



Post-exercise hot water immersion elicits heat acclimation adaptations in endurance trained and recreationally active individuals

Zurawlew, Michael; Mee, Jessica; Walsh, Neil

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26 **Abstract**

27 Hot water immersion (HWI) after exercise on 6 consecutive days in temperate conditions has
28 been shown to provide heat acclimation adaptations in a recreationally active population.
29 Endurance athletes experience frequent, sustained elevations in body temperature during
30 training and competition; as a consequence, endurance athletes are considered to be partially
31 heat acclimatized. It is therefore important to understand the extent to which endurance
32 trained individuals may benefit from heat acclimation by post-exercise HWI. To this end, we
33 compared the responses of eight endurance trained and eight recreationally active males
34 (habitual weekly endurance exercise: 9 h vs. 3 h) to a 6-day intervention involving a daily
35 treadmill run for 40 min (65% $\dot{V}O_{2\max}$) in temperate conditions followed immediately by
36 HWI (≤ 40 min, 40°C). Before (PRE) and after the intervention (POST), hallmark heat
37 acclimation adaptations were assessed during a 40-min treadmill run at 65% $\dot{V}O_{2\max}$ in the
38 heat (33°C, 40% RH). The 6 day, post-exercise HWI intervention induced heat acclimation
39 adaptations in both endurance trained and recreationally active individuals. Training status
40 did not significantly influence the magnitude of heat acclimation adaptations from PRE to
41 POST (interactions $P > 0.05$) for: the reduction in end-exercise rectal core temperature (T_{re} ,
42 mean, endurance trained -0.36°C; recreationally active -0.47°C); the reduction in resting T_{re}
43 (endurance trained -0.17°C; recreationally active -0.23°C); the reduction in T_{re} at sweating
44 onset (endurance trained -0.22°C; recreationally active -0.23°C); and, the reduction in mean
45 skin temperature (endurance trained -0.67°C; recreationally active -0.75°C: PRE to POST P
46 < 0.01). Furthermore, training status did not significantly influence the observed reductions in
47 mean $\dot{V}O_2$, mean metabolic energy expenditure, end-exercise physiological strain index,
48 perceived exertion or thermal sensation (PRE to POST $P < 0.05$). Only end-exercise heart
49 rate was influenced by training status ($P < 0.01$, interaction); whereby, recreationally active
50 but not endurance trained individuals experienced a significant reduction in end-exercise
51 heart rate from PRE to POST ($P < 0.01$). In summary, these findings demonstrate that post-
52 exercise hot water immersion presents a practical strategy to reduce thermal strain during
53 exercise-heat-stress in endurance trained and recreationally active individuals.

54

55 **Keywords:** heat, acclimation, hot water, thermal strain, training, running

56

57

58 **Introduction**

59 Exercise in the heat increases physiological strain, attenuates exercise capabilities and
60 increases susceptibility to exertional heat illness and the potentially fatal, exertional heat
61 stroke (Young et al., 1985; Binkley et al., 2002; Racinais et al., 2015). In the early twentieth
62 century, pioneering research on fatal heat stroke in the South African gold mines highlighted
63 a high mortality in the first four shifts worked by miners under high heat exposure;
64 particularly in those native to cold dry areas (Cluver, 1932; Dreosti, 1935). To mitigate the
65 risk of heat stroke, miners acclimatized by gradual introduction to the unfavorable working
66 conditions underground (Cluver, 1932). Current recommendations are for athletes, military
67 personnel and others in occupations involving high heat exposure to complete a period of
68 heat acclimation prior to competing or operating in the heat. Heat acclimation typically
69 involves exercising in the heat on 5–14 occasions for > 60-min, where core body temperature
70 and skin temperature are elevated and profuse sweating is initiated (Taylor, 2014; Periard et
71 al., 2015). The adaptive responses to exercise-heat-acclimation include, but are not limited to:
72 an earlier onset of cutaneous vasodilatation and sweating; an increase in sweating rate; a
73 reduction in resting and exercising core body temperature; a reduction in cardiovascular
74 strain and skin temperature; that in turn, improve thermal comfort and enhance endurance
75 performance in the heat (Gagge et al., 1967; Lorenzo et al., 2010; Taylor, 2014).

76
77 Routine endurance training performed in temperate conditions, which elevates body
78 temperature and initiates profuse sweating, shares common adaptive responses to exercise-
79 heat-acclimation such as; an earlier onset and an increase in sweating rate, a reduction in core
80 temperature and a reduction in cardiovascular strain during exercise-heat-stress; which in
81 turn, improves endurance performance in the heat (Piwonka et al., 1965; Strydom et al.,
82 1966; Gisolfi and Robinson, 1969; Shvartz et al., 1977). As such, endurance trained
83 individuals are considered to be partially heat acclimatized (Piwonka et al., 1965; Strydom et
84 al., 1966; Gisolfi and Robinson, 1969). By the same token, it has long been considered that
85 endurance trained individuals have less adaptation potential and require fewer exercise-heat-
86 exposures to achieve a plateau in heat acclimation responses, compared with untrained
87 individuals (Pandolf et al., 1977; Shvartz et al., 1977). For example, following constant work
88 rate heat acclimation, trained individuals acquired smaller thermal benefits during exercise-
89 heat-stress than untrained individuals (Shvartz et al., 1977). Soldiers of the highest aerobic
90 fitness required only four exercise-heat-acclimation exposures to achieve a plateau in the
91 reduction of end-exercise rectal core temperature (T_{re}); whereas, soldiers with the lowest
92 aerobic fitness required eight exercise-heat-acclimation exposures (Pandolf et al., 1977). A
93 limitation of these studies, reporting smaller and more rapid adaptations to heat acclimation
94 in trained individuals, is that the observed plateau in heat acclimation adaptations may simply
95 represent habituation to the constant exercise-heat-stress; resulting in a decline in the
96 adaptation stimulus (Taylor, 2014). Recent studies that have maintained the endogenous
97 thermal stimulus during controlled hyperthermia heat acclimation demonstrate comparable
98 thermal and cardiovascular adaptations in endurance trained (Neal et al., 2016) and
99 recreationally active individuals (Gibson et al., 2015).

