1	Title				
2	A sodium drink enhances fluid retention during 3 hours of post-exercise recovery when				
3	ingested with a standard meal				
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22					

Abstract

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The purpose of this study was to examine the efficacy of water and a 50 mmol/L NaCl solution on post-exercise rehydration when a standard meal was consumed during rehydration. Eight healthy participants took part in two experimental trials during which they 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 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 expenditure. Urine samples were collected before and after exercise and for three hours after 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 during the water compared to the NaCl trial respectively. A high degree of variability in results was observed with one participant producing 28% more urine and others ranging from 18 – 83% reduction in urine output during the NaCl trial. The results of this study suggest that after exercise induced dehydration, the ingestion of a 50 mmol/L NaCl solution leads to greater fluid retention compared with water, even when a meal is consumed post-exercise. Furthermore, ingestion of plain water may be effective for maintenance of fluid balance when food is consumed in the rehydration period.

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Key Words

43 Hypohydration, Recovery, Net Fluid Balance

Introduction

Exercise increases sweat rate and in many exercise settings, exercisers do not fully replace fluid lost through sweating (Sawka et al. 2007), meaning hypohydration might be present at the end of exercise. Starting exercise hypohydrated might impair strength (Minshull and 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. Consequently, in situations where exercise sessions are undertaken in close proximity, 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 dehydrating evening exercise session impaired exercise performance the following morning. Similarly, Wong et al. (1998) observed that the prescription of a set volume of fluid during a four hour recovery period results in more efficient recovery of endurance capacity than when fluid was ingested *ad libitum*.

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

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 the 4-7 h post-exercise. In real-world training/ competition scenarios it would be unusual not 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 fluid volume (i.e. fluid from foods and drinks) is matched (Maughan et al. 1996; Ray et al. 1998). Therefore it is scientifically and practically relevant to determine whether differences in the efficacy of different rehydration drinks persists when food is also consumed in the post-exercise period, as would likely be the case in sporting scenarios.

Sodium represents the major cation in the extracellular space and can be lost in large amounts 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 rehydration drink increases plasma osmolality (Nose et al. 1988) and enhances post-exercise rehydration in a dose-dependent manner (Maughan and Leiper 1995; Shirreffs and Maughan 1998; Merson et al. 2008). Sodium appears to enhance rehydration by effecting plasma osmolality and the consequent diuresis once the fluid has reached the circulation. However, the ingestion of food alongside drink after exercise, might slow the delivery of fluid to the circulation, thereby enhancing fluid retention (Evans et al. 2011; Clayton et al. 2014). Therefore, the purpose of the present study was to examine the effect of sodium on post-exercise rehydration when drinks were ingested along with a post-exercise meal.

Methods

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.

Procedure

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 $(33.3 \pm 1.7 \, ^{\circ}\text{C})$ and $53 \pm 13\%$ humidity) to induce dehydration of up to 1.8% body mass or until participants could exercise no longer. Participants then remained in the laboratory in a comfortable environment for a one hour rehydration/refeeding period and a three hour monitoring period without food or drink. Participants recorded their diet and physical activity patterns in the 24 hours preceding the first experimental trial and replicated these in the 24 hours prior to the second experimental trial. Experimental trials began at the same time of the morning and were preceded by fasting from 9 pm the previous evening, with the exception of ~500 ml water ingested one hour before arrival at the laboratory.

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.

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 minute intervals. The fluids ingested were either water (W) or a 50 mmol/l sodium chloride 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, participants were provided with a meal consisting of pasta, tomato pasta sauce, olive oil and mature cheddar cheese (Tesco, Cheshunt, UK) in a quantity sufficient to provide 30% of estimated daily energy expenditure. Participants were given the remaining 45 minutes of the rehydration period to consume the meal. Daily energy expenditure was estimated using the equations of Mifflin-St Jeor (1991) with a physical activity factor of 1.8. The meal provided 53, 33 and 14% of total energy from carbohydrate, fat and protein, respectively. The macronutrient and sodium chloride composition of the meal are presented in Table 1. Following completion of the rehydration/refeeding period, participants provided a further urine sample and remained in the laboratory for three hours. Urine samples were collected at hourly intervals during this three hour period.

