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Maughan, RJ, Watson, P, Cordery, PA, Walsh, NP, Oliver, SJ, Dolci, A, Rodriguez-Sanchez, N and Galloway, SD (2015) A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. American Journal of Clinical Nutrition. 103

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This article was accepted in its current form to American Journal of Clinical Nutrition on 24th November 2015. First published online December 23rd

2015.doi:10.3945/ajcn.115.114769

A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index.

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PubMed indexing; Maughan
Watson
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Source(s) of support: This study was funded by a grant from the European Hydration Institute (EHI)

Running head: Development of a beverage hydration index

Clinical Trial Registration: ISRCTN13014105, DOI 10.1186/ISRCTN13014105

Abbreviations:

BHI – beverage hydration index

ORS – oral rehydration solution
ICC – intra-class correlation coefficient
ANOVA – analysis of variance
Na – sodium
K – potassium
AVP – arginine vasopressin

1 **Abstract**

2 Background: Identification of beverages that promote longer term fluid retention and
3 maintenance of fluid balance is of real clinical and practical benefit in situations
4 where free access to fluids is limited, or when frequent breaks for urination are not
5 desirable. The post-ingestion diuretic response is likely to be influenced by several
6 beverage characteristics including the volume ingested, energy density, electrolyte
7 content, and the presence of diuretic agents. Objective: This study investigated the
8 effects of 13 different commonly-consumed drinks on urine output and fluid balance
9 when ingested in a euhydrated state, with a view to establishing a beverage
10 hydration index (BHI; i.e. volume of urine produced after drinking expressed relative
11 to a standard treatment [still water]) for each beverage. Design: Each subject (n=72,
12 euhydrated and fasted males) ingested 1 L of still water or one of three other
13 commercially-available beverages over a period of 30 minutes. Urine output was then
14 collected for the subsequent 4 h. The BHI was corrected for water content of drinks
15 and was calculated as the amount of water retained at 2 h after ingestion, relative to
16 that observed following ingestion of still water. Results: Total urine masses (mean
17 (SD)) over 4 h were smaller than the still water control (1337(330) g) after oral
18 rehydration solution (ORS, 1038(333) g, $P<0.001$), full-fat milk (1052(267) g,
19 $P<0.001$) and skimmed milk (1049(334) g, $P<0.001$). Cumulative urine output at 4 h
20 after ingestion of cola, diet cola, tea, cold tea, coffee, lager, orange juice, sparkling
21 water and a sports drink were not different from the response to water ingestion. The
22 mean BHI at 2 h was 1.54(0.74) for ORS, 1.50(0.58) for full-fat milk, and 1.58(0.60)
23 for skimmed milk. Conclusions: BHI may be a useful measure to identify the short-
24 term hydration potential of different beverages when ingested in a euhydrated state.

25 **Keywords:** fluid balance, dehydration, rehydration

26 **Introduction**

27 Water intake is episodic, while losses are continuous. Under normal free-living
28 conditions, homeostatic mechanisms mean that body water balance fluctuates over
29 the course of a normal day, but generally returns to the same point over a 24-hour
30 cycle (1). Consequently large fluid deficits are uncommon for the majority of the
31 population, but knowledge of beverages that can maintain hydration status over a
32 longer period may be of interest to those who wish to stay hydrated in situations
33 where free access to fluid is limited or when frequent breaks for urination are not
34 desirable (2, 3, 4, 5). While several studies have examined the effectiveness of
35 beverages for post-exercise rehydration (6), the protocols employed do not represent
36 a common situation for the majority of the population. Thus identification of
37 beverages that promote longer term fluid retention, and maintenance of fluid balance
38 for prolonged periods, under euhydrated conditions would be of real clinical and
39 practical benefit.

