

1 The effects of high intensity intermittent exercise compared to continuous exercise on
2 voluntary water ingestion

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16 Running head: High intensity exercise and voluntary water intake

17

18 Abstract

19 Water intake occurs following a period of high intensity intermittent exercise (HIIE) due to
20 sensations of thirst yet this does not always appear to be caused by body water losses. Thus,
21 the aim was to assess voluntary water intake following HIIE. Ten healthy males (22 ± 2 y,
22 75.6 ± 6.9 kg, $\dot{V}O_{2\text{peak}} 57.3\pm 11.4$ ml.kg⁻¹.min⁻¹) (mean \pm SD) completed two trials (7-14d apart).
23 Subjects sat for 30min then completed an exercise period involving 2min of rest followed by
24 1min at 100% $\dot{V}O_{2\text{peak}}$ repeated for 60min (HIIE) or 60min continuously at 33% $\dot{V}O_{2\text{peak}}$ (LO).
25 Subjects then sat for 60min and were allowed *ad libitum* water intake. Body mass was
26 measured at start and end of trials. Serum osmolality, blood lactate and sodium
27 concentrations, sensations of thirst and mouth dryness were measured at baseline, post-
28 exercise and after 5, 15, 30 and 60min of recovery. Vasopressin concentration was measured
29 at baseline, post-exercise, 5 and 30min. Body mass loss over the whole trial was similar
30 (HIIE: 0.77 ± 0.50 ; LO: $0.85\pm 0.55\%$) ($p=0.124$). Sweat lost during exercise (0.78 ± 0.22 v
31 0.66 ± 0.26 l) and voluntary water intake during recovery (0.416 ± 0.299 v 0.294 ± 0.295 l)
32 ($p<0.05$) were greater in HIIE. Serum osmolality (297 ± 3 v 288 ± 4 mOsmol.kg⁻¹), blood lactate
33 (8.5 ± 2.7 v 0.7 ± 0.4 mmol.l⁻¹), serum sodium (146 ± 1 v 143 ± 1 mmol.l⁻¹) and vasopressin
34 (9.91 ± 3.36 v 4.43 ± 0.86 pg.ml⁻¹) concentrations were higher after HIIE ($p<0.05$) and thirst
35 (84 ± 7 v 60 ± 21) and mouth dryness (87 ± 7 v 64 ± 23) also tended to be higher ($p=0.060$).
36 Greater voluntary water intake after HIIE was mainly caused by increased sweat loss and the
37 consequences of increased serum osmolality mainly resulting from higher blood lactate
38 concentrations.

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42 Key Words: osmolality, lactate, thirst

43

44 Introduction

45

46 An increase in serum osmolality causing an increased release of vasopressin has been
47 proposed as one of the mechanisms resulting in the sensation of thirst and water replacement
48 (Stricker & Verbalis 1988). Following the onset of exercise, loss of water from the vascular
49 space results in a rise in serum osmolality (Convertino, Keil, Bernauer, & Greenleaf, 1981).
50 During and following continuous exercise, the resultant effect of increased osmolality and
51 vasopressin release on voluntary water intake has been extensively studied (Cheuvront &
52 Haymes 2001; Dugas, Oosthuizen, Tucker, & Noakes, 2009; Wong, Williams, Simpson, &
53 Ogaki, 1998), yet the effect on water intake following a bout of high intensity intermittent
54 exercise (HIIE) is less well known. During and following HIIE, there is an increase in blood
55 lactate concentration, which has been linked to the prevention of serum sodium uptake from
56 the vascular space to the intracellular space, resulting in an increased serum osmolality (Nose
57 et al., 1991). Nose et al. (1991) explored the link between exercise intensity, plasma lactate
58 and plasma sodium concentrations. As submaximal exercise intensity increased, a significant
59 rise in plasma sodium concentrations was observed which correlated strongly with changes in
60 plasma lactate concentrations.

61

62 Bouts of high intensity exercise have also been shown to result in an increase in vasopressin
63 release (Hew-Butler, Noakes, Soldin, & Verbalis, 2008). For example, Hew-Butler et al.
64 (2008) found that on completion of a maximal oxygen uptake test, vasopressin concentrations
65 were significantly elevated compared to a submaximal bout of continuous exercise.
66 However, subsequent water intake was not assessed so the affect of increased vasopressin
67 release on sensations of thirst and voluntary water intake could not be established.

68

69 Following a period of high intensity exercise there is a shift in water from the vascular to the
70 interstitial and intracellular spaces (Convertino et al., 1981; Nose et al., 1991; Sjøgaard,
71 Adams, & Saltin, 1985). The movement of hypotonic water out of the vascular space will
72 contribute to the rise in plasma osmolality. Sjøgaard et al. (1985) analysed extra- and
73 intracellular muscle water shifts following one-legged dynamic knee-extensions in six males.
74 They attributed the movement of water to the interstitial space due to an increase in blood
75 pressure and to an increase in perfused capillaries, whilst an osmotic gradient caused by an
76 increase in lactate concentration was believed to cause water to move into the intracellular
77 space.

78

79 Despite the known effect of HIIE on the rise in serum osmolality and an increase in
80 vasopressin release the effect on subsequent voluntary water intake is unknown. A rise in
81 osmolality above the vasopressin release threshold of approximately $285 \text{ mOsmol.kg}^{-1}$
82 (Thompson, Bland, Burd, & Baylis, 1986) will lead to maximal anti-diuresis, resulting in an
83 osmotically driven thirst signal, thus facilitating water intake. Following a bout of HIIE,
84 increased serum osmolality above values experienced following continuous exercise of
85 matched work, may result in a greater osmotic signal, ultimately leading to increased water
86 intake. Excessive water intake may result in weight gain, which for weight bearing sports
87 such as running may impair performance. In addition water intake may lead to increased
88 urine output, which along with increased inconvenience may result in increased water losses
89 (Wong et al., 1998).

90

91 Depending on the duration of the HIIE, sweat losses may not be large enough to result in
92 sensations of thirst (Wolf, 1950) or result in a level of dehydration that will impair
93 performance (Sawka et al., 2007). Sensations of thirst have been shown to increase and result
94 in voluntary water intake when body mass losses, reach and increase beyond approximately
95 0.8% (Wolf, 1950), whilst body mass losses of less than 2% can be tolerated without
96 decrement in exercise performance (Sawka et al., 2007). However, despite this, water intake
97 will usually occur after HIIE and suggests a mechanism independent of water losses is acting
98 to increase sensations of thirst and subsequent voluntary water intake (Nose et al., 1991). As
99 increased blood lactate concentration has been shown to affect serum sodium concentration
100 and therefore serum osmolality (Nose et al., 1991), the question arises as to the influence of
101 increased blood lactate concentrations on sensations of thirst and subsequent voluntary water
102 intake.

