1   Title

2   Thirst responses following high intensity intermittent exercise when access to *ad libitum*

3   water intake was permitted, not permitted or delayed

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25 Abstract

26 An increase in subjective feelings of thirst and *ad libitum* drinking caused by an increase in

27 serum osmolality have been observed following high intensity intermittent exercise (HIIE)

28 compared to continuous exercise. The increase in serum osmolality is closely linked to the

29 rise in blood lactate and serum sodium concentrations. However, during an ensuing recovery

30 period after HIIE when serum osmolality will decrease, the resultant effect on sensations of

31 thirst and subsequent water intake is unclear. Therefore the aim of the study was to assess the

32 sensations of thirst and subsequent effect on *ad libitum* water consumption when water intake

33 was immediately allowed, delayed or prevented following a period of HIIE.

34

35 *Methods* Twelve males (26 ± 4 years, 80.1 ± 9.3 kg, 1.81 ± 0.05 m, $\dot{V}O_{2peak}$ 60.1 ± 8.9 ml.kg^{-}

36 ^{1}.min^{-1}) participated in three randomised trials undertaken 7–14 days apart. Participants

37 rested for 30 min then completed a 60 min HIIE exercise period (20 x 1 min at 100% $\dot{V}O_{2peak}$

38 with 2 min rest) followed by 60 min of recovery, during which *ad libitum* water intake was

39 provided immediately (W), delayed until the final 30 min (W30) or not permitted (NW).

40 Body mass was measured at the start and end of the trial. Blood lactate and serum sodium

41 concentrations serum osmolality and sensation of thirst were measured at baseline,

42 immediately post-exercise and during the recovery.

43

44 *Results* Body mass loss was different between all trials (W: 0.25 ± 0.45, W30: 0.49 ± 0.37,

45 NW: $1.29 \pm 0.37\%$; $p<0.05$). Sensations of thirst peaked post-exercise and decreased in W

46 and W30 following water ingestion ($p<0.05$). Total voluntary water intake was greater in W

47 trial (0.846 ± 0.417 v 0.630 ± 0.277 l; $p<0.05$) but was similar during the first 30 min period

48 of allowed drinking (0.618 ± 0.297 vs. 0.630 ± 0.277 l; $p>0.05$). Serum osmolality (299 ± 6

49 v 298 ± 5 vs. 298 ± 3 mOsmol.kg^{-1}), blood lactate (7.1 ± 1.1 vs. 7.2 ± 1.1 v 7.1 ± 1.2 mmol.l^{-}

50 ^{1}) and serum sodium concentrations (142 ± 2 vs. 145 ± 2 v 145 ± 2 mmol.l^{-1}) peaked post-

51 exercise (W vs. W30 vs. NW; $p<0.05$) but were not different between trials ($p>0.05$).

52

53 *Conclusions* Sensations of thirst were increased following HIIE and remained until satiated

54 by water intake. This was despite the likely primary stimulus, serum osmolality, decreasing

55 during the recovery period following a post-exercise peak. A combined effect of reduction in

56 blood lactate and serum sodium concentrations, restoration of plasma volume and water

57 intake contributed to the similar decrease in serum osmolality observed throughout the trials.

58   Highlights

59   - HIIE caused an increase in blood lactate concentrations, raising serum osmolality

60   - Despite decreased serum osmolality during recovery, thirst remained until satiated

61   - Delaying drinking 30min resulted in a similar volume consumed immediately post

62   HIIE

63

64   Key words:

65   Blood lactate; serum osmolality; thirst; water intake; satiation

66

1. Introduction

Thirst is an innate behaviour that drives an episodic desire to drink and is normally an adequate stimulus to maintain a state of euhydration under resting conditions [1]. However, when the body is placed under physiological stress, the thirst response often results in sufficient water consumed to satiate sensations of thirst but not to completely replace fluid losses (involuntary dehydration). Stricker and Verbalis [2] proposed two mechanisms relating to the generation of thirst sensations and desire to drink: hyperosmolality and hypovolaemia, whilst sensations of dry mouth have also been proposed as a mechanism of thirst [3,4,5].

In relation to hyperosmolality, serum osmolality thresholds at rest have been identified that drive arginine vasopressin (AVP) release (approximately 285 mOsmol.kg^{-1}; [6]) and sensations of thirst (approximately 290mOsmol.kg^{-1}; [7]), whilst it has also been suggested that changes in serum osmolality of approximately 5mOsmol.kg^{-1} will stimulate sensations of thirst [8]. Elevations in serum osmolality are detected by osmoreceptors in the organum vasculosum of the lamina terminalis and the subfornical region within the brain. Both of these circumventricular organs lack a blood-brain barrier, therefore allowing hormonal and osmotic stimuli to act [9]. Serum osmolality levels above the threshold for thirst will usually occur due to changes in cell tonicity, but can also arise due to the influence of blood lactate concentrations caused by a period of high intensity intermittent exercise (HIIE) [10]. Hypovolaemia is a common consequence of most exercise intensities, and will primarily occur due to ongoing sweat losses resulting from an effort to maintain body temperature. However, the relatively short duration of HIIE bouts may prevent water losses from reaching a sufficient level to stimulate sensations of thirst (approximately 0.8% body mass loss; [11]), therefore any change in blood volume following HIIE is likely to arise from changes in blood pressure and subsequent movement of water to the interstitial space [12].

Following HIIE, water moves from the vascular to the interstitial and intracellular spaces [12,13, 14]. Serum osmolality and subsequent arginine vasopressin release will increase in relation to the increase in blood lactate concentration [12,14, 15,16]. It has been hypothesised that the negatively charged lactate ions reduce sodium release from the vascular space thus increasing serum sodium concentrations and subsequent osmolality levels [14]. Therefore HIIE may in fact elevate serum osmolality above the threshold for thirst, and consequently influence drinking behaviour independent of associated water losses.

101 An increase in *ad libitum* drinking (total volume consumed) has been observed following a
102 period of HIIE compared to continuous exercise [10]. The observed increase in water intake
103 was associated with an increase in blood lactate, serum sodium and vasopressin
104 concentrations, an increase in serum osmolality and a tendency for greater subjective feelings
105 of thirst. During the recovery period access to water intake was allowed immediately after
106 exercise. It was therefore difficult to determine if thirst and subsequent drinking behaviour
107 was influenced by the reduction of factors that stimulated sensations of thirst (i.e. serum
108 osmolality and associated variables), the satiation of thirst sensations or a combination.
109 Although not measured, it was also possible that the increased respiration rate during the
110 HIIE may have contributed to the increases in mouth dryness and thirst observed by Mears &
111 Shirreffs [10]. By delaying and also preventing access to *ad libitum* water intake it is
112 possible the mechanisms relating to thirst and serum osmolality can be better understood and
113 a clearer insight into role played by HIIE on drinking behaviour can be established.