100

101 Despite compelling evidence that exercise-heat-acclimation alleviates thermal strain and
102 improves performance in the heat (Nielsen et al., 1997; Lorenzo et al., 2010), only 15% of
103 athletes competing at the 2015 World Athletics Championships in the heat and humidity of
104 Beijing heat acclimatized as part of their preparation (Periard et al., 2017). One possible
105 explanation is that athletes consider their high level of fitness confers adaptations similar to
106 heat acclimatization (Piwonka et al., 1965; Strydom et al., 1966; Gisolfi and Robinson,
107 1969); so they favor natural heat acclimatization in the few days preceding competition and

108 prioritise other strategies to combat the heat such as fluid replacement and pre-cooling
109 (Periard et al., 2017). Another explanation is that conventional exercise-heat-acclimation
110 protocols can be costly, impractical and may interfere with an athlete's training and taper:
111 exercise-heat-acclimation typically involves access to an environmental chamber and precise
112 control over exercising core temperature during endurance exercise. The completion of
113 alternative heat acclimation methods, such as post-exercise sauna bathing (Scoon et al., 2007)
114 and hot water immersion (HWI) (Zurawlew et al., 2016) have received increasing interest of
115 late (Casadio et al., 2017). These methods are; accessible, time efficient, simple to administer
116 and minimize disturbances to training and tapering. Recently, HWI after exercise in
117 temperate conditions on 6 consecutive days initiated hallmarks of heat acclimation in
118 recreationally active individuals (Zurawlew et al., 2016; Zurawlew et al., 2018). Heat
119 acclimation adaptations to post-exercise HWI included reductions in; T_{re} at rest, T_{re} at
120 sweating onset and T_{re} during exercise-heat stress; in turn, restoring endurance performance
121 in the heat to the level observed in temperate conditions (Zurawlew et al., 2016). Similar to
122 controlled hyperthermia heat acclimation, post-exercise HWI ensures a maintenance of the
123 daily thermal stimulus for adaptation (daily ΔT_{re} ; $\approx 2.1^{\circ}\text{C}$), since the termination of the HWI
124 relies primarily on participants removing themselves due to thermal discomfort (Zurawlew et
125 al., 2016; Zurawlew et al., 2018).

126
127 It remains unknown whether heat acclimation adaptations to post-exercise HWI in a
128 recreationally active population translate to an endurance trained population. As such, the
129 aim of the current study was to compare the adaptation responses of endurance trained and
130 recreationally active individuals following post-exercise HWI. We hypothesized that HWI
131 after submaximal exercise in temperate conditions on six consecutive days would induce
132 comparable heat acclimation adaptations in endurance trained and recreationally active
133 individuals.

134

135 **Methods**

136

137 **Participants**

138 In accordance with previously defined classifications (De Pauw et al., 2013), eight endurance
139 trained males (runners, $n = 6$ and triathletes, $n = 2$; age: 25 ± 4 years; body mass: 69 ± 4 kg;
140 self-reported weekly endurance exercise: 9 ± 3 h; $\dot{V}O_{2\max}$: 68 ± 6 mL·kg⁻¹·min⁻¹) and eight
141 recreationally active males (age: 21 ± 3 years; body mass: 71 ± 9 kg; self-reported weekly
142 endurance exercise: 3 ± 1 h; $\dot{V}O_{2\max}$: 54 ± 6 mL·kg⁻¹·min⁻¹), participated in the study. All
143 participants provided written informed consent to participate, were healthy, non-smokers,
144 free from any known cardiovascular or metabolic diseases and were not taking any
145 medication. Additionally, all participants had not been exposed to hot environmental
146 conditions in the past 3 months and were not regular hot bath or sauna users. The study
147 received local ethical approval and was conducted in accordance with the Declaration of
148 Helsinki (2013).

149

150 **Study design**

151 A mixed-methods (between and within) repeated measures (PRE to POST) design was used
152 to assess the effect of training status on heat acclimation adaptations. Endurance trained and
153 recreationally active participants completed a 40-min submaximal treadmill run at 65%
154 $\dot{V}O_{2\max}$ in the heat (33°C, 40% relative humidity; RH) before (PRE) and after (POST) heat
155 acclimation, as described previously (Zurawlew et al., 2016). Heat acclimation involved a
156 daily 40-min submaximal treadmill run at 65% $\dot{V}O_{2\max}$ in temperate conditions (19°C),
157 followed by a ≤ 40 -min HWI (40°C water) on six consecutive days, as described previously
158 (Zurawlew et al., 2016).

159

160 **Preliminary measurements**

161 In temperate conditions (19°C), a continuous incremental exercise test on a motorized
162 treadmill (HP Cosmos Mercury 4.0, Nussdorf-Traunstein, Germany) assessed $\dot{V}O_{2\max}$, as
163 previously described (Fortes et al., 2013). The interpolation of the running speed– $\dot{V}O_2$
164 relationship determined a running speed that elicited 65% $\dot{V}O_{2\max}$. This speed was verified
165 during steady state exercise with a 60-s expired gas sample collected by Douglas bag method,
166 30-min after the $\dot{V}O_{2\max}$ test. This individualized running speed was used during the
167 submaximal exercise in both the experimental trials and the daily intervention.

