

**Title:** Exercise heat acclimation and post-exercise hot water immersion improve resting and exercise responses to heat stress in the elderly.

**Running title:** Heat adaptation in the elderly

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**Figures:** One

**Tables:** Two

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**Key words:** Thermoregulation; Aging; Exercise; Climate Change; Heat Illness; Heat Adaptation

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16 **Abstract**

17 Objectives: To investigate the efficacy of heat acclimation (HA) in the young ( $Y_{EX}$ ) and elderly  
18 ( $E_{EX}$ ) following exercise-HA, and the elderly utilising post-exercise hot water immersion HA  
19 ( $E_{HWI}$ ).

20

21 Design: Cross-sectional study.

22

23 Method: Twenty-six participants ( $Y_{EX}$ :  $n=11$  aged  $22\pm 2$  years,  $E_{EX}$ :  $n=8$  aged  $68\pm 3$  years,  $E_{HWI}$ :  
24  $n=7$  aged  $73\pm 3$  years) completed two pre/post-tests, separated by five intervention days.  $Y_{EX}$   
25 and  $E_{EX}$  exercised in hot conditions to raise rectal temperature ( $T_{rec}$ )  $\geq 38.5^\circ\text{C}$  within 60 min,  
26 with this increase maintained for a further 60 min.  $E_{HWI}$  completed 30 min of cycling in  
27 temperate conditions, then 30 min of HWI ( $40^\circ\text{C}$ ), followed by 30 min seated blanket wrap.  
28 Pre and post-testing comprised 30 min rest, followed by 30 min of cycling exercise ( $3.5 \text{ W}\cdot\text{kg}^{-1}$   
29  $\dot{H}_{prod}$ ), and a six-minute walk test (6MWT), all in  $35^\circ\text{C}$ , 50% RH.

30

31 Results: The HA protocols did not elicit different mean heart rate (HR),  $T_{rec}$ , and duration  $T_{rec}$   
32  $\geq 38.5^\circ\text{C}$  ( $p>0.05$ ) between  $Y_{EX}$ ,  $E_{EX}$ , and  $E_{HWI}$  groups. Resting  $T_{rec}$ , peak skin temperature,  
33 systolic and mean arterial pressure, perceived exertion and thermal sensation decreased, and  
34 6MWT distance increased pre to post HA ( $p<0.05$ ), with no difference between groups.  $Y_{EX}$   
35 also demonstrated a reduction in resting HR ( $p<0.05$ ). No change was observed in peak  $T_{rec}$   
36 or HR, vascular conductance, sweat rate, or thermal comfort in any group ( $p>0.05$ ).

37

38 Conclusions: Irrespective of age or intervention, HA induced thermoregulatory, perceptual and  
39 exercise performance improvements. Both exercise-HA ( $E_{EX}$ ), and post-exercise HWI ( $E_{HWI}$ )  
40 are considered viable interventions to prepare the elderly for heat stress.

41 **Introduction**

42 A combination of an increasingly warmer global climate and sedentary behaviour leading to  
43 poor physiological conditioning, make the elderly (>65 years old) vulnerable to heat-related  
44 illness during the more frequent and longer-lasting periods of hot weather (i.e. heat waves).<sup>1-</sup>

45 <sup>3</sup> The elderly are at particular risk during heat wave due to specific age-related deteriorations  
46 in their thermoregulatory responses relative to the young. These include decreased cardiac  
47 output, a deterioration of autonomic responses and peripheral vascular responsiveness to  
48 heat stress, ultimately impairing skin blood flow (SkBF).<sup>3</sup> Furthermore, relative to the young  
49 the elderly demonstrate diminished sweat responses, eliciting increased heat storage, rises in  
50 core temperature and cardiovascular strain, and a concurrent risk of heat-related illness.<sup>3</sup> The  
51 elderly also demonstrate impaired perceptual responses to heat stress which may impair  
52 behavioural responses.<sup>4</sup> These factors contribute to multi-organ failure that leads to excess  
53 deaths in the elderly during heat waves.<sup>1,2</sup>

54

55 Heat acclimation (HA) is considered an effective strategy for mitigating against the previously  
56 stated responses to heat strain.<sup>5</sup> HA develops physiological adaptations, including decreased  
57 resting and exercise heart rate, and core and skin temperature, increased SkBF,  
58 hypervolemia, enhanced sweat sensitivity, output and efficiency.<sup>5</sup> HA also improves  
59 perceptual responses to heat, and can improve exercise capacity.<sup>6</sup> Despite HA being a  
60 prevalent strategy implemented in athletes and occupational/military personnel to mitigate  
61 heat stress,<sup>7-9</sup> and evidence that the elderly can adapt to seasonal temperature change,<sup>10</sup>  
62 elderly HA research remains limited.<sup>11-15</sup>

63

64 An experiment examining the benefits of three, one hour daily fixed intensity exercise-heat HA  
65 sessions in untrained elderly females deemed this approach insufficient (in dose) to induce  
66 adaptation.<sup>11</sup> A greater HA dose, specifically nine 90-120 min fixed intensity exercise-heat  
67 sessions, demonstrated that trained old participants made comparable adaptations to younger  
68 participants during passive heat stress.<sup>12</sup> Furthermore, whilst eight days of 90 min fixed  
69 intensity HA induced adaptation in trained young and elderly males, and untrained elderly  
70 males, sudomotor adaptations were greater in the young and trained elderly groups.<sup>13</sup> The  
71 untrained elderly cohort, whom represent the most at risk population during heat waves,  
72 demonstrating an inferior response.<sup>13</sup> This age-fitness interaction has been observed by  
73 others<sup>14</sup> who noted that temperature and sweat loss adaptations to six, one hour exercise HA  
74 sessions was not uniformly impacted by age, though age did inhibit the adaptive peripheral  
75 cardiovascular response, including cutaneous vascular conductance (CVC). It would also  
76 appear that peripheral adaptations to the sweat gland are limited in older individuals  
77 undertaking exercise HA (targeting a +0.9°C increase in core temperature) for nine days.<sup>15</sup>