114

115 The aim of the study was to assess the sensations of thirst and the subsequent effect on *ad*
116 *libitum* water intake during a recovery period following HIIE, when access to water was
117 allowed immediately, delayed or prevented. It was hypothesised that sensations of thirst
118 would increase, due to an increase in serum osmolality and that this would drive drinking
119 behaviours. Delaying or preventing drinking would not satiate sensations of thirst.

2. Methods

2.1 Participants

Twelve healthy male participants (age 26 ± 4 years, mass 80.1 ± 9.3 kg, height 1.81 ± 0.05 m, $\dot{V}O_{2peak}$ 60.1 ± 8.9 ml.kg^{-1}.min^{-1}) took part in three experimental trials, in a randomised order. The experimental protocol was explained to all participants verbally and in writing and written informed consent was provided. The experiment was approved by the Loughborough University Ethical Advisory Committee.

2.2 Experimental protocol

Participants visited the laboratory on five separate occasions for a peak oxygen uptake ($\dot{V}O_{2peak)}$ test, a familiarisation trial and three experimental trials differing in the time period during which *ad libitum* water intake was allowed following exercise; water permitted throughout the entire recovery period (W), water delayed until 30 minutes after exercise until the end of the recovery period (W30) and no water permitted at all during the recovery period (NW).

The first visit involved a discontinuous incremental test to volitional fatigue undertaken on an electrically braked cycle ergometer (Lode Corival; Lode BV, Groningen, Netherlands) was used to determine peak power and $\dot{V}O_{2peak}$. Expired gas was collected for 1 min at the end of each four minute stage. The familiarisation trial was identical to the W trial, and intended to inform the participants of the experimental procedures employed throughout the study. Participants were asked to record their dietary intake in the 24 hours prior to the first experimental trial (food and drink consumed, amount and method of preparation) and refrain from strenuous physical activity and consumption of alcohol. For each subsequent trial they were asked to repeat this. Participants were asked to arrive at the laboratory after an overnight fast with the exception of consumption of 500 ml of water ingested two hours before arrival at the laboratory to ensure they were in a euhydrated state.

Experimental trials began in the morning at the same time for each participant and were separated by a period of 7-14 days. A schematic outline of the trial is shown in Figure 1. Experimental trial order was randomised and participants were not aware of which trial they
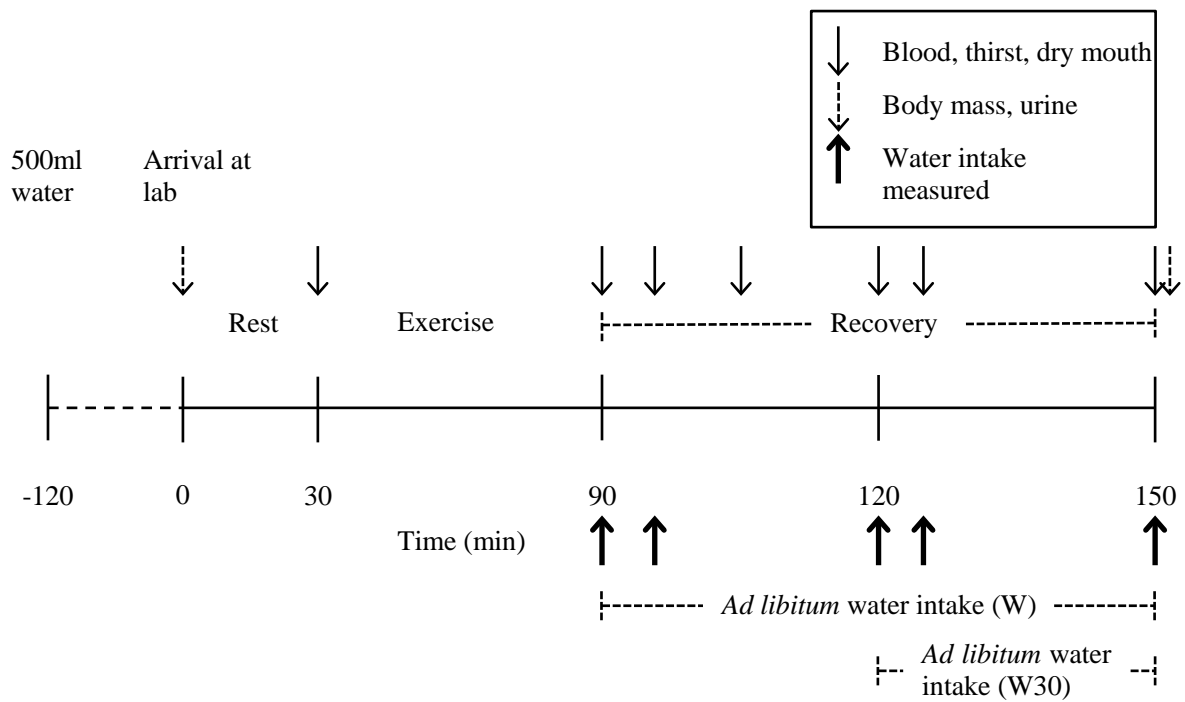
Figure 1. Schematic diagram indicating the testing protocol. Arrows represent sampling points.

were participating in when arriving at the laboratory for the first and second experimental trials.

On arrival at the laboratory, participants voided and asked to empty their bladder; total urine volume was measured and a 5 ml aliquot retained for analysis. Nude body mass was measured. Participants were asked to insert a rectal thermistor 10 cm past the anal sphincter, and a heart rate monitor (Polar Vantage; Kempele, Finland) was positioned. Throughout the trials, core (T_c) and skin (T_{sk}) temperature were measured continuously, and data were averaged every 10 min (BIOPAC MP100 System; BIOPAC, Santa Barbara, CA, USA). Mean weighted skin temperature was calculated using the formula outlined by Ramanathan [17]. Participants rested in a seated position for 30 minutes in a comfortable environment ($22.3 \pm 0.4°C$ and $47 \pm 9\%$ relative humidity; RH). Every 10 minutes during rest, exercise and recovery heart rate was recorded. Following the 30 minutes seated rest, participants completed two 100 mm visual analogue subjective feeling questionnaires relating to symptoms of thirst and dry mouth (0 mm = not at all thirsty/mouth not at all dry, 100 mm = very thirsty/mouth very dry). During the baseline period a 21 g cannula (Surflo, Terumo, Leuven, Belgium) was inserted into a superficial vein on the forearm to allow venous blood sampling. At the end of the rest period a baseline blood sample (7.5 ml) was collected.