168

169 **Experimental trials**

170 Participants were instructed to refrain from any exercise 24-h prior to, and on the day of
171 experimental trials. In addition, participants were instructed to refrain from alcohol, caffeine
172 or tobacco and to complete a diet diary 24-h prior to PRE. Twenty-four hours prior to POST,
173 participants were instructed to replicate this food and fluid intake. On the morning of
174 experimental trials, participants arrived at the laboratory fasted and were provided with a
175 standardized breakfast (0.03 MJ·kg⁻¹) and a bolus of water equivalent to 7 mL·kg⁻¹ of body
176 mass. Following a 20-min seated rest in temperate conditions (19°C), dressed in a T-shirt,
177 running shorts, socks and shoes, a venous blood sample was taken without stasis. A pre-
178 exercise nude body mass was taken using a digital platform scale (Model 705; Seca,
179 Hamburg, Germany) after voiding. A urine sample was provided and analyzed for urine
180 specific gravity to confirm that participants were hydrated (< 1.03) (Armstrong, 2005) using a
181 handheld refractometer (Atago Uricon-Ne refractometer, NSG Precision cells, New York,
182 USA). If participants did not meet the hydration criteria they were provided with a 500-mL
183 bolus of water and urine specific gravity was reanalyzed; exercise began only when urine
184 specific gravity < 1.03 ($n = 1$). Participants were instrumented for the exercise protocol, then

185 rested in a temperate laboratory to establish baseline measures prior to beginning the
186 exercise.

187

188 Dressed in running shorts, socks and shoes the participant entered the environmental chamber
189 ($33 \pm 0^\circ\text{C}$, $39 \pm 4\%$ RH; Delta Environmental Systems, Chester, UK) and completed a
190 submaximal treadmill run (40-min, $65\% \dot{V}O_{2\text{max}}$, 1% gradient). T_{re} , skin temperatures and
191 heart rate (Polar FT1, Polar Electro, Kempele, Finland) were monitored continuously and
192 local forearm sweat rate was measured every 20-s for the first 15-min of exercise.

193 Physiological strain index (PhSI) was calculated, as previously described (Tikusis et al.,
194 2002). Expired gas samples (60-s) were collected by Douglas bag method to assess for $\dot{V}O_2$
195 and respiratory exchange ratio (RER) immediately prior to the 10th, 20th, 30th and 40th min of
196 exercise. Metabolic energy expenditure was calculated using $\dot{V}O_2$ and RER as described
197 (Nishi, 1981). Rating of perceived exertion (RPE) (Borg, 1970) and thermal sensation
198 (Hollies and Goldman, 1977) were recorded every 10-min of exercise. On completion of the
199 exercise protocol, participants exited the environmental chamber and rested in temperate
200 conditions, dressed in running shorts, socks and shoes for 15-min. To estimate whole body
201 sweat rate (WBSR), participants towel dried and provided a nude body mass following the
202 seated rest. Participants were then provided with water equivalent to sweat losses and were
203 free to leave the laboratory when $T_{\text{re}} \leq 38.5^\circ\text{C}$.

204

205 **Post-exercise hot water immersion intervention**

206 Post-exercise HWI heat acclimation was completed on six consecutive days, as previously
207 described (Zurawlew et al., 2016). During the intervention, participants were instructed to
208 reduce their normal endurance exercise volume by that completed during the intervention in
209 the laboratory and to consume their normal diet and fluid intake, including caffeine and
210 alcohol (≤ 3 units per day). Participants arrived at the laboratory each day between 0600-h
211 and 1000-h. A heart rate monitor and a rectal thermistor were fitted and the participant rested
212 in temperate conditions (19°C) for 15-min. Following the seated rest, dressed in shorts, socks
213 and trainers, in a hydrated state (urine specific gravity < 1.03), participants completed a 40-
214 min submaximal run ($65\% \dot{V}O_{2\text{max}}$, 1% gradient) on a motorized treadmill in temperate
215 conditions (19°C). Within the first 20-min of exercise, participants consumed a bolus of
216 water ($5 \text{ mL} \cdot \text{kg}^{-1}$ of body mass). Following exercise, participants undertook a ≤ 40 -min HWI
217 (40°C), immersed to the neck dressed in shorts (2–3 min transition time). Immersion in hot
218 water was terminated either at 40 min, when participants removed themselves due to thermal
219 discomfort or when T_{re} exceeded the institutional ethical cut off (39.9°C). Following removal
220 from the hot water, participants rested in temperate laboratory conditions, dressed in shorts
221 for 15-min without fluids. Following which, participants towel dried and a nude body mass
222 was recorded and adjusted for fluid intake as a measure of WBSR. Participants were free to
223 leave the laboratory when $T_{\text{re}} \leq 38.5^\circ\text{C}$.

224

225 **Measurement and instrumentation**

226 **Body temperatures.** T_{re} was measured using a flexible, sterile rectal thermistor (Henleys
227 Medical Supplies Ltd., Herts, UK), self-inserted 10 cm beyond the rectal sphincter and
228 recorded using a data logger (YSI model 4000A, YSI, Dayton, USA). An area under the
229 curve (AUC) analysis was performed on T_{re} (time T_{re} was $> 38.5^\circ\text{C}$) during each post-
230 exercise HWI exposure to assess for cumulative hyperthermia, as previously described
231 (Cheuvront et al., 2008). Skin temperatures were measured during experimental trials using
232 insulated thermistors (Grant EUS-U, Cambridge, UK) secured on the right side of the body at
233 four locations: chest at a midpoint between the acromion process and the nipple; the lateral
234 mid-bicep; the anterior mid-thigh; and, lateral calf and recorded using a data logger (Grant

235 SQ2020, Cambridge, UK). Mean skin temperature (T_{sk}) was calculated from the four sites
236 using a weighted equation (Ramanathan, 1964).

237

238 **Sweating responses.** Changes in dry nude body mass estimated WBSR during experimental
239 trials and post-exercise HWI heat acclimation exposures. Dew point hygrometry measured
240 local forearm sweat rate during the experimental trials, as previously described (Fortes et al.,
241 2013). The individual relationships between local forearm sweat rate and T_{re} were used to
242 calculate the onset of sweating (Cheuvront et al., 2009).

243

244 **Blood sample collection and analysis**

245 During experimental trials, prior to exercise a venous blood sample (6 mL) was collected into
246 an EDTA vacutainer (BD, Oxford, UK) without stasis from an antecubital vein, following a
247 20-min seated rest to stabilize body fluids. Hemoglobin concentration ($\text{g}\cdot\text{dL}^{-1}$; Hemocue,
248 Sheffield, UK) in duplicate and hematocrit (%) in triplicate (capillary tube method) were
249 immediately assessed from aliquots of whole blood. The change in plasma volume was
250 estimated by correcting the initial plasma volume at PRE for the percentage change in plasma
251 volume at POST, as previously described (Dill and Costill, 1974).

