Effect of Permissive Dehydration on Induction and Decay of Heat Acclimation, and Temperate Exercise Performance.

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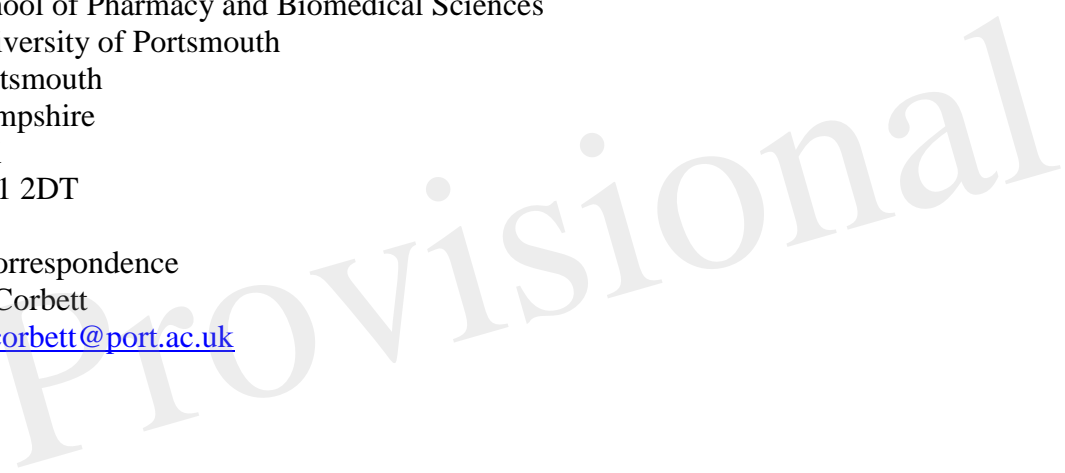
30 **Abstract**
31 **Purpose:** It has been suggested that dehydration is an independent stimulus for heat
32 acclimation (HA), possibly through influencing fluid-regulation mechanisms and increasing
33 plasma volume (PV) expansion. There is also some evidence that HA may be ergogenic in
34 temperate conditions and that this may be linked to PV expansion. We investigated: i) the
35 influence of dehydration on the time-course of acquisition and decay of HA; ii) whether
36 dehydration augmented any ergogenic benefits in temperate conditions, particularly those
37 related to PV expansion. **Methods:** Eight males (VO_{2max}: 56.9(7.2) mL·kg^{-1}·min^{-1}) undertook
38 two HA programmes (balanced cross-over design), once drinking to maintain euhydration
39 (HA_{Eu}) and once with restricted fluid-intake (HA_{De}). Days 1, 6, 11 and 18 were 60 min exercise-
40 heat stress tests (HST [40°C; 50%RH]), days 2-5 and 7-10 were 90 min, isothermal-strain
41 (T_{re}~38.5°C), exercise-heat sessions. Performance parameters (VO_{2max}, lactate threshold,
42 efficiency, peak power output [PPO]) were determined pre and post HA by graded exercise test
43 (22°C; 55 %RH). **Results:** During isothermal-strain sessions hypohydration was achieved in
44 HA_{De} and euhydration maintained in HA_{Eu} (average body mass loss -2.71(0.82)% *vs*. -
45 0.56(0.73)%, $P<0.001$), but aldosterone concentration, power output and cardiovascular strain
46 were unaffected by dehydration. HA was evident on day 6 (reduced end-exercise T_{re} [-
47 0.30°C(0.27)] and exercise heart rate [-12(15) beats.min^{-1}], increased PV [+7.2(6.4)%] and
48 sweat-loss [+0.25(0.22) L.hr^{-1}], $P<0.05$) with some further adaptations on day 11 (further
49 reduced end-exercise T_{re} [-0.25(0.19)°C] and exercise heart rate [-3(9) beats.min^{-1}], $P<0.05$).
50 These adaptations were not notably affected by dehydration and were generally maintained 7-
51 days post HA. Performance parameters were unchanged, apart from increased PPO (+16(20)
52 W, irrespective of condition). **Conclusions:** When thermal-strain is matched, permissive
53 dehydration which induces a mild, transient, hypohydration does not affect the acquisition and
54 decay of HA, or endurance performance parameters. Irrespective of hydration, trained
55 individuals require >5 days to optimise HA.
56
57 **Key words:** thermoregulation, fluid, acclimatization, hydration, hypohydration

1 Introduction

The heat acclimated phenotype has been extensively described (*e.g.* Armstrong and Maresh, 1991; Périard et al., 2015) and is characterised by adaptations enabling an individual to better accommodate a given thermal-stressor. Typically, heat acclimation (HA) is acquired by frequently and repeatedly elevating both core (T_C) and skin (T_{sk}) temperature (Regan et al., 1996) to a level challenging sudomotor and vasomotor thermoeffector responses for a sufficient duration (Fox et al., 1963). Although passive approaches have sometimes been employed (Beaudin et al., 2009), the increased thermal strain is often achieved through a combination of environmental heat-stress and increased metabolic heat-production through exercise (*e.g.* Lorenzo et al., 2010; Gibson et al., 2014: Gibson et al., 2015; Keiser et al., 2015). More recently, it has been suggested that dehydration, the process of losing fluid and achieving a state of hypohydration (lower-than-normal body water volume), may also represent an important stimulus for facilitating HA (Garrett et al., 2012; Garrett et al., 2014; Périard et al., 2015; Ackerman et al., 2016), although this may be controversial (Horowitz et al., 1999; Schwimmer et al., 2006) and in contrast to traditional guidelines for maintaining fluid and electrolyte balance (Armstrong and Maresh, 1991; Bergeron et al., 2012).

