1<u>Effect of drink carbohydrate content on post-exercise gastric emptying, rehydration</u> 2<u>and the calculation of net fluid balance</u>

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16Running head: Carbohydrate rehydration

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20Abstract

21The purpose of this study was to examine the gastric emptying and rehydration effects of 22hypotonic and hypertonic glucose-electrolyte drinks after exercise-induced dehydration. 23Eight healthy males lost ~1.8% body mass by intermittent cycling and rehydrated (150% of 24body mass loss) with a hypotonic 2% (2% trial) or a hypertonic 10% (10% trial) glucose-25electrolyte drink over 60 min. Blood and urine samples were taken at pre-exercise, post-26 exercise, and 60, 120, 180 and 240 min post-exercise. Gastric and test drink volume were 27determined 15, 30, 45, 60, 90 and 120 min post-exercise. At the end of the gastric sampling 28period 0.3% (2% trial) and 42.1% (10% trial) (P<0.001) of the drinks remained in the 29stomach. Plasma volume was lower (P < 0.01) and serum osmolality was greater (P < 0.001) at 3060 and 120 min during the 10% trial. At 240 min, 52% (2% trial) and 64% (10% trial) $31(P \le 0.001)$ of the drinks were retained. Net fluid balance was greater from 120 min during the 3210% trial (P < 0.001). When net fluid balance was corrected for the volume of fluid in the 33stomach, it was greater at 60 and 120 min during the 2% trial (P<0.001). These results 34suggest that the reduced urine output following ingestion of a hypertonic rehydration drink 35might be mediated by a slower rate of gastric emptying, but the slow gastric emptying of such 36solutions makes rehydration efficiency difficult to determine in the hours immediately after 37drinking, compromising the calculation of net fluid balance.

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39Key Words: glucose, exercise, dehydration, hypohydration

40Introduction

41Studies have frequently found that athletes finish exercise hypohydrated (Sawka et al., 2007), 42and when time availability between exercise bouts is short, fluid balance should be rapidly 43restored to avoid compromising subsequent exercise performance (Judelson et al., 2007).

44For rapid and complete rehydration, drink volume is vital (Shirreffs, Taylor, Leiper, & 45Maughan, 1996); but provided drink volume is sufficient, drink composition will determine 46drink retention, and manipulation of sodium (Shirreffs & Maughan, 1998; Merson, Maughan, 47& Shirreffs, 2008), carbohydrate (Evans, Shirreffs, & Maughan, 2009a; Osterberg, Pallardy, 48Johnson, & Horswill, 2010) and protein (Seifert, Harmon, & DeClercq, 2006; James, 49Clayton, & Evans, 2011) concentration has been shown to affect rehydration.

50Rehydration is composed of three inter-related processes: gastric emptying, intestinal 51absorption and fluid retention. Whilst one study (Mudambo, Leese, & Rennie, 1997) has 52suggested that gastric emptying might be slowed immediately post-exercise compared to after 53an overnight fast, little is known about what effects manipulating the energy or macronutrient 54content of drinks has on post-exercise gastric emptying. At rest, gastric emptying rate is 55closely related to drink energy density (Calbet & MacLean, 1997) and thus increasing drink 56carbohydrate concentration decreases the gastric emptying rate (Vist & Maughan, 1994). 57Additionally, drink osmolality can affect gastric emptying independently of carbohydrate 58content (Vist & Maughan, 1995). The rate of intestinal absorption is highly dependent on the 59rate of gastric emptying. Water uptake from the intestine is governed by osmotic gradients 60and the osmolality of ingested drinks, both of which determine the rate and direction of water 61flux (Leiper & Maughan, 1986). Shi et al. (1994) demonstrated that a hypotonic 62carbohydrate-electrolyte drink increased the rate of fluid absorption by 17% compared to a 63hypertonic carbohydrate-electrolyte drink. Similarly, Lieper and Maughan (1986) found that

64perfusion of an isotonic drink into the intestine promoted fluid and electrolyte uptake from 65the intestine, but that perfusion of a hypertonic drink led to a net efflux of water and 66electrolytes into the intestinal lumen.

67In situations where both post-exercise glycogen resynthesis and rehydration are required, the 68ingestion of hypertonic carbohydrate drinks might provide adequate carbohydrate for 69glycogen resynthesis whilst also providing water for rehydration. Rehydration studies have 70found that when a large volume of a hypotonic low sodium drink was ingested, substantial 71plasma volume expansion occurred, causing an acute reduction in serum osmolality and a 72large diuresis (Nose, Mack, Shi, & Nadel, 1988). In contrast, ingestion of a hypertonic 12% 73carbohydrate rehydration drink has been shown to attenuate the decline in serum osmolality, 74producing a less pronounced diuresis (Osterberg et al., 2010). The mechanism responsible for 75this effect is not fully understood, but might be related to the gastric emptying properties of 76the different drinks. The aim of this study was therefore to investigate the effect of hypotonic 77(2%) and hypertonic (10%) glucose-electrolyte drinks on gastric emptying and drink 78retention after exercise-induced dehydration.

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80Methods

81After approval by the Nottingham Trent University School of Science and Technology Ethics 82Committee, eight males (24 (3) y, 79.5 (9.3) kg, 1.80 (0.08) m) completed a medical 83screening questionnaire and gave their written consent. Using the data of Evans et al. (2009a) 84and an alpha of 5% and a beta of 50%, it was determined that 8 subjects would be required to 85observe a significant difference in urine output.