40

41 An adequate daily water intake is defined in the US by the Institute of Medicine (7) at
42 3.7 L for men and 3.0 L for women, and in Europe by the European Food Safety
43 Authority (8) as 2.5 L for men and 2.0 L for women. The distribution of fluids over the
44 course of the day and their composition may, however, also be important in
45 determining how well an individual is able to maintain an adequate hydration status.
46 The volume and composition of ingested drinks has a strong influence on the rates at
47 which they empty from the stomach and are absorbed in the small intestine, thus
48 affecting their entry into the body water pool (9). Beverage components are also
49 metabolised and excreted on different time scales (9). These various factors are
50 likely to result in different hydration status profiles in the first few hours after ingestion

51 of different beverages. It should therefore be possible to assign a Beverage
52 Hydration Index (BHI) to each drink that will define the hydration response to any
53 particular drink, in much the same way as the glycemic index defines the blood
54 glucose response to ingestion of foods (10). In the case of a BHI, the cumulative
55 volume of urine passed over a fixed period of time is in effect the area under the
56 curve for renal water excretion. The urine volume passed relative to a standard
57 treatment (still water) can therefore be calculated as the BHI of a beverage.

58
59 Therefore, the aim of the present study was to assess fluid balance responses to the
60 ingestion of a fixed volume of commonly-consumed beverages ingested when in a
61 euhydrated state, with a view to establishing the feasibility of a BHI. We hypothesized
62 that drinks containing a high electrolyte content or high energy content would have
63 greater fluid retention and thus a higher BHI compared to plain water. Conversely,
64 drinks containing nutrients with known diuretic actions, such as alcohol and caffeine,
65 may have lower BHI values.

66

67

68 **Methods**

69 *General Study Design*

70 Three separate laboratories (Loughborough, Bangor and Stirling) collaborated to test
71 72 recreationally active, healthy males. Ethics approval for the study was obtained
72 separately from the Ethics Committees of the three Institutions involved.

73

74 A randomization table was generated based on each participant undertaking a
75 maximum of four experimental trials, which included water plus three other test drinks

76 administered in a randomized fashion, and was based upon each experimental site
77 assessing all available test drinks (www.randomization.com). Rehydration study data
78 (11, 12) informed the sample size estimates and indicated a minimum sample size
79 for each test drink of $n=12$. Although not a cluster randomized trial we factored in an
80 additional sample size weighting to account for possible increased variance due to
81 data collection across three different sites. The final sample size estimate based on
82 80% power with mean total urine output of 900 ml, pooled SD of 300 ml, and a mean
83 difference detectable of 220 ml at an alpha level of 0.05 required a total of $n=15$
84 observations per drink. We therefore aimed to recruit $n=30$ at each site and allowing
85 for loss to follow-up this ensured completion of $n=24$ at each site, giving $n=17$
86 observations on any given test drink.

87

88 *Pre-Trial Standardization/Exclusion criteria*

89 At each site; 24 healthy, physically active men between 18 and 35 years old were
90 recruited. For the total sample of $n=72$ the mean (SD) characteristics were: age 24
91 (4) y; height 178 (6) cm; body mass 77.3 (9.9) kg; water intake 2.0 (0.8) L/d: **Table**
92 **1**). Those with a history of cardiovascular, renal, musculo-skeletal or metabolic
93 diseases, as determined from a pre-participation health screen questionnaire, were
94 excluded. As body mass was used as an index of euhydration, those currently
95 undertaking an energy-restricted diet and/or exercise plan were also excluded.
96 Participants were asked to record their diet including their fluid intake (household
97 measures technique) as well as any exercise performed, in a diary over the 2 days
98 before the first trial and asked to replicate this before their subsequent visits.
99 Participants were also asked not to perform any strenuous exercise or consume
100 alcoholic beverages in the 24 h preceding all trials.

101 *Experimental Procedures*

102 Following an overnight fast, of at least 8 h, participants emptied their bladder upon
103 waking; retaining an aliquot in a sterile collection tube. One hour before arriving at
104 the laboratory, volunteers were instructed to consume 500 ml of still water (Highland
105 Spring™, Perthshire, UK) over the course of 15 minutes. Upon arrival in the
106 laboratory, volunteers remained seated in a comfortable environment for 10 minutes.
107 A single 5 ml blood sample was collected via venipuncture from an antecubital vein
108 and blood was dispensed into a serum tube. Participants were then asked to void
109 their bowels and bladder before measurement of near-nude body mass (underwear
110 only) to the nearest 50 g behind a screen. Approximately 30 minutes after arrival at
111 the laboratory participants then ingested 1 L of the assigned test drink over a period
112 of 30 minutes (4 equal volumes administered 7.5 min apart). A fixed volume, rather
113 than a volume relative to body mass, was chosen as most drinks are served and
114 ingested in containers of a standard volume. Participants were asked to empty their
115 bladder at the end of the drinking period and again at the end of each hour of the
116 study period. If a participant requested to pass urine before the hour was complete,
117 this was collected and then added to any further urine produced at the end of the
118 corresponding hour. After the final urine sample was collected, near-nude body mass
119 was recorded once again.