103

104 It was hypothesised that the increase in lactate concentration, resulting from the high intensity
105 intermittent exercise, would increase serum sodium concentration and thus, serum osmolality,
106 in turn causing increased sensations of thirst and subsequent voluntary water intake and also
107 increased vasopressin release.

108

109 Methods

110

111 Subjects

112 Ten healthy male subjects (age 22 ± 2 years, mass 75.6 ± 6.9 kg, height 1.78 ± 0.08 m, \dot{V}
113 O_{2peak} 57.3 ± 11.4 ml.kg⁻¹.min⁻¹) (mean \pm SD) were recruited to take part in two trials,
114 undertaken in a counter-balanced order. All subjects had the experimental protocol explained
115 to them verbally and in writing. Subjects provided written informed consent and the
116 experiment was approved by the Loughborough University Ethical Advisory Committee.

117

118 Experimental protocol

119 Subjects were asked to visit the laboratory on four separate occasions for a $\dot{V}O_{2peak}$ test, a
120 familiarisation trial and two experimental trials; high intensity intermittent (HIIE) and
121 continuous (LO) exercise. During the first visit $\dot{V}O_{2peak}$ was measured using a discontinuous
122 incremental test to volitional fatigue on an electrically braked cycle ergometer (Lode Corival;
123 Lode BV, Groningen, Netherlands). During the final minute of each four minute incremental
124 stage, expired gas was collected in Douglas bags and analysed for oxygen and carbon dioxide
125 concentration (Servomex 1400 Oxygen and Carbon Dioxide Gas Analyser; Servomex,
126 Crowborough, UK). Gas volumes and temperature were measured using a Harvard dry gas
127 meter (Harvard Apparatus Ltd., Edenbridge, UK) and thermometer (Edale Digital
128 Thermometer D515: Edale instruments Ltd., Cambridge, UK) and corrected to STPD
129 (standard temperature and pressure, dry). Subjects visited the lab a further three times for the
130 familiarisation trial and two experimental trials. The familiarisation trial was identical to the
131 HIIE trial. Prior to each experimental trial subjects were asked to consume 500ml of water
132 two hours before arrival at the laboratory to ensure they were in a euhydrated state and to
133 arrive after an overnight fast. In the 24 hours prior to the first experimental trial, subjects
134 were asked to record their dietary intake (food and drink consumed, amount and method of
135 preparation) and refrain from strenuous physical activity and consumption of alcohol. They
136 were then asked to repeat this before each subsequent trial.

137

138 The experimental trials were separated by a period of 7-14 days and began in the morning at
139 the same time for each subject. Experimental trials were identical apart from the exercise
140 performed. A schematic outline of the experimental trial is presented in Figure 1.

141 Experimental trial order was decided by incomplete Latin square design and subjects did not
142 know which trial they were participating in when arriving at the laboratory for the first trial.

143

144 In each trial, on arrival, subjects voided and the whole urine volume was measured and a 5 ml
145 sample retained for later analysis and had nude body mass measured. Subjects were asked to
146 insert a rectal thermistor 10 cm past the anal sphincter. Skin thermistors were attached at the
147 chest, tricep, thigh and calf and a heart rate monitor was positioned (Polar Vantage; Kempele,
148 Finland). Core (T_c) and skin temperature (T_{sk}) were measured continuously throughout the
149 trials and a minute average was taken every 10 min (BIOPAC MP100 System; BIOPAC,
150 Santa Barbara, CA, USA). Mean weighted skin temperature was calculated using the
151 formula outlined by Ramanathan (1964). Subjects sat for 30 min to account for postural
152 alterations in blood volume at $19.7 \pm 1.1^\circ\text{C}$ and $30.7 \pm 10.5\%$ relative humidity (RH).
153 Baseline heart rate values every 10 min were recorded and a 100 mm visual analogue
154 subjective feelings questionnaire comprising of thirst and dry mouth scales was administered
155 at the completion of the 30 min seated rest (0 mm = not all thirsty/mouth not at all dry, 100
156 mm = very thirsty/mouth very dry). During the rest period a 21g cannula (Surflo, Terumo,
157 Leuven, Belgium) was inserted into a superficial vein on the forearm to allow venous blood
158 sampling. The line was flushed with 2-3 ml of heparinised saline. A baseline (B) blood
159 sample (7.5 ml) was collected at the end of the rest period. Subjects then cycled for a period
160 of 60 min in $24.9 \pm 0.7^\circ\text{C}$ and $51.1 \pm 2.1\%$ RH. In the HIIE trial, they rested for 2 min and
161 then performed 1 minute of cycling at a power output attempted to equal the maximum
162 power achieved when recording $\dot{V}O_{2peak}$ (305 ± 55 W), however exact total work performed
163 during the HIIE trial was not measured. This was repeated 20 times during the 60 minute
164 period. In the LO trial, subjects cycled continuously at 33% of their peak power output for
165 60 min (102 ± 18 W). Every 10 min in the LO trial, heart rate was recorded and subjects
166 were asked to provide a rating of their perceived exertion (RPE) and thermal sensation. In
167 the HIIE trial, this was performed at the end of a HIIE bout closest to the completion of a 10
168 min period. Immediately following completion of exercise (post-exercise, PE), a blood
169 sample (7.5 ml) was collected and thirst and dry mouth subjective feelings questionnaires
170 were completed. Subjects were then seated for 60 min in $21.2 \pm 1.8^\circ\text{C}$ and $29.5 \pm 10.3\%$ RH
171 with tap water ($11 \pm 3^\circ\text{C}$) intake measured during each 30 minute period. The amount of
172 water consumed was measured but the subject was not made aware of the volume or that the
173 volume was being measured. They were provided with no external cues to drink and

174 informed at the commencement of the recovery period that they could drink as they wanted
175 and that the bottle would be refilled if necessary. Heart rate and thermal sensation were
176 measured every 10 min. At 5, 15, 30 and 60 min a blood sample (7.5 ml) was collected and
177 thirst and dry mouth subjective feelings questionnaires were completed. Subjects voided, the
178 volume was measured and a 5 ml sample was retained for later analysis and they then had
179 nude body mass measured. After completion of the body mass measurement, subjects were
180 allowed to leave the laboratory. Ambient temperature and relative humidity was measured at
181 10 minute intervals (RH85 Digital Thermo-Hygrometer; Omega, Manchester, UK).