86Preliminary Testing

87During the first preliminary visit, subjects orally inserted a 14 g gastric tube (Sonde Gastro-88Duodenal Type Levin, Vygon Ltd, Cirencester, UK) and the stomach was emptied as much 89as possible. A recovery test was then performed, which involved instillation of 100 ml 90distilled water into the stomach, before the stomach contents were mixed by aspirating and 91re-injecting 30-50 ml of stomach contents continuously for 1 minute. Stomach contents were 92removed, and the gastric tube was considered to be positioned at the base of the stomach if 9380-110 ml was recovered. The second visit involved an incremental exercise test on an 94electrically braked cycle ergometer (Excalibur Sport, Lode B.V., Groningen, Netherlands) to 95determine subject's peak aerobic power. Exercise started at 105 W and the intensity was 96increased by 35 W every 3 min until volitional fatigue. The third preliminary visit involved 97familiarisation with the dehydration and rehydration phases of the protocol described below, 98after which subjects remained in the laboratory for 1 h.

99Experimental Protocol

100All subjects completed two experimental trials in a randomised counterbalanced order, 101separated by at least 7 days. Trials commenced after an overnight fast, except 500 ml water 102consumed 1.5 h before arrival. In the 24 h preceding the first experimental trial, subjects 103recorded their dietary intake and physical activity, and repeated these dietary and physical 104activity patterns in the 24 h before the second experimental trial. Subjects were asked to 105avoid strenuous physical activity and alcohol ingestion during this 24 h.

106Upon arrival at the laboratory a blood sample was obtained by venepuncture of an antecubital 107vein (pre-exercise), after which a urine sample was collected and body mass was measured 108(wearing dry boxer shorts only) (Adam CFW150; Adam Equipment Co Ltd, Milton Keynes, 109UK). Subjects then commenced exercise in a temperature (35 (0.2)°C) and humidity (68.4 110(6.1)% relative humidity) controlled environment (Design Environmental Ltd, Ebbw Vale, 111UK) until a body mass loss (BML) of 1.6% was achieved. Exercise began at an intensity of 11250% peak aerobic power and subjects exercised in 10 min blocks separated by 5 min rest, 113during which they were weighed. After the desired BML was achieved, subjects were given 11410 minutes to shower, followed by 5 min rest in a comfortable environment, before body 115mass was measured (wearing dry boxer shorts only) to determine the total BML.

116A 21 gauge cannula was then inserted into an antecubital vein and subjects inserted the117gastric tube, before a blood sample (0 min) was taken and subjects provided a urine sample.118The stomach was then emptied and the recovery test was performed.

119Subjects then ingested a volume of drink (1) equivalent to 150% of their total BML (kg) over 120a period of 1 h, in 4 aliquots of equal volume. Subjects ingested the first aliquot of drink, 121which contained 10 mg·l⁻¹ phenol red dye (VWR International, Lutterworth, UK), at 0 min 122and had 1 min to finish the drink. Further aliquots provided at 15, 30 and 45 min, contained 123no phenol red, and were consumed in 1, 5 and 5 min, respectively. Drinks were made up ~1 h 124before consumption. Drinks contained either 2% (2% trial) or 10% (10% trial) glucose, 125additional sodium chloride to give a sodium concentration of ~30 mmol·l⁻¹, and 100 ml·l⁻¹ of 126sugar free squash (Table 1).

127Following rehydration, subjects rested in the laboratory (24.2 (0.9)°C; 42.6 (11.0)% relative 128humidity) for 3 h, with urine and blood samples obtained every hour (60, 120, 180 and 240 129min). If subjects needed to urinate before the hourly time point they could, except in the 15 130min before each blood sample. This urine was then pooled with the urine produced at the next 131hourly time point. All blood samples (5 ml) were taken after at least 15 min seated in a 132comfortable environment. Immediately before each urine sample, subjects were asked to rate 133their subjective feelings of thirst, stomach fullness, bloating, hunger, tiredness, alertness, 134concentration, head soreness, dryness of mouth, refreshedness and energy using a 100 mm 135visual analogue scale, with 0 mm representing 'not at all' and 100 mm representing 'very'.136Additional questions using 100 mm visual analogues scale on sweetness, saltiness, bitterness137and pleasantness of drinks were asked at 60 min.

138Gastric Sampling

139Gastric volume was measured using double sampling gastric aspiration (Beckers, Rehrer, 140Brouns, Ten Hoor, & Saris, 1988). A sample of the initial aliquot of drink was retained for 141analysis. Following ingestion of the initial aliquot of drink, the stomach contents were mixed 142as previously described and a sample (2.5 ml) was retained. At each sampling point, the 143stomach contents were mixed, before a sample (2.5 ml) was aspirated. Five ml of phenol red 144solution was then added to the stomach, and the stomach contents were mixed again before a 145further sample (2.5 ml) was taken. Gastric and drink volume were calculated from changes in 146phenol red concentrations. Gastric samples were also obtained after each drink had been 147ingested, to calculate the dilution of dye concentration at each drinking point.

148Gastric volume was measured at 15, 30, 45, 60, 90 and 120 min. The concentration of the 149phenol red in the 5 ml aliquot added to the stomach was $0.5 \text{ g} \cdot 1^{-1}$ at 15, 30, 45 and 60 min and 1501 g $\cdot 1^{-1}$ at 90 and 120 min.