120

121 *Drinks and drink preparation*

122 Each participant consumed still water (Highland Spring™, Perthshire, UK) and three
123 of the following drinks in a randomized, counter-balanced order: sparkling water
124 (Highland Spring™, Perthshire, UK), cola (Coca-Cola®, Uxbridge, UK), diet cola
125 (Diet Coke®, Uxbridge, UK), sports drink (Powerade®, Coca-Cola®, Uxbridge, UK),

126 oral rehydration solution (ORS: Dioralyte™, Sanofi. One, Surrey, UK), orange juice
127 (Tesco Everyday Value, Hertfordshire, UK), Lager beer (Carling®, Staffordshire, UK),
128 hot black coffee (Nescafe® Original, York, UK), hot black tea (PG tips®, Unilever,
129 London, UK), cold black tea (PG tips®, Unilever, London, UK), full fat milk (3.6% fat;
130 Tesco, Hertfordshire, UK) or skimmed milk (0.1% fat; Tesco, Hertfordshire, UK). The
131 nutrient composition of the test drinks is presented in **Table 2**.

132

133 All cold drinks were stored at standard refrigerated temperature (4-6°C) until serving.
134 Tea, coffee and ORS were prepared according to manufacturer's instructions, being
135 prepared with still water (Highland Spring™ still water). Hot black coffee and black
136 tea were brewed with freshly boiled still water (Highland Spring™ still water) and
137 served at 60°C, with the temperature being maintained in a hot water bath. Cold
138 black tea was brewed in the same manner, then stored and served at 4-6°C. The
139 ORS was prepared and stored, and also served at 4-6°C. A 5 ml sample of each
140 drink preparation was aliquoted into plain tubes. All drinks were tested for osmolality,
141 sodium (Na) and potassium (K) after preparation within 48 h and 5 days after
142 collection, respectively.

143

144 *Urine and Serum Analysis*

145 All urine collected during the study was passed into a 1 L plastic container. The
146 volume of each urine pass was determined by measuring the mass on an electronic
147 balance (to the nearest 0.1 g), with the mass of the empty plastic container
148 subtracted to enable the estimation of urine volume. From each urine sample a 5 ml
149 aliquot was dispensed into a plain screw-capped tube. This was stored at 4°C for the
150 analysis of urine osmolality, sodium (Na) and potassium (K) concentrations. Urine

151 and serum osmolality was measured in duplicate using freezing-point depression
152 method (either Gonotec Osmomat, Berlin, Germany or Advanced Instruments, MA,
153 USA) within 48 h of collection. Urine Na/K concentrations were measured in duplicate
154 using flame photometry (Corning Flame Photometer, Cambridge, UK) within 5 days
155 of collection. Collection, handling and storage of urine and serum were in accordance
156 with the Human Tissues Act. Stored samples were discarded once satisfied analysis
157 was completed.

158

159 Whole blood in the serum tube was allowed to stand for 1 h at room temperature to
160 clot before centrifugation (10 min, 4°C, 2000-3000 g). Serum was then dispensed
161 into an appropriate storage tube (e.g. eppendorf) and stored at 4°C for measurement
162 of osmolality.

163

164 To help ensure consistency in the data analyzed across sites, seven independently
165 prepared quality control solutions were also analyzed in replicates of ten by each
166 research group. These contained undisclosed concentrations of Na/K and a
167 measured osmolality. Two-way random effects intra class correlation coefficient
168 analysis (ICC) suggested good agreement between the different institutions for
169 osmolality, Na/K analysis where ICC were all 0.999 or greater. In addition, Bland-
170 Altman limits of agreement analysis indicated that bias between any two institutions
171 was less than 2% for osmolality, less than 1% for Na and less than 2% for K.