252

253 **Statistical analysis**

254 A sample size calculation (G*Power 3.1.2), using an alpha level of 0.05, power of 0.80 and a
255 strong correlation of 0.7, was performed using data from a study comparing heat acclimation
256 responses in endurance trained and untrained individuals (Shvartz et al., 1977). For a two-
257 way (group \times time) repeated measures ANOVA, a sample size of eight participants per group
258 were calculated to detect a significant difference in the magnitude of reduction in end-
259 exercise T_{re} ($\Delta 0.3^\circ\text{C}$), between endurance trained and untrained individuals following heat
260 acclimation. All data were checked for normality and sphericity and statistical significance
261 was accepted at $P < 0.05$. Two-way repeated measures analysis of variance (ANOVA) with
262 Greenhouse Geisser correction to the degrees of freedom (where necessary) were used to
263 assess for main effects, i.e. differences between groups (endurance trained vs. recreationally
264 active) and changes from PRE to POST during the experimental trials and from day 1 to day
265 6 of the intervention, as well as interaction effects (group \times time). Bonferroni-adjusted
266 pairwise comparisons were used where appropriate to determine where differences occurred.
267 Independent t -tests assessed for differences in total HWI time and total AUC between
268 endurance trained and recreationally active. The magnitude of effect was reported using
269 Cohen's d , where 0.2, 0.5 and 0.8 represent small, medium and large effects, respectively
270 (Cohen, 1988). Pearson's correlations were used to determine the strength of the relationship
271 between training status or aerobic fitness (habitual weekly endurance exercise and $\dot{V}O_{2\max}$)
272 and the reduction in end-exercise T_{re} and heart rate, and between the thermal stimulus (total
273 AUC) during the heat acclimation intervention and the reduction in end-exercise T_{re} and heart
274 rate. Data are presented as mean \pm standard deviation (SD) and were analyzed using SPSS
275 version 24 (IBM Corporation, NY, USA), or GraphPad Prism Version 5.02 (GraphPad
276 Software Inc. La Jolla, USA).

277

278

279 **Results**

280

281 **Intervention**

282 All participants completed a 40-min treadmill run at 65% $\dot{V}O_{2\max}$ followed by HWI (≤ 40
283 min) on six consecutive days. Compared to their typical endurance exercise volume, during
284 the intervention, weekly endurance exercise volume was unchanged for endurance trained (-1
285 ± 2 h; $P > 0.05$) and increased in recreationally active individuals ($+2 \pm 0$ h; $P < 0.01$).
286 During the 6-day intervention, HWI duration increased from day 1 to day 6 ($P < 0.05$);
287 ensuring a maintenance of the endogenous stimulus for adaptation, with a similar AUC and
288 end-HWI T_{re} between day 1 and day 6 ($P > 0.05$; Table 1). In addition, total immersion time
289 ($P = 0.08$, $d = 1.0$) and total AUC ($P = 0.08$, $d = 0.8$) during the 6-day intervention tended to
290 be greater in endurance trained than recreationally active individuals (Table 1).

291

292 ***Table 1 near here***

293

294 **Experimental trials**

295 No significant interaction effects (group \times time; $P > 0.05$) demonstrate that training status did
296 not influence the observed adaptations to the 6-day post-exercise HWI intervention, for
297 measures of: end-exercise T_{re} (Figure 1A); resting T_{re} ; T_{re} at sweating onset; ΔT_{re} during
298 exercise; end-exercise T_{sk} ; end-exercise $T_{re}-T_{sk}$ gradient; end-exercise PhSI; end-exercise
299 RPE, end-exercise thermal sensation, mean $\dot{V}O_2$, mean RER or mean metabolic energy
300 expenditure (Table 2). Training status did not relate strongly to the magnitude of thermal
301 adaptation; since the reduction in end-exercise T_{re} during exercise-heat-stress was not
302 strongly correlated with either habitual endurance exercise volume ($r = 0.35$, $P > 0.05$) or
303 $\dot{V}O_{2\max}$ ($r = 0.29$, $P > 0.05$). In endurance trained individuals, a larger thermal stimulus
304 during the HWI intervention (total AUC $63-320^\circ\text{C}\cdot\text{min}^{-1}$) was strongly associated with a
305 larger reduction in end-exercise T_{re} ($r = -0.71$; $P < 0.05$). Moreover, post-exercise HWI
306 reduced thermal strain during exercise-heat stress in all 16 participants, supported by a main
307 effect of time (PRE vs. POST) for end-exercise T_{re} (PRE; $38.85 \pm 0.49^\circ\text{C}$, POST; $38.43 \pm$
308 0.42°C , $P < 0.01$, $d = 0.9$; Figure 1B); albeit, one recreationally active participant
309 experienced only a 0.08°C reduction in end-exercise T_{re} . Contrary to the notion that the most
310 highly trained would benefit the least from the HWI intervention, the most accomplished
311 endurance trained participant, an international marathon runner ($\dot{V}O_{2\max}$: $81 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$
312 and road half-marathon PB: 66 min) experienced a meaningful reduction in end-exercise T_{re}
313 during exercise-heat-stress (PRE; 38.94°C , POST; 38.62°C).