151Calculations

152Net fluid balance (NFB) was calculated from sweat losses during exercise, estimated from 153BML (kg), drink volume ingested during rehydration (ml) (D) and cumulative urine output 154(ml) (U) (Maughan, Shirreffs, & Leiper, 2007).

$$155NFB_n = 0 - (BML*1000) + D - U$$

156Corrected net fluid balance was calculated to account for the volume of fluid remaining in the 157stomach (GV).

158Corrected NFB $_{n} = NFB _{n} - GV _{n}$

159Sample Analysis

160Of each blood sample, 1.3 ml was mixed with EDTA (1.75 mg·l⁻¹) and placed in ice, before 161plasma was separated by centrifugation (3000 g, 10 min, 3°C). A further 1.3 ml blood was 162mixed with EDTA (1.75 mg·l⁻¹) and was used for analysis of hemoglobin by the 163cyanmethemoglobin method and hematocrit by microcentrifugation. Hemoglobin and 164hematocrit values were used to estimate changes in blood, red cell and plasma volume, 165relative to pre-exercise (Dill & Cositll, 1974). The remainder of the blood sample was 166allowed to clot, before serum was separated by centrifugation (3000 g, 10 min, 3°C). Serum 167was analyzed for osmolality by freezing point depression (Gonotec Osmomat 030 Cryoscopic 168Osmomter; Gonotec, Berlin, Germany), whilst plasma was stored at -80°C and analyzed for 169glucose concentration by the glucose oxidase peroxidase amino antipyrine phenol method 170(Randox Daytona, Randox, Crumlin, UK).

171Volume and osmolality were determined for each urine sample. Drink samples were analysed 172for osmolality, as well as sodium and potassium concentration by flame photometry (Corning 173Clinical Flame Photometer 410C; Corning Limited, Essex, UK).

174Phenol red concentrations in gastric aspirates and drink samples were analyzed by 175spectrophotometry after dilution (1:20) with NaOH-NaHCO₃ (200:500 mmol·l⁻¹) buffer.

176Statistical Analysis

177Data was analyzed using SPSS 18.0 (Chicago, IL, USA). All data were checked for normality 178of distribution using a Shapiro-Wilk test. Data containing two factors were then analyzed 179using two-way repeated measures ANOVA. Significant differences were located using 180Bonferroni adjusted paired t-tests for normally distributed data or Bonferroni-adjusted 181Wilcoxon signed-ranked tests for non-normally distributed data. Data containing one factor 182were analyzed using paired t-tests or Wilcoxon signed-ranks tests as appropriate. Differences 183were considered significant at $P \leq 0.05$. Normally distributed data are presented as means 184(SD), while non-normally distributed data are presented as median (range).

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186Results

187There was no difference in any of the measured pre-exercise variables (Table 2a) or any of 188the variables measured during exercise (Table 2b) or rehydration (Table 2c).

189Gastric volumes

190Residual gastric volume was 30 (8-117) ml and was not different between trials (P=0.607). 191Total gastric volume (Fig. 1a) was greater during the 10% trial than during the 2% trial from 19215 min after ingestion of the initial bolus of test drink until 120 min (P<0.001). The volume 1930f drink remaining in the stomach (Fig. 1b) followed the same pattern, and the volume of 194drink present in the stomach at the end of the gastric sampling period was 7 (5) ml (2% trial) 195and 893 (175) ml (10% trial) (P<0.001), representing 0.3 (0.2)% (2% trial) and 42.1 (6.6)% 196(10% trial) of the total drink volume ingested. The mean volume of gastric secretions present 197in the stomach at each time point was greater after ingestion of 10% drink (79 (36) ml) 198compared to the 2% drink (40 (5) ml) (P=0.005).

199Urine output, osmolality, drink retention and net fluid balance

200Urine output (Fig. 2) showed main effects of trial (P=0.001), time (P<0.001) and trial x time 201interaction (P=0.005). Urine output was greater at 60 min (P=0.046) and 120 min (P=0.005) 202on the 2% trial compared to the 10% trial. Total urine output after drinking was greater

203during the 2% trial (1025 (219) ml) compared to the 10% trial (746 (210) ml) (*P*=0.003), 204therefore a greater amount of the 10% drink was retained (64 (11)% vs. 52 (10)%) (*P*<0.001).

205The dehydration phase of the experiment increased urine osmolality (Table 3a) compared to 206pre-exercise during both trials (P<0.001). Urine osmolality was greater during the 10% trial 207than during the 2% trial 60 min after exercise (P=0.015). Additionally, urine osmolality at 208120 min was lower than pre-exercise during the 2% trial (P=0.002) and tended to be lower 209during the 10% trial (P=0.054).

210Whole body net fluid balance (Fig. 3) was significantly negative on both trials (P<0.001) at 211the end of exercise (0 min) and had become significantly positive on both trials at the end of 212rehydration (60 min) (P<0.001). From the end of rehydration, net fluid balance declined 213during both trials, but was only significantly negative compared with pre-exercise during the 2142% trial from 180 min onwards (P<0.003). Whole body net fluid balance was also greater 215during the 10% trial compared to the 2% trial from 120 min onwards (P<0.003).

216Corrected net fluid balance (Fig. 3, dashed lines) was calculated to account for volumes of 217fluid that remained in the stomach and was significantly more negative at 60 and 120 min 218during the 10% trial compared to the 2% trial (P<0.001). Corrected net fluid balance was 219significantly lower than pre-exercise at 0 min during the 2% trial (P<0.005), and at 0, 60 and 220120 min during the 10% trial (P<0.01).

