deprivation heightened thermal comfort rating compared to PSD and non-sleep deprived
344 tests under the same heat stress (40°C, 40% RH) (Muginshtein-Simkovitch et al., 2015). This
345 highlights a potential issue with the sensitivity of the TS scale utilised in the current study, as
346 participants' peak TS was 8.0 ± 0.5 in both tests, the maximum score achieved in just 30 min
347 running. It has been previously stated that T_{skin} is the driver for TS (Schlader et al., 2011),
348 reinforced by the findings of this study which indicated no differences in exercising or peak
349 T_{skin} with no differences observed in TS. These conflicting results surrounding perception and
350 sleep deprivation have been attributed to a large variation in sleep deprivation durations,
351 exogenous factors of the experimental design (e.g. duration and intensity of exercise,
352 temperature and humidity of environment) and a vast array of effects on emotional regulation
353 (e.g. mood) following sleep deprivation (Fullagar et al., 2015).

354 Previous literature has suggested sleep deprivation (33 hrs) decreases sudomotor function (-
355 27% sweat rate) (Sawka et al., 1984) induced by a reduction in reflex cutaneous vasodilation
356 and peripheral blood flow (Kolka & Stephenson, 1988). An explanation of this alteration is
357 due to participants exercising at relative exercise intensities evoking different heat productions
358 and evaporative heat loss requirements as a consequence of the experimental protocol (Cramer
359 & Jay, 2014). In contrast, there were no difference in whole body sweat rate in the current
360 study (Table 2), similar to the findings by Moore et al. (2013), who demonstrated PSD to have
361 no effect on sweat rate (1.30 ± 0.41 vs. 1.26 ± 0.4 L.hr⁻¹ [PSD]). Hom et al. (2012) reported

362 an increased sweat rate after 28 hrs sleep deprivation, although, this followed 10 days heat
363 acclimation, where improved sudomotor function is likely attributed to heat adaptation not
364 sleep deprivation. Sudomotor responses are primarily initiated by increased T_{re} and T_{skin}
365 (Kolka & Stephenson, 1988), though human abdominal receptors may also be relevant (Morris
366 et al., 2016) and contribute to the afferent neural signals integrated at the hypothalamus
367 (Shibasaki et al., 2006). T_{re} and T_{skin} did not differ between conditions and as expected, no
368 difference in sweat rate occurred (Table 2). In light of this, controlling for the factors that alter
369 thermoregulatory responses in this study (e.g. circadian rhythm, hydration status, \dot{H}_{prod} ,
370 menstrual cycle) (Sawka et al., 2007), it is suggested sleep deprivation does not alter
371 sudomotor function during an acute bout of exercise-heat stress in females.

372 It has been proposed that sleep deprivation may compromise cardiovascular regulation,
373 primarily associated with a reduced sympathetic activity, however, there is also research that
374 has reported HR to decrease or be unchanged following sleep deprivation (Oliver et al., 2009;
375 Sawka et al., 1984). The current study found a significantly reduced exercising HR following
376 SDHST (-2 ± 6 beats.min⁻¹, $P=0.01$). However, other studies have reported larger, more
377 meaningful reductions (Muginshtein-Simkovitch et al., 2015; Vaara et al., 2009). This is
378 emphasised by only a small effect found in the current study for this 2 beats.min⁻¹ reduction
379 ($d = 0.44$). A downregulated sympathetic cardiac autonomic activity, increased vagal outflow
380 after 30 and 60 hrs sleep deprivation has been shown (Vaara et al., 2009), while HR is reported
381 to reduce with chronic sleep deprivation, shorter acute periods do not induce meaningful
382 cardiovascular reductions.

383

384 **Limitations and future recommendations**

385 As sleep was evaluated using self-reported diaries (Carney et al., 2012), it is recommended
386 these are validated alongside a quantitative method for analysing sleep data (e.g. actigraphs),
387 as seen in previous literature (Muginshtein-Simkovitch et al., 2015). Results from this study
388 follow the controls aforementioned and are constrained to females in the follicular phase of