314

315 ***Figure 1 near here***

316

317 ***Table 2 near here***

318

319 Recreationally active individuals experienced a smaller thermal stimulus during the HWI
320 intervention (total AUC $58-197^\circ\text{C}\cdot\text{min}^{-1}$) than endurance trained individuals, since they
321 terminated HWI sooner due to thermal discomfort (Table 1). The thermal stimulus during the
322 post-exercise HWI intervention was not strongly related to the reduction in end-exercise T_{re}
323 in recreationally active individuals ($r = 0.12$, $P > 0.05$). As such, there appear to be other
324 drivers contributing to the observed adaptations in recreationally active individuals, beyond
325 the total AUC. An interaction effect (group \times time; $P < 0.01$) was observed for end-exercise
326 heart rate, with a significant reduction from PRE to POST in recreationally active (PRE; 178
327 $\pm 12 \text{ beats}\cdot\text{min}^{-1}$, POST; $163 \pm 9 \text{ beats}\cdot\text{min}^{-1}$, $P < 0.01$, $d = 1.4$), but not endurance trained
328 individuals (PRE; $167 \pm 15 \text{ beats}\cdot\text{min}^{-1}$, POST; $163 \pm 16 \text{ beats}\cdot\text{min}^{-1}$, $P > 0.05$, $d = 0.2$; Table

329 2). Correlations suggest that the decrease in end-exercise heart rate during exercise-heat-
330 stress after the HWI intervention was relatively strongly related to habitual exercise volume
331 ($r = 0.68$, $P < 0.01$; Figure 2A) and aerobic fitness ($\dot{V}O_{2\max}$; $r = 0.57$, $P < 0.05$; Figure 2B);
332 whereby, those with a higher habitual exercise volume and aerobic fitness demonstrated a
333 smaller reduction in end-exercise heart rate after the 6-day post-exercise HWI intervention. In
334 contrast, the thermal stimulus during the HWI intervention (total AUC) was not strongly
335 related to the decrease in end-exercise heart rate during exercise-heat-stress ($r = 0.14$, $P >$
336 0.05).

337

338 ***Figure 2 near here***

339

340 Other hallmark heat acclimation adaptations were achieved following post-exercise HWI
341 (main effect of time, PRE vs. POST, $n = 16$), including reductions in: resting T_{re} (PRE; 36.91
342 $\pm 0.31^\circ\text{C}$, POST; $36.71 \pm 0.35^\circ\text{C}$, $P < 0.01$, $d = 0.6$); T_{re} at sweating onset ($P < 0.01$, $d = 0.6$);
343 ΔT_{re} during exercise ($P < 0.05$, $d = 0.5$); end-exercise heart rate ($P < 0.01$, $d = 0.7$); T_{sk} ($P <$
344 0.01 , $d = 0.8$); $T_{re}-T_{sk}$ gradient ($P < 0.05$, $d = 0.3$); PhSI ($P < 0.01$, $d = 0.9$); RPE ('fairly
345 hard' to 'fairly light', $P < 0.05$, $d = 0.7$); thermal sensation ('hot' to 'uncomfortably warm', P
346 < 0.01 , $d = 1.0$); mean $\dot{V}O_2$ ($P < 0.01$, $d = 0.2$) and mean energy expenditure (PRE; $1037 \pm$
347 160 W, POST; 1003 ± 160 W, $P < 0.01$, $d = 0.2$; Table 2). No main effect for time (PRE vs.
348 POST; $n = 16$) was observed for WBSR (1.02 ± 0.35 L·h⁻¹ to 1.07 ± 0.33 L·h⁻¹, $P > 0.05$,
349 Table 2) or mean RER ($P > 0.05$) and the relative changes in plasma volume were not
350 significantly different in endurance trained ($4 \pm 8\%$) or recreationally active participants ($3 \pm$
351 7% ; $P > 0.05$, $d = 0.6$). There was no main effect for training status (endurance trained vs.
352 recreationally active), for measures of resting T_{re} , T_{re} at sweating onset, mean exercising RER
353 and measures taken at end-exercise including: T_{re} ; heart rate; T_{sk} ; $T_{re}-T_{sk}$ gradient; RPE and
354 thermal sensation ($P > 0.05$). However, ΔT_{re} during exercise, end-exercise PhSI, WBSR,
355 mean $\dot{V}O_2$ and mean metabolic energy expenditure were greater in endurance trained
356 compared with recreationally active individuals ($P < 0.05$).

357

358 Discussion

359

360 The present study sought to compare heat acclimation adaptations in endurance trained and
361 recreationally active individuals after a 6-day post-exercise HWI intervention. In agreement
362 with our hypothesis, the new and noteworthy finding is that HWI brought about comparable
363 heat acclimation adaptations in endurance trained and recreationally active individuals.

364 Hallmark heat acclimation adaptations, observed in endurance trained and recreationally
365 active individuals, included reductions in resting T_{re} and reductions in: end-exercise T_{re} ; end-
366 exercise PhSI; T_{re} at sweating onset and T_{sk} during exercise-heat-stress. Furthermore, training
367 status did not significantly influence observed reductions in thermal sensation or RPE during
368 exercise-heat-stress after the HWI intervention. The observed benefits were achieved by
369 exposure to a large thermal stimulus for adaptation during the daily heat acclimation sessions
370 (change in $T_{re} \approx 2^{\circ}\text{C}$; $T_{sk} = 40^{\circ}\text{C}$); despite no significant changes in WBSR or plasma volume.

371

372 The heat acclimation benefit of the HWI intervention for endurance trained individuals is
373 emphasized by the association between the thermal stimulus (total AUC $^{\circ}\text{C}\cdot\text{min}^{-1}$) and the
374 reduction in end-exercise T_{re} during exercise-heat-stress ($r = -0.71$); whereby, thermal strain
375 during exercise-heat-stress was reduced most in endurance trained individuals who
376 experienced the greatest thermal stimulus during the HWI intervention. Contrary to the
377 notion that the most highly trained individuals would benefit the least (Pandolf et al., 1977;
378 Shvartz et al., 1977), our most accomplished endurance performer, an international marathon
379 runner, experienced a meaningful reduction in end-exercise T_{re} after the HWI intervention
380 (0.32°C). Nevertheless, endurance trained individuals tended to require a greater thermal
381 stimulus during the HWI intervention to achieve a similar reduction in thermal strain as
382 recreationally active individuals. This was likely a consequence of the endurance trained
383 individuals' partial heat acclimatization status and associated increased heat tolerance
384 (Piwonka et al., 1965; Strydom et al., 1966; Gisolfi and Robinson, 1969; Selkirk and
385 McLellan, 2001). The responsible mechanism(s) for the reduction in resting T_{re} with post-
386 exercise HWI (Zurawlew et al., 2016) and exercise-heat-acclimation (Tyler et al., 2016)
387 require elucidation. Reductions in resting T_{re} have been related to a lowering of metabolic
388 rate in seasonal heat acclimatization (Buguet et al., 1988) and to endurance training
389 adaptations (Baum et al., 1976). The coupling of the reduction in resting T_{re} (-0.20°C) and T_{re}
390 at sweating onset (-0.22°C) likely accounts for the further reduction in thermal strain during
391 exercise heat stress after the HWI intervention (end exercise $T_{re} -0.42^{\circ}\text{C}$): heat loss via
392 sweating and cutaneous vasodilation are initiated at lower thermoregulatory thresholds after
393 heat acclimation (Buono et al., 1998).