78 That study reported sudomotor responses to iontophoresis during passive heating as  
79 unchanged after HA, and whilst maximal ion absorption improved, this decayed within seven  
80 days.<sup>15</sup> The success of the longer duration protocols i.e. six to nine sessions<sup>12-15</sup>, over shorter  
81 protocols i.e. three sessions<sup>11</sup>, points to the need to investigate the minimum dose required  
82 for adaptation.

83

84 Further to the additional investigation of the timecourse of adaptations arising from exercise-  
85 HA interventions, real-world implementation of HA in the elderly necessitates consideration of  
86 accessibility and efficacy issues. The use of passive heating/hot water immersion (HWI) is not  
87 a new concept for adapting people to the heat, though it has not been well investigated in the  
88 elderly. Exercise is a potent stimuli to improve the physiological profile of older individuals and  
89 seems important to retain in an intervention where the participant will be subsequently  
90 exercising in the heat.<sup>9</sup> Recently six days of post-exercise HWI was reported to have improved  
91 thermoregulatory responses during submaximal exercise in hot conditions in the young.<sup>16</sup>  
92 From a practical perspective, post-exercise passive HWI is likely achievable for the majority  
93 of the elderly population, and safety considerations aside has the potential to be completed at  
94 home. Therefore in addition to further understanding adaptations to exercise-HA, a HWI HA  
95 intervention also warrants investigation.

96

97 This study sought to investigate the efficacy of HA in the young ( $Y_{EX}$ ) and elderly ( $E_{EX}$ ) following  
98 exercise-HA, and the elderly utilising post-exercise HWI HA ( $E_{HWI}$ ). The hypothesis was that  
99 the younger exercise HA group, elderly exercise HA group, and post exercise HWI HA in the  
100 elderly would induce heat adaptation.

101 **Methods**

102 Twenty-six recreationally active participants volunteered for the study (for group  
103 characteristics, see Table 1), which was completed outside of the UK summer months. The  
104 experimental protocol was approved by the University ethics committee and conducted in  
105 accordance with the principles of the 2013 revision of the Declaration of Helsinki. *A priori*  
106 power analysis selecting conventional  $\alpha$  (0.05) and  $\beta$  (0.20) levels observed that based on  
107 previous work,<sup>17</sup> eight participants in each group were required to detect pre-post HA  
108 differences in resting and peak rectal temperature ( $T_{rec}$ ), and resting and peak heart rate (HR).  
109 Participants provided written informed consent, passed a medical questionnaire and  
110 subsequently refrained from caffeine (12h), alcohol/strenuous exercise (24h) and arrived  
111 euhydrated (urine osmolality  $<700$  mOsm.kg<sup>-1</sup>) to testing sessions which were conducted in a  
112 climate-controlled chamber (TISS, UK).

113

114 **[Add Table 1 near here please]**

115

116 Participants completed two preliminary visits separated by 48 hours (pre-test 1, and pre-test  
117 2), five consecutive intervention days, and two visits commencing 48 hours following the  
118 intervention (post-test 1, and 24 hr later post-test 2). During pre-test 1, anthropometric (height,  
119 nude body mass [NBM], and four site skin fold thickness for body fat [BF] (Harpenden, UK)<sup>4</sup>  
120 and baseline measurements (Electro cardio-gram [ECG, elderly],  $T_{rec}$ , [Henley Medical, UK],  
121 skin temperature [ $T_{skin}$ , Eltek Ltd, UK] and HR [Polar Electro, RS800, Finland]) were recorded  
122 in line with previously reported techniques.<sup>4</sup> To familiarise participants with the forearm SkBF  
123 technique, a 30 min resting exposure to 35°C/50% relative humidity (RH) followed. Systolic  
124 and diastolic blood pressure (SBP, DBP, Omron, M4, Japan) were recorded after 20 min to  
125 calculate CVC. After rest, a graded exercise test (GXT) was completed.<sup>4</sup> In brief, ~45 sec  
126 respiratory gas was collected during rest and the end of each incremental cycling stage (initial  
127 stage: young; 50W, elderly; 25W, increments: young; 25W, elderly; 15W) to calculate  
128 individualised resting metabolic equivalents (MET) and exercise intensities eliciting 6 METs  
129 and 3.5 W.kg<sup>-1</sup> metabolic heat production ( $\dot{H}_{prod}$ ) for use during pre-test 2. Participants then  
130 completed a six-minute walk test (6MWT) on a treadmill (Woodway Pro, Germany), for  
131 familiarisation. The 6MWT commenced at 3km.h<sup>-1</sup>, with participants subsequently adjusting  
132 the speed in  $\pm 0.2$ km.h<sup>-1</sup> increments. These procedures were all repeated during post-test 1.  
133 For pre/post-test 2, participants completed a 'simulated activities of daily living protocol'<sup>4</sup> i.e.  
134 30 min rest and 30 min recumbent cycling at 6 METs/3.5 W.kg<sup>-1</sup>  $\dot{H}_{prod}$ , followed by the 6MWT  
135 as a measure of exercise performance that could be undertaken by all participants, all within  
136 a 35°C/50% RH environment. During rest, laser doppler (moorVMS-LDF, Moor Instruments,  
137 UK) was used to calculate SkBF. At baseline and throughout testing HR,  $T_{rec}$ , and  $T_{skin}$  were

138 recorded every 5 min and rating of perceived exertion (RPE), thermal sensation (TS), thermal  
139 comfort (TC) were recorded every 10 min as outlined in our previous work.<sup>4</sup> To further  
140 characterise the dose of each intervention, area under the curve at  $T_{rec} = 38.5^{\circ}\text{C}$  (AUC)<sup>18</sup> and  
141 thermal impulse<sup>5</sup> were calculated from  $T_{rec}$  data.