143 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 030 144 Cryoscopic Osmometer, Gonotec, Berlin, Germany). Analysis was performed in duplicate. 145 146 147 Water content of food was determined from manufacturer values. Retention of the drinks was 148 calculated from total water intake (drink volume plus water from food) and the volume of 149 urine produced at individual time points. Net fluid balance was calculated from sweat loss 150 (assuming all body mass loss during exercise was water and that all water loss during 151 exercise was in the form of sweat), total water intake (calculated as described above) and 152 urine volume. 153 154 Statistical analyses Data was found to be normally distributed using the Kolmogorov-Smirnov test and are 155 156 presented as Mean \pm SD. Data was analysed using two factor repeated measures ANOVA, 157 one factor repeated measures ANOVA with post-hoc analysis and dependent t-tests as 158 appropriate. Bonferonni corrections were applied for multiple comparisons where appropriate. Critical value of significance was taken as 0.05 and all analysis was performed 159 160 using IBM SPSS Version 21. 161 162 **Results** 163 Pre-exercise and sweat loss measurements 164 Participants pre-exercise body mass was (W: 71.15 ± 13.61 kg; NaCl: 71.11 ± 13.69 kg;

P=0.872) and urine osmolality (W: 644 ± 334 mOsmol/ kg; NaCl: 593 ± 317 mOsmol/ kg;

P=0.663) were not different between trials. Additionally, total sweat loss (W: 1040 ± 440

mL; NaCl: 1040 ± 290 mL; P=0.988) and percentage body mass loss (W: 1.44 ± 0.52 %;

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NaCl: 1.47 ± 0.39 %; P=0.793) were not different between trials. Fluid and food intake in the post-exercise refeeding period were not different between trials (Table 1).

Urine output and net fluid balance

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 produced 28% more urine during the NaCl trial while other participants ranged from 18 - 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 significantly greater than post-exercise values one (P=0.004) and three hours (P=0.028) after 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 rehydration. At the end of the recovery period the percentage of fluid retained was greater (P=0.017) during the NaCl trial ($70 \pm 21\%$) than during trial W ($50 \pm 20\%$).

There were time (P<0.001) effects and a tendency (P=0.055) for interaction effects for net fluid balance (Figure 2). Net fluid balance was significantly negative (P<0.05) on both trials after exercise and significantly positive (P<0.05) on both trials after rehydration. Participants 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 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 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.

Discussion

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 $(70 \pm 21 \%)$ compared to the water trial $(50 \pm 20\%)$.

Despite the large volume of research available on post-exercise rehydration, few studies have included a post-exercise meal within their study design and therefore possibly lack ecological valididy. Most previous studies have compared solutions containing different micro- or macro-nutrients without ingestion of food or fluid after the rehydration period and these have provided the basis for rehydration recommendations post-exercise. In practice, recreational exercisers and athletes are likely to consume food during the recovery period and/or ingest fluid after the initial period of rehydration. Both of these practices could potentially alter the rehydration characteristics of an ingested solution. Studies that have included the ingestion of food during the post-exercise period have not specifically investigated whether this practice alters the efficacy of a solution that has been ingested to promote rehydration. Maughan, Leiper and Shirreffs (1996) compared the ingestion of a commercially available sports drink during a rehydration period with the ingestion of water with a standard meal. They observed

that a greater proportion of fluid was retained when a standard meal was ingested with water than when only a sports drink was ingested. Ray et al. (1998) investigated the effects of water, a carbohydrate electrolyte solution, chicken broth and chicken noodle soup on fluid retention after exercise in the heat. It was reported that urine volume following ingestion of 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 compared to the carbohydrate electrolyte solution trial. These studies suggest that the 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 consumed during the post-exercise period and provides further support for the importance of considering the influence of food on recovery of water balance after exercise.

To ensure a return to positive fluid balance following exercise-induced dehydration, it is necessary to ingest a volume of fluid greater than that lost through sweating during exercise (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 reluctant to ingest the large volumes of fluid that may be required over a short period of time (Davis et al. 2014; O'Neal et al. 2014). If a sufficient volume of fluid is ingested, the retention of fluid is principally determined by the composition of the solution ingested. In particular, the composition of the ingested solution affects fluid balance mechanisms by altering plasma osmolality. Change in plasma osmolality is the main factor that affects AVP secretion while it is less sensitive to reductions in blood volume (Robertson 1974). Robertson (1974) reports that a change in plasma osmolality of approximately 3 mOsm/kg is sufficient to induce a change in plasma AVP of 1 pg/mL which, in turn, has a significant effect on urine 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.