Dehydration through combined exercise and heat-stress causes hyperosmotic hypovolemia, reducing thermoeffector function (lower sweating and skin blood flow (Sawka, 1992)), and increasing thermal, cardiovascular and fluid-regulatory strain (Kenefick et al., 2007; Sawka, 1992). Whilst impaired thermoeffector activity might possibly be maladaptive in terms of sudomotor and vasomotor function, the resultant increased tissue-temperature is important; for T_Cs between 37.3 and 38.5°C the magnitude of HA is proportional to the thermal forcing-function (Fox et al., 1963), although increasing T_C beyond 38.5°C may not confer any additional benefit (Gibson et al., 2014; Gibson et al., 2015). Indeed, because dehydration and heat are often inter-linked in their causation and the strain they induce, demarcating their individual effects can be difficult (Ackerman et al., 2016). Recent research employing an isothermal strain (target rectal temperature (T_{re})=38.5°C) HA programme suggests that dehydration can provide a thermally-independent adaptation stimulus (Garrett et al., 2014). Restricting fluid ingestion (permissive dehydration) during the five, daily, exercise-heat exposures (90 min·day^{-1}) increased plasma aldosterone concentration ([aldo]$_p$) over the HA programme, relative to euhydration; this correlated with an increased plasma volume (PV), while increased resting forearm perfusion and reduced exercise heart rate were also observed during a subsequent heat stress test (HST). The adaptations that appear to be most affected by permissive dehydration (*e.g.* PV expansion and cardiovascular stability) are among the most rapidly acquired during HA (~4-5 days) and also the quickest to decay upon cessation of HA (Williams et al., 1967; Périard et al., 2015). It remains to be established whether permissive dehydration positively influences the adaptive responses to heat over the longer timescales (~10 days) typically necessary to optimise HA (Périard et al., 2015), or whether permissive dehydration affects the retention of the heat acclimated phenotype following HA. Evidence from rodent studies indicates that severe (10% body mass loss) acute hypohydration can adversely affect the longer-term adaptive response to heat (Horowitz et al., 1999; Schwimmer et al., 2006), although the relevance of this work to humans repeatedly dehydrating to a milder hypohydration (<3% body mass loss) over the course of HA is unclear.

The ergogenic potential of HA under more temperate conditions is currently under debate (Minson and Cotter 2016; Nybo and Lundby, 2016). Lorenzo et al. (2010) demonstrated improved exercise performance in a cool environment (13°C; 30% RH) following a 10-day exercise-heat acclimation programme (40°C; 30% RH) compared to the same training in the cool conditions, possibly related to PV expansion and its influence on VO$_{2max}$ by a Frank-

108 Starling effect. Studies also provide indication that HA elicits improvements in VO_{2max} (Sawka
109 et al., 1985; Lorenzo et al., 2010), exercise economy (Sawka et al., 1983) and lactate threshold
110 (Lorenzo et al., 2010) in temperate conditions; together these are key determinants of
111 endurance performance (Joyner and Coyle, 2008). However, many of these studies have been
112 criticised for inadequate control (Corbett et al., 2014) and this ergogenic effect has not been
113 replicated in recent experiments employing more appropriate controls (Karlsen et al., 2015;
114 Keiser et al., 2015). Moreover, the influence of PV expansion on VO_{2max} depends on the
115 balance between increased cardiac output and the haemodilution effect on O_2-carrying
116 capacity, which may be unfavourable in an already hypervolemic population. Recently, Keiser
117 et al. (2015) showed no effect of PV expansion on VO_{2max} or exercise performance among a
118 well-trained cohort, whether induced through HA, or by albumin-solution infusion, although
119 there was considerable inter-individual variation. Given that dehydration may augment the
120 hypervolemic aspect of HA (Garrett et al., 2014), understanding the resultant effects on VO_{2max}
121 and exercise performance is important, particularly as these programmes are often used by
122 athletes and individuals undertaking heavy physical work. Interestingly, there is some evidence
123 of an ergogenic effect of short-term HA programmes with permissive dehydration amongst
124 trained individuals in hot (Garrett et al., 2014) and temperate conditions (Neal et al., 2016), but
125 these studies must be interpreted cautiously due to the lack of an appropriate comparison group.
126
127 Accordingly, the primary aim of this study was to investigate the influence of permissive
128 dehydration on the time-course and magnitude of the acquisition and decay of HA over a short-
129 and longer-term, using a matched thermal strain HA programme. An ancillary aim was to
130 investigate the ergogenic potential of HA and specifically to examine whether permissive
131 dehydration augmented any ergogenic effects of HA, particularly those effects related to PV
132 expansion.
133
2 Method
2.1 Participants
136 Eight trained male athletes participated in this study which was approved by the University's
137 Ethics Committee (Mean(SD) age: 21(3) years; height: 1.81(0.05) m; mass: 77.31(4.88) kg;
138 body fat: 10.0(3.5)%; VO_{2max}: 56.9(7.2) mL·kg^{-1}·min^{-1}; peak power output (PPO): 338(46) W).
139 This sample size is consistent with previous work in this area that has identified between-
140 conditions differences in key thermo-physiological indices (Garrett et al., 2014). Participants
141 were all engaged in recreational endurance exercise (running, cycling, triathlon). All
142 participants provided written informed consent.
143
2.2 Experimental design
145 A within-participant, balanced cross-over design was employed, with participants undertaking
146 both control (euhydrated heat acclimation [HA_{Eu}]) and intervention (permissive dehydration
147 [HA_{De}]) HA programmes (target ambient conditions: 40°C; 50% RH). Each HA programme
148 lasted 11-days and consisted of three bouts of exercise at a fixed external work rate (heat stress
149 test [HST]), undertaken on day 1 (HST_{pre}), day 6 (HST_{mid}) and day 11 (HST_{post}), interspersed
150 with eight isothermal heat strain exercise-heat exposures (ISO). The ISO approach was used to
151 induce HA so as to avoid the potential for a dehydration-induced elevation in T_{re}, which would
152 provide an additional thermal stimulus for adaptation and the HSTs enabled assessment of the
153 induction of short- and longer-term adaptations. A temperate (target ambient conditions: 20°C;
154 55% RH) graded exercise test (GXT) was completed before (GXT_{pre}) and after (GXT_{post}) HA
155 for assessment of performance parameters and thermoregulatory responses during temperate
156 exercise. To obtain an index of decay the HST was repeated one week after the HA programme
157 (HST_{decay}). HA programmes were identical apart from the fluid consumption during ISO, where