142

143 Daily HA commenced at the same time each day. Following hydration and baseline  
144 physiological measurements, on day 1 and day 5 of HA, triplicate capillary blood samples were  
145 taken from willing participants ( $Y_{EX}$   $n=10$ ,  $E_{EX}$   $n=7$ ,  $E_{HWI}$   $n=6$ ) to determine plasma volume (PV)  
146 changes from haemoglobin [Hb] and haematocrit [Hct]. Exercise HA [young ( $Y_{EX}$ ), elderly ( $E_{EX}$ )]  
147 occurred within a  $35^{\circ}\text{C}/50\%$  RH environment. Participants exercised on a cycle ergometer  
148 (Monark 824E, Sweden) using relative exercise intensities ( $Y_{EX}$ ; men= $2.3 \text{ W}\cdot\text{kg}^{-1}$ , women= $2.0$   
149  $\text{W}\cdot\text{kg}^{-1}$  [ $T_{rec}+0.014\pm 0.003^{\circ}\text{C}\cdot\text{min}^{-1}$ ],  $E_{EX}$ ; men= $1.5 \text{ W}\cdot\text{kg}^{-1}$  and women= $1.2 \text{ W}\cdot\text{kg}^{-1}$   
150 [ $T_{rec}+0.010\pm 0.003^{\circ}\text{C}\cdot\text{min}^{-1}$ ]) to increase  $T_{rec}$  to  $38.5^{\circ}\text{C}$  (or  $+1.5^{\circ}\text{C}$  if baseline  $T_{rec} = <36.5^{\circ}\text{C}$ )  
151 within 60 min and maintain the increase for an additional 60 min. If the prescribed exercise  
152 intensity could not be maintained, then it was reduced to enable exercise continuation. Elderly  
153 participant BP was checked throughout and a rest period of 5 min occurred after 30 min of  
154 exercise for all participants. Participants were supported as they dismounted the cycle  
155 ergometer and were encouraged to move slowly to avoid dizziness. During the HWI  
156 intervention ( $E_{HWI}$ ), identical relative exercise intensities were used during 30 min of cycle  
157 ergometry within normothermic conditions ( $\sim 23^{\circ}\text{C}$ , 60% RH) though it was not an experimental  
158 objective to increase  $T_{rec}$  as within the  $E_{EX}$  group, end exercise  $T_{rec}$  in the  $E_{HWI}$  group was  
159  $\sim 37.9^{\circ}\text{C}$  ( $+0.7$ - $1.0^{\circ}\text{C}$ ;  $T_{rec}+0.008\pm 0.003^{\circ}\text{C}\cdot\text{min}^{-1}$ ). After exercise,  $E_{HWI}$  participants completed  
160 30 min of HWI ( $40^{\circ}\text{C}$ ) within an inflatable bath filled so that water level was approximately to  
161 the sternum (mean  $T_{rec} +0.5^{\circ}\text{C}$ ). For safety, upon completion of HWI, BP was assessed, and  
162 participants remained in the bath during emptying. Participants exited the bath slowly with  
163 assistance, after drying and changing, they sat covered in blankets for 30 min.

164

165 All data are presented as mean  $\pm$  SD and were assessed for normality and sphericity prior to  
166 further statistical analyses. When the assumption of sphericity was violated the Greenhouse-  
167 Geisser adjustment was used. For parametric data, one-way between groups ANOVA, and  
168 Bonferroni pairwise comparisons were used to determine differences in baseline  
169 characteristics between groups, intervention data, and the pre-post change in dependent  
170 variables between groups. The Kruskal-Wallis test and Wilcoxon signed ranks were used in  
171 place of one-way ANOVA for non-parametric data. Mixed two-way ANOVA [time (2; pre and  
172 post)\*group (3;  $Y_{EX}$ ,  $E_{EX}$ ,  $E_{HWI}$ )] were used to analyse changes before and after the intervention.  
173 Bonferroni-corrected post-hoc comparisons were completed if interaction and main effects

174 were observed. Following a main effect for time, two-one-sided t-tests' equivalence testing  
175 (TOST)<sup>19</sup> utilising individual Hedges *g* effect sizes (per dependent variable) from a published  
176 meta-analysis<sup>6</sup> were used alongside 95% confidence intervals to identify similarity. For all  
177 analyses, significance was set at  $p < 0.05$ .

178 **Results**

179 *Participants.* By design, participants in the E<sub>EX</sub> and E<sub>HWI</sub> groups were older ( $p < 0.05$ ) than Y<sub>EX</sub>  
180 with effective matching for NBM, BMI and BSA across groups. In addition to age, there was a  
181 significant difference between Y<sub>EX</sub> and E<sub>HWI</sub> for height and body fat, and between E<sub>EX</sub> and E<sub>HWI</sub>  
182 for age and height (Table 1).

