1	Title
2	A sodium drink enhances fluid retention during 3 hours of post-exercise recovery when
3	ingested with a standard meal
4	
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23 Abstract

The purpose of this study was to examine the efficacy of water and a 50 mmol/L NaCl 24 solution on post-exercise rehydration when a standard meal was consumed during 25 26 rehydration. Eight healthy participants took part in two experimental trials during which they 27 lost 1.5 ± 0.4 % of initial body mass via intermittent exercise in the heat. Participants then rehydrated over a 60 minute period with water or a 50 mmol/L NaCl solution in a volume 28 29 equivalent to 150% of their body mass loss during exercise. In addition, a standard meal was ingested during this time which was equivalent to 30% of participants predicted daily energy 30 expenditure. Urine samples were collected before and after exercise and for three hours after 31 32 rehydration. Cumulative urine volume (981 \pm 458 mL and 577 \pm 345 mL; P = 0.035) was greater, whilst percentage fluid retained (50 \pm 20% and 70 \pm 21 %; P = 0.017) was lower 33 during the water compared to the NaCl trial respectively. A high degree of variability in 34 results was observed with one participant producing 28% more urine and others ranging from 35 18 – 83% reduction in urine output during the NaCl trial. The results of this study suggest 36 that after exercise induced dehydration, the ingestion of a 50 mmol/L NaCl solution leads to 37 38 greater fluid retention compared with water, even when a meal is consumed post-exercise. 39 Furthermore, ingestion of plain water may be effective for maintenance of fluid balance when 40 food is consumed in the rehydration period.

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42 Key Words

43 Hypohydration, Recovery, Net Fluid Balance

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45 Introduction

Exercise increases sweat rate and in many exercise settings, exercisers do not fully replace 46 fluid lost through sweating (Sawka et al. 2007), meaning hypohydration might be present at 47 48 the end of exercise. Starting exercise hypohydrated might impair strength (Minshull and 49 James 2013) or endurance (Kenefick et al. 2010) performance although the extent of hypohydration that may negatively affect performance is a matter of some debate. 50 51 Consequently, in situations where exercise sessions are undertaken in close proximity, 52 adequate post-exercise rehydration is vital for ensuring optimal recovery between sessions. Consistent with this, Davis et al. (2015) demonstrated that suboptimal rehydration after a 53 54 dehydrating evening exercise session impaired exercise performance the following morning. 55 Similarly, Wong et al. (1998) observed that the prescription of a set volume of fluid during a 56 four hour recovery period results in more efficient recovery of endurance capacity than when fluid was ingested ad libitum. 57

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59 When time between training sessions is short (i.e. less than 12 hours) the volume and 60 composition of a rehydration drink are the critical factors influencing the success of a 61 rehydration strategy (Shirreffs and Sawka, 2011). Studies have shown that a volume of fluid 62 in excess of that lost must be ingested to allow complete rehydration (Mitchell et al. 1994; 63 Shirreffs et al. 1996). If a sufficient volume of fluid is ingested, it is the composition that 64 determines how much of the fluid is retained, and consequently the success of the rehydration 65 strategy. Studies have systematically investigated the impact of different drink compositions and identified that the sodium (Maughan and Leiper 1995; Shirreffs and Maughan 1998; 66 Merson et al. 2008), carbohydrate (Evans et al. 2009; Osterberg et al. 2010; Clayton et al. 67 68 2014) and protein (Seifert et al. 2006; James et al. 2011; James et al. 2013) content can all enhance rehydration. 69

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71 Whilst these studies are adequately designed to detect (possibly small) differences between rehydration drinks, one limitation of these studies is that usually only fluids are provided in 72 73 the 4-7 h post-exercise. In real-world training/ competition scenarios it would be unusual not 74 to eat, at least some, food over this time period. This is particularly pertinent as consuming food with a rehydration drink enhances rehydration compared to the drink alone when total 75 76 fluid volume (i.e. fluid from foods and drinks) is matched (Maughan et al. 1996; Ray et al. 77 1998). Therefore it is scientifically and practically relevant to determine whether differences 78 in the efficacy of different rehydration drinks persists when food is also consumed in the 79 post-exercise period, as would likely be the case in sporting scenarios.