182

183 Sample analysis

184 For each 7.5 ml venous blood sample, 1.0 ml was aliquoted and mixed with anticoagulant (K^+
185 EDTA; 1.5 mg.ml^{-1}) for analysis of haemoglobin concentration, haematocrit and glucose
186 concentration. A further 5.0ml was aliquoted and mixed with anticoagulant (K^+ EDTA; 1.5
187 mg.ml^{-1}) and from this, plasma was separated and frozen at -80°C for later analysis of
188 hormone concentration. The remaining blood ($\sim 2.0 \text{ ml}$) was allowed to clot and was
189 centrifuged at 3000 rpm for 15 min at 4°C before the serum was removed and later analysed
190 for potassium and sodium concentration by flame photometry (Corning Clinical Flame
191 Photometer 410C; Corning Ltd., Halstead, Essex, UK) and osmolality analysis by freezing
192 point depression (Gonotec Osmomat auto Cryoscopic Osmometer; Gonotec, Berlin,
193 Germany). Haemoglobin concentration was measured in duplicate using the
194 cyanmethaemoglobin method. Haematocrit was determined by micro-centrifugation and
195 measured in triplicate. Using the method of Dill and Costill (1974), blood and plasma
196 volume changes were calculated from haemoglobin concentrations and haematocrit values.
197 A $100 \mu\text{l}$ sample of anticoagulated blood was pipetted into 0.3 M perchloric acid in a ratio of
198 1:10 in duplicate for analysis of glucose by the GOD-PAP method (Randox Laboratories
199 Ltd., Crumlin, UK) and lactate by fluorimetry using the method outlined by Maughan
200 (1982). Plasma arginine vasopressin and aldosterone concentrations were measured by
201 enzyme immunoassay (Enzyme Immunoassay; Enzo Life Sciences, Ann Arbor, MI, USA)
202 using $100\mu\text{l}$ samples. Samples were measured in duplicate.

203

204 The total volume of each urine sample was measured and a 5 ml sample was retained. This
205 was analysed for osmolality through freezing point depression (Gonotec Osmomat auto
206 Cryoscopic Osmometer; Gonotec, Berlin, Germany).

207

208 Statistical analysis

209 Data were checked for normality of distribution using Shapiro-Wilks tests. All samples were
210 normally distributed and subsequently, either paired samples t-tests or repeated measures
211 ANOVA was performed. Post-hoc paired samples t-tests with Bonferroni correction were
212 performed to identify where statistical differences occurred when significant main or
213 interaction effects were observed. Linear regression values and Pearson's product moment
214 correlation coefficients were calculated when appropriate. Linear regression was used to
215 examine the change in plasma vasopressin associated with the change in serum osmolality.
216 Correlation analysis was calculated between variables deemed to be closely related in terms
217 of physiological and behavioural mechanisms related to water balance. Statistical
218 significance was accepted when $p < 0.05$. Data expressed as mean \pm SD.

219

220

221 Results

222

223 Blood samples were collected from eight subjects due to cannulation problems in two
224 subjects. Despite not being continuous data, the time points in Figures 3, 4, 5 and 6 are
225 joined as the points represent progressive time points throughout the trials.

226

227 Baseline values

228 There was no difference in baseline body mass between the HIIE (75.57 ± 7.28 kg) and LO
229 trial (75.71 ± 6.98 kg) ($p=0.496$). Similar baseline values for urine osmolality (510 ± 248 v
230 507 ± 270 mOsmol.kg⁻¹ for HIIE and LO trials respectively), serum osmolality (285 ± 4 v
231 284 ± 3 mOsmol.kg⁻¹ for HIIE and LO trials respectively) and subjective feelings of thirst (53
232 ± 21 v 40 ± 15 for HIIE and LO trials respectively) and mouth dryness (52 ± 22 v 42 ± 16 for
233 HIIE and LO trials respectively) were observed, suggesting subjects arrived in a similar state
234 of euhydration (Sawka et al. 2007) ($p>0.05$).

235

236 Water balance

237 Body mass loss from the beginning of the trial until after the recovery period following
238 voluntary water intake was similar between trials (0.77 ± 0.50 v $0.85 \pm 0.55\%$ for HIIE and
239 LO trials respectively) ($p=0.124$). Sweat loss was greater in the HIIE trial (0.78 ± 0.22 l)
240 compared to the LO trial (0.66 ± 0.26 l) ($p=0.009$). In the HIIE trial, subjects consumed more
241 water during the recovery period ($p<0.0001$) (Figure 2) but this difference was solely due to a
242 higher water intake during the first 30 min of recovery ($p=0.006$) whilst during the final 30
243 min of the recovery period, water intake was similar ($p=0.094$). The increase in water intake
244 between the LO and HIIE trials was positively correlated with the increased sweat losses that
245 also occurred ($r=0.731$, $p=0.534$). Expressed as a percentage, the amount of water lost that
246 was replaced was higher in the HIIE trial compared to the LO trial (44 ± 29 v $35 \pm 34\%$;
247 $p=0.012$). In the HIIE trial, one subject drank more than the water lost (104%), and the next
248 highest replacement value was 77%. In the LO trial, two subjects replaced 90-100% with the
249 remaining subjects replacing less than 51% of the water lost during exercise. Negating water
250 intake, body mass losses from baseline would have been similar: $1.34 \pm 0.36\%$ in the HIIE
251 trial and $1.26 \pm 0.39\%$ in the LO trial ($=0.205$), with only one subject in both trials losing
252 enough water to elicit a greater than 2% body mass loss. The difference in percentage body
253 mass lost when water was included and negated in the calculation was greater in the HIIE

254 trial ($p < 0.0001$). There was no difference in urine output at the end of the trial (0.23 ± 0.12 v
255 0.28 ± 0.12 l for HIIE and LO trials respectively; $p = 0.203$).