183

184 *Heat acclimation intervention.* Mean HR ( $\%HR_{max}$ ), mean  $T_{rec}$ , mean duration  $T_{rec} \geq 38.5^{\circ}C$ ,  $T_{rec}$   
185 AUC $38.5^{\circ}C$  and mean whole body sweat rate (WBSR) ( $\%NBM \cdot hr^{-1}$ ) were not different  
186 between groups/interventions ( $p > 0.05$ ). Group differences ( $p < 0.05$ ) were observed between  
187 Y<sub>EX</sub>, and E<sub>EX</sub> and E<sub>HWI</sub> for mean exercise duration and mean HR ( $b \cdot min^{-1}$ ), mean RPE, mean  
188 TC, and mean TS, **and Y<sub>EX</sub> and E<sub>HWI</sub> for mean thermal impulse**. E<sub>EX</sub> and E<sub>HWI</sub> differed ( $p < 0.05$ )  
189 in mean exercise duration, **mean thermal impulse** and mean HR ( $b \cdot min^{-1}$ ) (Table 1).

190

191 **[Add Table 2 near here please]**

192

193 *Pre and Post testing.* An effect of time ( $p < 0.05$ ) was observed between pre-post HA  
194 highlighting a reduction in resting  $T_{rec}$ , peak  $T_{skin}$ , resting HR, SBP and mean arterial pressure  
195 (MAP), RPE, and TS, and an increased 6MWT distance. Interaction effects were observed  
196 whereby resting HR was lower at post HA in Y<sub>EX</sub> only (Table 2). Between group differences  
197 ( $p < 0.05$ ) were observed for the magnitude of change in resting  $T_{skin}$ , DBP, MAP, and 6MWT  
198 distance. Post hoc analysis observed group differences whereby resting and peak  $T_{skin}$ , peak  
199 HR, and DBP and MAP were different between Y<sub>EX</sub> and E<sub>HWI</sub>. Resting  $T_{skin}$  was different  
200 between E<sub>EX</sub> and E<sub>HWI</sub>. When comparing pre-post changes between groups (Figure 1), a group  
201 effect was observed for resting HR where Y<sub>EX</sub> was different to E<sub>EX</sub> ( $p < 0.001$ ). No difference in  
202 pre-post HA change was observed in the for rest  $T_{rec}$  or  $T_{skin}$ , peak  $T_{rec}$ ,  $T_{skin}$ , HR, PV, WBSR,  
203 SBP, DBP, MAP, CVC, RPE, TS, TC or 6MWT distance between groups ( $p > 0.05$ ) (Figure 1).  
204 TOST analysis identified no significant differences ( $p > 0.05$ ), thus whilst the change was *not*  
205 *different* following ANOVA between groups, it is not possible to state that the response was  
206 *equivalent*.

207

208 **[Add Figure 1 near here please]**

209 **Discussion**

210 This study implemented an exercise-HA protocol with the elderly for five consecutive days and  
211 examined adaptations to a young group completing a comparable exercise-HA protocol. Also,  
212 examined were the benefits of post exercise HWI in the elderly. All groups demonstrated  
213 acclimation via a reduction in resting  $T_{rec}$  during the post-test simulated activities of daily living  
214 protocol, and improved performance during the subsequent 6MWT. Peak  $T_{rec}$  and HR was not  
215 reduced at the end of simulated activities of daily living protocol, therefore the protocol did not  
216 completely reduce the thermal strain of participants. Further consideration of intensity,  
217 duration and application of thermal stress to induce adaptations is therefore required in the  
218 elderly.

219

220 Central to understanding the efficacy of a heat adaptation intervention is evoking the  
221 potentiating stimuli for adaption i.e. elevated  $T_{rec}$  and  $T_{skin}$ , and elevated sweat rates.<sup>5</sup> Though  
222  $T_{skin}$  was not measured during intervention visits, **and thermal impulse differed between EX  
223 and HWI trials**, mean HR,  $T_{rec}$ , duration  $T_{rec} \geq 38.5^{\circ}\text{C}$ , AUC $38.5^{\circ}\text{C}$  and WBSR responses did  
224 not differ between groups during the intervention. To support efficacy considerations between  
225  $E_{EX}$  and  $E_{HWI}$  these two groups also did not differ with regards to mean duration  $T_{rec} +1.5^{\circ}\text{C}$   
226 and RPE, TC, TS. The mean thermal,<sup>16,20-22</sup> and sudomotor<sup>16,20-22</sup> stimuli (Table 1) was similar  
227 to previous work which have demonstrated heat adaptation within a five-day isothermic/HWI  
228 HA interventions. The similarity in thermal stimuli between our groups and the cited previous  
229 experimental work gives confidence that an effective dose of HA was administered.