221Plasma volume and serum osmolality

222Exercise induced a similar reduction in plasma volume (Table 3b) between trials (P=0.385). 223Plasma volume was greater during the 2% trial than during the 10% trial at 60 and 120 min 224(P<0.01). Compared to pre-exercise, plasma volume was reduced at 0 min during the 2% trial 225(P=0.001) and at 0 and 60 min during the 10% trial (P<0.01) and was greater than pre226exercise from 60 to 120 min (P<0.05) during the 2% trial. The estimated change in blood 227volume mirrored those of plasma volume, whilst there was no change in red cell volume 228(P>0.05).

229Serum osmolality (Table 3c) was greater during the 10% trial than during the 2% trial at 60 230and 120 min post-exercise (P<0.01). Additionally, serum osmolality was greater than pre-231exercise at 0 min during the 2% trial (P=0.017) and at 60 min during the 10% trial (P=0.001), 232and was lower than pre-exercise at 120, 180 and 240 min during the 2% trial (P<0.01).

233Plasma glucose concentration

234Pre-exercise plasma glucose concentration (Table 3d) was not different between trials 235(P=0.412), but was greater during the 10% trial compared to the 2% trial at 120 and 180 min 236(P<0.05). Compared to pre-exercise, plasma glucose concentration at 60 min was increased 237during the 2% trial (P=0.016) and tended to be increased during the 10% trial (P=0.064) and 238at 180 min was decreased during the 2% trial (P=0.030).

239Subjective feelings questionnaires

240There was a main effect of trial for stomach fullness (P < 0.001) (Fig. 4a), bloatedness 241(P < 0.001) (Fig. 4b) and hunger (P=0.002) (Fig. 4c), with these subjective feelings greater 242during the 10% trial compared to during the 2% trial from 60 min onwards (P < 0.01). There 243were significant main effects of time for subjective feelings of thirst (P < 0.001), stomach 244fullness (P < 0.001), bloatedness (P < 0.001), hunger (P < 0.001) and dryness of mouth 245(P < 0.001). At the end of the rehydration period, the 10% glucose drink was perceived to be 246more sweet (P=0.002), and tended to be perceived as more bitter (P=0.058) than the 2% 247glucose drink, but there was no difference in the perceived saltiness (P=0.409) or 248pleasantness (P=0.147) of the drinks.

250Discussion

251The results of this investigation demonstrate that following exercise-induced dehydration, a 252hypertonic 10% glucose-electrolyte drink emptied from the stomach at a slower rate, led to a 253slower restoration of plasma volume and over the duration of this study resulted in a reduced 254urine output compared to a hypotonic 2% glucose-electrolyte drink, when a volume of 150% 255of BML was ingested.

256Post-exercise nutritional requirements are often multifactorial in nature and following 257dehydrating endurance exercise, rehydration as well as resynthesis of glycogen stores is 258likely to be required. For maximal resynthesis of muscle glycogen a carbohydrate ingestion 259rate of ~1.2 g·kg⁻¹·h⁻¹ is required (Betts & Williams, 2010). When rehydration is also 260required, carbohydrate based drinks provide carbohydrate for muscle glycogen resynthesis, as 261well as water for rehydration. Evans, Maughan, and Shirreffs (2009b) reported that following 262cycling exercise in the heat, similar amounts of a 2% glucose drink (2539 (436) ml) and 10% 263glucose drink (2173 (252) ml) were ingested over a 2 h rehydration period. In their subjects, 264this equated to carbohydrate ingestion rates of ~0.3 g·kg⁻¹·h⁻¹ and 1.4 g·kg⁻¹·h⁻¹ with the 2% 265and 10% glucose drinks, respectively (Evans et al., 2009b). This suggests that hypertonic 266carbohydrate drinks might help facilitate post-exercise muscle glycogen resynthesis and 267rehydration in situations where both are required and food is not consumed.

268It is generally accepted that in order to achieve complete rehydration after exercise, it is 269necessary to consume a volume of fluid in excess of that lost to account for on-going water 270losses through sweat and urine production (Mitchell, Grandjean, Pizza, Starling, & Holtz, 2711991; Shirreffs et al., 1996). As long as a sufficient volume of a rehydration drink is 272consumed, the rate of ingestion (Jones, Bishop, Green, & Richardson, 2010) and the 273composition (Shirreffs & Maughan, 1998; Seifert et al., 2006; Merson et al., 2008; Evans et 274al., 2009a; Osterberg et al., 2010; James et al., 2011) of the drink determine how well the 275drink is retained. The addition of electrolytes, specifically sodium, has been shown to 276increase the fraction of the ingested drink that is retained (Shirreffs & Maughan, 1998), and 277more recent investigations have shown that increasing the carbohydrate content of a 278rehydration drink resulted in an increased drink retention (Osterberg et al., 2010). The results 279of the present study are in line with these findings, as despite consuming a similar volume of 280rehydration drink on each trial, total urine output was 278 (175) ml greater during the 2% 281trial compared to the 10% trial, and at the end of the study, whole body net fluid balance was 282more negative during the 2% trial. These studies have investigated the effects of mild 283hypohydration (~2% body mass loss) at level commonly reported in an applied setting 284(Sawka et al., 2007), but it remains to be seen whether these effects remain at higher levels of 285hypohydration. Furthermore, the difference in fluid balance between the trials of the present 286study (~0.3% body mass) and that of other rehydration studies (<1% body mass) is unlikely 287to have any effect on exercise performance (Sawka et al., 2007). At present post-exercise 288rehydration has only been investigated in response to a one off acute bout of dehydrating 289exercise and it remains to be seen whether training athletes might accrue significant levels of 290hypohydration over multiple training sessions sufficient to impair performance.