394

395 As a consequence of their habitual exercise training, endurance trained individuals are
396 considered to be further along the heat adaptation continuum than recreationally active
397 individuals; reducing their adaptation potential (Taylor, 2014). It's perhaps not surprising
398 then, and in keeping with exercise-heat-acclimation findings (Shvartz et al., 1977), that a
399 greater reduction in exercising heart rate was observed in recreationally active than
400 endurance trained individuals after the HWI intervention (-15 vs. -4 beats $\cdot\text{min}^{-1}$ in endurance
401 trained). Indeed, the magnitude of the reduction in heart rate was associated with habitual
402 endurance exercise volume ($r = 0.68$) and aerobic fitness ($r = 0.57$); whereby, heart rate was
403 reduced most in those completing less habitual endurance exercise and those with lower
404 aerobic fitness. Likely, the passive heat stimulus during HWI elicited the notable reduction in
405 cardiovascular strain in recreationally active individuals in the present study; in agreement
406 with the findings of others (Brebner et al., 1961; Brazaitis and Skurvydas, 2010). It's unlikely
407 that the reduction in heart rate was due to the daily exercise as we have previously shown no

408 improvements in cardiovascular fitness (i.e. no reduction in heart rate or $\dot{V}O_2$) in
409 recreationally active individuals who performed the same daily exercise intervention
410 followed by a thermoneutral bath (Zurawlew et al., 2016). The more notable reduction in
411 heart rate in recreationally active individuals after the HWI intervention likely relates to
412 alterations in cardiac autonomic regulation (Periard et al., 2016); adaptations already
413 possessed by the endurance trained individuals (Carter et al., 2003). We show no obvious
414 plasma volume expansion and a similar widening of the $T_{re}-T_{sk}$ gradient in recreationally
415 active and endurance trained after the intervention; both mechanisms are often posited to
416 reduce cardiovascular strain with heat acclimation (Periard et al., 2015).

417
418 Hallmark adaptations to the heat have long been considered to include an expansion in
419 resting plasma volume (Greenleaf et al., 1983) and an increase in WBSR during exercise-
420 heat-stress (Wyndham and Strydom, 1969). Corroborating our recent work in recreationally
421 active individuals (Zurawlew et al., 2016; Zurawlew et al., 2018), the current findings
422 demonstrate that post-exercise HWI also reduces thermal strain during exercise-heat-stress in
423 endurance trained individuals; despite no obvious increase in plasma volume or WBSR. It's
424 noteworthy that a recent meta-analysis highlighted the rather modest and variable plasma
425 volume expansion ($+4 \pm 5\%$) and increase in WBSR ($+5 \pm 11\%$) in short-term heat
426 acclimation studies (< 7 exposures) (Tyler et al., 2016). Typically, > 7 exercise-heat-
427 acclimation exposures are required to initiate an increase in WBSR; but even then, these
428 responses are highly variable ($+29 \pm 29\%$) (Tyler et al., 2016). The semi-recumbent body
429 position and hydrostatic forces during HWI may maintain central vascular volume and in-
430 turn reduce fluid regulatory stress and the stimulus for plasma volume expansion (Nagashima
431 et al., 1999; Bradford et al., 2015). However, the absence of an expansion in plasma volume
432 may be associated with errors in estimating relative changes in plasma volume using
433 hemoglobin and hematocrit; therefore, future research should verify this finding using tracer
434 techniques. Immersing the skin in hot water has been shown to reduce sweat gland activity
435 and the stimulus for an increase in sweating (Hertig et al., 1961; Brebner and Kerslake,
436 1968). However, a more likely explanation for the lack of an increase in WBSR with HWI is
437 that the decrease in T_{re} at sweating onset (-0.22°C) was offset by the decrease in resting T_{re} ($-$
438 0.20°C). No increase in WBSR during exercise-heat-stress after the HWI intervention may
439 provide additional thermoregulatory and performance benefits during exercise-heat-stress, by
440 constraining dehydration and preserving central blood volume (Montain and Coyle, 1992).
441 Heat acclimation by post-exercise HWI may limit the 'wasteful overproduction of sweat'
442 (Mitchell et al., 1976); particularly important in high humidity conditions when evaporative
443 heat loss is limited and when sweat may drip from the skin. Notwithstanding, we recognize
444 that the relatively modest exercise-heat stress for experimental trials ($65\% \dot{V}O_{2max}$, 33°C ,
445 $40\% \text{RH}$) may have masked an increase in WBSR (Poirier et al., 2015); as such, studies
446 should investigate the influence of heat acclimation by post-exercise HWI on WBSR during a
447 more uncompensable exercise scenario.