Water is absorbed relatively quickly from the gastrointestinal tract resulting in a relatively large reduction in plasma osmolality (Nose et al. 1988) leading to a marked diuresis. For this reason, water is often not considered an adequate rehydration solution as participants are often returned to negative net fluid balance relatively quickly (Shirreffs et al. 1996; Shirreffs and Maughan 1998; Evans et al. 2009; James et al. 2014). The results of the present study 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 likely that the addition of food in the rehydration period reduces the overall rate of fluid absorption leading to less urine output than if food is not consumed. This observation would suggest that water may be considered an adequate rehydration solution in certain situations where food is also consumed, although further studies are required to confirm this hypothesis and the appropriate mechanisms.

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

of a diuresis seen following the ingestion of plain water (Nose et al. 1988). Similarly, Merson 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 ingestion of the NaCl solution in this study resulted in less urine output than when water was ingested and a greater degree of fluid retention suggesting that, even with co-ingestion of food, the addition of sodium is still beneficial for water retention in the post-exercise period. It is likely that the mechanism for this observation is similar to those observed previously. It 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 to the water trial whereas other participants exhibited much lower effects of the intervention. 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.

The addition of carbohydrate (Evans et al. 2009; Osterberg et al. 2010) and milk protein (James et al. 2011) to rehydration solutions and the ingestion of milk (Shirreffs et al. 2007) have been shown to be effective for maintaining fluid balance after exercise-induced dehydration. It has been suggested that the success of altering macronutrient composition of rehydration solutions is due to a reduced rate of fluid uptake which will consequently effect the rate of change of plasma osmolality (Clayton et al. 2014). Similarly, a study by Wong and Chen (2011) reported that a carbohydrate-electrolyte solution was more effective at maintaining euhydration following exercise induced dehydration than distilled water or lemon tea. While the results of the present study demonstrate that rehydration solutions that influence plasma sodium concentration provide beneficial effects on fluid retention when coingested with food, it is not clear whether similar results would be obtained if food is coingested 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.

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

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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Table 1: Characteristics of the standard meal during the post-exercise refeeding period during

W and NaCl trials. Values are Mean ± SD.

	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

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

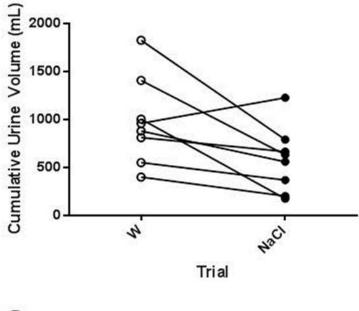
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Figure legends

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.



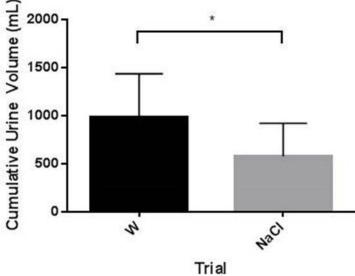


Figure 2: Net fluid balance (mL) following ingestion of water (W) or 50 mmol/L NaCl (NaCl). "*" indicates significant difference from "Pre-" time point on both trials. "#" indicates significant difference from "Pre-" time point on the NaCl trial.

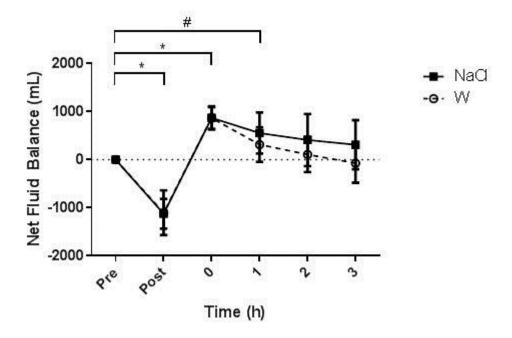


Figure 3: Urine osmolality (mOsm/kg) following ingestion of water (W) or 50 mmol/L NaCl (NaCl). "*" indicates significant difference from "Pre-" time point on trial W.