291The reduced total urine output observed during the 10% trial is likely due to acute changes in 292circulating arginine vasopressin (AVP) concentration. Plasma osmolality has been shown to 293have a profound effect on concentrations of AVP, with linear regression analysis showing 294AVP concentrations change by 0.41 pmol·l⁻¹ per 1 mosmol·kg⁻¹ change in plasma osmolality 295(Bayliss, 1987). Serum osmolality was lower during the 2% trial than during the 10% trial at 29660 and 120 min, and consequently urine output at these time points was greater during the 2% 297trial. This suggests that the greater total urine output during the 2% trial, was likely due to

298lower concentrations of circulating AVP, particularly during the first 2 h after ingestion of the 299initial bolus of test drink.

300Changes in blood and plasma volume can also have an influence on drink retention, and the 301carbohydrate concentration of rehydration drinks has been observed to affect plasma volume 302response (Evans et al., 2009a; Evans et al., 2011). Evans, Shirreffs, and Maughan (2009c) 303demonstrated that ingestion of a hypertonic drink causes a reduction in plasma volume. 304whereas ingestion of a hypotonic drink results in the expansion of plasma volume. Previous 305 investigations have demonstrated that hypotonic drinks are quickly absorbed in the small 306intestine, resulting in a rapid appearance in the extracellular fluid (Hunt, Elliott, Fairclough, 307Clark, & Farthing, 1992; Shi et al., 1994), whereas hypertonic drinks cause a transient 308secretion of water into the intestinal lumen to establish an osmotic gradient suitable for fluid 309uptake from the intestine (Leiper & Maughan, 1986). The present study supports these 310findings, as compared to pre-exercise, plasma volume immediately after rehydration was 311reduced during the 10% trial (-5.9 (2.8)%), but was increased during the 2% trial (+6.7 312(2.7)%). This suggests that during the 10% trial, water may have moved into the intestinal 313 lumen to reduce the osmolality of the drink, temporarily delaying the restoration of plasma 314volume. This likely resulted in an increased serum osmolality, which would be expected to 315result in an increase in AVP concentration and an attenuation of the diuretic response. In 316contrast, the hypotonic drink appeared rapidly in the extracellular fluid, reduced serum 317osmolality and stimulated a greater diuretic response.

318Whilst the data for whole body net fluid balance suggest that restoration of fluid balance was 319enhanced following ingestion the 10% glucose-electrolyte drink compared to a 2% glucose-320electrolyte drink, the determination of corrected net fluid balance which takes into account 321the volume of fluid in the stomach shows that this is not the case, at least for the 2 hours after 322exercise (Fig. 3). The finding that 42.1 (6.6) % of the 10% drink remained in the stomach 120 323min after exercise suggests that some drink was likely to still be present in the stomach and/ 324or intestines at 180 and 240 min and that whole body net fluid balance is likely over 325estimated at these time points in the 10% trial. Although gastric volume is a powerful 326 regulator of gastric emptying, the rate of gastric emptying of hypertonic solutions appears to 327be relatively linear (Vist & Maughan, 1995; Maughan, Leiper, & Vist, 2004; Evans, 328Shirreffs, & Maughan, 2011), at least when gastric volume is greater than 400 ml. In the 329present study, gastric volume decreased in a linear manner between 60 and 120 min $330(r^2=0.997)$. Using the rate of gastric emptying of the hypertonic drink between 60 and 120 331min for each individual subject and assuming that gastric emptying remained linear, it would 332have taken an estimated 249 (54) min for the 10% drink to completely empty from the 333subjects' stomach. This is still likely to be an overestimate of how quickly the 10% drink 334 emptied from the stomach as the rate of gastric emptying of a solution is well known to 335decrease exponentially as gastric volume decreases (Vist & Maughan, 1994; Vist & 336Maughan, 1995). The data demonstrate that although a hypertonic 10% glucose-electrolyte 337drink reduced urine output after drinking compared to a hypotonic 2% glucose-electrolyte 338 solution, whether this infers enhanced fluid balance is questionable as a large proportion of 339drink remained in the gastrointestinal tract for several hours after drinking.

340The rate of gastric emptying and intestinal absorption of ingested drinks are essential for the 341maintenance of plasma volume and a prompt return to euhydration (Leiper, 1998). It is 342currently recommended that after exercise, a volume in excess of BML is consumed to ensure 343complete rehydration (Sawka et al., 2007). Evidence suggests that slightly hypotonic drinks 344are ideal in situations when swift replacement of fluid losses is desirable, as rapid gastric 345emptying and intestinal absorption mean that ingested fluid reaches the peripheral circulation 346quickly to restore plasma volume (Leiper & Maughan, 1986; Hunt et al., 1992). However, 347when large volumes of drink are consumed, substantial plasma volume expansion is likely, 348which results in an acute reduction in serum osmolality and consequently a reduction in AVP 349concentration, causing a large diuresis (Nose et al., 1988), unless a high concentration of 350sodium is present (Shirreffs & Maughan, 1998). In contrast, ingestion of large volumes of 351hypertonic drinks is likely to cause an efflux of water into the intestinal lumen, leading to a 352delayed restoration of plasma volume and the maintenance of serum osmolality (Evans et al., 3532009c), which might attenuate the decline in AVP concentration and reduce the observed 354diuresis. The results of the current study suggest that the short-term reduction in urine output 355observed when a hypertonic carbohydrate drink was ingested after exercise-induced 356dehydration might be mediated by a reduced rate of gastric emptying. Given that a proportion 357of a hypertonic solution will remain in the gastrointestinal tract for some time after drinking, 358this reduced urine output does not necessarily imply enhanced rehydration. Evans et al. 359(2011) have recently shown that following repeated ingestion of a 10% hypertonic glucose-360electrolyte drink after an overnight fast, gastric emptying and fluid uptake were slower than 361 following ingestion of a 2% hypotonic glucose-electrolyte drink. The results of the present 362 investigation are consistent with these findings, as the volume of test drink remaining in the 363stomach was greater during the 10% trial compared to the 2% trial from 15 min after 364ingestion until the end of the gastric sampling period, and this reduced the rate of fluid 365uptake.