230

231 The ability for young healthy individuals to adapt to heat has been well evidenced.<sup>6</sup> This  
232 experiment adds to the comparatively small body of work examining the capacity for  
233 acclimation in elderly adults. The maximal observed changes in resting  $T_{rec}$  within both  $E_{EX}$  (-  
234  $0.33^{\circ}\text{C}$ ) and  $E_{HWI}$  ( $-0.39^{\circ}\text{C}$ ) interventions are similar to  $Y_{EX}$  ( $-0.30^{\circ}\text{C}$ ) and that which might be  
235 expected from equivalent duration interventions in the young.<sup>7</sup> It should be noted that the  
236 greatest change occurred on day 5 of HA, rather than during the post test. The timing and  
237 impact of the other intense post-tests on  $T_{rec}$  e.g. GXTs, may therefore be relevant  
238 considerations for future experimental work in the elderly. Reductions in resting HR in  $Y_{EX}$  (-  
239  $11 \pm 8 \text{ b.min}^{-1}$ ) were not observed in  $E_{EX}$  and  $E_{HWI}$  though this is unsurprising as *i*) the variability  
240 in PV expansion led to insignificant group changes<sup>5</sup> and *ii*) age-related impairments in  
241 cardiovascular adaptations to HA have been established.<sup>14</sup> Likewise CVC did not change,  
242 suggesting that no intervention modified the baseline SkBF response in the same manner as  
243 evidenced following longer interventions in younger vs older trained individuals.<sup>14</sup> Together  
244 these data indicate the likely need to increase the intervention duration to improve  
245 cardiovascular responses to heat stress in the elderly effectively. The reduction of

246 physiological strain likely facilitated the improved TS and RPE during the 6MWT, and exercise  
247 capacity for the same cardiovascular strain occurred to improve 6MWT performance by ~10%  
248 in  $E_{EX}$  and  $E_{HWI}$  compared to  $Y_{EX}$  (+4%). The null WBSR response may be an artefact of the  
249 short-term intervention, with longer interventions demonstrating larger magnitudes of  
250 sudomotor adaptation.<sup>9</sup> The null WBSR response, and the relatively short duration (30 min  
251 exercise) of the fixed intensity post test protocol<sup>22</sup>, are probable reasons for the null response  
252 in peak  $T_{rec}$ . An interesting additional finding was the improvement in SBP and MAP, which  
253 adds to the increasing body of support for heat as a supplemental stimuli to exercise for  
254 improving cardiovascular health.<sup>23</sup>

255

256 Given logistical challenges in administering exercise-HA in the elderly, this study sought to  
257 understand whether post exercise HWI would be an appropriate alternative (at a physiological  
258 level). With the exceptions of resting  $T_{skin}$ , few statistical differences occurred between  
259 adaptations observed in  $E_{EX}$  and  $E_{HWI}$  groups supporting this proposal. **The ability for post  
260 exercise HWI to induce adaptations which do not differ in magnitude to EX protocols for a  
261 lesser impulse is a noteworthy experimental consideration for future HA work.** Irrespective of  
262 intervention type, this experiment enhances our understanding of the number of sessions  
263 required to induce heat adaptations. With three days of HA proving insufficient in females,<sup>11</sup>  
264 the use of a five day exercise HA/post exercise HWI protocol now appears to have some  
265 efficacy, though it remains likely that more complete adaptations will be achieved with longer  
266 protocols e.g. as observed using a nine day intervention.<sup>12</sup> In addition to clarifying the delayed  
267 induction peripheral cardiovascular adaptations, our data supports the notion that longer  
268 protocols should also be implemented to induce substantial changes in the sudomotor  
269 response, particularly in untrained elderly individuals.<sup>13</sup> To enable the elderly to engage with  
270 prolonged HA interventions, scheduling adjustments, for example intermittent day protocols  
271 may also be considered.<sup>7</sup> Further to known intraindividual variability in HA,<sup>24</sup> which may further  
272 increase with age, this study utilised a mixed-sex cohort which may have impacted the  
273 observed magnitude of adaptive response to a five day intervention given females may display  
274 a delayed temporal pattern to adaptation.<sup>21</sup> Visual inspection of the responses in females  
275 participants (Figure 1), does not necessarily support this within our cohort. Although some  
276 classical HA adaptations were evident after  $E_{EX}$  and  $E_{HWI}$ , further heat/exercise stimulus  
277 maybe required to develop greater magnitudes of adaptation and develop significant  
278 adaptation in other pertinent heat illness markers such as renal function<sup>25</sup> and gut-  
279 permeability.<sup>26</sup> Future work should investigate these variables, alongside cellular/molecular  
280 responses including heat shock proteins (HSPs) given the importance of HSPs in heat  
281 adaptation,<sup>27</sup> and age-related declines in HSP inducibility.<sup>28</sup>

282

283 Given increased exercise prescription is required for exercise HA, elderly HA using the HWI  
284 model could be the focus for future work to provide a more logistically viable intervention. This  
285 intervention should be assessed against younger individuals, with the absence of this direct  
286 comparison a limitation of the current study. Further consideration of the intervention data  
287 (Table 1) also acknowledges that despite a lack of statistical difference, the  $Y_{EX}$  and  $E_{HWI}$  group  
288 experienced numerically different stimuli, yet comparable adaptive responses were induced.  
289 This may be an artefact of the elderly possessing a lower threshold for adaptation, thus  
290 adapting effectively to 'reduced' stimuli relative to the young. This may be advantageous for  
291 this cohort, and examination of the required absolute thresholds for adaptation in this  
292 population would provide mechanistic and applied insight. In addition, future elderly HA  
293 research should investigate the possibility of age-related differences in the time-course of heat  
294 adaptation/retention/decay/re-acclimation. In a young population, re-acclimation develops the  
295 same or greater magnitude of adaptations to the heat when compared to the original HA.<sup>29</sup>  
296 Therefore, re-acclimation/acclimation memory is a pertinent future direction for elderly HA.  
297 Furthermore, chronic interventions such as HA should be examined against, and in  
298 conjunction with, acute heat alleviation interventions<sup>30</sup> in the elderly to further guide health  
299 policy.