158 a regimen was prescribed to either maintain hydration or facilitate dehydration. A minimum
159 three-month wash-out period was prescribed between HA programmes (see figure 1). All
160 testing was completed in the UK winter months (November-February) with an average ambient
161 temperature of 2°C during the data collection periods. The average temperature in the three
162 months preceding the data collection period was 8°C.
163
164 ************Insert figure 1 near here***
165
166 **2.3 Experimental procedures**
167 **2.3.1 Heat stress test**
168 Participants cycled in the hot environment on a calibrated CompuTrainer cycle ergometer
169 (RacerMate Inc., Seattle, Washington, USA) for 60 minutes at 35% of PPO reached in the GXT
170 (described subsequently). 1.25 L of 3.6% carbohydrate solution (drink temperature 20°C) was
171 ingested to replace fluid losses, divided into five equal boluses (0.25 L) and consumed
172 immediately prior to commencing exercise and every 15 minutes thereafter. Convective
173 cooling was provided at a rate of 3.5 m·s^{-1}; this prevented most participants from reaching the
174 T_{re} withdrawal criteria of 40°C, whilst maintaining an acceptably high mean skin temperature
175 ($\bar{T}_{sk}$) and allowing thermoeffector responses to be assessed.
176
177 **2.3.2 Isothermal heat strain sessions**
178 During each ISO participants exercised in the hot environment on a calibrated CompuTrainer
179 cycle ergometer (RacerMate Inc., Seattle, WA, USA), initially selecting a work rate eliciting a
180 rating of perceived exertion (RPE (Borg, 1982)) of 15. This was maintained until T_{re}=38.3°C,
181 at which point external power output was adjusted as appropriate to maintain the target T_{re}
182 (38.5°C) and a small amount of convective cooling (~2-3 m·s^{-1}) was used to facilitate the
183 exercise component and provide some perceptual benefit whilst maintaining a high $\bar{T}_{sk}$. During
184 HA$_{Eu}$ participants consumed 1.75 L of 3.6 % carbohydrate-electrolyte fluid (Science In Sport,
185 Nelson, UK) in 0.25 L boluses every 15 minutes (drink temperature 20 °C), including
186 immediately prior to and at the end of each ISO. After the exercise, participants were
187 encouraged to drink *ad libitum* to ensure similar hydration for the following days. Permissive
188 dehydration is defined as purposefully allowing a person to dehydrate through restricting fluid
189 intake (Garrett et al., 2014); during HA$_{De}$ no fluid consumption was permitted during each ISO,
190 or for 10 minutes after. Thereafter, participants consumed 1.75 L of the aforementioned
191 beverage and were subsequently encouraged to drink *ad libitum* to ensure adequate hydration
192 on arrival the following day. The drinking regimens that we employed were used in a previous
193 study where a clear separation of hydration state was achieved and an influence of permissive
194 dehydration on (short-term) HA was demonstrated (Garrett et al., 2014).
195
196 **2.3.4 Graded exercise test**
197 All GXTs were performed on a Lode Excalibur cycle ergometer (Lode, Groningen, The
198 Netherlands) in a temperate environment. Participants exercised for 20 minutes at 85 or 110
199 W, dependent upon the estimated fitness of the participant (fixed within-participant).
200 Thereafter, work-rate was incremented by 25 W every 3 minutes until blood lactate
201 concentration [Lac] was ≥4 mmol·L^{-1}, following which, the participant was given a five minute
202 break before beginning cycling again at 100 W for five minutes. Work-rate was then increased
203 25 W·min^{-1} until volitional exhaustion. [Lac] was determined from fingertip capillary blood
204 obtained at the end of each exercise stage (Biosen C-line, EKF Diagnostic, Cardiff, UK).
205 Convective cooling was provided at a rate of 3.5 m·s^{-1}.
206
207 **2.4 General procedures**

208 Participants wore the same clothes (cycling shorts, shoes, socks) on each day, abstained from
209 alcohol throughout the experimental period or caffeine for 12 hours before exercise, consumed
210 a similar diet before each test and drank 0.5 L of water two hours before every attendance.
211 Participants were instructed to maintain their normal high-intensity training (except 24 hours
212 before HSTs or GXTs) and replace an equivalent duration of low/moderate training with that
213 completed in the laboratory to maintain usual training volume; this was reiterated throughout
214 the study and verbally verified.
215
216 To ensure similar hydration before HSTs and to ascertain the extent to which participants were
217 able to maintain hydration status across the course of each HA regime, urine osmolality was
218 assessed from daily pre-exercise urine samples (Osmometer 3320, Advanced Instruments Inc.,
219 Norwood, MA, USA). This equipment was also used to determine plasma osmolality. Nude
220 body mass (dry) was measured pre- and post- each test session (Industrial Electronic Weight
221 Indicator, Model I10, Ohaus Corporation, Parsippany, NJ, USA); body mass changes were
222 used to determine whole-body sweat rate (SR), adjusted for fluid ingested. Ambient conditions
223 were measured by a WBGT logger (Squirrel 1000, Grant Instruments, Cambridge, UK), T_{re} by
224 a thermistor (Grant Instruments, Cambridge, UK) self-inserted 15 cm beyond the anal sphincter
225 and cardiac frequency (f_c) by short range telemetry (Polar RS800, Polar Electro, Kempele,
226 Finland). During HSTs and GXTs, skin temperature (T_{sk}) was measured using thermistors on
227 the chest, biceps, thigh and calf (Grant Instruments, Cambridge, UK) and local SR (upper-right
228 back [Q-Sweat, WR Medical Electronics, Maplewood, MN, USA]) and forearm skin blood
229 flow (MoorLAB, Moor Instruments, Devon, UK) were recorded. During HSTs expired gases
230 (Douglas bag method), RPE (Borg, 1982), thermal sensation and thermal comfort (Zhang,
231 2003) were measured at 15 minute intervals; a sample of sweat was collected using a custom
232 patch constructed from Parafilm® (Bemis NA, Neenah, WI, USA) for determining sodium
233 concentration [Na^+] by flame photometry (Corning 400, Essex, UK). During GXTs VO_2 was
234 measured breath-by-breath throughout (Quark B2, COSMED, Rome, Italy).
235
236 Immediately before and after HSTs and ISO 1 a 10 mL venous blood samples was obtained
237 (K2 EDTA blood collection tubes, Beckton Dickson & Company, Plymouth, UK) from the
238 antecubital vein following 10 minutes of seated rest for the measurement of haemoglobin
239 concentration [Hb] (201^+ HemoCue, Sweden) and haematocrit (Hct) (Hawksley, Lancing,
240 UK). Whole blood samples were centrifuged (1500 g for 15 minutes at 4°C, Heraeus™
241 Multifuge™ 3 S-R, Thermo Electron Corporation, Germany) and the resultant plasma stored
242 at -80°C for subsequent biochemical analyses using enzyme linked immunosorbent assays for
243 [aldo]$_p$ (ELISA Kit #ADI-900-173, Enzo Life Sciences, Exeter, UK) and extracellular heat
244 shock protein 70 concentration (e[HSP70])(Amp'd® HSP70 High Sensitivity ELISA Kit
245 #ENZ-KIT-101, Enzo Life Sciences, Exeter, UK).
246
247 **2.5 Data analysis**
248 Mean skin temperature was calculated according to Ramanathan (1964) and mean body
249 temperature ($\overline{T}_b$) as the weighted mean of T_{re} and $\overline{T}_{Sk}$ according to Parsons (1993). For GXT
250 data the lactate threshold was defined as the power output at [Lac] of 4 mmol·L^{-1}, gross
251 mechanical efficiency (GME) was calculated at 185 W (highest work rate below lactate
252 threshold achieved by all participants), and VO_2max was defined as the highest 15 s VO_2.
253 Physiological strain index (PSI) was determined according to Moran et al. (1998) and plasma
254 and blood volume shifts were determined according to Dill and Costill (1974). Metabolic heat
255 production (MHP) was determined as:
256 MHP (W·m^{-2}) = MHP/BSA
257 MHP (W) = (heat production × 1000)/60