256

257 Serum osmolality was higher in the HIIE trial post-exercise ($p = 0.006$) and after 5 ($p = 0.048$)
258 and 30 min ($p < 0.001$) of the recovery period (Figure 3a). Serum osmolality values were
259 similar across all sample points in the LO trial ($p > 0.05$) but were elevated above baseline and
260 the recovery period samples post-exercise in the HIIE trial ($p \leq 0.015$). In the HIIE trial values
261 had returned to baseline following 5 min of recovery ($p > 0.05$).

262

263 Serum sodium concentrations post-exercise were higher in the HIIE trial compared to the LO
264 trial ($p = 0.018$) (Figure 3b). In the HIIE trial, post-exercise concentrations were greater
265 compared to baseline and during the recovery period ($p \leq 0.015$) and had returned to baseline
266 after 5 min of the recovery period ($p > 0.05$). In the LO trial serum sodium concentrations did
267 not increase above baseline ($p > 0.05$). Serum potassium concentrations were the same at
268 baseline and similar between trials and sample points (baseline: 4.4 ± 0.3 v 4.4 ± 0.3 mmol.l⁻¹;
269 ¹; post-exercise: 5.1 ± 0.3 v 4.9 ± 0.4 mmol.l⁻¹; 5min: 4.4 ± 0.3 v 4.5 ± 0.3 mmol.l⁻¹; 15min:
270 4.6 ± 0.3 v 4.5 ± 0.3 mmol.l⁻¹; 30min: 4.5 ± 0.3 v 4.5 ± 0.3 1mmol.l⁻¹ and 60min: 4.6 ± 0.3 v
271 4.4 ± 0.2 mmol.l⁻¹) ($p > 0.05$).

272

273 Blood analysis

274 At baseline, blood lactate concentrations were similar between trials ($p = 0.914$) but increased
275 during exercise and remained elevated throughout the recovery period ($p \leq 0.006$) (Figure 4).
276 In the HIIE trial, blood lactate concentrations peaked post-exercise and remained elevated
277 above baseline values until 30 min of the recovery period ($p \leq 0.015$).

278 Plasma vasopressin concentrations were higher in the HIIE trial at post-exercise and after 5
279 and 30 min of the recovery period ($p < 0.05$) (Figure 5a). In the HIIE trial, post-exercise
280 vasopressin concentrations increased from baseline ($p = 0.048$), had a tendency to remain
281 elevated above baseline after 5 min of recovery ($p = 0.054$) and were elevated above baseline
282 values after 30 min of the recovery period ($p < 0.05$). In the LO trial concentrations did not
283 change from baseline ($p > 0.05$). In both the HIIE and LO trials, aldosterone concentration did
284 not change from baseline ($p > 0.05$) (Figure 5b) but after 30 minute of the recovery period
285 aldosterone concentrations were greater in the HIIE trial compared to the LO trial ($p = 0.048$).

286 Haemoglobin concentrations increased from baseline (156 ± 7 and 158 ± 7 g.l⁻¹ for HIIE and
287 LO respectively) to post-exercise (171 ± 7 and 163 ± 8 g.l⁻¹ for HIIE and LO respectively) in
288 both trials ($p < 0.05$). Haemoglobin concentrations were higher post-exercise in the HIIE trial
289 ($p < 0.05$) but had returned to baseline concentrations after 15 min (HIIE) and 5 min (LO) of
290 the recovery period. A similar response was found for haematocrit values with an increase
291 from baseline to post-exercise in the HIIE (44.0 ± 2.5 to 48.2 ± 2.3 %) ($p < 0.05$) and LO (44.5
292 ± 2.0 to 45.7 ± 2.4 %) ($p < 0.05$) trials. Haematocrit values were higher post-exercise in the
293 HIIE trial ($p < 0.05$) and returned to baseline at the same rate as haemoglobin concentrations.
294 Plasma volume change from baseline was greater in the HIIE trial compared to the LO trial at
295 post-exercise and after 5, 15 and 30 min of the recovery period ($p < 0.05$) (Figure 6a). In the
296 HIIE trial plasma volume was different compared to baseline at post-exercise and after 5 and
297 15. In the LO trial plasma volume changes from baseline at each sample point were similar
298 ($p > 0.05$). Blood volume changes from baseline were greater in the HIIE trial compared to
299 the LO trial at post-exercise and after 5, 15 and 30 min of the recovery period ($p < 0.05$)
300 (Figure 6b). In the HIIE trial, blood volume decreased from baseline at post-exercise and
301 after 5 and 15 min of the recovery period ($p < 0.0001$) before returning to baseline values. In
302 the LO trial blood volume had decreased from baseline at post-exercise ($p < 0.05$) but had
303 returned to baseline values by 5 min of the recovery period ($p > 0.05$).

304

305 Subjective feeling questionnaires

306 Reported sensations of thirst peaked in both trials post-exercise (Figure 7a) and tended to be
307 higher in the HIIE trial compared to the LO trial at the post-exercise sample ($p = 0.060$). In
308 the HIIE trial, post-exercise reported sensations of thirst were greater than baseline and
309 during the recovery period ($p < 0.0001$). Reported sensations of mouth dryness peaked in
310 both trials post-exercise (Figure 7b) and tended to be higher in the HIIE trial compared to the
311 LO trial at post-exercise ($p = 0.060$). There was no difference between trials at the other
312 sample points ($p > 0.05$). In the HIIE trial post-exercise reported sensations of mouth dryness
313 were greater than baseline and during the recovery period ($p < 0.0001$).

314

315 Correlations

316 In the HIIE trial serum sodium concentrations were positively correlated to blood lactate
317 concentrations and serum osmolality (Table 1). Serum osmolality was positively correlated
318 to blood lactate concentrations, vasopressin and aldosterone concentrations and sensations of

319 thirst and mouth dryness. Vasopressin concentrations were positively correlated to blood
320 lactate concentrations but there was no correlation with aldosterone concentrations. In the
321 LO trial correlations were not found between serum osmolality, serum sodium
322 concentrations, blood lactate concentrations and vasopressin and aldosterone concentrations.