448
449 Despite evidence that exercise-heat-acclimation alleviates thermal strain and improves
450 performance in the heat (Nielsen et al., 1997; Lorenzo et al., 2010), practical barriers limit
451 athlete engagement with current exercise-heat-acclimation recommendations (Tyler et al.,
452 2016; Casadio et al., 2017; Periard et al., 2017). As such, there has been an increasing interest
453 of late in practical heat acclimation methods; including, training in temperate conditions
454 whilst wearing additional clothing (Stevens et al., 2018) and post-exercise HWI (Zurawlew et
455 al., 2016; Zurawlew et al., 2018). Exercise in temperate conditions wearing additional
456 clothing may provide the necessary elevations in core and skin temperature for adaptation
457 (Dawson et al., 1989; Ely et al., 2018). However, a recent field study in triathletes showed

458 that training in temperate conditions (18°C) wearing additional clothing was not an effective
459 heat acclimation strategy (Stevens et al., 2018). For endurance trained athletes residing and
460 training in temperate conditions, the current findings support the recommendation that
461 incorporating a hot bath (lasting up to 40 min) in the post-exercise washing routine represents
462 an effective and accessible heat acclimation strategy to prepare for competition in the heat.
463 Taking a hot bath after temperate exercise limits interference with an athlete's training and
464 taper and does not require access to an environmental chamber or precise control over
465 exercising core temperature. The current findings (delta end exercise T_{re} -0.42°C), and our
466 previous work (Zurawlew et al., 2016; Zurawlew et al., 2018), show that the magnitude of
467 adaptations following post-exercise HWI compare favorably with exercise-heat-acclimation
468 interventions, as reported in a recent meta-analysis (delta exercise core temperature -0.34°C)
469 (Tyler et al., 2016). Therefore, the findings from the current study, considered alongside the
470 extant literature, do not support the notion that exercise-heat-acclimation evokes superior
471 adaptation and should be recommended in favour of passive heat acclimation (Periard et al.,
472 2016; Tyler et al., 2016). Notwithstanding, future studies should directly compare post-
473 exercise HWI and exercise-heat-acclimation and confirm whether the observed adaptations in
474 endurance trained individuals translate to improved aerobic performance; as was previously
475 observed in recreationally active individuals (Zurawlew et al., 2016). A small handful of
476 studies provide evidence of heat acclimation with repeated HWI alone (without prior
477 exercise) (Brebner et al., 1961; Bonner et al., 1976; Brazaitis and Skurvydas, 2010); as such,
478 studies may wish to compare the efficacy of HWI with and without prior exercise. However,
479 unpublished observations in our laboratory show a larger thermal stimulus (daily AUC
480 °C·min⁻¹) for post-exercise HWI than for the HWI alone strategies used in two of these
481 studies (Brebner et al., 1961; Bonner et al., 1976). Moreover, we have concerns regarding
482 participant safety and tolerance to the unpleasantly high water temperature (44°C) used in
483 another of these studies (Brazaitis and Skurvydas, 2010). On the one hand, studies should
484 determine whether meaningful heat acclimation can be achieved by fewer and/or shorter
485 post-exercise HWI exposures. On the other hand, mindful of safety and practical constraints,
486 exploring whether the observed adaptations can be further augmented by increasing the
487 intensity of the prior exercise, or increasing the number and/or duration of HWI exposures
488 requires investigation. Finally, studies should also determine the rate of decay of heat
489 acclimation after the 6-day post-exercise HWI intervention and whether the adaptations
490 observed herein translate to females.

491

492 **Conclusion**

493 Hot water immersion after exercise in temperate conditions on six consecutive days reduced
494 thermal strain during exercise-heat-stress in endurance trained and recreationally active
495 individuals. For high level athletes residing and training in temperate conditions,
496 incorporating a hot bath in the post-exercise washing routine represents an effective heat
497 acclimation strategy to prepare for major competition in the heat.

498 **Author contributions**

499 NW had primary responsibility for the final content. NW, JM and MZ were involved in the
500 conception of the project and development of the research plan. MZ led the data collection.
501 NW, JM and MZ performed the data analysis, interpreted the data and prepared the
502 manuscript.

503

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510

511 *Conflicts of interest:* The authors of the study declare that they have no conflicts of interest.

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727

728 **Figure legends**

729

730 FIGURE 1. Effect of 6-day post-exercise hot water immersion heat acclimation on end-
731 exercise rectal core temperature (T_{re}) following a 40-min submaximal treadmill run at 65%
732 $\dot{V}O_{2max}$ in the heat (33°C, 40% RH) in endurance trained and recreationally active
733 individuals. Bars represent mean \pm SD of the PRE to POST change (A) and mean at PRE and
734 POST (B) for end-exercise T_{re} . Lines between bars represent individual participant responses.
735 ** $P < 0.01$ denotes POST lower than PRE (main effect of time).

736

737 FIGURE 2. Relationship between habitual endurance exercise volume (A) and aerobic fitness
738 (B) and the change in heart rate response to exercise-heat-stress after 6-day post-exercise hot
739 water immersion heat acclimation.

740

TABLE 1. The influence of 40-min submaximal running at 65% $\dot{V}O_{2max}$ in temperate conditions followed by post-exercise hot water immersion in 40°C water on thermoregulatory variables, heart rate and immersion time in endurance trained and recreationally active participants.

	Endurance trained		Recreationally active	
	Day 1	Day 6	Day 1	Day 6
Submaximal exercise				
End-exercise T_{re} (°C)	38.37 ± 0.48	38.27 ± 0.43	38.34 ± 0.32	38.22 ± 0.23
End-exercise heart rate (beats·min ⁻¹) *	147 ± 13	144 ± 10	150 ± 9	144 ± 9
Hot water immersion				
End-immersion T_{re} (°C)	39.44 ± 0.44	39.36 ± 0.31	39.15 ± 0.18	39.21 ± 0.20
Immersion time (min) **	35 ± 8	40 ± 0	28 ± 5	40 ± 1
<i>n</i> completing 40-min immersion	5 of 8	8 of 8	0 of 8	7 of 8
Submaximal exercise and hot water immersion				
WBSR (L·h ⁻¹) * #	1.08 ± 0.34	1.25 ± 0.26	0.72 ± 0.17	0.95 ± 0.18
AUC (°C·min ⁻¹)	33 ± 24	29 ± 15	18 ± 7	20 ± 7

T_{re} ; rectal temperature, AUC; area under the curve. * $P < 0.05$ and ** $P < 0.01$, main effect of time. # $P < 0.05$, main effect of group. Data displayed as mean ± SD.