172

173 *Data and statistical analysis*

174 Participant characteristics, pre-trial participant preparation and urine responses to the
175 still water trial from each institution were initially compared by an ordinary one-way

176 analysis of variance (ANOVA). To confirm that hydration status was similar before
177 each trial, serum and urine osmolality were compared between drinks by repeated
178 measures ANOVA.

179

180 The main outcome measure was cumulative urine mass after ingestion of each drink.
181 This was also expressed as a BHI for each beverage by dividing each individual's
182 cumulative urine mass after still water with cumulative urine mass for each other test
183 drink consumed. Individual hour cumulative urine mass and BHI of each drink was
184 compared by paired t-test to determine which drinks differed from still water.

185

186 To assess the practical meaning of the BHI differences observed between still water
187 and each of the test drinks, the difference was compared to the normal variation
188 determined from a separate repeatability analysis. For this purpose twelve
189 participants ingested the same drink on two occasions. The drinks used for this
190 repeatability analysis were the same as those used in the present study. The
191 repeatability of the BHI was equal to a coefficient of variation of 18% (~180 ml). In
192 addition, the meaningfulness of group differences was also calculated using Cohen's
193 *d* effect size (13) and 95%CI of differences between means.

194

195 Even though a fixed volume of each of the test drinks was consumed, the presence
196 of other components in some of these drinks means the water content of drinks
197 varied from 88% to 100% (Table 2). It might therefore be argued that the BHI should
198 be corrected for the differences in water intake. If, however, the aim was to estimate
199 the effects of the different drinks on body water content, then the uncorrected values
200 would be more appropriate. For clarity the data have been expressed both ways.

201 All other secondary outcome measures (net fluid balance, BHI corrected for water
202 content, cumulative urine electrolyte loss) were analyzed by paired t test.

203

204 All statistical analyses were completed using a computerized statistical software
205 package (GraphPad Prism version 6 for Windows, GraphPad Software, La Jolla
206 California USA). Statistical significance was accepted at $P < 0.05$. Data are
207 presented as mean (SD).

208

209

210 **Results**

211 The study was conducted between February and August 2014. The study was
212 completed when the target number of participants (n=72) had finished the study,
213 providing n=17 observations on each test drink in total across the three sites, with
214 n=72 observations on water. In total n=86 participants were recruited, pre-
215 participation screening excluded n=1 participant, and n=85 were randomized. Loss
216 to follow-up occurred due to vomiting following ingestion of the tea (n=6) and ORS
217 (n=1), or voluntary withdrawal from the study due to external factors (n=6).

218

219 *Institutional comparison of pre-trial standardization and urine output response to a*
220 *standard drink*

221 Before ingestion of drinks on the still water trial body mass, serum osmolality and
222 urine osmolality were not different suggesting that participants' preparation before
223 trials was similar at each institution (**Table 3**). We also confirmed that cumulative
224 urine mass after the still water drink trial was similar at each institution, which further
225 suggests that the participants in the three institutions had similar fluid regulation

226 **(Table 3)**. It was therefore deemed reasonable to combine the data from the three
227 institutions for the main study.

228

229 *Pre-drink ingestion hydration status*

230 Serum osmolality (293(6) mmol/kg, $P = 0.88$) and urine osmolality (582(265)
231 mmol/kg, $P = 0.56$) was similar immediately before drinks were ingested on each
232 trial.