7

171

172   Participants then completed 60 minutes of HIIE, comprising of repeated cycles of 1 min of

173   cycle exercise at a power output equal to the maximum power achieved during the $\dot{V}O_{2peak}$

174   test, followed by 2 min rest. This was undertaken in $23.0 \pm 0.4°C$ and $48 \pm 10\%$ RH. During

175   the 60 minute period this pattern of activity was repeated 20 times. A blood sample (7.5 ml)

176   was collected immediately following the completion of exercise and the two subjective

177   feelings questionnaire were repeated. Participants were then seated at rest for 60 minutes in

178    $22.7 \pm 0.3°C$ and $47 \pm 10\%$ RH.

179

180   In the W trial *ad libitum* water intake ($10 \pm 3°C$) was allowed for the whole duration of the

181   recovery period, with the volume of water ingested recorded between 0-5 minutes, 5-30

182   minutes, 30-35 minutes and 35-60 minutes. In the W30 trial, *ad libitum* water intake was

183   delayed until 30 minutes of the recovery period had passed. Water intake was then measured

184   between 30-35 minutes and 35-60 minutes. In the NW trial no water was permitted during

185   the 60 min recovery period. The participant was not made aware of the volume consumed, or

186   that the volume was being measured. Participants were informed at the start that they could

187   drink as they wanted, that the bottle would be refilled if necessary and were provided with no

188   external cues to drink. Blood samples were collected at 5, 15, 30, 35 and 60 minutes and

189   thirst and dry mouth subjective feelings questionnaires were completed. At the end of the

190   recovery period following the final blood sample, participants voided, the urine volume was

191   measured and a 5 ml sample was retained for later analysis. Nude body mass was then

192   measured, after which, participants were allowed to leave the laboratory. Ambient

193   temperature and relative humidity was measured at 10 minute intervals (RH85 Digital

194   Thermo-Hygrometer; Omega, Manchester, UK).

195

196   2.3 Sample analysis

197   For each 7.5 ml venous blood sample, a 1.0 ml aliquot was mixed with an anticoagulant (K^+

198   EDTA; 1.5 $mg.ml^{-1}$) for analysis of haemoglobin concentration (Cyanmethaemoglobin

199   method; Sigma, St Louis, MO, USA), haematocrit (micro-centrifugation; Hawksley,

200   Worthing, UK) and glucose concentration. A further 5.0ml was mixed with anticoagulant

201   (K^+ EDTA; 1.5 $mg.ml^{-1}$) and from this, plasma was separated and frozen at $-80°C$ for later

202   analysis of hormone concentrations. The remaining blood (~2.0 ml) was allowed to clot at

203   room temperature before being centrifuged at 3000 rpm for 15 min at $4°C$ to yield serum.

204 This was later analysed for sodium concentration by flame photometry (Corning Clinical
205 Flame Photometer 410C; Corning Ltd., Halstead, Essex, UK) and osmolality by freezing
206 point depression (Gonotec Osmomat auto Cryoscopic Osmometer; Gonotec, Berlin,
207 Germany).   Blood and plasma volume changes were calculated from haemoglobin
208 concentrations and haematocrit values using the method of Dill and Costill [18].
209 Anticoagulated blood (100 µl) was added to 0.3 M perchloric acid in a ratio of 1:10 in
210 duplicate for analysis of glucose by the GOD-PAP method (Randox Laboratories Ltd.,
211 Crumbin, UK) and lactate by fluorimetry using the method outlined by Maughan [19].
212 Enzyme immunoassay (Enzyme Immunoassay; Enzo Life Sciences, Ann Arbor, MI, USA)
213 was used to measure plasma arginine vasopressin and aldosterone concentrations using 100µl
214 samples. Samples were measured in duplicate.

215

216 The volume of each urine void was measured and a 5 ml sample retained and analysed for
217 osmolality by freezing point depression (Gonotec Osmomat auto Cryoscopic Osmometer;
218 Gonotec, Berlin, Germany).

219

220 2.4 Statistical analysis
221 Data were checked for normality of distribution using Shapiro-Wilks tests.   Normally
222 distributed data with one factor (overall fluid balance variables) were analysed using a one-
223 way ANOVA and data with two factors (time dependent fluid balance variables, blood
224 variables, thirst and mouth dryness, temperatures, heart rate and RPE) were analysed using a
225 two-way repeated measures ANOVA design. If a significant ANOVA result was found, to
226 identify where the statistical differences occurred, paired samples t-tests with Bonferroni
227 correction were performed. Using Friedman's ANOVA and Wilcoxon signed-rank tests non-
228 parametric data was examined (aldosterone concentration). On non-parametric data post-hoc
229 tests were performed when significant and non-significant interaction effects were found.
230 Linear regression values and Pearson's product moment correlation coefficients and
231 Spearmon's ranked correlation coefficients were calculated when appropriate. Correlation
232 analysis was performed between variables that were deemed to be related in terms of water
233 balance and the mechanism identified by Nose *et al.* [14] (serum osmolality/ serum sodium/
234 blood lactate). Statistical significance was accepted when $p < 0.05$. When post-hoc tests were
235 conducted, p values presented were multiplied to correct for repeated samples.   Data is
236 expressed as mean $\pm$ SD except for aldosterone concentration, which is median (range).
237 Error bars plotted above time points represent a group standard deviation of all samples in all

238   trials at that time point. This was to improve clarity of the figures and reduce potential

239   confusion of overlapping error bars. Statistical analysis was conducted using Statistical

240   Package for the Social Sciences for Windows, version 18.0 (SPSS *inc,* Chicago, IL, USA).

3. Results

3.1 Baseline measures

There was no difference in body mass measured at baseline between trials (Table 1; p>0.05). Similar results between trials for serum osmolality (284 ± 3 vs. 284 ± 3 v 285 ± 3 mOsmol.kg^{-1} for W, W30 and NW respectively) and urine osmolality (409 ± 221 vs. 434 ± 256 vs. 454 ± 238 mOsmol.kg^{-1} for W, W30 and NW respectively; p>0.05) suggests that participants arrived in a similar state of hydration, interpreted as euhydrated [20, 21].