389 the menstrual cycle (Stachenfeld & Taylor, 2014), reinforced in Table 1. During the luteal
390 phase progesterone concentrations are elevated ($\sim 10 \text{ ng.mL}^{-1}$) increasing resting T_{re} by ~ 0.3 -
391 0.6°C , onset threshold for cutaneous vasodilation by 0.2 - 0.3°C and sweating threshold by
392 0.3°C (Pivarnik et al., 1992,. It would therefore, be of interest to conduct testing in the luteal
393 phase, to offer comparison and investigate how the different phases of the menstrual cycle
394 may affect how females respond in the heat when sleep deprived. As highlighted by Coris et
395 al. (2006) the main limiting factor was that HISI scores were not correlated to a physiological
396 measure. It is reported in the literature a higher T_{re} to contribute to HRI and **to be** associated
397 with more extreme heat illnesses (e.g. heat stroke) (Moran et al., 2004). Therefore, assuming
398 this correlation exists, a higher T_{re} should ensure a higher reported HISI score, however
399 empirical evidence is still required. As such, future research allied to the HISI should focus
400 on identifying the association of symptom with T_{re} and adjust the index accordingly. The
401 highest score reached was 58, under half of the potential maximum (130), where the
402 participants were reaching near maximal HR ($\geq 180 \text{ beats.min}^{-1}$) and high T_{re} ($\geq 39.2^\circ\text{C}$).
403 Therefore, the validity and sensitivity of the HISI requires further examination during high
404 intensity exercise, passive heat exposures and long term interventions (e.g. heat acclimation).
405 Further multidisciplinary research is required to determine how acute, intermittent and
406 prolonged sleep deprivation disrupts cognition and how it may alter aerobic or occupational
407 performance under heat stress, especially for athletic or military individuals where perception,
408 pacing and decision making is critical.

409

410 **Conclusion**

411 This is the first study investigating acute sleep deprivation, while controlling for individual
412 alterations to a stressor accurately through \dot{H}_{prod} , under uncompensable heat stress. It was
413 reported that 24 hrs sleep deprivation increased the perception of symptoms related to HRI,
414 but had no effect on thermoregulatory function. These novel findings emphasise that contrary
415 to previous literature, younger (< 30 years) female athletes, occupational workers or military

416 personnel, who experience an acute bout of 24 hrs sleep deprivation during shift work or
417 traveling to a hot climate, will not incur an enhanced physiological strain during high intensity
418 exercise.

419

420 **Acknowledgements**

421 The author would like to thank the volunteers for their participation in this investigation, as
422 well as Aimee Jones, Claire Carroll and Emily Watkins for their assistance in data collection.

423

424

425

426 **Conflict of Interest**

427 The authors declare that they have no competing interests such as funding or personal financial
428 interest.

429 **References**

430

431 Arciero, P. J., Goran, M. I., & Poehlman, E. T. (1993). Resting metabolic rate is lower in
432 women than in men. *Journal of Applied Physiology*, 75(6), 2514-2520

433 Belenky, G., Wesensten, N. J., Thorne, D. R., Thomas, M. L., Sing, H. C., Redmond, D. P., .
434 . . Balkin, T. J. (2003). Patterns of performance degradation and restoration during sleep
435 restriction and subsequent recovery: a sleep dose-response study. *Journal of Sleep Research*,
436 12(1), 1-12

437 Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sports*
438 *Exercise*, 14(5), 377-381

439 Carney, C. E., Buysse, D. J., Ancoli-Israel, S., Edinger, J. D., Krystal, A. D., Lichstein, K. L.,
440 & Morin, C. M. (2012). The consensus sleep diary: standardizing prospective sleep self-
441 monitoring. *Sleep*, 35(2), 287-302. doi: 10.5665/sleep.1642