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81 Sodium represents the major cation in the extracellular space and can be lost in large amounts 82 in sweat (Baker et al. 2016). Alterations in plasma osmolality influence arginine vasopressin secretion, which ultimately dictates urine production rates. The addition of sodium to a 83 84 rehydration drink increases plasma osmolality (Nose et al. 1988) and enhances post-exercise 85 rehydration in a dose-dependent manner (Maughan and Leiper 1995; Shirreffs and Maughan 86 1998; Merson et al. 2008). Sodium appears to enhance rehydration by effecting plasma 87 osmolality and the consequent diuresis once the fluid has reached the circulation. However, 88 the ingestion of food alongside drink after exercise, might slow the delivery of fluid to the 89 circulation, thereby enhancing fluid retention (Evans et al. 2011; Clayton et al. 2014). 90 Therefore, the purpose of the present study was to examine the effect of sodium on post-91 exercise rehydration when drinks were ingested along with a post-exercise meal.

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93 Methods

94 *Participants*

Five male and three female non heat acclimatised, recreationally active participants (Age = 21 ± 2 y, Height 179 ± 8 cm and Body Mass 71.1 ± 13.2 kg) volunteered for this investigation which had prior approval from the Institutional Ethics Committee. All participants provided written informed consent and completed a standard medical screening questionnaire prior to participation.

100

101 **Procedure**

102 Participants completed two experimental trials in a randomized counterbalanced order separated by a period of at least seven days. Experimental trials involved exercise in the heat 103 104 $(33.3 \pm 1.7 \text{ °C and } 53 \pm 13\% \text{ humidity})$ to induce dehydration of up to 1.8% body mass or 105 until participants could exercise no longer. Participants then remained in the laboratory in a 106 comfortable environment for a one hour rehydration/refeeding period and a three hour 107 monitoring period without food or drink. Participants recorded their diet and physical activity 108 patterns in the 24 hours preceding the first experimental trial and replicated these in the 24 109 hours prior to the second experimental trial. Experimental trials began at the same time of the 110 morning and were preceded by fasting from 9 pm the previous evening, with the exception of 111 ~500 ml water ingested one hour before arrival at the laboratory.

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Following arrival at the laboratory, participants were instructed to empty their bladder and collect all urine produced before measurement of body mass (Adam Equipment, Multon Keynes, UK) to the nearest 10 g wearing minimal clothing. Participants exercised in 10 min blocks, separated by 5 min rest in the chamber. Exercise workload was initially 2 W/ kg body mass and participants could reduce the workload if required. Body mass was measured during the 5 min rest periods and exercise continued until participants lost ~1.8% of their initial body mass or until participants could exercise no longer. If a participant was unable to continue and terminated exercise before reaching the required body mass loss during their first experimental trial this level of body mass loss was replicated in their second trial. Following the cessation of exercise, participants showered before a urine sample was collected and a final body mass measurement obtained. Exercise time was 44 ± 12 and 48 ± 9 minutes (P = 0.285) and exercise intensity was 106 ± 19 and 108 ± 22 watts (P = 0.178) on the water and sodium chloride trials respectively.

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127 Participants then underwent a 60 minute rehydration/refeeding period, during which they drank a volume of fluid equal to 150% BM lost during exercise in four aliquots given at 15 128 129 minute intervals. The fluids ingested were either water (W) or a 50 mmol/l sodium chloride 130 solution (NaCl). The test solution was prepared by weighing out the required NaCl and dissolving in commercially available water. Fifteen minutes into the rehydration period, 131 participants were provided with a meal consisting of pasta, tomato pasta sauce, olive oil and 132 133 mature cheddar cheese (Tesco, Cheshunt, UK) in a quantity sufficient to provide 30% of 134 estimated daily energy expenditure. Participants were given the remaining 45 minutes of the 135 rehydration period to consume the meal. Daily energy expenditure was estimated using the 136 equations of Mifflin-St Jeor (1991) with a physical activity factor of 1.8. The meal provided 137 53, 33 and 14% of total energy from carbohydrate, fat and protein, respectively. The 138 macronutrient and sodium chloride composition of the meal are presented in Table 1. 139 Following completion of the rehydration/refeeding period, participants provided a further 140 urine sample and remained in the laboratory for three hours. Urine samples were collected at 141 hourly intervals during this three hour period.

For each urine sample collected, total volume produced was measured and a 5 mL sample
retained for analysis of osmolality using freezing point depression (Gonotec Osmomat 030
Cryoscopic Osmometer, Gonotec, Berlin, Germany). Analysis was performed in duplicate.