3.2 Subjective feelings questionnaires (thirst and mouth dryness)

Peak sensations of thirst were reported in all trials immediately post-exercise compared to baseline (p<0.05; Figure 2a), with no differences apparent between trials (p>0.05). Following the onset of water intake when *ad libitum* fluid was available, thirst sensations decreased (p<0.05). Baseline and peak values of sensations of mouth dryness reported at post-exercise were similar between all trials (p>0.05; Figure 2b). In the W and W30 trials, sensations of mouth dryness decreased following consumption of water. Thirst and mouth dryness were strongly positively correlated in all three trials (p<0.001; Table 2).
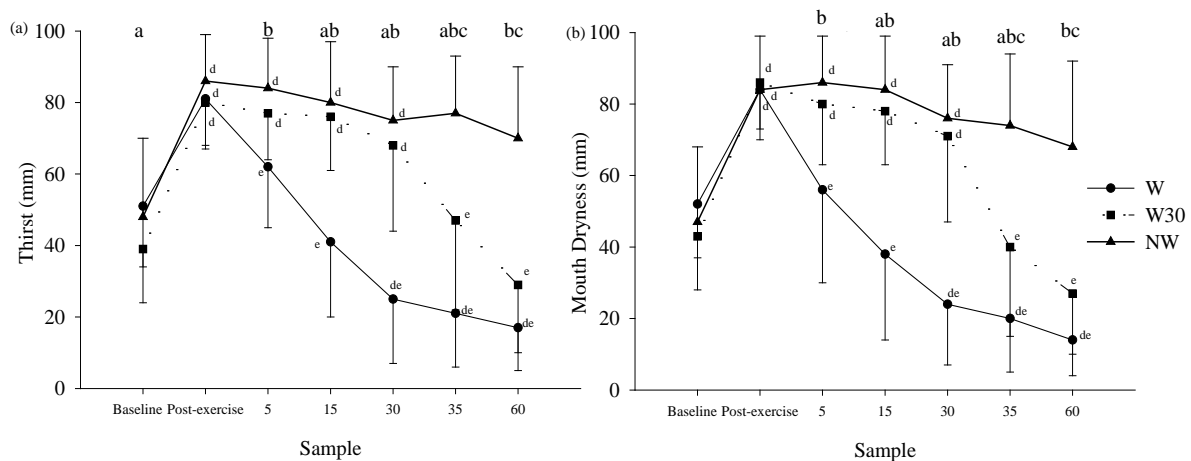

Figure 2. Subjective feeling questionnaire responses for (a) thirst and (b) mouth dryness over the duration of each trial. [a] denotes difference between W and W30 trials, [b] denotes difference between W and NW trials, [c] denotes difference between W30 and NW trials, [d] denotes difference within the trial compared to baseline and [e] denotes difference within the trial compared to post-exercise (p<0.05).
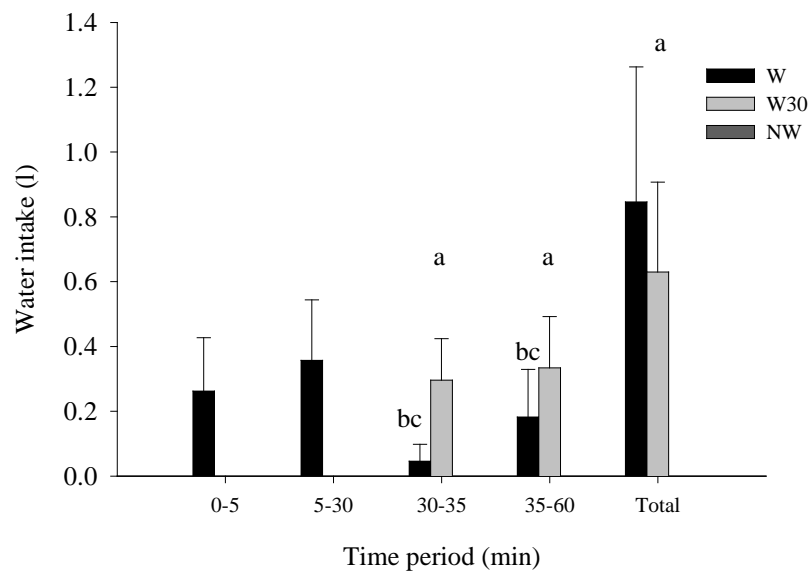
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Figure 3. Water intake (l) during each trial. Comparison between W and W30 trials. [a] denotes difference between trials, [b] denotes difference to 0-5 min (W) and [c] denotes different to 5-30 min (W) ($p<0.05$).

259

260   3.3 Body water balance

261   Body mass decreased from the start to the end of the trial in the W30 and NW trial ($p<0.05$),

262   whilst percentage decrease in body mass was greater in the NW trial compared to the W and

263   W30 trials ($p<0.05$; Table 1). Sweat losses and post-exercise urine output were similar

264   between trials ($p>0.05$; Table 1). Total water intake was greater in the W trial compared to

265   the W30 trial ($p=0.009$; Figure 3) with similarities observed between volumes consumed

266   when initial drinking periods were compared ($p>0.05$). The proportion of water lost through

267   sweating that was subsequently replaced tended to be greater in the W trial compared to the

268   W30 trial ($p=0.08$). Four participants ingested sufficient water during the recovery period to

269   replace more than 100% of water lost in the W trial.