300

### 301 **Conclusion**

302 This study showed exercise HA develops improvements in physiological (reduced resting  $T_{rec}$ ,  
303 peak  $T_{skin}$ , resting HR, SBP and MAP), perceptual (reduced RPE and TS) and exercise  
304 performance (increased 6MWT distance) responses to heat stress in the young and elderly.  
305 Furthermore, a novel post-exercise HWI protocol elicits improved responses to heat stress  
306 suggests its also a viable intervention to prepare the elderly for heat waves.

307

### 308 **Practical implications**

- 309 • Five sessions of exercise heat acclimation induced improvements in physiological,  
310 perceptual and exercise performance responses during heat stress in young and elderly  
311 participants.
- 312 • The implementation of post exercise hot water immersion also induced thermoregulatory,  
313 perceptual and functional improvements in the elderly.
- 314 • Future work should investigate practical heat acclimation strategies across a broad  
315 spectrum of variables aligned to heat illness to further our understanding of the capacity  
316 for heat adaptation in older age, and guide mitigating strategies for heat illness risk in the  
317 vulnerable.

318

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408

409 **Legends**

410 Table 1. Mean  $\pm$  SD Participant characteristics and summary data from each intervention by  
411 group ( $Y_{EX}$ ; young exercise-HA,  $E_{EX}$ ; elderly exercise-HA,  $E_{HWI}$ ; elderly HWI). \*= $\neq$ difference from  
412  $Y_{EX}$ . †= $\neq$ different from  $E_{EX}$ .

413

414 Table 2. Mean  $\pm$  SD (95% CI). Physiological and perceptual variables pre and post  
415 intervention for young exercise-HA ( $Y_{EX}$ ), elderly exercise-HA ( $E_{EX}$ ), and elderly HWI ( $E_{HWI}$ )  
416 groups.\* represents a significant ( $p<0.05$ ) with group difference from Pre. ^ represents a  
417 significant ( $p<0.05$ ) overall difference from Pre. # represents a significant ( $p<0.05$ ) difference  
418 compared to  $Y_{EX}$  within timepoint. \$ represents a significant ( $p<0.05$ ) overall difference from  
419  $Y_{EX}$ . † represents a significant ( $p<0.05$ ) difference compared to  $E_{EX}$  within timepoint. ‡  
420 represents a significant ( $p<0.05$ ) overall difference from  $E_{EX}$ .

421

422 Figure 1. Mean  $\pm$  95% CI. Change in physiological and perceptual variables pre and post  
423 intervention for young exercise-HA ( $Y_{EX}$ ; circles), elderly exercise-HA ( $E_{EX}$ ; diamonds), and  
424 elderly HWI ( $E_{HWI}$ ; triangles) groups. Female participants are identified by open symbols. \*  
425 Denotes individual group difference ( $p<0.05$ ), ^ represents a significant ( $p<0.05$ ) overall  
426 difference from Pre.

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Table 1.

Participant characteristics	Young (Y <sub>EX</sub> )	Elderly (E <sub>EX</sub> )	Elderly HWI (E <sub>HWI</sub> )	Statistical summary
Sex (M/F)	8M, 3F	7M, 1F	3M, 4F	
Age (yrs)	22 ± 2	68 ± 3 *	73 ± 3 *†	F= 912.3, p < 0.001
Height (cm)	175 ± 6	175 ± 9	162 ± 7 *†	F= 8.8, p = 0.001
NBM (kg)	74.0 ± 13.5	74.1 ± 11.1	71.7 ± 20.4	F= 0.1, p = 0.942
BMI (kg.m <sup>2</sup> )	23.9 ± 3.5	24.2 ± 2.8	27.1 ± 5.8	F= 0.5, p = 0.621
BSA (m <sup>2</sup> )	1.89 ± 0.18	1.89 ± 0.17	1.75 ± 0.26	F= 1.2, p = 0.333
Body fat (%)	18 ± 7	18 ± 7	29 ± 10 *	F= 4.4, p = 0.025
Intervention summary				
Mean exercise duration (min)	109 ± 12	120 ± 0 *	30 ± 0 *†	F= 271.0, p < 0.001
Mean HR (b.min <sup>-1</sup> )	144 ± 11	113 ± 9 *	100 ± 12 *†	F= 42.1, p < 0.001
Mean HR (%HR <sub>max</sub> )	73 ± 6	74 ± 5	68 ± 8	F= 2.3, p = 0.119
Mean T <sub>rec</sub> (°C)	38.11 ± 0.15	37.91 ± 0.36	38.01 ± 0.13	F= 1.8, p = 0.191
Mean T <sub>rec</sub> AUC 38.5°C (°C.min <sup>-1</sup> )	4.5 ± 3.4	2.4 ± 3.4	3.1 ± 2.7	F= 1.0, p = 0.381
Mean thermal impulse (°C.min <sup>-1</sup> )	2546 ± 459	2564 ± 549	1874 ± 464 *†	F= 4.9, p = 0.016
Mean duration T <sub>rec</sub> ≥ 38.5°C (min)	31 ± 20	23 ± 24	15 ± 11	F= 1.5, p = 0.245
Mean duration T <sub>rec</sub> ≥ +1.5°C (min)	53 ± 15	14 ± 12 *	5 ± 6 *	F= 37.7, p < 0.001
Mean WBSR (%NBM.hr <sup>-1</sup> )	1.2 ± 0.3	1.0 ± 0.3	1.1 ± 0.7	F= 0.7, p = 0.936
Mean RPE	12 ± 2	10 ± 3 *	9 ± 4 *	χ <sup>2</sup> = 10.8, p = 0.005
Mean TC	5 ± 1	2 ± 2 *	3 ± 2 *	χ <sup>2</sup> = 8.8, p = 0.012
Mean TS	6.3 ± 0.5	5.3 ± 0.5 *	5.0 ± 0.5 *	χ <sup>2</sup> = 11.1, p = 0.004

Table 1.