323

324 Core and skin temperature

325 Core temperature peaked at the end of exercise in both the HIIE ($38.2 \pm 0.3^{\circ}\text{C}$) and LO (37.6
326 $\pm 0.3^{\circ}\text{C}$) trials. Core temperature was greater at 30, 40, 50 and 60 min of the exercise period
327 and remained elevated after the first 10 min of the recovery period in the HIIE trial compared
328 to the LO trial ($p < 0.05$). Skin temperatures were similar between trials at all time points and
329 mean skin temperature over the duration of the trials was similar (31.6 ± 1.1 v $31.6 \pm 1.2^{\circ}\text{C}$
330 for HIIE and LO trials respectively) ($p > 0.05$).

331

332 Heart rate, rating of perceived exertion and thermal sensation

333 During the exercise period of the trials, heart rate was significantly higher during the HIIE
334 trial (158 ± 12 v 110 ± 10 beats.min⁻¹, $p < 0.0001$), with differences occurring at 10, 20, 30,
335 40, 50 and 60 min of exercise. Thermal sensation was higher after 20 (5 ± 1 v 3 ± 1 ,
336 $p < 0.0001$), 30 (6 ± 1 v 4 ± 1 , $p < 0.0001$), 40 (6 ± 1 v 4 ± 1 , $p < 0.0001$), 50 (6 ± 1 v 4 ± 1 ,
337 $p < 0.0001$) and 60 min (6 ± 2 v 4 ± 1 , $p < 0.0001$) of the exercise period in the HIIE trial.
338 There was no difference in thermal sensation during the baseline and recovery periods
339 ($p > 0.05$). Ratings of perceived exertion were higher in the HIIE trial after 10 (14 ± 2 v $10 \pm$
340 2 , $p = 0.006$), 20 (15 ± 1 v 11 ± 2 , $p < 0.0001$), 30 (16 ± 2 v 11 ± 2 , $p < 0.0001$), 40 (16 ± 2 v 11
341 ± 3 , $p < 0.0001$), 50 (17 ± 2 v 11 ± 2 , $p < 0.0001$) and 60 min (17 ± 2 v 11 ± 2 , $p < 0.0001$) of the
342 exercise period.

343

344 Discussion

345 The aim of the study was to assess voluntary water intake following either a period of HIIE
346 or continuous exercise. The exercise conditions were chosen to generate significant
347 differences in blood lactate concentrations in order to examine the effects on the
348 physiological mechanisms responsible for voluntary water intake. The 60 minutes of
349 exercise under the two conditions achieved approximately the same work loads (366 kJ) and
350 a clearly significant difference in blood lactate concentrations.

351

352 Water intake in the HIIE trial was greater during the first 30 min after exercise. Despite the
353 greater water intake, body mass loss was similar between trials primarily due to the increased
354 sweat loss recorded in the HIIE trial. It appeared that the increased water intake could be
355 mainly related to the increased sweat losses. Body mass loss in both trials was greater than
356 0.8% and so would have stimulated sensations of thirst (Wolf, 1950) yet were not greater
357 than 2% suggesting that rapid rehydration through water intake may have not been necessary
358 (Sawka et al., 2007).

359

360 However, there was a wide range in the individual response to water intake with the amount
361 of water replaced (12-104% in the HIIE trial and 0-94% in the LO trial), indicating that water
362 intake replacement was highly variable. Nevertheless the water replacement helped alleviate
363 thirst sensations in the HIIE trial indicated by the reduction in reported sensations of thirst
364 following the onset of the water intake period. This adds strength to the notion that when *ad*
365 *libitum* water is allowed then individuals consume sufficient amounts to alleviate sensations
366 of thirst despite not replacing all of the water lost during exercise (Noakes, 2007). It must be
367 noted that the sensation of thirst is complicated and can be affected and influenced by
368 additional variables not measured in the current study. In addition to those measured,
369 oropharyngeal reflexes, absorption rate and fluid capacity of the gastrointestinal system
370 (Greenleaf, 1992) and stomach distension (Rolls et al., 1980) have all been linked to
371 sensations of thirst. Therefore, it would seem that in the current study, the variables
372 measured would not completely explain all of the variance in thirst and voluntary water
373 intake.

374

375 The sensation of thirst has been suggested to arise when serum osmolality is greater than 290
376 mOsmol.kg⁻¹ (Phillips, Rolls, Ledingham, Forsling, & Morton, 1985). This may provide
377 explanation as to why subjects in the present study consumed water despite body mass losses

378 not greater than 2%. In the HIIE trial, osmolality first increased above the threshold value
379 post-exercise before decreasing below this value between 15 and 30 min of the recovery
380 period, whilst in the LO trial, osmolality values did not increase above 290 mOsmol.kg⁻¹. In
381 the HIIE trial, despite elevated serum osmolality after 5 and 15 min of the recovery period,
382 sensations of thirst had decreased from peak post-exercise values following the onset of
383 drinking. It appears that the premise of a threshold for thirst works only to initiate drinking
384 and once this has occurred then the effect of the threshold for thirst is diminished. As a result,
385 water intake during the final 30 min of the HIIE trial was similar to the LO trial confirming
386 that satiation of water intake occurs quickly following an initial bout of drinking (Rolls et al.,
387 1980). Closer monitoring of the water intake period, particularly during the first 5 min would
388 have allowed greater interrogation of water intake behaviour in response to exercise.

389

390 It has been hypothesised that the efflux of sodium ions from the vascular space to the
391 intracellular space is reduced by the negatively charged lactate ions produced following HIIE,
392 thus causing an increase in serum sodium concentration and subsequently, serum osmolality.
393 In the current study the difference in serum osmolality at post-exercise between trials was
394 approximately 10 mOsmol.kg⁻¹, whilst the difference in serum sodium concentration was
395 approximately 3 mmol.l⁻¹. Using the formula assessed by Worthley, Guerin, and Pain (1987),
396 the change in serum osmolality (12 mOsmol.kg⁻¹) in the HIIE trial from baseline to after
397 exercise was not completely accounted for by the change in serum sodium concentration (2*4
398 mmol.l⁻¹). Therefore it appeared that the change in blood lactate concentration was a direct
399 contributing factor to the increase in serum osmolality (contribution of 4 mOsmol.kg⁻¹). The
400 effect of blood lactate concentrations on serum osmolality values, both directly and
401 indirectly through the increase of serum sodium concentrations, would have contributed to
402 the osmotically driven release of vasopressin and potentially contributed to the increased
403 consumption of water in the HIIE trial (Phillips et al., 1985).