270

271

272   3.3 Blood analysis

273   No difference in serum osmolality was observed between trials at all sample points ($p>0.05$;

274   Figure 4a). Peak osmolality values occurred immediately post-exercise, before decreasing

275   during the recovery period ($p<0.05$). Similar to serum osmolality, blood lactate (Figure 4b)

276   and serum sodium concentrations (Figure 4c) were similar between trials ($p>0.05$) with peak

277   concentrations occurring immediately post-exercise ($p<0.05$). Blood lactate concentrations
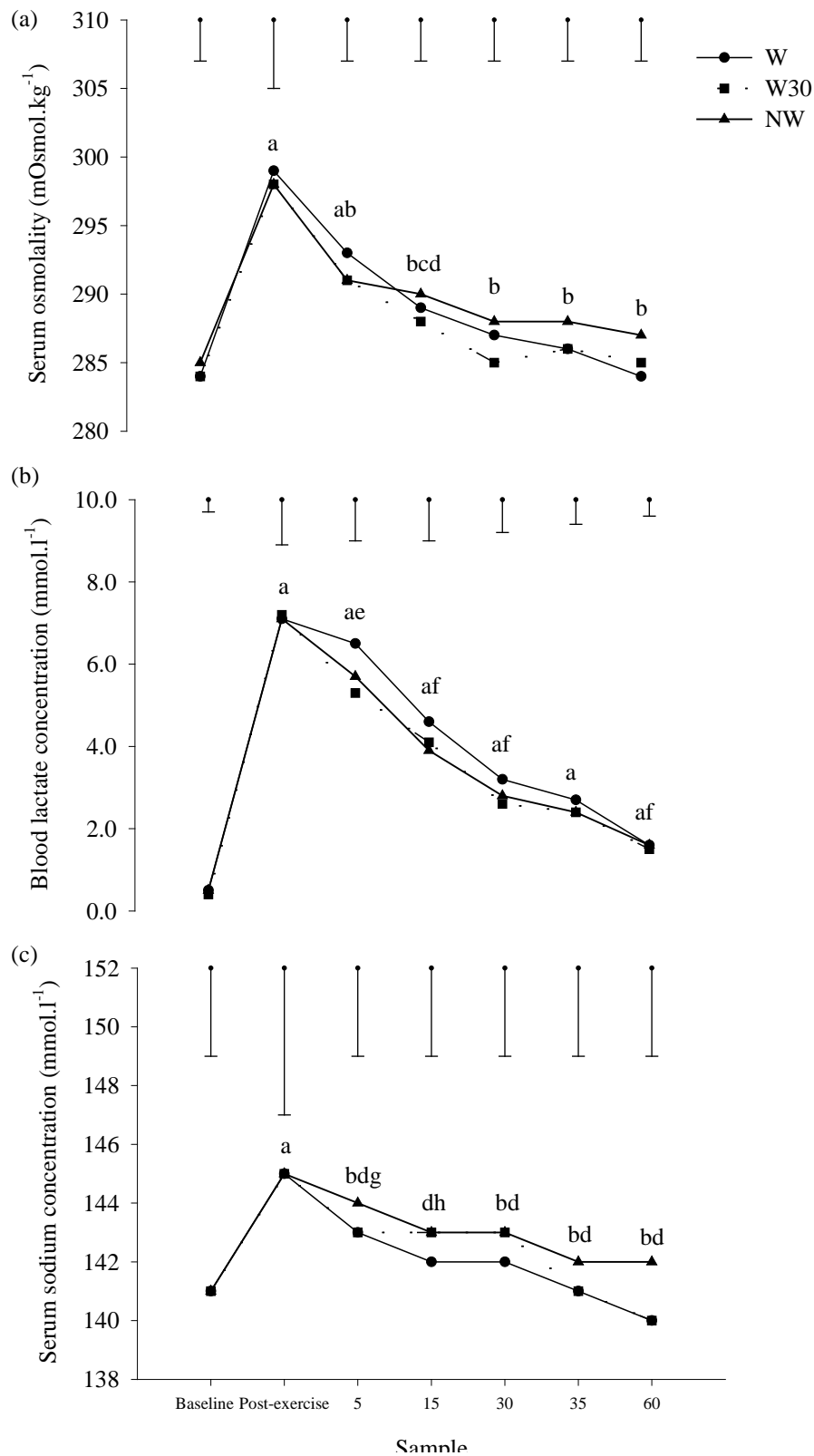
Figure 4. (a) Serum osmolality, (b) blood lactate concentration and (c) serum sodium concentration over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. [a] denotes different to baseline in all trials, [b] denotes different to post-exercise in all trials, [c] denotes different to baseline in W trial, [d] denotes different to baseline in NW trial, [g] denotes different to baseline in W30 trial, [e] denotes different to previous sample in W30 and NW trials, [f] denotes different to previous sample in all trials, [h] denotes different to post-exercise sample in W and NW trial ($p<0.05$).

278   gradually decreased throughout the recovery period in all trials.   Serum sodium

13

concentrations in the NW trial remained elevated above baseline throughout the recovery period, whilst in the in the W and W30 trials, serum sodium concentrations returned to baseline after 5 and 15 minutes respectively. AVP concentrations were similar between trials ($p > 0.05$; Figure 5a) with peak concentrations found post-exercise ($p < 0.05$). During the W30, the onset of water intake did not appear to cause further changes in any of the blood variables measured ($p > 0.05$).
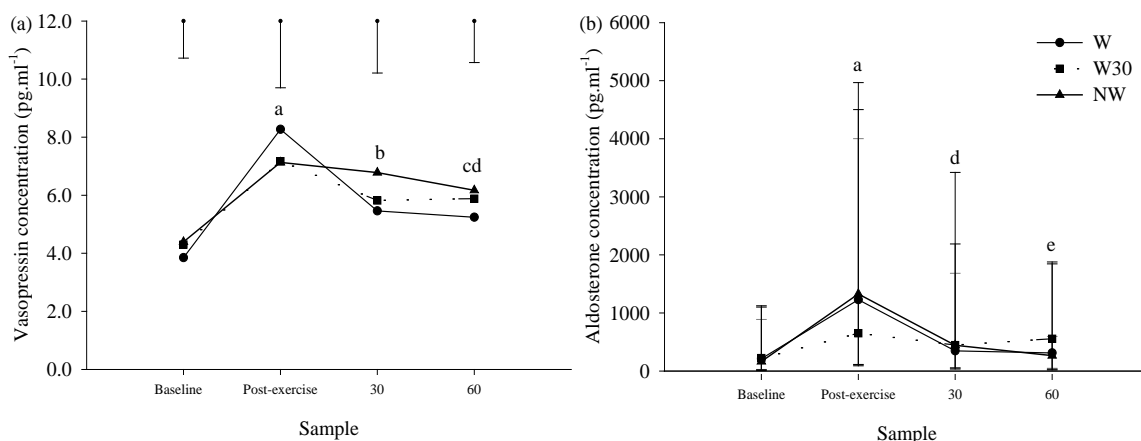

Figure 5. (a) Vasopressin (mean ± SD) and (b) aldosterone (median (range)) concentrations over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. [a] denotes different to baseline values in all trials, [b] denotes different to baseline in W, [c] denotes different to post-exercise in W, [d] denotes different to baseline in W and W30,[e] denotes different to post-exercise in all trials ($p < 0.05$).