442 Cohens, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale, NJ:
443 Lawrence Erlbaum Associates.

444

- 445 Coris, E. E., Ramirez, A. M., & Van Durme, D. J. (2004). Heat illness in athletes: the
446 dangerous combination of heat, humidity and exercise. *Sports Medicine*, 34(1), 9-16
- 447 Coris, E. E., Walz, S. M., Duncanson, R., Ramirez, A. M., & Roetzheim, R. G. (2006). Heat
448 illness symptom index (HISI): a novel instrument for the assessment of heat illness in athletes.
449 *Southern Medical Journal*, 99(4), 340-345. doi: 10.1097/01.smj.0000209285.96906.0f
- 450 Cramer, M. N., & Jay, O. (2014). Selecting the correct exercise intensity for unbiased
451 comparisons of thermoregulatory responses between groups of different mass and surface
452 area. *Journal of Applied Physiology*, 116(9), 1123-1132. doi:
453 10.1152/jappphysiol.01312.2013
- 454 Fujita, M., Lee, D., Ismail, M. S., & Tochihara, Y. (2003). Seasonal effects of sleep
455 deprivation on thermoregulatory responses in a hot environment. *Journal of Physiology*
456 *Anthropology Applied Human Science*, 22(6), 273-278
- 457 Fullagar, H. H., Skorski, S., Duffield, R., Hammes, D., Coutts, A. J., & Meyer, T. (2015).
458 Sleep and athletic performance: the effects of sleep loss on exercise performance, and
459 physiological and cognitive responses to exercise. *Sports Medicine*, 45(2), 161-186. doi:
460 10.1007/s40279-014-0260-0
- 461 Galloway, S. D., & Maughan, R. J. (1997). Effects of ambient temperature on the capacity to
462 perform prolonged cycle exercise in man. *Medicine & Science in Sports & Exercise*, 29(9),
463 1240-1249
- 464 Garcia-Garcia, F., Juarez-Aguilar, E., Santiago-Garcia, J., & Cardinali, D. P. (2014). Ghrelin
465 and its interactions with growth hormone, leptin and orexins: Implications for the sleep-wake
466 cycle and metabolism. *Sleep medicine reviews*, 18(1), 89-97
- 467 Heled, Y., Rav-Acha, M., Shani, Y., Epstein, Y., & Moran, D. S. (2004). The “golden hour”
468 for heatstroke treatment. *Military medicine*, 169(3), 184-186
- 469 Hom, L. L., Lee, E. C., Apicella, J. M., Wallace, S. D., Emmanuel, H., Klau, J. F., . . . Maresh,
470 C. M. (2012). Eleven days of moderate exercise and heat exposure induces acclimation
471 without significant HSP70 and apoptosis responses of lymphocytes in college-aged males.
472 *Cell Stress Chaperones*, 17(1), 29-39. doi: 10.1007/s12192-011-0283-5
- 473 Horne, J. A., & Reyner, L. A. (1995). Sleep related vehicle accidents. *British Medical Journal*,
474 310(6979), 565-567
- 475 James, C. A., Richardson, A. J., Watt, P. W., & Maxwell, N. S. (2014). Reliability and validity
476 of skin temperature measurement by telemetry thermistors and a thermal camera during
477 exercise in the heat. *Journal of Thermal Biology*, 45, 141-149. doi:
478 10.1016/j.jtherbio.2014.08.010
- 479 Jay, O., Bain, A. R., Deren, T. M., Sacheli, M., & Cramer, M. N. (2011). Large differences in
480 peak oxygen uptake do not independently alter changes in core temperature and sweating
481 during exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative*
482 *Physiology*, 301(3), R832-R841
- 483 Jones, A. M., & Doust, J. H. (1996). A 1% treadmill grade most accurately reflects the
484 energetic cost of outdoor running. *Journal of Sports Sciences*, 14(4), 321-327