404

405 Prior to the onset of thirst stimulated water intake, vasopressin is released to increase water
406 reabsorption in the kidneys (Bankir, 2001). Vasopressin release will increase until maximum
407 anti-diuresis has been reached (Thompson et al., 1986). In the current study there was a large
408 increase in plasma vasopressin concentration following the high intensity exercise when
409 serum osmolality values were above the reported threshold value. Vasopressin concentration
410 remained elevated above baseline values throughout the HIIE trial, consistent with serum
411 osmolality remaining above the threshold value outlined. Vasopressin concentration has been

412 widely shown to decrease quickly (2.5 – 15 min) following initiation of drinking (Burrell,
413 Lambert, & Baylis, 1991; Figaro & Mack, 1997; Geelen et al., 1984; Seckl, Williams, &
414 Lightman, 1986). However in these latter studies serum osmolality decreased at either a
415 similar rate (Burrell et al., 1991) or at a slightly delayed rate (30-60 min) (Seckl et al., 1986).
416 In the present study vasopressin concentration remained elevated, whilst thirst sensations
417 decreased after the initial post-exercise peak. Again, this is perhaps related to sensations of
418 thirst becoming quickly satiated once water intake occurs and also suggests that vasopressin
419 is not a direct stimulus of thirst. Increased serum osmolality increases vasopressin
420 concentrations and sensations of thirst, however it appeared that in the current study, water
421 intake satiated sensations of thirst quickly, whereas serum osmolality and vasopressin
422 concentrations were more delayed in returning to baseline levels following water intake. In
423 conjunction with the decrease in water intake during the final 30 minute period it would also
424 suggest that the increased blood lactate concentration and serum osmolality relationship may
425 have had an effect on maintaining vasopressin concentrations.

426

427 Although the total work rates performed throughout the trials were similar they were not
428 matched precisely. However, the study was effective in achieving its purpose of generating
429 significant differences in blood lactate concentrations between exercise conditions.

430

431 During the recovery period it appeared that two main variables were influencing the decrease
432 in serum osmolality from post-exercise peak values. The reduction of blood lactate
433 concentration and the intake of water both contributed to decreasing serum osmolality. As
434 the effect of no water intake was not assessed, determining the relative contributing effect of
435 each variable was difficult. However, when the effect of preventing or delaying water intake
436 following HIIE was assessed, similar decreases in serum osmolality, blood lactate and serum
437 sodium concentrations were found (Mears and Shirreffs, unpublished data). Delaying water
438 intake resulted in a similar voluntary water intake despite reduced serum osmolality values,
439 suggesting that once the desire to drink arose, sensations remained until satiated.

440

441 Conclusion

442 In conclusion, water intake following a period of HIIE was greater than an exercise period of
443 low intensity continuous exercise. The increased water intake in the HIIE trial was mainly
444 attributed to the increased water losses. In addition, the result of an increase in serum
445 osmolality and subsequent vasopressin release caused by an increased blood lactate

446 concentration in combination with an increased serum sodium concentration may have also
447 contributed to the increased water intake.
448

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451

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520 List of figures

521 **Figure 1** Schematic diagram indicating the testing protocol. Arrows represent sampling
522 points. SFQ denotes subjective feelings questionnaire

523 **Figure 2** Voluntary water intake during each trial. † denotes difference between trials, *
524 denotes different from 30-60 min ($p < 0.05$)

525 **Figure 3** Serum osmolality (a) and serum sodium concentrations (b) over the duration of each
526 trial. † different between trials, * different from baseline, # different to post-exercise ($p < 0.05$).
527 B denotes baseline sample, PE denotes post-exercise sample

528 **Figure 4** Blood lactate concentrations over the duration of each trial. † different between
529 trials, * different to baseline, # different to post-exercise, ^ different to 5min, § different to
530 15min ($p < 0.05$). B denotes baseline sample, PE denotes post-exercise sample

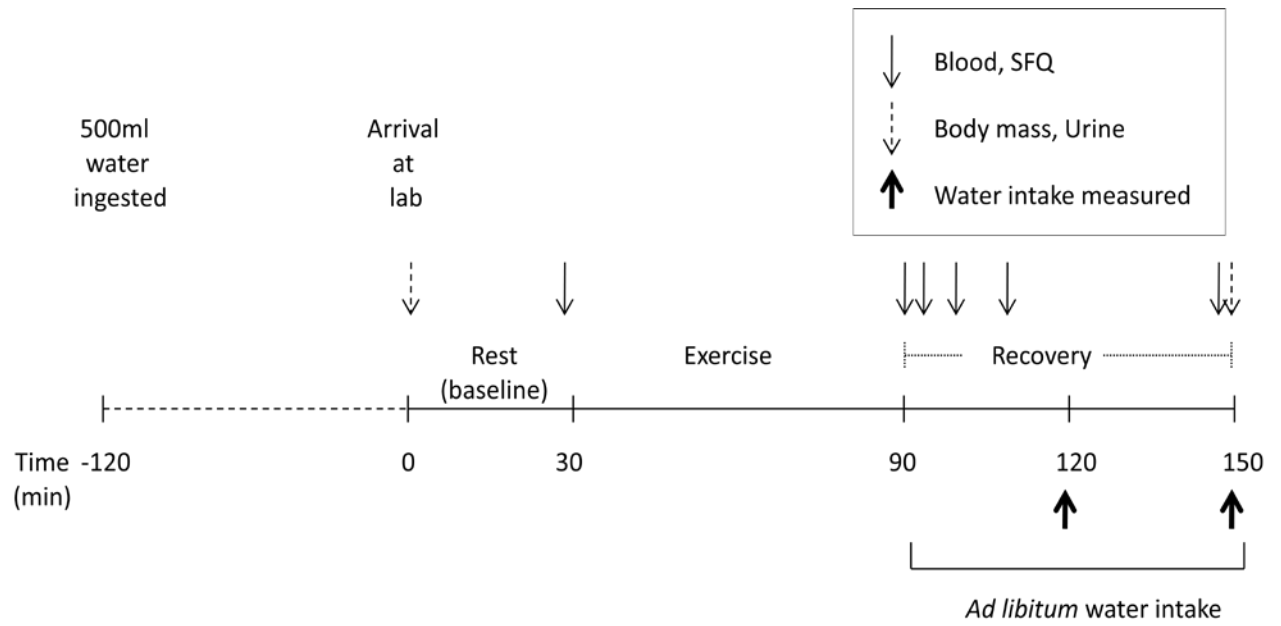
531 **Figure 5** Vasopressin (a) and aldosterone (b) concentration over the duration of each trial. †
532 different between trials, * different to baseline, # different to post exercise ($p < 0.05$). B denotes
533 baseline sample, PE denotes post-exercise sample