258   heat production (kcal) = ((100 – GME/100) × energy input.
259   BSA = body surface area (m^2)
260   GME = Gross mechanical efficiency (%).
261
262   **2.6 Statistical analysis**
263   Statistical analyses were undertaken using SPSS (IBM Version. 22, IBM, New York, NY,
264   USA). Significance was set at $P \leq 0.05$; data are presented as mean(SD) unless otherwise stated.
265   Following tests for normality, two-way repeated measures ANOVA was used to analyse the
266   main effects, *i.e.* changes in responses over time and between condition (HA_{Eu} *vs*. HA_{De}), as
267   well as the interaction effect (*i.e.* time × condition). The Greenhouse-Geisser statistic was
268   employed to account for violations of sphericity; Bonferroni adjusted Students *t*-tests were used
269   *post-hoc* for analysis of main and interaction effects. *Post-hoc* analysis of significant time
270   effects for ISO sessions were made relative to ISO1 only, with alpha adjusted accordingly. The
271   Wilcoxon sign ranked test was used to analyse ordinal (RPE) data. Relationships between the
272   change in PPO and thermoregulatory parameters were assessed by Pearson's correlation
273   coefficient.
274
275   **3 Results**
276   **3.1 Isothermal heat strain sessions**
277   Ambient conditions for ISO sessions were 39.3(0.5)°C, 56.2(5.1)% RH. All participants
278   completed each ISO, in both conditions, with the daily exercise responses to each HA
279   programme summarised in table 1. A main effect for the influence of condition on mean session
280   body weight loss indicted that hypohydration was achieved in HA_{De} and euhydration
281   maintained in HA_{Eu} (body mass loss -2.71(0.82)% *vs*. -0.56(0.73)%, $P < 0.001$). This effect was
282   supported by the plasma osmolality changes within ISO1 whereby a significant condition
283   ($P = 0.013$) and interaction effect were evident ($P = 0.016$), with *post-hoc* analysis indicating that
284   plasma osmolality did not differ between conditions at baseline and was unchanged over HA_{Eu}
285   (Pre=290(4) *vs*. Post=287(4) mOsmo·kg^{-1}), but increased over the course of the ISO session
286   for HA_{De} (Pre=293 (5) *vs*. Post=297(7) mOsmo·kg^{-1}, $P = 0.006$). Aldosterone concentration
287   increased over ISO1 ($P = 0.001$), but the extent of any increase was not different between
288   conditions and there was no interaction effect (HA_{Eu} Pre=2651(2700) *vs*. Post=5859(4044)
289   pmol·L^{-1}; HA_{De} Pre=2686(2496) *vs*. Post=7741(4763) pmol·L^{-1}).
290
291   Over the course of each HA programme the time to reach the target T_{re} did not differ between
292   conditions and the same elevated average T_{re} was maintained over the final 60 minutes of each
293   session. Average power over the ISO sessions increased, but to a similar extent in both
294   conditions; *post-hoc* analysis identified significant increases from the first day (ISO1) to the
295   final day (ISO 8). Conversely, f_C reduced over time, particularly at ISO3, but again, this did
296   not differ between conditions. Whole-body SR was augmented with HA irrespective of
297   condition, with *post-hoc* comparisons to the initial ISO session indicating that this occurred
298   from ISO4 onwards. Participants managed to maintain a stable pre-exercise body mass and
299   urine osmolality over the course of the intervention, in both conditions, despite an increased
300   sweat rate and temporary hypohydration during HA_{De}.
301
302   *********************Insert table 1 near here*********************************
303
304   **3.2 Heat acclimation**
305   The ambient conditions (39.4(0.3)°C, 52.8(2.8)% RH) and the external work rate (Mean
306   122(14) W) were the same across all HSTs. The thermophysiological, metabolic, biochemical
307   and perceptual changes over the course of each HA programme, as measured during the HSTs,

308 are summarised in Table 2 (supplementary material), with select thermophysiological
309 adaptations shown in figure 2. A number of main effects for time were identified, with *post-*
310 *hoc* analysis showing that some HA was evident by HST_{mid}, as indicated by significantly
311 reduced thermal strain at rest and during exercise, lower exercise cardiovascular strain,
312 increased whole-body SR and increased blood volume and PV. However improved thermal
313 comfort and sensation and reduced PSI were only becoming evident at HST_{post} and there were
314 further improvements in a number of thermal parameters from HST_{mid} to HST_{post}. These
315 adaptations were well maintained during the decay period with no significant changes in any
316 parameter from HST_{post} to HST_{decay}, with the exception of a reduced whole-body SR and RER,
317 whereas MHP was reduced relative to HST_{pre} and suggests improved metabolic efficiency,
318 given that external work rate was unchanged. Plasma aldosterone concentration was not
319 assessed during HST_{decay} but a time effect was evident over the time points assessed (P=0.048).
320 Although the location of this effect could not be identified *post-hoc*, numerically, $[aldo]_p$
321 increased over the HA programme, but this did not differ between conditions and there was no
322 interaction effect.
323
324 The only significant differences between HA conditions was for Δ blood volume, which was
325 lower in HA_{De}, and also demonstrated a significant time × condition interaction. Although the
326 location of any differences could not be located *post-hoc,* there was a trend for a between-
327 conditions difference in HST_{decay} (P=0.06). An interaction effect was also noted for Δ plasma
328 volume, but again, the location of any differences could not be located *post-hoc*, although
329 numerically, the greatest difference between conditions was also in the decay period.
330
331 *********************Insert figure 2 near here*********************************
332
3.3 Temperate exercise
334 Ambient conditions for the GXT were 22.0(0.2)°C, 54.6(5)% RH. Both of the heat acclimation
335 programmes reduced the thermo-physiological burden under temperate conditions, as
336 evidenced by a significant time effect (GXT_{pre} *vs*. GXT_{post}) for resting and exercise T_{re} and
337 heart rate, end exercise $\bar{T}_b$ (all reduced), and skin blood flow (increased). The only significant
338 condition effect was for RER, which was higher in HA_{Eu} than HA_{De}, but there were no
339 significant interaction effects (see table 3 [supplementary material]). With regard to parameters
340 related to endurance performance, there were no significant main effects for time or condition,
341 or the time × condition interaction for VO_{2max}, lactate threshold or GME (see figure 3). There
342 was a significant main effect of time on PPO achieved during the GXT (P=0.033), but the
343 condition and interaction effects were not significant (see figure 3) and the increase in PPO
344 was not correlated with any of the improvements in thermoregulatory function. Likewise,
345 maximum heart rate (f_{Cmax}) reached in the GXT was significantly reduced following HA (from
346 187(7)b·min^{-1} to 183(7) beats·min^{-1} in HA_{Eu} and from 189(10) to 181(9) beats·min^{-1} in HA_{De},
347 P=0.003) but, the condition and interaction effects were, again, not significant.
348
349 *********************Insert figure 3 near here*********************************
350
4 Discussion
352 The main findings of the present study were: i) there was substantial evidence of adaptation to
353 heat over both the short- and longer-term phases of the present study, but when thermal strain
354 is matched, the time course and magnitude of the acquisition and decay of HA are largely
355 unaffected by permissive dehydration, compared to maintaining euhydration; ii) permissive
356 dehydration did not notably influence the effect of HA on key parameters related to endurance