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Water content of food was determined from manufacturer values. Retention of the drinks was calculated from total water intake (drink volume plus water from food) and the volume of urine produced at individual time points. Net fluid balance was calculated from sweat loss (assuming all body mass loss during exercise was water and that all water loss during exercise was in the form of sweat), total water intake (calculated as described above) and urine volume.

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154 Statistical analyses

Data was found to be normally distributed using the Kolmogorov-Smirnov test and are presented as Mean \pm SD. Data was analysed using two factor repeated measures ANOVA, one factor repeated measures ANOVA with post-hoc analysis and dependent t-tests as appropriate. Bonferonni corrections were applied for multiple comparisons where appropriate. Critical value of significance was taken as 0.05 and all analysis was performed using IBM SPSS Version 21.

161

162 **Results**

163 **Pre-exercise and sweat loss measurements**

Participants pre-exercise body mass was (W: 71.15 \pm 13.61 kg; NaCl: 71.11 \pm 13.69 kg; P=0.872) and urine osmolality (W: 644 \pm 334 mOsmol/ kg; NaCl: 593 \pm 317 mOsmol/ kg; P=0.663) were not different between trials. Additionally, total sweat loss (W: 1040 \pm 440 mL; NaCl: 1040 \pm 290 mL; P=0.988) and percentage body mass loss (W: 1.44 \pm 0.52 %; 168 NaCl: 1.47 ± 0.39 %; P=0.793) were not different between trials. Fluid and food intake in the 169 post-exercise refeeding period were not different between trials (Table 1).

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171 Urine output and net fluid balance

172 Total urine output (Figure 1) was greater during W (981 \pm 458 mL) than NaCl (577 \pm 345 mL; P=0.035). There were time (P<0.001) effects for urine volume. One participant 173 174 produced 28% more urine during the NaCl trial while other participants ranged from 18 -175 83% less urine production during the NaCl trial. The greatest volume of urine produced during each trial occurred one hour after completion of the rehydration and urine volume was 176 177 significantly greater than post-exercise values one (P=0.004) and three hours (P=0.028) after 178 ingestion of W and one (P=0.012) and two (P=0.008) hours after ingestion of NaCl. Urine volumes were greater on W than NaCl one (P=0.017) and three (P=0.026) hours after 179 180 rehydration. At the end of the recovery period the percentage of fluid retained was greater 181 (P=0.017) during the NaCl trial ($70 \pm 21\%$) than during trial W ($50 \pm 20\%$).

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183 There were time (P < 0.001) effects and a tendency (P = 0.055) for interaction effects for net 184 fluid balance (Figure 2). Net fluid balance was significantly negative (P < 0.05) on both trials 185 after exercise and significantly positive (P < 0.05) on both trials after rehydration. Participants 186 remained in positive fluid balance (P=0.04) 1 hour after the end of the rehydration period during the NaCl trial, with no other difference (P>0.05) from pre-exercise values were 187 188 observed during trial W. No differences (P < 0.05) were observed between trials at any time point however there was a tendency for net fluid balance to be greater on the NaCl trial on W 189 190 1 (P = 0.071) and 3 (P = 0.061) hours after rehydration.

There was a main effect of time (P=0.001), but no trial (P=0.288) or interaction effects (P=0.461) for urine osmolality (Figure 3). Urine osmolality was reduced one (P=0.02) and three (P=0.03) hours after rehydration on trial W. No differences (P>0.05) were observed from pre-exercise values on the NaCl trial or between the trials at any time point.

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197 **Discussion**

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The main finding of this study was that when food is ingested following exercise induced dehydration, fluid retention is greater when a 50 mmol/L NaCl solution is consumed compared to water. Following the three hour recovery period, cumulative urine volume was less during the NaCl trial compared to the water trial, meaning a greater proportion of drink was retained during the NaCl trial (70 ± 21 %) compared to the water trial (50 ± 20%).