366Rate of gastric emptying is an important consideration for rehydration drinks (Leiper, 1998). 367Results of the current study confirm that if the aim of rehydration is rapid fluid replacement, 368hypotonic drinks should be consumed, as they are quickly emptied from the stomach, leading 369to a rapid appearance in the extracellular fluid. However, this leads to an acute decrease in 370serum osmolality and stimulates diuresis (Nose et al., 1988). Although hypertonic glucose-371electrolyte drinks decrease total urine output after rehydration (Osterberg et al., 2010), the 372location of the fluid is an important consideration. Gastric emptying has been identified as the 373main reason for the reduced rate of fluid uptake (Evans et al., 2011), so therefore by 374monitoring the rate of gastric emptying in the current study an estimate was formulated as to 375how much of the drink remained in the stomach. Corrected net fluid balance (Fig. 3) was 376calculated to account for the volume of test drink remaining in the stomach at each sampling 377point. Whilst fluid remains in the stomach, it is not contributing to rehydration, so assessing 378net fluid balance by conventional methods may be impractical. Corrected net fluid balance 379 gives a much clearer perspective of how much of the ingested drink is available for 380restoration of body water loss. Although it must be noted that the volume of drink contained 381 within the intestines could not be determined during the present investigation. As shown in 382Figure 3, corrected net fluid balance did not become positive over the 120 min after the onset 383of drinking and given the slow rate of gastric emptying of the 10% drink, it is unlikely that 384corrected net fluid balance would have become positive at any time point during the 10% 385trial. In contrast, during the 2% trial, corrected net fluid balance became positive immediately 386after rehydration (60 min). It should also be noted that ingestion of hypertonic drinks could 387lead to the secretion of water into the intestinal lumen, delaying plasma volume restoration, 388which may not be considered ideal in situations where rapid restoration of plasma volume 389and rehydration is the main objective. Furthermore, ingestion of the 10% drink resulted in 390greater subjective feelings of bloating, stomach fullness and reduced feeling of hunger. In a 391 practical setting, where *ad libitum* drink ingestion will take place, this may lead to the 392cessation of drinking before a positive net fluid balance has been attained, although a recent 393study found no differences in *ad libitum* drink ingestion between 0, 2 and 10% glucose-394electrolyte drinks (Evans et al., 2009c).

395In conclusion, compared to the ingestion of a hypotonic 2% glucose-electrolyte drink, 396ingestion of a hypertonic 10% glucose-electrolyte drink after exercise-induced dehydration 397resulted in a slower rate of gastric emptying and a reduction in total urine output after 398drinking. It appears that the delayed rate of gastric emptying of the hypertonic glucose-399electrolyte drink might explain the increased drink retention observed in this and previous 400experiments. The delayed rate of gastric emptying of the 10% glucose-electrolyte drink 401augmented a slower recovery of plasma volume, consequently attenuating the decline in 402serum osmolality and reducing urine output in the 2 h after the onset of drink ingestion. 403However, whilst ingestion of a large volume of a hypertonic 10% glucose-electrolyte drink 404resulted in an acute reduction in urine output compared to a hypotonic 2% glucose-electrolyte 405drink, the location of the fluid retained needs to be given careful consideration when 406determining net fluid balance. The slower rate of gastric emptying of hypertonic drinks 407means ingestion of such drinks in situations where recovery periods are short (2-3 h) might 408not be appropriate.

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420References

421Baylis, P.H. (1987). Osmoregulation and control of vasopressin secretion in healthy humans. 422*American Journal of Physiology 253*, R671-R678.

423Beckers, E.J., Rehrer, N.J., Brouns, F., Ten Hoor F. & Saris, W.H. (1988). Determination of 424total gastric volume, gastric secretion and residual meal using the double sampling technique 425of George. *Gut 29*, 1725-1729.

426Calbet, J.A., & MacLean, D.A. (1997). Role of caloric content on gastric emptying in 427humans. *Journal of Physiology 498*, 553-559.

428Dill, D., & Costill, D.L. (1974). Calculation of percentage changes in volumes of blood, 429plasma, and red cells in dehydration. *Journal of Applied Physiology 37*, 247-248.

430Evans, G.H., Shirreffs, S.M., & Maughan, R.J. (2009a). Postexercise rehydration in man: the 431effects of osmolality and carbohydrate content of ingested drinks. *Nutrition 25*, 905-913.