357 performance (VO_{2max}, LT, GME) and although there was a small ergogenic effect (4.6(5.8)%
358 increased PPO), this was not affected by the drinking regimen.
359
360 Our primary finding does not support the suggestion that dehydration provides an additional
361 stimulus for the induction of HA (Garrett et al., 2012; Garrett et al., 2014; Périard et al., 2015;
362 Ackerman et al., 2016). The data from the short-term phase are somewhat at odds with recent
363 work indicating that dehydrating during 90 minute daily exercise-heat stress within a 5-day
364 isothermal HA programme facilitated some aspects of HA (Garrett et al., 2014), but the reason
365 for these discrepant findings is unclear. Aerobic fitness reduces the strain induced by mild
366 hypohydration (Merry et al., 2010) and aerobically fit individuals require a greater stimulus to
367 challenge the fluid-regulatory processes than less fit individuals (Merry et al., 2008). However,
368 the fitness of our participants (VO_{2max} 57(7) mL·kg^{-1}·min^{-1}; PPO 338(49) W)) was comparable
369 to Garrett et al. (2014) (VO_{2max} 60(7) mL·kg^{-1}·min^{-1}; PPO 340(30) W) and greater
370 hypohydration lacks ecological validity, could impair some training adaptations (Judelson et
371 al., 2008) and, in rodents at least, might impair aspects of the genomic (Schwimmer et al.,
372 2006) and phenotypic (Horowitz et al., 1999) adaptation to heat. A more sustained stimulus
373 might be required to optimise the rebound hypervolemic response (Ackerman et al., 2016), but
374 the drinking regimes were virtually identical and earlier, rather than later, carbohydrate-
375 electrolyte fluid replacement is crucial for recovering PV following ~3% body weight loss
376 (Kovacs et al., 2002). Alternatively, because fluid consumption may need to exceed fluid losses
377 by ~50% to restore euhydration in a hypohydrated individual (Shirreffs and Maughan, 1998),
378 the *ad libitum* intake of fluid, electrolyte and protein following the permissive dehydration may
379 have been insufficient to enable any additional hypervolemic adaptation (Kay et al., 2005), but
380 this is not supported by the stable daily baseline body mass and (euhydrated) urine osmolality
381 and while there was some evidence for reduced blood volume change in HA_{De}, this appeared
382 to be during the decay, rather than induction, phase.
383
384 A clear separation of hydration state was achieved; in HA_{Eu} body mass was maintained
385 consistent with euhydration (-0.56(0.71)% body mass change); in HA_{De} body mas was reduced
386 (-2.71(0.82)% body mass change) to a degree consistent with hypohydration (Cheuvront et al.,
387 2010; Cheuvront et al., 2015) and similar to previous studies employing a HA_{De} programme (-
388 1.8 to -3.1% average body mass change (Garrett et al., 2012; Garrrett et al., 2014; Neal et al.,
389 2016). Likewise, baseline plasma osmolality was within the normative range (Cheuvront et al.,
390 2010) and was maintained in HA_{Eu}, but increased in HA_{De} to a level consistent with mild
391 dehydration (Cheuvront et al., 2010), although this was not measured in all ISO sessions.
392 Nevertheless, assuming a constant sweating rate, hypohydration (body mass change >-2%) will
393 only have been achieved for the final ~23 minutes of each ISO and maintained for a further 10
394 minute rest period before fluid consumption, which may have been insufficient to influence the
395 fluid-regulatory mechanisms that are hypothesised to be integral to any effects on HA (Garrett
396 et al., 2012; Garrett et al., 2014; Périard et al., 2015; Ackerman et al., 2016), although once
397 dehydration is achieved [aldo]$_p$ does not appear to further increase with time (Kenefick et al.,
398 2007). Nevertheless, the increased plasma osmolality in HA_{De} did not surpass the threshold 2%
399 increase in osmolality that may be obligatory for compensatory renal water conservation
400 (Cheuvront et al., 2015) and although [aldo]$_p$ was increased by the exercise-heat stress, this was
401 not affected by permissive dehydration, at least within ISO 1. Overall, given the substantial
402 similarities in study-design, the reasons for differences between Garrett et al. (2014) and the
403 short-term phase of the present study remain largely unclear. The lack of effect of permissive
404 dehydration over a longer-term HA is, perhaps, less surprising given the modest degree of
405 hypohydration induced, the minimal influence that this likely had on fluid-regulatory
406 mechanisms (Cheuvront et al., 2015), the rapid time-course over which haematological and