534 **Figure 6** Plasma volume (a) and blood volume (b) changes from baseline values over the
535 duration of each trial. † different between trials, * different to baseline values, # different to
536 the post exercise change, ^ different to the baseline to 5 min change ($p < 0.05$). B denotes
537 baseline sample, PE denotes post-exercise sample

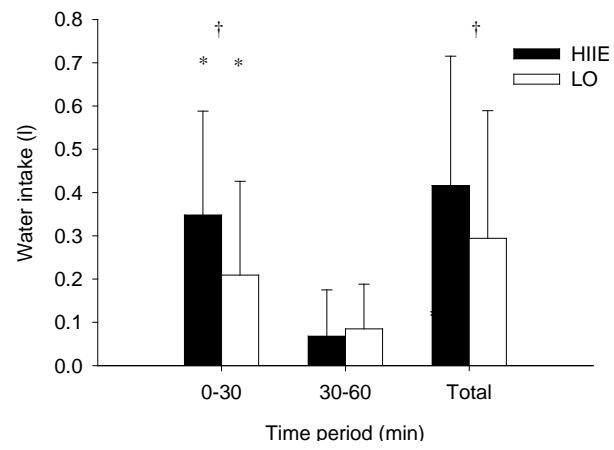
538 **Figure 7** Sensations of thirst (a) and mouth dryness (b) over the duration of each trial. *
539 different to baseline in the HIIE trial, # different to post exercise in the HIIE trial, ^ different
540 to 5 min in the HIIE trial ($p < 0.05$). B denotes baseline sample, PE denotes post-exercise
541 sample