Thirst sensations were positively correlated to serum osmolality, serum sodium and blood lactate concentrations in all three trials ($p < 0.05$; Table 2). Similar responses were observed for sensations of mouth dryness except in the NW trial for serum osmolality. Sensations of thirst and mouth dryness were positively correlated to AVP concentrations in the W and W30 trials ($p < 0.05$).

Plasma volume changes from baseline were similar between trials at all sample points ($p > 0.05$) (Figure 6a). In all trials there was a decrease in plasma volume from baseline values at post-exercise and after 5 minutes of the recovery period ($p < 0.05$) before plasma volume returned to baseline ($p > 0.05$). Blood volume changes from baseline values were similar between trials at all sample points except at 30 min when there was a decrease in blood volume in the W30 trial compared to an increase in the NW trial (Figure 6b). Decreases from
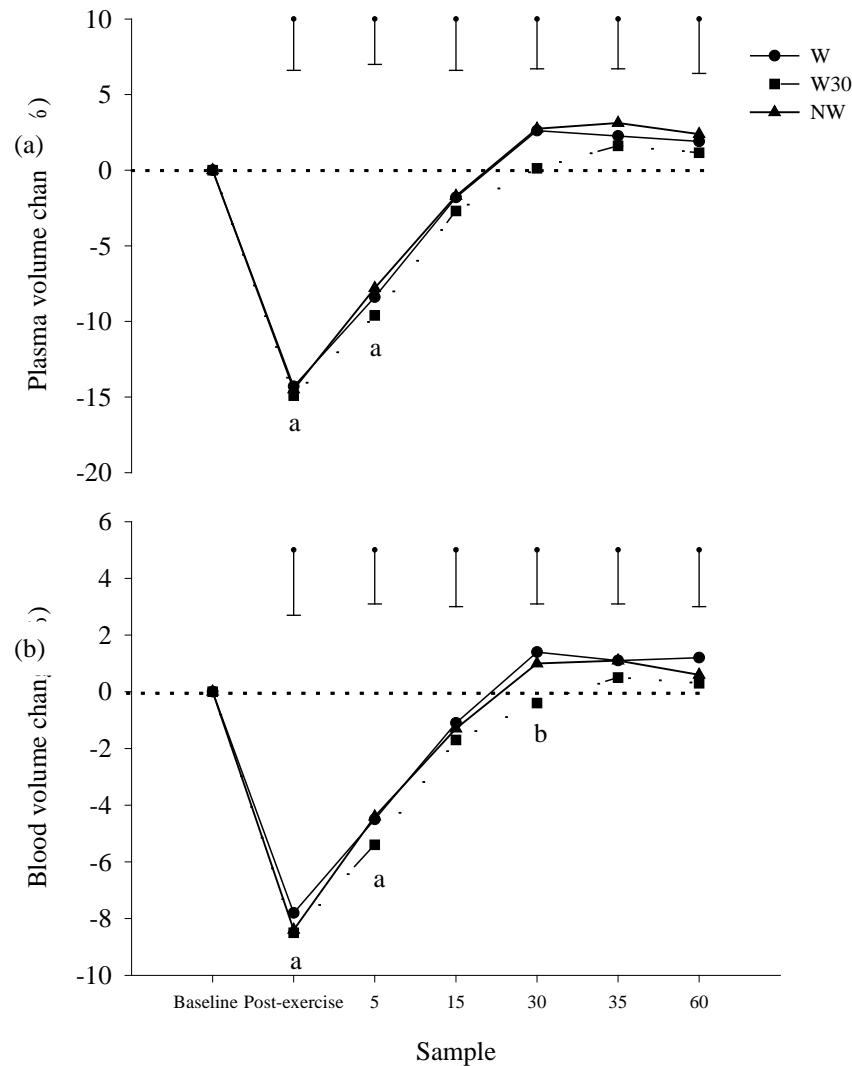
Figure 6. (a) Plasma and (b) blood volume changes compared to baseline values over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. [a] denotes different to baseline values in all trials, [b] denotes NW trial different to W30 (p<0.05).

298   baseline values were observed in all trials at post-exercise and after 5 min of the recovery

299   period (p<0.001) before blood volumes returned to baseline values.   Blood glucose

300   concentrations were similar between trials and sample points (mean concentrations for W:

301   4.20 ± 0.30, W30: 4.20 ± 0.25, NW: 4.37 ± 0.37 mmol.l^{-1}; p>0.05).

302

303   Similar concentrations of aldosterone were found between trials at all sample points (p>0.05;

304   Figure 5b) with peak values observed post-exercise (p<0.05). Sensations of thirst and mouth

305   dryness were not significantly correlated to aldosterone concentrations (p>0.05).

306

307   3.6 Core and skin temperature

308   Core temperature increased throughout the HIIE bout in all trials, peaking at the end of the

309   exercise period (W; 38.24 ± 0.17, W30: 38.16 ± 0.36, NW: 38.04 ± 0.33°C). No differences

310   between trials were recorded ($p > 0.05$). During each trial core temperature was elevated above

311   baseline values after 20 minutes of the exercise period had elapsed and remained elevated

312   above baseline values until 10 minutes into the recovery period in the W trial and 40 minutes

313   in the W30 and NW trial ($p < 0.05$). No differences were observed within and between trials

314   for skin temperature measurements ($p > 0.05$).

315

316   3.7 Heart rate

317   Heart rate was similar between trials at all time points except between the W30 and NW trial

318   after 50 minutes of the recovery period when a higher heart rate was recorded in the NW trial

319   (63 ± 8 vs. 70 ± 9 beats.min^{-1} W30 and NW trials respectively). Peak heart rates were

320   observed during the exercise period of the trials (163 ± 13 vs. 163 ± 12 vs. 162 ± 13

321   beats.min^{-1} for W, W30 and NW trials respectively) and were elevated from baseline values

322   (61 ± 10 vs. 57 ± 8 vs. 63 ± 7 beats.min^{-1} for W, W30 and NW trials respectively; $p < 0.05$).

323   4. Discussion

324   The aim of the study was to assess the sensations of thirst and the subsequent effect on *ad*

325   *libitum* water intake during a recovery period following HIIE, when access to water was

326   allowed immediately, delayed by 30 min or prevented. The main finding was that sensations

327   of thirst remained until satiated by voluntary water intake. Despite a delay voluntary water

328   intake was similar between comparable time periods. HIIE increased blood lactate and serum

329   sodium concentrations, therefore increasing serum osmolality providing a large driver for the

330   increases in sensations of thirst.