407 cardiovascular adaptations to heat manifest (Armstrong and Maresh, 1991; Périard et al., 2015),
408 and the isothermal strain.
409
410 Because some aspects of HA develop rapidly (Périard et al., 2015), there has been considerable
411 interest in short-term HA programmes (Garret et al., 2012; Garrett et al., 2014; Neal et al.,
412 2016), particularly for trained individuals who are typically partially heat acclimated and may
413 adapt more rapidly (Périard et al., 2015), as well as for logistical reasons. A recent meta-
414 analysis suggests there is little difference in some aspects of HA over the short and longer-time
415 scales that we studied (Tyler et al., 2106), although few of the studies included repeated
416 measures on the same participants and most employed a controlled work-rate regimen (66%),
417 rather than isothermal-exercise approach (11%), meaning that the adaptation stimulus would
418 have reduced over time. In the present study, which employed an isothermal exercise-heat
419 stress approach, significant hypervolemia, increased whole-body sweat rate and reductions in
420 indices of thermal and cardiovascular strain were evident at HST_{mid}, indicating that notable
421 adaptation was achieved within this brief timescale, as others have also demonstrated (Garret
422 et al., 2012; Garrett et al., 2014; Neal et al., 2016). For some indices, such as plasma volume
423 expansion, exercise heart rate and whole body SR, there was no further significant change
424 beyond HST_{mid}. In contrast, further reduction in thermal strain, including exercise T_{re}, $\overline{T}_{sk}$ and
425 $\overline{T}_b$, was evident from HST_{mid} to HST_{post}, whereas reduced PSI and perceptual benefits (improved
426 thermal comfort and sensation) did not manifest until HST_{post}. Taken together, this indicates
427 that the HA phenotype was not fully developed by HST_{mid}. The temporal pattern of adaptation
428 was broadly consistent with the general consensus regarding the time-course of human heat
429 acclimation, particularly with respect to the rapid accrual of plasma volume and associated
430 improvement in cardiovascular function (Armstrong and Maresh, 1991; Périard et al., 2015).
431 In contrast, sudomotor adaptations are typically regarded as being slower to develop
432 (Armstrong and Maresh, 1991; Périard et al., 2015), but in the present study whole body sweat
433 rate was unchanged beyond HST_{mid}. However, the reducing sweat [Na$^+$] will have facilitated
434 sweat evaporation and the progressive reductions in T_{re} and $\overline{T}_b$ observed in the HSTs would
435 reduce the thermoafferent sudomotor drive. Moreover, our participants displayed high initial
436 sweating rates, presumably as a consequence of frequent exposure to high endogenous thermal
437 load through their habitual training; fitter individuals have smaller scope for adaptation, but
438 tend to adapt more rapidly than less fit individuals (Périard et al., 2015) and pronounced
439 sudomotor adaption has previously been documented with short-term HA (Neal et al., 2016).
440 Resting [aldo]$_p$ also increased over the HA regimen, which is in keeping a recent meta-analysis
441 indicating a small effect of HA on resting [aldo]$_p$, (Tyler et al., 2016) but e[HSP70] was
442 unchanged following HA. The e[HSP70] response was somewhat surprising since we
443 repeatedly exceeded the proposed endogenous temperature threshold for e[HSP70] release
444 (Gibson et al., 2014), although results from meta-analysis suggests that the effect of HA on
445 e[HSP] is trivial, relative to intracellular [HSP] (Tyler et al., 2016) and basal values may be
446 unchanged during HA (Magalhães et al., 2015). Moreover, the responses could have been
447 blunted by the aerobic training habitually undertaken by our participants and the associated
448 frequent elevations in T_C, which would likely render them partially heat acclimated.
449
450 The present study also sought to investigate the extent to which any adaptation to heat was
451 maintained over a 7-day decay period, and whether this was affected by the fluid consumption
452 regimen employed during the HA. Relative to the time-course of induction, the decay in
453 adaptation following HA is poorly documented, but it is generally believed that the
454 haematological and cardiovascular adaptions are among the quickest to decay (Williams et al.,
455 1967; Périard et al., 2015); aspects of the adaptive response most likely to be affected by
456 permissive dehydration (Garrett et al., 2014). Nevertheless, the multitude of approaches used

for the induction and assessment of HA and use of limited sample sizes of varying fitness means that there is considerable variation within the published literature regarding the time course of decay of HA. For instance, Williams et al. (1967) reported that, among a group of South African miners who had undertaken a 16 day HA regimen in hot-humid conditions, adaptations in heart rate and mean sweat rate declined by ~50% within 1 week, with a 25% loss in the adaptation in T_{re}. In contrast, Pandolf et al. (1977) showed little decline in heart rate or T_{re} in fit young men up to 18 days after a 9-day dry-heat acclimation regime and Weller et al. (2007) showed little decay in T_{re} or heart rate 12 days after completing a 14 day dry-heat acclimation regimen. Indeed, it has been suggested that the retention of HA benefits is superior in aerobically fit individuals and with acclimating to dry heat (Pandolf, 1998). The results of the present study are broadly in keeping with this assertion as there was no significant decay in most of the typical indices of physiological strain HA over the 7-day decay period; although SR and RER were diminished relative to HST$_{post}$, they remained above baseline values and no differences were evident between the drinking conditions. However, these assertions should be tempered by reduced metabolic heat production evident at HST$_{post}$ (discussed subsequently), which occurred despite a fixed external work rate and would have reduced heat-loss requirements during the HST. Moreover, there was a trend for blood volume to decay to a greater extent with HA$_{De}$, but this did not notably influence indices of thermophysiological strain and should be interpreted cautiously given that it was under free-living conditions.

An ancillary aim of the present study was to investigate the ergogenic potential of HA and whether permissive dehydration augmented any ergogenic effects of HA. However, irrespective of drinking regimen, there was no effect of HA on VO$_{2max}$, LT, or GME, but given the similarity in the adaptive response to heat, the lack of between-groups differences is unsurprising. This finding is in contrast to a number of studies that have shown an effect of HA on these parameters (Sawka et al., 1983; Sawka et al., 1985; Lorenzo et al., 2010), although these studies have often lacked adequate control and often a simple training effect cannot be excluded (Corbett et al., 2014). The possibility of a training effect was reduced in the present study by the recruitment of competitive athletes, although this may have diminished the adaptation potential due to a ceiling effect, whilst the perception based prescription of work rate during the ISO session and modest hypohydration resulted in similar cardiovascular strain and training stimulus in each group. Although pronounced PV expansion was evident in both drinking conditions, there was no evidence of any change in VO$_{2max}$. This is in contrast to Lorenzo et al. (2010), who demonstrated increased VO$_{2max}$ concomitant with HA induced PV expansion, but is consistent with recent work showing no effect of HA induced PV expansion on VO$_{2max}$ (Karlsen et al., 2015; Keiser et al., 2015). The reason for these equivocal findings is not entirely clear, although in Lorenzo et al. (2010) the relative intensity of training sessions in the heat was higher than for a control group undertaking training under cool conditions and the possibility of an additional training stimulus cannot be excluded. Cardiovascular strain was matched between control and experimental groups in Keiser et al. (2015), although it may have been higher in the experimental group of Karlsen et al. (2015). Alternatively, while the effect of PV expansion on VO$_{2max}$ appears unfavourable at the population level for trained individuals, there appears to be substantial inter-individual variation (Keiser et al., 2015), possibly due to individuality in the balance between increased cardiac output and the haemodilution effect on O$_2$-carrying capacity. When pronounced inter-individual variation is combined with relatively small sample sizes, the data may not reflect population characteristics, although at the elite performance level these individual differences may be important.