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205 Despite the large volume of research available on post-exercise rehydration, few studies have 206 included a post-exercise meal within their study design and therefore possibly lack ecological 207 valididy. Most previous studies have compared solutions containing different micro- or 208 macro-nutrients without ingestion of food or fluid after the rehydration period and these have 209 provided the basis for rehydration recommendations post-exercise. In practice, recreational 210 exercisers and athletes are likely to consume food during the recovery period and/or ingest 211 fluid after the initial period of rehydration. Both of these practices could potentially alter the 212 rehydration characteristics of an ingested solution. Studies that have included the ingestion of 213 food during the post-exercise period have not specifically investigated whether this practice 214 alters the efficacy of a solution that has been ingested to promote rehydration. Maughan, 215 Leiper and Shirreffs (1996) compared the ingestion of a commercially available sports drink 216 during a rehydration period with the ingestion of water with a standard meal. They observed 217 that a greater proportion of fluid was retained when a standard meal was ingested with water 218 than when only a sports drink was ingested. Ray et al. (1998) investigated the effects of 219 water, a carbohydrate electrolyte solution, chicken broth and chicken noodle soup on fluid 220 retention after exercise in the heat. It was reported that urine volume following ingestion of 221 the carbohydrate electrolyte solution was greater than following ingestion of chicken broth and that urine osmolalitites were higher on the chicken broth and chicken noodle soup trials 222 223 compared to the carbohydrate electrolyte solution trial. These studies suggest that the 224 ingestion of food is an important consideration during post-exercise rehydration. The present study is the first to investigate the efficacy of rehydration solutions when a standard meal is 225 226 consumed during the post-exercise period and provides further support for the importance of 227 considering the influence of food on recovery of water balance after exercise.

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229 To ensure a return to positive fluid balance following exercise-induced dehydration, it is 230 necessary to ingest a volume of fluid greater than that lost through sweating during exercise 231 (Shirreffs et al. 1996), in order to account for ongoing urine losses during and after rehydration. It is important to note, however, that in real world situations athletes may be 232 233 reluctant to ingest the large volumes of fluid that may be required over a short period of time 234 (Davis et al. 2014; O'Neal et al. 2014). If a sufficient volume of fluid is ingested, the 235 retention of fluid is principally determined by the composition of the solution ingested. In 236 particular, the composition of the ingested solution affects fluid balance mechanisms by 237 altering plasma osmolality. Change in plasma osmolality is the main factor that affects AVP 238 secretion while it is less sensitive to reductions in blood volume (Robertson 1974). Robertson 239 (1974) reports that a change in plasma osmolality of approximately 3 mOsm/kg is sufficient 240 to induce a change in plasma AVP of 1 pg/mL which, in turn, has a significant effect on urine 241 production. Rehydration solutions appear to impact on plasma osmolality in two ways.

Firstly, by affecting the ionic composition of the extracellular fluid; and secondly, by affecting the rate of fluid delivery to the extracellular fluid via alterations in gastric emptying or intestinal absorption rates. Both of these mechanisms will influence plasma osmolality and consequently urine production.

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247 Water is absorbed relatively quickly from the gastrointestinal tract resulting in a relatively 248 large reduction in plasma osmolality (Nose et al. 1988) leading to a marked diuresis. For this 249 reason, water is often not considered an adequate rehydration solution as participants are 250 often returned to negative net fluid balance relatively quickly (Shirreffs et al. 1996; Shirreffs 251 and Maughan 1998; Evans et al. 2009; James et al. 2014). The results of the present study 252 suggest, however, that the ingestion of water alongside food ensures that individuals remain euhydrated for up to 3 hours after fluid ingestion. Although not measured in this study, it is 253 254 likely that the addition of food in the rehydration period reduces the overall rate of fluid 255 absorption leading to less urine output than if food is not consumed. This observation would 256 suggest that water may be considered an adequate rehydration solution in certain situations 257 where food is also consumed, although further studies are required to confirm this hypothesis 258 and the appropriate mechanisms.

259

The addition of sodium to a rehydration solution has been shown on numerous occasions to have a positive effect on maintenance of rehydration throughout a recovery period. Shirreffs and Maughan (1998) observed that participants were only in positive fluid balance six hours after rehydration when a 100 mmol/L sodium solution was ingested however they were essentially in fluid balance when a 50 mmol/L sodium solution was ingested. As sodium is the main ion in the extracellular fluid, addition of sodium to a rehydration solution maintains plasma osmolality and, therefore, plasma AVP concentrations which leads to the avoidance 267 of a diuresis seen following the ingestion of plain water (Nose et al. 1988). Similarly, Merson 268 et al. (2008) observed that the addition of 40 or 50 mmol/L NaCl to a rehydration solution resulted in reduced urine output compared to solutions containing 1 or 31 mmol/L NaCl. The 269 ingestion of the NaCl solution in this study resulted in less urine output than when water was 270 271 ingested and a greater degree of fluid retention suggesting that, even with co-ingestion of 272 food, the addition of sodium is still beneficial for water retention in the post-exercise period. 273 It is likely that the mechanism for this observation is similar to those observed previously. It 274 should be noted, however, that the results of the present study demonstrate that three of the eight participants exhibited markedly lower urine production during the NaCl trial compared 275 276 to the water trial whereas other participants exhibited much lower effects of the intervention. 277 Although not measured in this study, it is unlikely that the difference in fluid retention between the trials is likely to confer any benefit on subsequent exercise performance. 278