331

332   Sensations of thirst peaked post-exercise in all three trials and remained elevated until

333   immediately after *ad libitum* water intake was permitted (W and W30 trials). Thirst is a

334   multifactorial sensation that can be caused by a multitude of factors [1, 2], many examined

335   within the context of this study. The main driver appeared to be the increase in serum

336   osmolality as this was significantly raised above suggested thresholds [7]. Sensations of thirst

337   and mouth dryness were also significantly correlated with several other physiological

338   measurements (many interlinking and also contributing to the increase in serum osmolality)

339   including serum sodium and blood lactate concentrations and fluid balance hormones. These

340   factors appeared to contribute to the stimulation of thirst, but despite varying reductions in

341   the measured variables during the recovery period, it was apparent that only through water

342   consumption were sensations of thirst satiated.

343

344   Water intake was strongly governed by sensations and the satiation of thirst. Despite the

345   delay in water provision, the volume of water consumed in the W and W30 trials to satiate

346   thirst was similar during the first 30 minutes of permitted drinking. In the W30 trial,

347   physiological responses associated with water intake at the onset of drinking (i.e. serum

348   osmolality and plasma vasopressin concentrations) were lower than in the W trial when

349   drinking was allowed, yet thirst remained elevated. Drinking to alleviate sensations of thirst

350   was strongly correlated with mouth dryness (W: r=0.959, W30: r=0.921, NW: r=0.775) and

351   probably suggests that water intake volume was likely driven primarily by oropharyngeal

352   factors. The breakdown of each drinking period providing further indication of this.

353   Consumption of water during the first 5 minutes and the subsequent 25 minute period was

354   comparable in the W and W30 trials. In studies examining voluntary water intake following a

355   period of dehydration through exercise or water restriction, water intake predominantly

356   occurs at the onset of the drinking period to reduce sensations of mouth dryness and thirst

357 commonly detected by osmoreceptors in the oropharyngeal region [3, 4, 22]. Figaro and
358 Mack [4] observed 54% of total fluid consumed during the initial 5 minute period; largely
359 governed by oropharyngeal stimuli. By infusing fluid directly into the stomach they were able
360 to prevent the satiation of thirst sensations and the reduction in vasopressin concentration.
361 Despite infusion of fluid to return plasma osmolality and plasma volume levels to resting
362 values, when *ad libitum* fluid intake was permitted, subjects drank to alleviate thirst as
363 detected by dryness of the oropharyngeal region. In the current study there were significant
364 decreases in self-reported sensations of mouth dryness and thirst after *ad libitum* water intake
365 was allowed. These differences, combined with similar water intake volumes between both
366 0-5 and 5-30 min in the W trial and 30-35 and 35-60 min in the W30 trial, would confirm that
367 that the osmoreceptors play an important role in governing voluntary water intake behaviour.

368

369 Rather than drinking to replace fluid losses, participants appeared to consume a volume
370 sufficient to relieve the possible unpleasantness and discomfort associated with mouth
371 dryness and thirst. This resulted in a fluid deficit apparent at the end of the recovery period
372 and involuntary dehydration. Voluntary water intake replaced only $82 \pm 39\%$ and $63 \pm 27\%$
373 of the water lost in the W and W30 trials respectively, similar to volumes commonly replaced
374 in the literature [23,24]. Rapid rehydration is required if body mass losses are large (>2%)
375 and/ or there is a need to rehydrate effectively for another bout of exercise within a short
376 period of time [25]. Otherwise a return to a state of euhydration can be achieved gradually
377 through normal meals, snacks and plain water intake. In the current study body mass losses
378 of just over 1% would not be indicative of a requirement for rapid rehydration.

379

380 In addition to mouth dryness sensations of thirst were likely driven by the increase in serum
381 osmolality post-exercise. At rest an increase in serum osmolality above ~285 mOsmol.kg^{-1}
382 will stimulate vasopressin release [6], whilst a further increase to above ~290 mOsmol.kg^{-1}
383 will lead to the sensations of thirst [7] and subsequent water intake. In addition a rise in
384 serum osmolality of approximately 5 mOsmol.kg^{-1} has been deemed sufficient to induce
385 sensations of thirst [8]. However in the model outlined by the authors, a rise in serum
386 osmolality is usually caused by intracellular losses when total body water losses are around
387 2% [8]. In the present study, the period of HIIE increased serum osmolality above 290
388 mOsmol.kg^{-1} and the increase was greater than 5 mOsmol.kg^{-1} but body mass losses were
389 approximately 1.3%. The peak in post-exercise serum osmolality caused an increase in
390 vasopressin concentrations and the stimulation of thirst sensation. The rise in vasopressin

391 concentration was likely due to osmotically driven signals although it has been shown that

392 high intensity exercise will drive vasopressin release through non-osmotic stimuli [15].

393 Release of vasopressin will increase renal water reabsorption, thereby reducing serum

394 osmolality and as a result, sensations of thirst [2]. However, in the current study, despite an

395 increase in vasopressin and decrease in serum osmolality, sensations of thirst remained until

396 satiated suggesting that key driver in water intake following a delay was behavioural.

397 Correlation analysis indicated that the decrease in serum osmolality following peak values

398 was closely related to the decrease in blood lactate and serum sodium concentrations. It is

399 likely there was also contribution from restoration of plasma volume, and reduction in

400 hydrostatic pressure in the capillary beds [13,26].

401

402 The cause of an increase in serum osmolality following HIIE has been positively related to an

403 increase in blood lactate and serum sodium concentrations alongside haemoconcentration

404 [13, 14, 26]. As a key contributor [10], an increase in blood lactate concentrations will

405 prevent serum sodium release into the vascular space, resulting in an increase in osmolality

406 levels [14]. The relative contribution of blood lactate concentrations to the increase in serum

407 osmolality was calculated using the formula assessed by Worthley *et al.* [27] (Serum

408 osmolality = 2[Na$^+$] + [BUN] + [Glucose] + [lactate]). The change in osmolality from

409 baseline to post-exercise in the current study of 13-15 mmol.l^{-1} would have been caused by

410 the increase in serum sodium concentration (contribution of 2 * 4 mOsmol.kg^{-1}) and also

411 blood lactate concentration (contribution of 5-7 mOsmol.kg^{-1}). It would therefore appear that

412 increased blood lactate concentrations following HIIE contribute both directly and indirectly

413 (through serum sodium concentrations) to the increase in serum osmolality and therefore, the

414 subsequent desire to drink.