432Evans, G.H., Shirreffs, S.M., & Maughan, R.J. (2009b). Postexercise rehydration in man: the 433effects of carbohydrate content and osmolality of drinks ingested ad libitum. *Applied* 434*Physiology Nutrition and Metababolism* 34, 785-793.

435Evans, G.H., Shirreffs, S.M., & Maughan, R.J. (2009c). Acute effects of ingesting glucose 436drinks on blood and plasma volume. *British Journal of Nutrition 101*, 1503-1508.

437Evans, G.H., Shirreffs, S.M., & Maughan, R.J. (2011). The effects of repeated ingestion of 438high and low glucose–electrolyte drinks on gastric emptying and blood 2H2O concentration 439after an overnight fast. *British Journal of Nutrition 106, 1732-1739*.

440George, J.D. (1968). New clinical method for measuring the rate of gastric emptying: the 441double sampling test meal. *Gut 9*, 237-242.

442Hunt, J.B., Elliott, E.J., Fairclough, P.D., Clark, M.L, & Farthing, M.J. (1992). Water and 443solute absorption from hypotonic glucose-electrolyte drinks in human jejunum. *Gut 33*, 479-444483.

445James, L.J., Clayton, D., & Evans, G.H. (2011). Effect of milk protein addition to a 446carbohydrate-electrolyte rehydration drink ingested after exercise in the heat. *British Journal* 447*of Nutrition 105*, 393-399.

448Jones, E.J., Bishop, P.A., Green, J.M., & Richardson, M.T. (2010). Effects of metered versus 449bolus water consumption on urine production and rehydration. *International Journal of Sport* 450*Nutrition and Exercise Metabolism 20*, 139-144.

451Judelson, D.A., Maresh, C.M., Farrell, M.J., Yamamoto, L.M., Armstrong, L.E., Kraemer, 452W.J., ... & Anderson, J.M. (2007). Effect of hydration state on strength, power, and 453resistance exercise performance. *Medicine and Science in Sports and Exercise* 39, 1817-4541824.

455Leiper, J.B. (1998). Intestinal water absorption-implications for the formulation of 456rehydration drinks. *International Journal of Sports Medicine 19*, S129-S132.

457Leiper, J.B., & Maughan, R.J. (1986). Absorption of water and electrolytes from hypotonic, 458isotonic and hypertonic drinks. *Journal of Physiology 373*(Suppl), 90P.

459Maughan, R.J., Shirreffs, S.M., & Leiper, J.B. (2007). Errors in the estimation of hydration 460status from changes in body mass. *Journal of Sports Sciences* 25, 797-804.

461Maughan, R.J., Leiper, J.B., & Vist, G.E. (2004). Gastric emptying and fluid availability after 462ingestion of glucose and soy protein hydrolysate drinks in man. *Experimental Physiology 89*, 463101-108.

464Merson, S.J., Maughan, R.J., & Shirreffs, S.M. (2008). Rehydration with drinks differing in 465sodium concentration and recovery from moderate exercise-induced hypohydration in man. 466*European Journal of Applied Physiology 103*, 585-594.

467Mitchell, J.B., Grandjean, P.W., Pizza, F.X., Starling, R.D., & Holtz, R.W. (1994). The effect 468of volume ingested on rehydration and gastric emptying following exercise-induced 469dehydration. *Medicine and Science in Sports and Exercise 26*, 1135-1143.

470Mudumbo, K.S., Leese, G.P., & Rennie, M.J. (1997). Gastric emptying in soldiers during and 471after field exercise in the heat measured with the [13C]acetate breath test method. *European* 472*Journal of Applied Physiology and Occupational Physiology* 75, 109-114.

473Nose, H., Mack, G.W., Shi, X.R., & Nadel, E.R. (1988). Shift in body fluid compartments 474after dehydration in humans. *Journal of Applied Physiology* 65, 318-324.

475Osterberg, K.L., Pallardy, S.E., Johnson, R.J., & Horswill, C.A. (2010). Carbohydrate exerts 476a mild influence on fluid retention following exercise-induced dehydration. *Journal of* 477*Applied Physiology 108*, 245-250.

478Sawka, M.N., Burke, L.M., Eichner, E.R., Maughan, R.J., Montain, S.J., & Stachenfeld, N.S.
479(2007). American College of Sports Medicine position stand. Exercise and fluid replacement.
480Medicine and Science in Sports and Exercise 39, 377-390.

481Seifert, J., Harmon, J., & DeClercq, P. (2006). Protein added to a sports drink improves fluid 482retention. *International Journal of Sport Nutrition and Exercise Metabolism 16*, 420-429. 483Shi, X., Summers, R.W., Schedl, H.P., Chang, R.T., Lambert, G.P, & Gisolfi, C.V. (1994).
484Effects of drink osmolality on absorption of select fluid replacement drinks in human
485duodenojejunum. *Journal of Applied Physiology* 77, 1178-1184.

486Shirreffs, S.M., & Maughan, R.J. (1998). Volume repletion after exercise-induced volume 487depletion in humans: replacement of water and sodium losses. *American Journal of* 488*Physiology 274*, F868-F875.

489Shirreffs, S.M., Taylor, A.J., Leiper, J.B., & Maughan, R.J. (1996). Post-exercise rehydration 490in man: effects of volume consumed and drink sodium content. *Medicine and Science in* 491*Sports and Exercise 28*, 1260-1271.

492Vist, G.E., & Maughan, R.J. (1995). The effect of osmolality and carbohydrate content on the 493rate of gastric emptying of liquids in man. *Journal of Physiology* (London) *486*, 523-531.