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280 The addition of carbohydrate (Evans et al. 2009; Osterberg et al. 2010) and milk protein 281 (James et al. 2011) to rehydration solutions and the ingestion of milk (Shirreffs et al. 2007) 282 have been shown to be effective for maintaining fluid balance after exercise-induced 283 dehydration. It has been suggested that the success of altering macronutrient composition of 284 rehydration solutions is due to a reduced rate of fluid uptake which will consequently effect 285 the rate of change of plasma osmolality (Clayton et al. 2014). Similarly, a study by Wong and 286 Chen (2011) reported that a carbohydrate-electrolyte solution was more effective at 287 maintaining euhydration following exercise induced dehydration than distilled water or 288 lemon tea. While the results of the present study demonstrate that rehydration solutions that 289 influence plasma sodium concentration provide beneficial effects on fluid retention when co-290 ingested with food, it is not clear whether similar results would be obtained if food is co-291 ingested with solutions that influence rate of fluid uptake. Future investigations in this area should, therefore, focus on whether the ingestion of food during the post-exercise period effects the efficacy of solutions that exert their effect on plasma osmolality primarily via reducing fluid absorption rather than effects on extracellular fluid composition.

295

296 The extent of body mass loss exhibited in the present study is similar to other studies of this 297 nature, and representative of levels reported in elite athletes after typical training sessions 298 (Maughan et al. 2004), but represents only a relatively modest level of hypohydration. The 299 results described are applicable to such scenarios however extrapolation of these findings to 300 those athletes, and exercisers, that exhibit significantly greater degrees of hypohydration 301 should be avoided until further study has been undertaken in these situations. If a further 302 exercise session is not anticipated within a relatively short time frame, normal mechanisms of 303 restoring fluid balance will ensure a return to euhydration and a rehydration strategy 304 involving rapid ingestion of large fluid volumes, as described in this and other studies, is 305 unlikely to be necessary. The results of this study suggest that if the time between exercise 306 sessions is up to 3 hours then co-ingestion of food and a sodium solution enhances fluid 307 retention in comparison to food and water however further study should be undertaken to 308 determine whether differences are still observed over longer recovery periods.

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In conclusion, the results of this study demonstrate that when food is ingested with a 50 mmol/L NaCl solution following exercise-induced dehydration a greater fraction of fluid is retained when compared to water however participants remained euhydrated 3 hours after food was co-ingested with water.

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	W	NaCl	P Value
Food water (mL)	463 ± 68	465 ± 65	0.244
Energy Intake (Kcal)	896 ± 132	899 ± 125	0.528
Carbohydrate (g)	118 ± 17	119 ± 17	0.453
Fat (g)	33 ± 5	33 ± 5	0.448
Protein (g)	32 ± 5	32 ± 4	0.560
NaCl (g)	1.8 ± 0.3	1.9 ± 0.3	1.000

Table 1: Characteristics of the standard meal during the post-exercise refeeding period during
W and NaCl trials. Values are Mean ± SD.

430 The water content of the food was calculated as $463 \pm 68 \text{ mL}$ (W) and $465 \pm 65 \text{ mL}$ resulting 431 in a total water intake of 2013 ± 689 and $2014 \pm 469 \text{ mL}$ (P = 0.991) on the W and NaCl 432 trials respectively.

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434 Figure legends

Figure 1: Cumulative urine volume (mL) produced by (a) each participant and (b) all
participants following ingestion of water (W) or 50 mmol/L NaCl (NaCl). "*" indicates
significant difference between the trials.



Figure 2: Net fluid balance (mL) following ingestion of water (W) or 50 mmol/L NaCl
(NaCl). "*" indicates significant difference from "Pre-" time point on both trials. "#"
indicates significant difference from "Pre-" time point on the NaCl trial.

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Figure 3: Urine osmolality (mOsm/kg) following ingestion of water (W) or 50 mmol/L NaCl
(NaCl). "*" indicates significant difference from "Pre-" time point on trial W.