415

416 During the recovery period serum osmolality progressively decreased in all three trials.

417 Despite significant decreases from peak values, serum osmolality remained above

418 approximately 290 mOsmol.kg^{-1} in all three trials until around the 30 minute timepoint

419 suggesting that despite the initial decreases following cessation of exercise serum osmolality

420 was likely still contributing to sensations of thirst. During the recovery period, the longer

421 that thirst remained elevated (i.e. the amount of time that water was withheld), the weaker the

422 correlation between thirst and serum osmolality. This suggests that serum osmolality is a key

423 initial driver of thirst and will still contribute to sensations following a period of HIIE, but

424   perhaps the relative influence is reduced, particularly when the rise in osmolality is not

425   directly influenced by body water losses.

426

427   The HIIE period was a prolonged period of exercise, involving 20 minutes of exercise over

428   an hour period. Throughout the trials, body mass losses were approximately 1.3% (based on

429   sweat losses and negating for water intake), enough to stimulate sensations of thirst [11] but

430   often lower than would typically be associated with a sufficient rise in serum osmolality [8].

431   Although it appears a large contribution to increased serum osmolality and subsequent thirst

432   was caused by increased blood lactate concentrations, it is likely that the hypovolemia

433   experienced would also stimulate sensations of thirst particularly as aldosterone

434   concentrations increased [2, 8]. To determine this contribution, further work is required to

435   assess the effect of a shorter period of HIIE when body mass losses were minimal.

436

437   During a HIIE session, water intake will likely be permitted throughout the exercise period.

438   Thirst is therefore likely to occur throughout a portion of the exercise period resulting in

439   water intake. Although water intake is likely to be small in volume, due to the time available

440   to drink and the inference that water intake is to satiate thirst and reduce mouth dryness, it is

441   possible that volumes similar to the first 5 minutes in the W trial could be consumed between

442   exercise intervals. Over prolonged periods, this could potentially lead to a gain in body mass

443   and/ or increased frequency of urination [28], which may interfere with exercise. In addition,

444   in many exercise settings, a reduced body mass may be advantageous, particularly in weight

445   bearing sports [29]. Therefore any increase in body mass through water consumption,

446   however slight, may serve to increase the metabolic cost of exercise.

447

448   As shown in the study, thirst is a multi-factorial sensation arising from numerous stimuli;

449   however it is possible that factors such as stomach distension may have influenced sensations

450   of thirst and subsequent water intake [30]. Unfortunately this was not measured in the current

451   study; this may have improved the determination and relative contribution of factors resulting

452   in thirst.

453

454   5. Conclusion

455   In conclusion, sensations of thirst and mouth dryness increased following a period of HIIE

456   and remained until satiated by voluntary fluid intake. Sensations of thirst appeared to be

457   largely driven by an increase in serum osmolality, caused by an increase in blood lactate and

458   serum sodium concentrations.

459

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536

537

539 Table 1. Body mass (BM) and water variables for all trials. † denotes pre to post measurement difference, *

540 denotes different to W trial, # denotes different to W and W30 trials (p<0.05). BM = body mass.

Trial	Pre BM (kg)	Post BM (kg)	BM change (%)	Water intake (l)	Sweat loss (l)	Urine Output (l)	Water replaced (%)
	79.9	79.7	-0.25	0.85	0.83	0.21	82
W	± 8.9	± 8.9	± 0.54	± 0.42	± 0.18	± 0.10	± 39
	79.8	79.4	-0.49	0.63	0.82	0.21	63
W30	± 8.9	± 8.9†	± 0.37	± 0.28*	± 0.23	± 0.08	± 27*
	80.2	79.2	-1.29		0.84	0.21	
NW	± 9.0	± 9.0†*	± 0.37$^{\#}$	-	± 0.24	± 0.10	-

541

542 Table 2. Correlation coefficients (r) and significance levels (p) for measured variables in each trial. (conc.
543 denotes concentration). *denotes significant (p<0.05). Correlations involving aldosterone are Spearmon's rank
544 correlations, remaining correlations are Pearson's product moment correlations.

Variables		W		W30		NW	
		r	p	r	p	r	p
Thirst	Mouth dryness	0.959	<0.001*	0.921	<0.001*	0.775	<0.001*
	Serum osmolality	0.562	<0.001*	0.314	0.005*	0.292	0.01*
	Serum sodium conc.	0.535	<0.001*	0.554	<0.001*	0.499	<0.001*
	Blood lactate conc.	0.494	<0.001*	0.528	<0.001*	0.421	<0.001*
	AVP conc.	0.376	0.013*	0.456	0.003*	0.226	0.150
	Aldosterone conc.	0.268	0.079	0.173	0.261	0.099	0.524
Mouth dryness	Serum osmolality	0.567	<0.001*	0.406	<0.001*	0.119	0.301
	Serum sodium conc.	0.518	<0.001*	0.610	<0.001*	0.560	<0.001*
	Blood lactate conc.	0.466	<0.001*	0.525	<0.001*	0.373	0.001*
	AVP conc.	0.398	0.008*	0.328	0.037*	0.115	0.469
	Aldosterone conc.	0.271	0.076	0.131	0.396	0.070	0.653
Serum osmolality	Serum sodium conc.	0.646	<0.001*	0.555	<0.001*	0.424	<0.001*
	Blood lactate conc.	0.824	<0.001*	0.773	<0.001*	0.813	<0.001*
	AVP conc.	0.621	<0.001*	0.218	0.170	0.443	0.003*
	Aldosterone conc.	0.321	0.034*	0.313	0.039*	0.416	0.005*
Serum sodium conc.	Blood lactate conc.	0.607	<0.001*	0.648	<0.001*	0.616	<0.001*
	AVP conc.	0.501	0.001*	0.218	0.171	0.183	0.247
	Aldosterone conc.	0.412	0.005*	0.194	0.206	0.197	0.200
Blood lactate conc.	AVP conc.	0.720	<0.001*	0.471	0.002*	0.470	0.002*
	Aldosterone conc.	0.431	0.004*	0.379	0.011*	0.446	0.002*
AVP conc.	Aldosterone conc.	0.380	0.012*	0.174	0.276	0.313	0.044*

545