TABLE 2. Effect of 6-day post-exercise hot water immersion heat acclimation on thermal, cardiovascular, metabolic and perceptual responses at rest and to 40-min submaximal treadmill running at 65% $\dot{V}O_{2\max}$ in the heat (33°C, 40% RH) in endurance trained and recreationally active participants.

	Endurance Trained	Recreationally Active
T_{re} at sweating onset (°C) **	-0.22 ± 0.24	-0.23 ± 0.29
ΔT_{re} during exercise (°C) * ##	-0.19 ± 0.35	-0.25 ± 0.27
End-exercise T_{sk} (°C) **	-0.67 ± 0.38	-0.75 ± 0.70
End-exercise $T_{re}-T_{sk}$ gradient (°C) *	0.31 ± 0.42	0.27 ± 0.62
WBSR (L·h ⁻¹) ##	0.13 ± 0.02	-0.03 ± 0.25
End-exercise heart rate (beats·min ⁻¹) **	-4 ± 5	-15 ± 7 ††
End-exercise PhSI (0–10) ** #	-1 ± 1	-1 ± 1
Mean $\dot{V}O_2$ (L·min ⁻¹) ** ##	-0.1 ± 0.1	-0.1 ± 0.2
Mean metabolic energy expenditure (W) ** ##	-28 ± 31	-40 ± 50
End-exercise RPE (6–20) *	-1 ± 1	-2 ± 3
End-exercise thermal sensation (1–13) **	-1 ± 1	-1 ± 1

T_{sk} , mean skin temperature; T_{re} , rectal temperature; WBSR, whole body sweat rate; PhSI, physiological strain index; RPE, rating of perceived exertion. Data displayed as mean ± SD of the PRE to POST change. * $P < 0.05$ and ** $P < 0.01$, denotes POST different than PRE (main effect of time). # $P < 0.05$ and ## $P < 0.01$, denotes endurance trained different than recreationally active (main effect of group). †† $P < 0.01$, denotes POST lower than PRE within group (post hoc time effect).

Figure 1

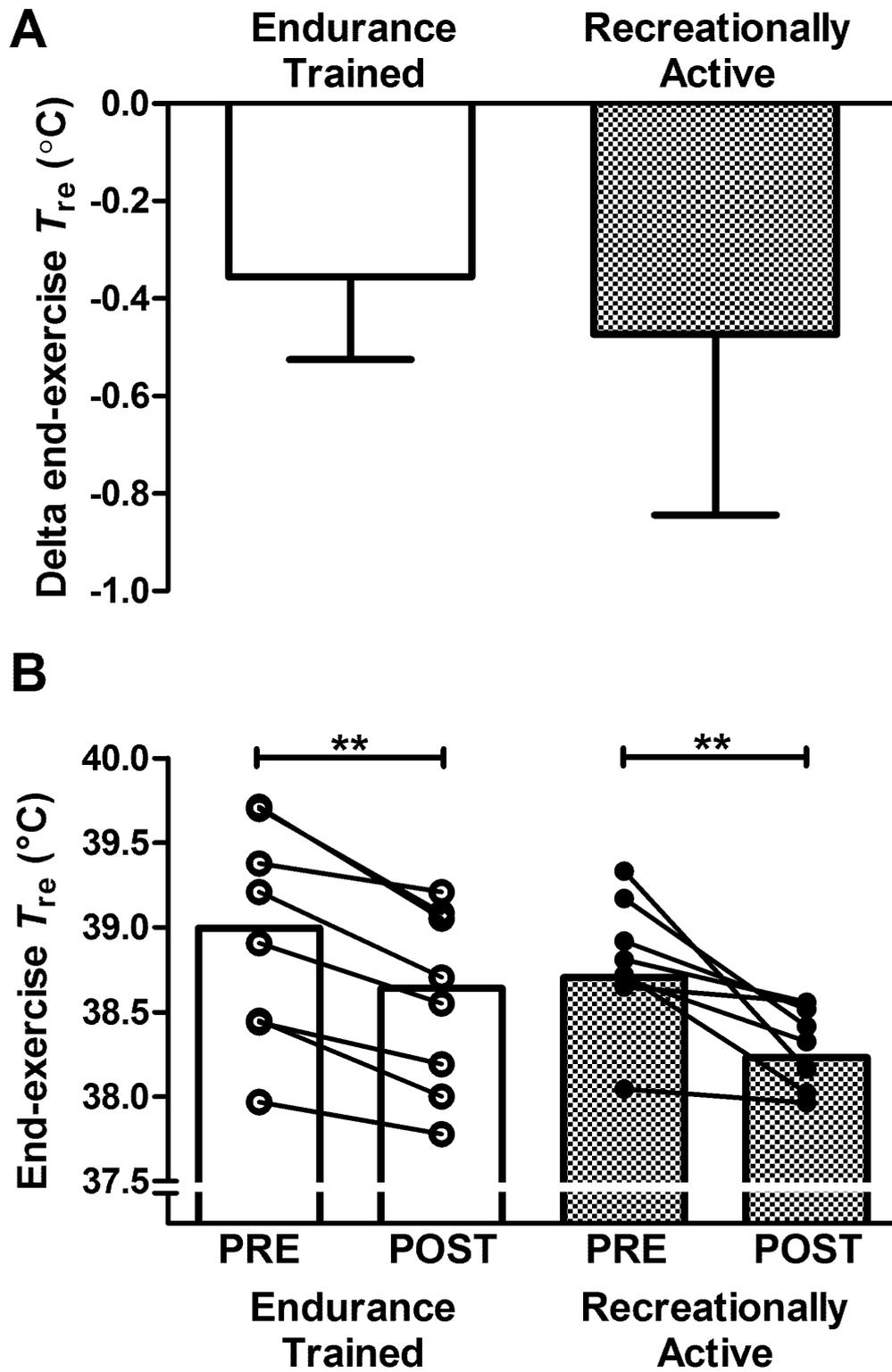


Figure 2