Although our data from the HST indicate that the O_2 cost of exercise was diminished 1 week post exercise, this was not evident in the GME data obtained during the GXT. Because the improved economy was specific to performance in a hot environment it could simply represent the effect of reduced thermal strain. Alternatively, a move to a more efficient phenotype as has been demonstrated in rodents undergoing prolonged HA (Kodesh et al., 2011); this could explain why this effect had not developed at HST_{mid} or HST_{post}. Results from a recent meta-analysis have also concluded that there may be a small effect of HA on GME during exercise in the heat (Tyler et al., 2016), but with the exception of studies lacking appropriate control (Sawka et al., 1983), there appears to be little evidence for an effect of HA on GME in humans under temperate conditions (Karlsen et al., 2015). Nevertheless, a small ergogenic effect was apparent as indicated by a 4.6% increase in PPO achieved at the end of the GXT, irrespective of drinking condition, but the mechanisms underpinning this ergogenic effect are unclear given the lack of change in VO_{2max}, LT and GME. The effect of ambient temperature on aerobic exercise is a continuum, with an exponential performance decline at temperatures above ~10°C (Galloway and Maughan, 1997). Although it is clear that HA attenuates the performance decrement in hot environments, it has been hypothesised that the improved thermoregulatory capability with HA should also attenuate the heat-related performance decrement evident under more temperate conditions (Corbett et al., 2014). Indeed significant reductions in thermal-strain were evident in the sub-maximal exercise preceding the GXT, but none of these changes were correlated with the performance improvement, and the T_{re} at exercise termination was similar pre *vs*. post HA, and below the levels associated with impaired performance. Alternatively, we cannot exclude a simple placebo or learning effect on PPO, as we did not include a sham treatment or temperate training group; the primary purpose of the present study was to examine the influence of hydration on HA and performance, rather than the effect of HA *per se*. This assertion is strengthened by our (unpublished) observation of a similar magnitude of improvement in PPO (6.0%) for 8 trained individuals following an identical protocol to the present study, but with all ISO session undertaken with exercise at a matched RPE, under cool conditions (13°C; 60% RH).

In summary, the present study is the first to examine the influence of dehydration on short- and longer-term HA and its subsequent decay, as well as the effect of a longer-term HA regimen with permissive dehydration on key endurance performance parameters. Our data demonstrate that, when thermal strain is matched, the time course and magnitude of the acquisition and decay of HA are largely unaffected by permissive dehydration, compared to maintaining euhydration. Furthermore, neither HA regimen affected VO_{2max}, LT, or GME. PPO was increased consistent with a small ergogenic effect of HA, but this was not affected by the drinking regimen and should be interpreted cautiously in the absence of a plausible mechanism. However, it is important to note that no notable negative effects of permissive dehydration were evident either, and traditional guidance to maintain hydration during HA (Armstrong and Maresh, 1991; Bergeron et al., 2012) may be unnecessary when trained individuals commence exercise in a euhydrated state, when thermal strain is matched, and where a transient mild hypohydration is induced.

5 Contributions

RN, HM, MT, JY, and JC were involved in conceptual design, data collection, interpretation, and manuscript preparation. All authors approve the submission of this work and agree to be accountable for all aspects of the work.

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9 Figure legends

Figure 1: Experimental protocol for examining the effect of hydration on the adaptive responses to exercise in the heat. GXT = Graded Exercise Test; HST = Heat Stress Test; ISO = Isothermal strain acclimation session; Eu = Euhydration; De = Dehydration.

Figure 2: Select thermophysiological variables showing time course of heat acclimation with (HA$_{De}$: black) and without (HA$_{Eu}$: grey) permissive dehydration as determined from standard heat stress tests (HST). Data are mean(SD) and n= 8 unless otherwise stated. Panel A: End exercise rectal temperature (T_{re}); Panel B: End exercise mean skin temperature ($\bar{T}_{Sk}$); Panel C: End exercise mean heart rate (f_c); Panel D: Mean HST whole-body sweat rate (n=7). Significant *post-hoc* time effects ($P<0.05$) are denoted by superscripted letter ([a]=HST$_{pre}$ *vs*. HST$_{mid}$; [b]=HST$_{pre}$ *vs*. HST$_{post}$; [c]=HST$_{pre}$ *vs*. HST$_{decay}$; [d]=HST$_{mid}$; *vs*. HST$_{post}$; [e]=HST$_{mid}$; *vs*. HST$_{decay}$; [f]=HST$_{post}$ *vs*. HST$_{decay}$).

Figure 3: Mean(SD) results from temperate (22°C, 55% RH) graded exercise test performed Pre- and Post- heat acclimation, with (HA$_{De}$: black) and without (HA$_{Eu}$ grey) permissive dehydration (*n*=8). Panel A: Lactate Threshold; Panel B: Gross Mechanical Efficiency (GME); Panel C: Maximal Oxygen Uptake (VO$_{2max}$); Panel D: Peak Power Output (PPO) . *= Significant main effect for time ($P<0.05$)

766 **10 Tables**

767 **Table 1:** Mean(SD) daily responses during 90 min isothermal strain heat acclimation sessions, with (HA$_{De}$) and without (HA$_{Eu}$) permissive

768 dehydration (*n*=8). Significant difference=$P<0.05$. Significant *post-hoc* time effects are relative to ISO1 only and denoted by superscripted letter

769 (^{2}=ISO1 *vs*. ISO2; ^{3}=ISO1 *vs*. ISO3; ^{4}=ISO1 *vs*. ISO4 etc.).* *Post-hoc* comparisons not significant relative to ISO1.