233

234 *Urine output and fluid balance*

235 Urine mass did not differ between trials immediately after the ingestion of the drinks
236 ($P > 0.19$). One hour after the ingestion of the drinks cumulative urine mass was
237 lower, and net fluid balance was higher, than the still water drink after the ingestion of
238 full fat milk ($P < 0.01$), skimmed milk ($P < 0.01$), ORS ($P < 0.01$, **Figure 1**). Two and
239 three hours after drink ingestion cumulative urine mass was lower, and net fluid
240 balance was higher, than the still water drink after the ingestion of full fat milk ($P <$
241 0.01), skimmed milk ($P < 0.01$), ORS ($P < 0.01$) and orange juice ($P < 0.05$). Four
242 hours after drinks were ingested, cumulative urine mass was lower, and net fluid
243 balance was higher, for full fat milk ($P < 0.01$), skimmed milk ($P < 0.01$), and ORS (P
244 < 0.01), but not orange juice ($P = 0.06$). The effect sizes at 4 h for cumulative urine
245 output in comparison to still water were 1.04 for full fat milk, 0.85 for skimmed milk,
246 and 1.09 for ORS (all large effects) with an effect size of 0.65 for orange juice (a
247 medium effect). The mean differences (95%CI) in cumulative urine output were 294 g
248 (154 to 434) for full fat milk, 339 g (190 to 489) for skim milk, and 362 g (222 to 505)
249 for ORS.

250

251 *Beverage Hydration Index*

252 After 2 h full fat milk, skimmed milk, ORS and orange juice had a higher BHI than still
253 water (All differences $P < 0.05$, **Figure 2**). The effect sizes at 2 h were 1.22 for full fat
254 milk, 1.37 for skim milk, 1.03 for ORS, and 0.87 for orange juice (all large to very
255 large effects). The higher BHI between still water and full fat milk, skimmed milk,
256 ORS and orange juice also exceeded twice the CV of the BHI measure. Mean
257 differences (95%CI) for 2 h BHI values were 0.50 (0.20 to 0.80) for full fat milk, 0.58
258 (0.28 to 0.89) for skimmed milk, 0.54 (0.16 to 0.93) for ORS and 0.39 (0.05 to 0.73)
259 for orange juice. Additionally, full fat milk, skimmed milk, ORS and orange juice
260 beverage hydration indexes were greater than still water at 3 and 4 h after drink
261 consumption ($P < 0.05$).

262

263 *Beverage Hydration Index corrected for water content*

264 The water content of the drinks used in this study varied from 100% to 88% (Table
265 2), and consequently the amount of water ingested varied between drinks. It might be
266 appropriate therefore to recalculate the BHI to take account of the different volumes
267 of water ingested on the different trials. The BHI values presented in **Figure 3** have
268 been normalized by the drinks' water content to reflect the effect of the drink itself on
269 hydration status excluding the differences in water content. As was the case without
270 the correction for drink water content, the corrected BHI for full fat milk ($P = 0.02$),
271 skimmed milk ($P < 0.01$) and ORS ($P = 0.01$) were higher than that for still water. The
272 effect sizes for corrected BHI data at 2 h were 0.89 for full fat milk, 1.14 for skimmed
273 milk, and 0.98 for ORS (all large effects). The mean differences (95%CI) for
274 corrected 2 h BHI were 0.32 (0.06 to 0.58) for full fat milk, 0.44 (0.16 to 0.72) for
275 skimmed milk, and 0.50 (0.13 to 0.87) for ORS. The BHI for orange juice was,

276 however, no longer different than still water ($P = 0.11$) with an effect size of 0.60 (a
277 medium effect) and a mean difference (95%CI) of 0.24 (-0.06 to 0.54).

278

279 *Urinary electrolyte excretion and balance*

280 Several drinks had greater Na or K balances than still water 2 h after drinks were
281 consumed (**Figure 4**). Drinks with positive Na or K balances were typically those with
282 the highest BHI. That is, ORS had a positive Na balance (**Figure 4A**), whilst orange
283 juice, full fat and skimmed milk had positive K balances (**Figure 4B**).

284

285

286 **Discussion**

287 Adequate hydration status may be associated with a decreased risk of a range of
288 adverse outcomes, including urological, gastrointestinal, circulatory, and neurological
289 disorders (14, 15). In addition, maintenance of euhydration is important for the
290 preservation of physical and mental function (4, 5, 15). Consequently, identification of
291 beverages that promote longer term fluid retention, and maintenance of fluid balance
292 for prolonged periods, would be of real clinical and practical benefit in situations
293 where free access to fluids is limited, or when frequent breaks for urination are not
294 desirable (2, 3, 4, 5). In this study we propose a novel tool to enable the objective
295 assessment of a beverage's effectiveness to maintain hydration status over a period
296 of time post-ingestion. The calculated beverage hydration index revealed that drinks
297 containing the highest macronutrient and electrolyte contents were the most effective
298 at maintaining fluid balance.