Abbreviations: M; male, F; female, NBM; nude body mass, BMI; body mass index, BSA; body surface area, BF; body fat, HR; heart rate,  $T_{rec}$ ; rectal temperature, RPE; rating of perceived exertion, TC; thermal comfort, TS; thermal sensation. \* represents a significant ( $p < 0.05$ ) difference from  $Y_{EX}$ . † represents a significant ( $p < 0.05$ ) difference from  $E_{EX}$ . Note RPE, TC and TS reported as median  $\pm$  interquartile range.

Table 2.

	Pre Heat Acclimation			Post Heat Acclimation			Pre-Post Heat Acclimation Change			Statistical outcomes		
	Y <sub>EX</sub>	E <sub>EX</sub>	E <sub>H<sub>WI</sub></sub>	Y <sub>EX</sub>	E <sub>EX</sub>	E <sub>H<sub>WI</sub></sub>	Y <sub>EX</sub>	E <sub>EX</sub>	E <sub>H<sub>WI</sub></sub>	Time	Group	Group*Time
Rest T <sub>rec</sub> (°C)	37.07 ± 0.42	37.08 ± 0.54	37.27 ± 0.29	37.02 ± 0.37	37.16 ± 0.45	37.16 ± 0.16	-0.05 ± 0.34 (-0.26 - 0.15)	+0.08 ± 0.23 (-0.08 - 0.24)	-0.12 ± 0.24 (-0.29 - 0.06)	F = 0.3, p = 0.607	F = 0.4, p = 0.647	F = 0.9, p = 0.419
Rest T <sub>rec</sub> HA (°C)	36.96 ± 0.38	37.20 ± 0.43	37.40 ± 0.30	36.91 ± 0.30 ^	36.92 ± 0.50 ^	37.08 ± 0.20 ^	-0.04 ± 0.32 (-0.23 - 0.15)	-0.28 ± 0.26 (-0.46 - 0.10)	-0.33 ± 0.35 (-0.58 - 0.07)	F = 12.0, p = 0.002	F = 1.8, p = 0.182	F = 2.2, p = 0.133
Rest T <sub>rec</sub> MAX (°C)	37.15 ± 0.35	37.25 ± 0.48	37.40 ± 0.30	36.85 ± 0.33 ^	36.92 ± 0.50 ^	37.02 ± 0.17 ^	-0.30 ± 0.29 (-0.47 - 0.12)	-0.33 ± 0.25 (-0.50 - 0.16)	-0.39 ± 0.25 (-0.58 - 0.20)	F = 40.2, p < 0.001	F = 0.8, p = 0.471	F = 0.3, p = 0.777
Peak T <sub>rec</sub> (°C)	37.38 ± 0.25	37.52 ± 0.45	37.81 ± 0.33	37.41 ± 0.28	37.62 ± 0.38	37.77 ± 0.24	+0.03 ± 0.20 (-0.10 - 0.14)	+0.09 ± 0.22 (-0.06 - 0.25)	-0.04 ± 0.22 (-0.20 - 0.12)	F = 0.3, p = 0.581	F = 3.4, p = 0.052	F = 0.7, p = 0.490
Rest T <sub>skin</sub> (°C)	30.35 ± 0.82	30.74 ± 0.73	31.24 ± 0.62 \$‡	30.38 ± 0.58	30.63 ± 0.97	31.71 ± 0.58 \$‡	0.04 ± 1.14 (-0.64 - 0.71)	-0.10 ± 0.82 (-0.67 - 0.46)	0.47 ± 0.69 (-0.05 - 0.98)	F = 0.5, p = 0.484	F = 8.5, p = 0.002	F = 0.7, p = 0.488
Peak T <sub>skin</sub> (°C)	36.19 ± 0.31	36.25 ± 0.13	35.97 ± 0.44 \$	36.03 ± 0.28 ^	35.98 ± 0.50 ^	35.69 ± 0.71 ^\$	-0.17 ± 0.27 (-0.33 - 0.01)	-0.27 ± 0.58 (-0.55 - 0.15)	-0.28 ± 0.40 (-0.60 - 0.01)	F = 7.9, p = 0.010	F = 5.1, p = 0.14	F = 0.2, p = 0.786
Rest HR (b.min <sup>-1</sup> )	72 ± 11	62 ± 13	71 ± 11	61 ± 8 *	64 ± 13	67 ± 12	-11 ± 8 (-16 - 6)	+2 ± 5 (-2 - 5)	-4 ± 5 (-8 - 0)	F = 11.0, p = 0.003	F = 0.6, p = 0.572	F = 9.2, p = 0.001
Peak HR (b.min <sup>-1</sup> )	129 ± 15	110 ± 19	122 ± 20	123 ± 14	110 ± 22	118 ± 21	-6 ± 10 (-12 - 1)	+0 ± 5 (-4 - 4)	-4 ± 5 (-8 - 2)	F = 2.8, p = 0.107	F = 2.0, p = 0.156	F = 1.1, p = 0.361
Peak HR (%HR <sub>max</sub> )	64.9 ± 7.5	72.2 ± 12.7	83.0 ± 14.3 \$	62.1 ± 7.3	72.0 ± 14.6	80.2 ± 14.5 \$	-2.8 ± 5.3 (-8 - 3)	-0.2 ± 3.4 (-1 - 3)	-2.8 ± 3.6 (-5 - 1)	F = 2.4, p = 0.132	F = 5.1, p = 0.015	F = 0.8, p = 0.446