- 485 Kolka, M. A., & Stephenson, L. A. (1988). Exercise thermoregulation after prolonged
486 wakefulness *Journal of Applied Physiology*, 64(4), 1575-1579
- 487 McDermott, B. P., Casa, D. J., YeARGIN, S. W., Ganio, M. S., Armstrong, L. E., & Maresh, C.
488 M. (2007). Recovery and return to activity following exertional heat stroke: considerations for
489 the sports medicine staff. *Journal of Sport Rehabilitation*, 16(3), 163-181
- 490 Moore, J. P., Harper Smith, A. D., Di Felice, U., & Walsh, N. P. (2013). Three nights of sleep
491 deprivation does not alter thermal strain during exercise in the heat. *European Journal of*
492 *Applied Physiology*, 113(9), 2353-2360. doi: 10.1007/s00421-013-2671-2
- 493 Moran, D. S., Heled, Y., Still, L., Laor, A., & Shapiro, Y. (2004). Assessment of heat tolerance
494 for post exertional heat stroke individuals. *Medical Science Monitor*, 10(6), Cr252-Cr257
- 495 Morris, N. B., Coombs, G., & Jay, O. (2016). Ice Slurry Ingestion Leads to a Lower Net Heat
496 Loss during Exercise in the Heat. *Medicine and Science in Sports and Exercise*, 48(1), 114-
497 122
- 498 Muginshtein-Simkovitch, E., Dagan, Y., Cohen-Zion, M., Waissengrin, B., Ketko, I., &
499 Heled, Y. (2015). Heat tolerance after total and partial acute sleep deprivation. *Chronobiology*
500 *International*, 32(5), 717-724. doi: 10.3109/07420528.2015.1030409
- 501 Nielsen, B. (1996). Olympics in Atlanta: a fight against physics. *Medicine and Science in*
502 *Sports and Exercise*, 28(6), 665-668
- 503 Nishi, Y. (1981). Measurement of thermal balance of man. *Studies in environmental science*,
504 10, 29-39
- 505 Oliver, S. J., Costa, R. J., Laing, S. J., Bilzon, J. L., & Walsh, N. P. (2009). One night of sleep
506 deprivation decreases treadmill endurance performance. *European Journal of Applied*
507 *Physiology*, 107(2), 155-161. doi: 10.1007/s00421-009-1103-9
- 508 Pivarnik, J. M., Marichal, C. J., Spillman, T., & Morrow, J. (1992). Menstrual cycle phase
509 affects temperature regulation during endurance exercise. *Journal of Applied Physiology*,
510 72(2), 543-548
- 511 Ramanathan, N. L. (1964). A New Weighting System for Mean Surface Temperature of the
512 Human Body. *Journal of Applied Physiology*, 19, 531-533
- 513 Rav-Acha, M., Hadad, E., Epstein, Y., Heled, Y., & Moran, D. S. (2004). Fatal exertional heat
514 stroke: a case series. *The American Journal of the Medical Sciences*, 328(2), 84-87
- 515 Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld,
516 N. S. (2007). American College of Sports Medicine position stand. Exercise and fluid
517 replacement. *Medicine and Science in Sports and Exercise*, 39(2), 377-390
- 518 Sawka, M. N., Gonzalez, R. R., & Pandolf, K. B. (1984). Effects of sleep deprivation on
519 thermoregulation during exercise. *American Journal of Physiology*, 246(1 Pt 2), R72-77
- 520 Schlader, Z. J., Simmons, S. E., Stannard, S. R., & Mündel, T. (2011). Skin temperature as a
521 thermal controller of exercise intensity. *European Journal of Applied Physiology*, 111(8),
522 1631-1639

- 523 Shibasaki, Wilson, T. E., & Crandall, C. G. (2006). Neural control and mechanisms of eccrine
524 sweating during heat stress and exercise. *Journal of Applied Physiology* (1985), *100*(5), 1692-
525 1701. doi: 10.1152/jappphysiol.01124.2005
- 526 Short, M. A., & Banks, S. (2014). The functional impact of sleep deprivation, sleep restriction,
527 and sleep fragmentation *Sleep Deprivation and Disease* (pp. 13-26): Springer.
- 528 Spurway, N., & Jones, A. (1997). *Guidelines for the Physiological Testing of Athletes*. Leeds,
529 UK: British Association of Sport and Exercise Sciences.
- 530 Stachenfeld, N. S., & Taylor, H. S. (2014). Challenges and methodology for testing young
531 healthy women in physiological studies. *American Journal of Physiology. Endocrinology and*
532 *Metabolism*, *306*(8), E849-853. doi: 10.1152/ajpendo.00038.2014
- 533 Toner, M. M., Drolet, L. L., & Pandolf, K. B. (1986). Perceptual and physiological responses
534 during exercise in cool and cold water. *Perceptual and Motor Skills*, *62*(1), 211-220
- 535 Vaara, J., Kyrolainen, H., Koivu, M., Tulppo, M., & Finni, T. (2009). The effect of 60-h sleep
536 deprivation on cardiovascular regulation and body temperature. *European Journal of Applied*
537 *Physiology*, *105*(3), 439-444. doi: 10.1007/s00421-008-0921-5
- 538 World Medical Association. (2013). World Medical Association Declaration of Helsinki:
539 ethical principles for medical research involving human subjects. *Journal of the American*
540 *Medical Association*, *310*(20), 2191.

541
542
543
544
545
546
547

548 **Figure and Table legends**

549

550 **Figure 1.** Heat illness symptom index (HISI) scores for the heat stress test (HST) and sleep
551 deprived HST (SDHST) for each individual participant. Mean and SD also represented for
552 HST and SDHST.

553

554 **Figure 2.** Each heat illness symptom index (HISI) symptom reported for all participants
555 comparing both heat stress tests (mean \pm SD).

556

557 **Figure 3.** The time course of core temperature [T_{re}] ($^{\circ}$ C) during both heat stress tests; HST
558 and SDHST. Data presented in mean \pm SD.

559

560 **Table 1.** Participants resting characteristics before main heat stress tests (mean \pm SD).

561

562 **Table 2.** Peak and average values represented as mean \pm SD across both heat stress tests
563 (HST), where * indicates statistical significance between tests.