| | ISO1 | | ISO2 | | ISO3 | | ISO4 | | ISO5 | | ISO6 | | ISO7 | | ISO8 | | | *P* value | |
	HA$_{Eu}$	HA$_{De}$	HA$_{Eu}$	HA$_{De}$	HA$_{Eu}$	HA$_{De}$	HA$_{Eu}$	HA$_{De}$	HA$_{Eu}$	HA$_{De}$	HA$_{Eu}$	HA$_{De}$	HA$_{Eu}$	HA$_{De}$	HA$_{Eu}$	HA$_{De}$	Time	Condition	Interaction
Time to 38.5°C T_{re} (min)	29(5)	31(10)	31(6)	28(7)	31(8)	28(8)	31(5)	33(7)	32(8)	29(5)	37(12)	32(6)	39(15)	36(11)	34(11)	32(7)	0.018*	0.335	0.812
Average T_{re} (final 60 min) (°C)	38.68 (0.07)	38.65 (0.18)	38.56 (0.16)	38.62 (0.09)	38.60 (0.08)	38.59 (0.20)	38.60 (0.16)	38.59 (0.08)	38.58 (0.16)	38.60 (0.12)	38.50 (0.19)	38.56 (0.11)	38.43 (0.20)	38.48 (0.11)	38.56 (0.20)	38.57 (0.10)	0.063	0.684	0.899
Average f_c (beats·min^{-1})	148 (10)	146 (13)	146 (8)	146 (11)	141 (10)	139 (10)	141 (9)	136 (7)	143 (9)	142 (9)	140 (7)	142 (9)	143 (11)	143 (10)	138 (8)	147 (10)	0.019 ^{3}	0.918	0.154
External work rate (W)	80 (19)	70 (22)	105 (19)	88 (20)	90 (22)	81 (25)	93 (18)	92 (19)	97 (26)	91 (17)	97 (28)	97 (19)	109 (28)	98 (16)	108 (29)	106 (18)	<0.001 ^{8}	0.485	0.649
Pre-exercise mass (kg)	76.8 (4.7)	75.9 (4.8)	77.3 (4.3)	76.4 (5.1)	77.4 (4.7)	76.4 (5.2)	77.4 (4.7)	76.4 (5.3)	77.5 (5.0)	76.4 (5.1)	77.3 (5.0)	76.7 (5.1)	77.1 (4.5)	76.5 (5.0)	77.3 (4.3)	76.7 (4.7)	0.186	0.263	0.800
Whole-body SR (L·hr^{-1})	1.21 (0.41)	1.18 (0.40)	1.33 (0.31)	1.27 (0.41)	1.33 (0.33)	1.25 (0.34)	1.43 (0.34)	1.29 (0.37)	1.49 (0.35)	1.42 (0.38)	1.48 (0.34)	1.46 (0.35)	1.58 (0.37)	1.56 (0.45)	1.60 (0.40)	1.57 (0.38)	<0.001 $^{4-8}$	0.229	0.066
Urine osmolality (mOsmo·kg^{-1})	329 (188)	487 (273)	277 (152)	408 (243)	325 (168)	432 (219)	420 (209)	304 (103)	294 (115)	415 (320)	348 (209)	404 (190)	337 (122)	335 (210)	249 (144)	292 (212)	0.649	0.287	0.442
Body mass loss (%)	-0.26 (0.81)	-2.35 (0.89)	-0.45 (0.69)	-2.51 (0.89)	-0.32 (0.68)	-2.46 (0.75)	-0.54 (0.70)	-2.56 (0.82)	-0.64 (0.72)	-2.80 (0.83)	-0.62 (0.71)	-2.88 (0.77)	-0.78 (0.78)	-3.04 (0.84)	-0.86 (0.82)	-3.09 (0.81)	<0.001 $_{5,7,8}$	<0.001	0.756

770 T_{re}=rectal temperature; SR=sweat rate.

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Figure 01.JPEG

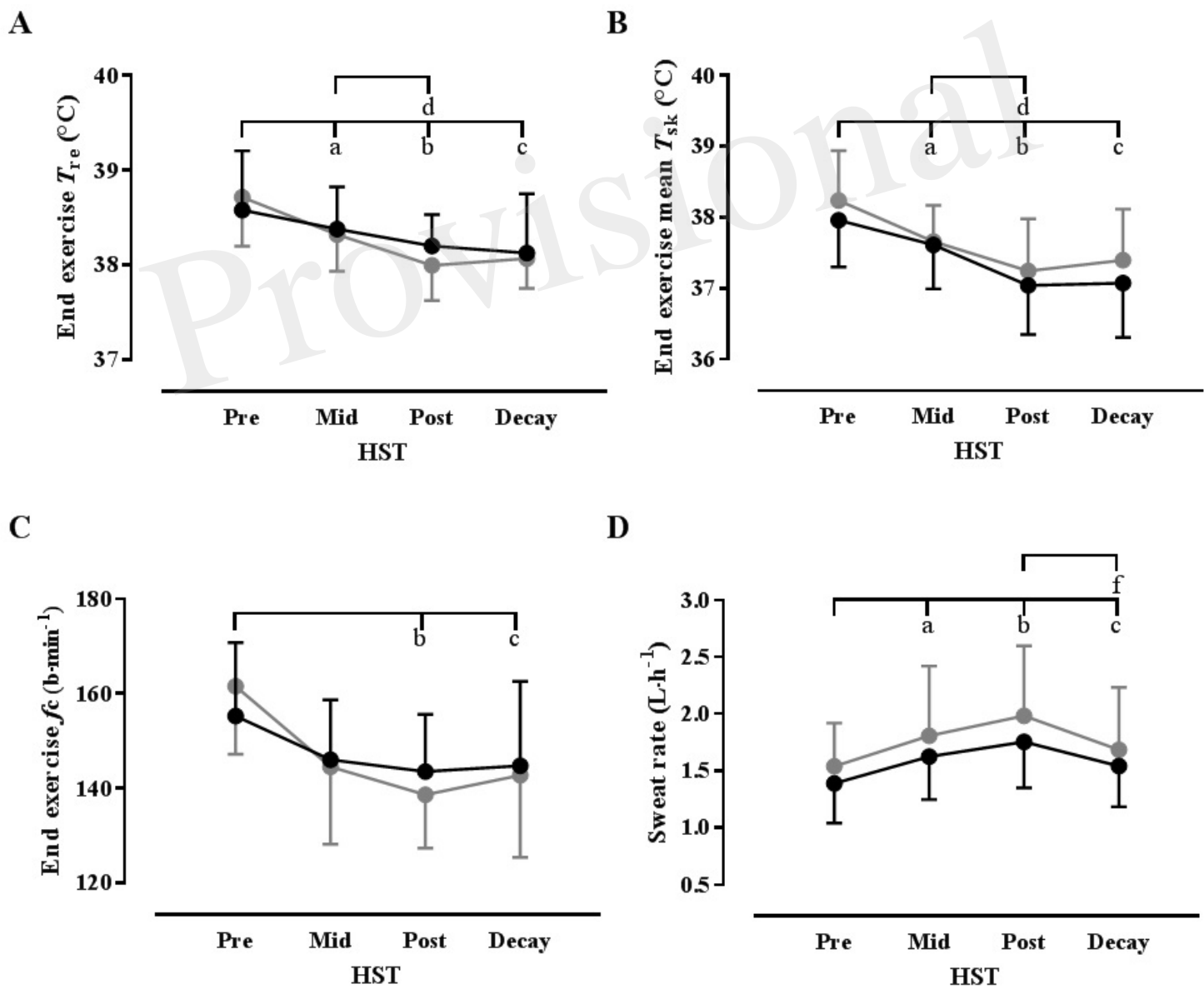| Day	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Test	GXT pre	Off	HST pre	ISO1 Eu or De	ISO2 Eu or De	ISO3 Eu or De	ISO4 Eu or De	HST mid	ISO5 Eu or De	ISO6 Eu or De	ISO7 Eu or De	ISO8 Eu or De	HST post	Off	GXT post	Off	Off	Off	Off	HST decay

Figure 02.JPEG

Figure 03.JPEG