PV (%)	-	-	-	-	-	-	+ 3.7 ± 7.1 (-0.5 - 7.9)	+ 4.1 ± 3.5 (1.7 - 6.5)	+ 0.1 ± 3.2 (-2.2 - 2.5)		F = 1.3, p = 0.304	
WBSR (L.h <sup>-1</sup> )	1.07 ± 0.35	0.73 ± 0.14	1.07 ± 0.58	1.03 ± 0.38	0.92 ± 0.20	0.96 ± 0.41	-0.04 ± 0.48 (-0.32 - 0.24)	+0.18 ± 0.11 (0.11 - 0.26)	-0.10 ± 0.41 (-0.41 - 0.21)	F = 0.0, p = 0.873	F = 1.3, p = 0.281	F = 1.2, p = 0.320
SBP (mmHg)	122 ± 19	121 ± 10	139 ± 28	119 ± 14 ^	114 ± 13 ^	130 ± 30 ^	-3 ± 12 (-10 - 4)	-7 ± 10 (-14 - 0)	-7 ± 15 (-18 - 4)	F = 5.3, p = 0.031	F = 0.7, p = 0.491	F = 0.3, p = 0.783
DBP (mmHg)	66 ± 9	71 ± 9	85 ± 16 \$	64 ± 6	70 ± 10	77 ± 13 \$	-2 ± 8 (-7 - 3)	-1 ± 3 (-3 - 2)	-8 ± 21 (-24 - 7)	F = 2.3, p = 0.145	F = 7.0, p = 0.005	F = 0.7, p = 0.486
MAP (mmHg)	85 ± 9	88 ± 7	103 ± 15 \$	72 ± 8 ^	85 ± 10 ^	95 ± 18 ^\$	-3 ± 7 (-7 - 1)	-3 ± 4 (-6 - 0)	-8 ± 18 (-21 - 6)	F = 4.3, p = 0.050	F = 4.3, p = 0.027	F = 0.6, p = 0.572
CVC (%peak)	51.7 ± 20.2	57.8 ± 19.3	52.1 ± 21.7	48.9 ± 17.4	74.9 ± 37.5	56.2 ± 62.9	-2.8 ± 23.8 (-17 - 11)	+6.7 ± 21.8 (-8 - 22)	+7.3 ± 44.7 (-26 - 40)	F = 0.3, p = 0.578	F = 0.6, p = 0.542	F = 0.3, p = 0.743
Peak RPE	12 ± 2	12 ± 2	14 ± 3	10 ± 2 ^	11 ± 2 ^	14 ± 4 ^	-2 ± 2 (-3 - 1)	-1 ± 2 (-2 - 1)	-1 ± 2 (-2 - 1)	F = 8.7, p = 0.007	F = 2.5, p = 0.102	F = 0.7, p = 0.517
Peak TS	5.7 ± 0.5	5.9 ± 0.5	6.1 ± 0.9	5.3 ± 0.3 ^	5.6 ± 0.4 ^	6.1 ± 1.1 ^	-0.4 ± 0.5 (-0.8 - 0.1)	-0.3 ± 0.4 (-0.5 - 0.0)	0.0 ± 0.4 (-0.6 - 0.4)	F = 6.0, p = 0.022	F = 2.2, p = 0.133	F = 1.2, p = 0.334
Peak TC	3 ± 1	3 ± 1	4 ± 1	2 ± 1	3 ± 1	4 ± 2	-1 ± 1 (-1 - 0)	0 ± 1 (-1 - 1)	-1 ± 1 (0 - 0)	F = 1.7, p = 0.208	F = 3.0, p = 0.69	F = 1.9, p = 0.170
6MWT distance (m)	746 ± 42	589 ± 140 #	497 ± 134 #	778 ± 67 *	639 ± 115 *#	544 ± 137 *#	+32 ± 39 (16 - 55)	+50 ± 30 (12 - 68)	+47 ± 16 (7 - 57)	F = 46.5, p < 0.001	F = 12.2, p < 0.001	F = 0.4, p = 0.413

Abbreviations: T<sub>re</sub>; rectal temperature, T<sub>skin</sub>; skin temperature, HR; heart rate, PV; plasma volume WBSR; whole body sweat rate, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, CVC; cutaneous vascular conductance, RPE; rating of perceived exertion, TS; thermal sensation, TC; thermal comfort, 6MWT; six minute walk test. Notes: All variables analysed during pre/post test 2 with the exception of Rest T<sub>rec</sub> HA which compares day 1 and day 5 of HA, and Rest T<sub>rec</sub> MAX which calculates the greatest change in T<sub>rec</sub> irrespective of timepoint.

## Table 2.

\* represents a significant ( $p < 0.05$ ) with group difference from Pre. ^ represents a significant ( $p < 0.05$ ) overall difference from Pre

# represents a significant ( $p < 0.05$ ) difference compared to Y<sub>EX</sub> within timepoint. \$ represents a significant ( $p < 0.05$ ) overall difference from Y<sub>EX</sub>

† represents a significant ( $p < 0.05$ ) difference compared to E<sub>EX</sub> within timepoint. ‡ represents a significant ( $p < 0.05$ ) overall difference from E<sub>EX</sub>