494Vist, G.E., & Maughan, R.J. (1994). Gastric emptying of ingested drinks in man: effect of 495beverage glucose concentration. *Medicine and Science in Sports and Exercise 26*, 1269-1273.

Table 1. Composition of test drinks. Values are means (SD).

	2% drink	10% drink	
Energy (kJ·l ⁻¹) Osmolality (mosmol·kg ⁻¹) Carbohydrate (g·l ⁻¹) Sodium (mmol·l ⁻¹)	400 193 (2) 20 29 (0)	1760 656 (3) 100 29 (0)	
Potassium (mmol·l ⁻¹)	0.4 (0.1)	0.4 (0.1)	
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Table 2. Pre-exercise variables (a), exercise variables (b) and rehydration variables (c). 518Values are means (SD).

	2% Trial	10% Trial	P value
a) Pre-trial variables			
Body mass (kg)	79.5 (9.3)	79.4 (8.9)	0.808
Serum osmolality (mosmol·kg ⁻¹)	290 (4)	290 (4)	0.931
Urine osmolality (mosmol·kg ⁻¹)	377 (175)	414 (216)	0.248
b) Exercise variables			
Body mass loss (%)	1.8 (0.1)	1.8 (0.1)	0.280
Workload (W)	142 (18)	142 (16)	0.871
Exercise time (min)	57 (6)	57 (7)	0.624
Heat exposure (min)	87 (11)	89 (10)	0.625
c) Rehydration variables			
Drink volume (ml)	2129 (171)	2108 (188)	0.332
Drink temperature (°C)	20 (1)	20 (1)	0.600

Table 3. Urine osmolality (mosmol·kg⁻¹) (a), change in plasma volume relative to pre-526exercise (%) (b), serum osmolality (mosmol·kg⁻¹) (c) and blood glucose (mmol·l⁻¹) (d) at each 527hour after exercise. Values are means (SD).

		Time after exercise (min)					
	Pre-exercise	0	60	120	180	240	
a) Urine Osmolality (mosmol·kg ⁻¹)							
2%	377 (175)	770 (115) ‡	383 (236) *	81 (50) ‡	315 (219)	634 (242)	
10%	414 (216)	770 (93) †	554 (237)	192 (226)	297 (239)	436 (305)	
b) Change in plasma volume (%)							
2%	0 (0)	-3.8 (2.2) ‡	6.7 (2.7) ‡ *	4.2 (2.0) ‡ *	2.5 (3.0)	2.5 (2.1)	
10%	0 (0)	-4.3 (2.0) †	-5.9 (2.8) †	-1.9 (4.3)	-1.3 (4.7)	3.3 (3.8)	

c) Serum Osmolality (mosmol·kg⁻¹)

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10%	4.9 (0.3)	4.9 (0.6)	8.4 (3.1)	6.3 (2.1)	5.7 (1.2)	4.4 (0.8)
2%	5.1 (0.7)	4.8 (0.3)	7.7 (1.5) ‡	4.1 (0.4) *	4.1 (0.3) ‡ *	4.3 (0.2)
d) Bloo	d Glucose (mr	$\operatorname{mol} \cdot 1^{-1}$)				
10%	290 (4)	292 (4)	296 (4) †	292 (4)	287 (3)	286 (3)
2%	290 (4)	294 (4) ‡	287 (5) *	285 (4) ‡ *	284 (5) ‡	286 (3) ‡

528* mean values were different between trials (P < 0.05). $\ddagger 2\%$ trial different to pre-exercise 529(P < 0.05). $\ddagger 10\%$ trial different to pre-exercise (P < 0.05).

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532Figure legends

533**Fig. 1.** Total volume of fluid in the stomach (ml) (a) and the total volume of drink in the 534stomach (ml) (b) during the 2% (\circ) and 10% (\blacktriangle) trials. * mean values were different 535between trials (*P*<0.05). Points are mean values with vertical error bars representing standard 536deviations.

537**Fig. 2.** Urine output (ml) at each hour after exercise during the 2% (\circ) and 10% (\blacktriangle) trials. * 538mean values were different between trials (*P*<0.05). ‡ 2% trial different to 0 min (*P*<0.05). † 53910% trial different to 0 min (*P*<0.05). Points are mean values with vertical error bars 540representing standard deviations.

541Fig. 3. Net fluid balance (ml) during the 2% (\circ) and 10% (\blacktriangle) trials, as well as when 542corrected for volume of drink in the stomach during the 2% (\bullet) and 10% (\triangle) trials. * mean 543values for net fluid balance different between trials (P<0.05). ‡ 2% trial different to pre-544exercise for net fluid balance (P<0.05). † 10% trial different to pre-exercise for net fluid 545balance (P<0.05). # mean values for corrected net fluid balance different between trials 546(P<0.05). § 10% trial for corrected net fluid balance different to pre-exercise (P<0.05). Points 547are mean values with vertical error bars representing standard deviations.

Fig. 4. Subjective feelings (mm) of stomach fullness (a), bloating (b) and hunger (c) during 549the 2% (\circ) and 10% (\blacktriangle) trials. * mean values different between trials (*P*<0.05). ‡ 2% trial 550different to pre-exercise (*P*<0.05). ‡ 10% trial different to pre-exercise (*P*<0.05). Points are 551median values.

555Figure 1.







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584Figure 3.



Time after exercise (min)

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