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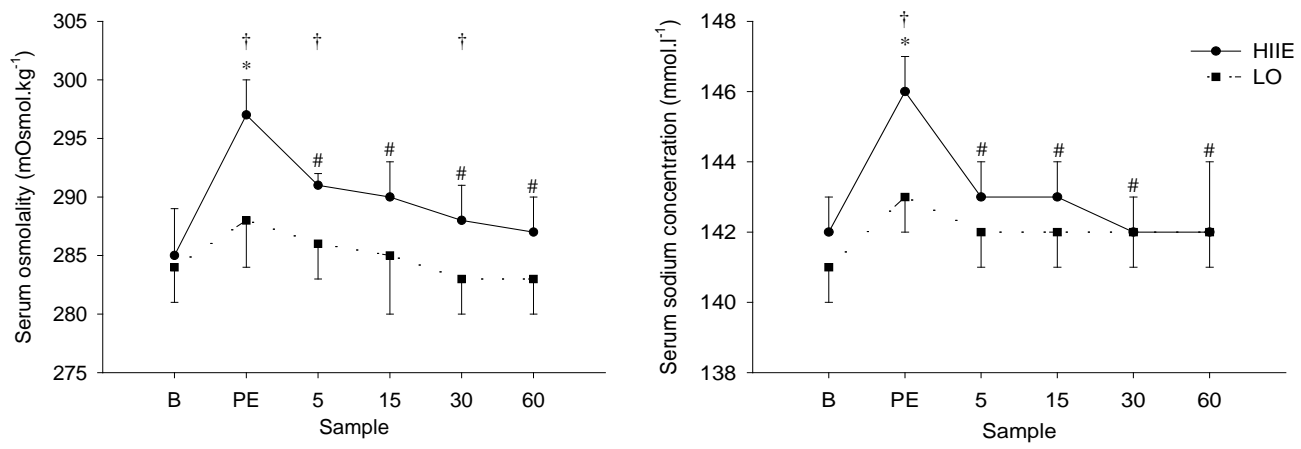
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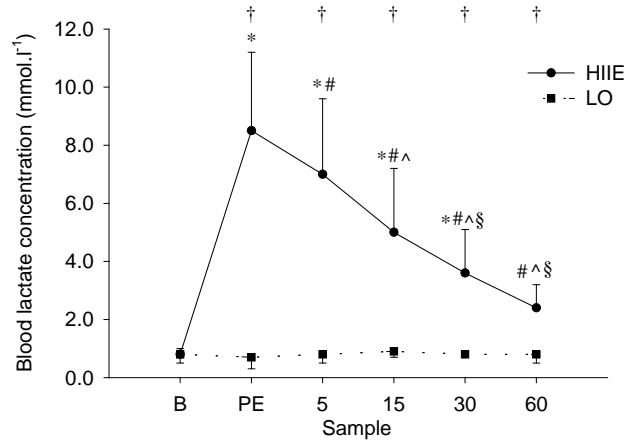
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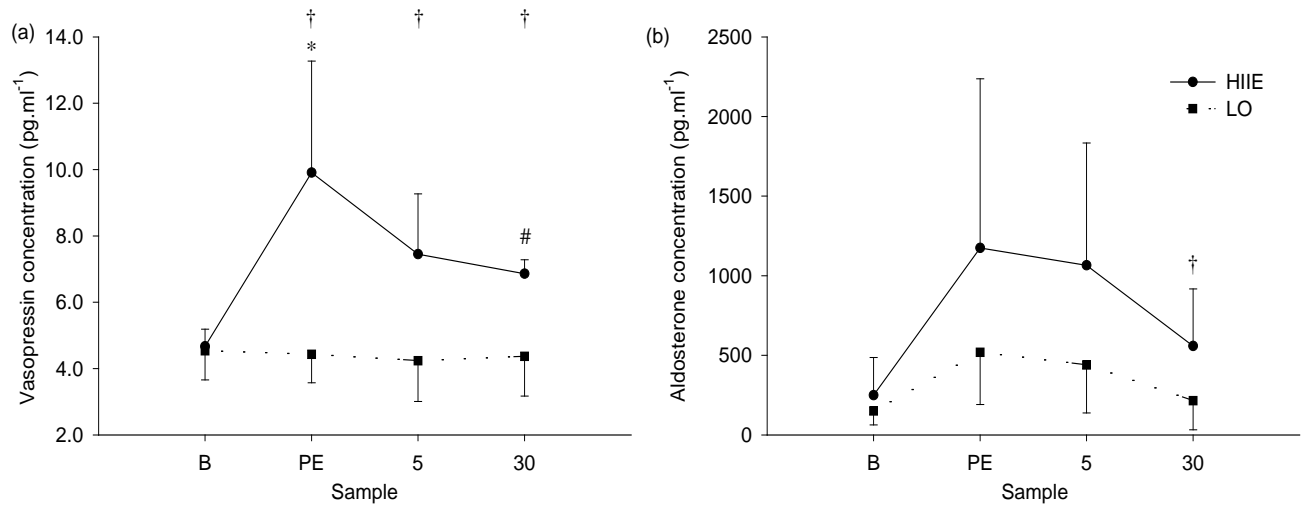
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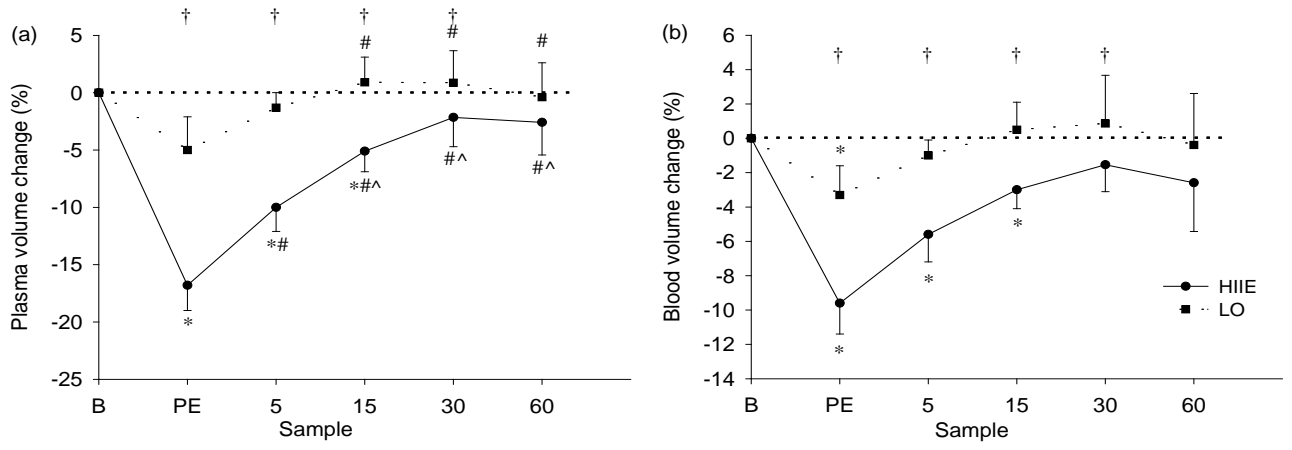
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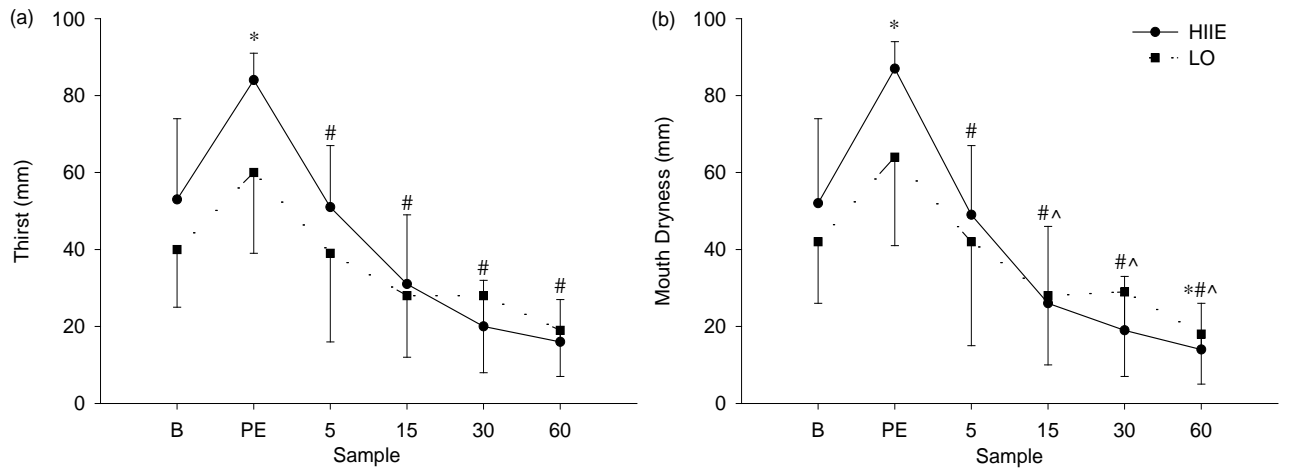
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Table 1 Correlation coefficients between measured variables in each trial (*p<0.05; **p<0.0001)

Variables		HIE		LO	
		r	p	r	p
Serum osmolality	Serum sodium concentration	0.470	0.001*	0.223	0.142
	Blood lactate concentration	0.655	<0.0001**	0.170	0.265
	Vasopressin concentration	0.661	<0.0001**	-0.195	0.320
	Aldosterone concentration	0.545	0.003*	0.373	0.050
	Thirst	0.419	0.004*	0.293	0.051
	Mouth dryness	0.411	0.005*	0.211	0.165
Serum sodium concentration	Blood lactate concentration	0.608	<0.0001**	0.201	0.184
	Vasopressin concentration	0.663	<0.0001**	0.131	0.506
	Aldosterone concentration	0.415	0.028*	0.207	0.506
	Thirst	0.521	<0.0001**	0.264	0.079
	Mouth dryness	0.552	<0.0001**	0.243	0.108
Blood lactate concentration	Vasopressin concentration	0.657	<0.0001**	-0.307	0.112
	Aldosterone concentration	0.476	0.010*	-0.096	0.628
	Thirst	0.518	<0.0001**	0.270	0.073
	Mouth dryness	0.468	0.001*	0.265	0.078
Vasopressin concentration	Aldosterone concentration	0.317	0.100	-0.119	0.547
	Thirst	0.517	0.005*	-0.242	0.214
	Mouth dryness	0.517	0.004*	-0.270	0.164
Aldosterone concentration	Thirst	0.226	0.248	0.309	0.110
	Mouth dryness	0.159	0.419	0.262	0.177
Thirst	Mouth dryness	0.976	<0.0001**	0.972	<0.0001**