299

300 The differences noted in the urine volume and calculated BHI during the monitoring
301 period might be attributed in part to differences in the water content of the different
302 drinks. Stahl et al. (17) recognized that the amount of water present in a fixed volume
303 of beverage varies due to the presence of other nutrients, meaning the amount of
304 water available to influence hydration status can markedly differ; an observation
305 these authors termed the 'post-absorptive hydration index'. The water content of the
306 the test beverages in the present study ranged from 100% for still water to 88% for
307 full fat milk. Correction of the urine output to account for differences in the volume of
308 water ingested made little difference to the relative BHI responses (Figures 2 & 3),
309 suggesting that such a correction may not be required when considering drinks with
310 characteristics similar to those used in the present study.

311
312 In addition to variations in the water content of a beverage, the present BHI model
313 recognizes that the presence of additional nutrients in a beverage will also
314 significantly influence the retention of fluid, meaning that beverages with similar water
315 contents may display markedly different effects on long term hydration status. There
316 are several elements of a beverage that might affect fluid balance in the hours
317 following ingestion: the macronutrient content, the electrolyte (primarily Na and K)
318 content, and the presence of diuretic agents (primarily caffeine and alcohol). Ingested
319 drinks with a high-energy content, whether in the form of carbohydrate, fat, protein or
320 alcohol will empty from the stomach more slowly than energy-free drinks and will thus
321 potentially reduce or delay the diuresis that follows compared with the ingestion of a
322 bolus of still water (11, 18). This effect has the potential to contribute to the retention
323 of ingested fluids within the body water space. The drinks in the present study with
324 the highest energy density were full-fat milk 640 kcal/L; orange juice 470 kcal/L; lager

325 330 kcal/L; cola 420 kcal/L; skimmed milk 350 kcal/L. A high-energy content was
326 generally associated with a high BHI, but a comparison of the responses to cola,
327 lager and orange juice suggest that other factors also play a significant role (e.g.
328 electrolytes, alcohol).

329

330 In the present study, no water or salt deficit was induced before the beginning of the
331 study. Acute administration of a bolus of water plus sodium chloride or other sodium
332 salts results in a transient increase in total body water: this hyperhydration is
333 prolonged relative to that observed after the intake of still water (19). In the present
334 study, the ORS and milk drinks contained relatively high concentrations of Na and K,
335 the orange juice contained a moderate amount of K, while the remaining drinks
336 contained relatively trivial concentrations of these electrolytes. It is notable that the
337 drinks with the highest electrolyte content tended to have the highest BHI.

338

339 The known diuretic effects of caffeine and alcohol, because of their action in
340 inhibiting the release of arginine vasopressin (AVP; 20, 21), would influence the
341 response to ingested drinks that contain caffeine or alcohol. An acute dose of less
342 than 250-300 mg of caffeine is unlikely to have a measurable effect on urine output,
343 though such an effect is likely to be seen when the dose exceeds about 300 mg (22).
344 In line with these observations, we did not observe an impact of moderate caffeine
345 intake (96-212mg) on net fluid balance in the present study. Furthermore, the alcohol
346 content of the lager did not increase diuresis over other drinks, but the alcohol may
347 have countered the hypothesized positive influence of energy density on the BHI.
348 Perhaps surprisingly, only one study has examined fluid balance responses to
349 alcohol in a euhydrated state (23). These authors reported a 12% greater diuresis

350 following the ingestion of 1L of lager beer containing 4% alcohol, compared to the
351 ingestion of the same volume of a non-alcoholic control beer.

352

353 The beverage hydration index values presented here are based on the net fluid
354 balance at 2 h after the end of the drink ingestion period. This time point was chosen
355 for 4 reasons. Firstly, this was the time at which drinks began to show differences.
356 Secondly, the majority (82%) of urine output over the 4 hour period had been passed
357 by this point. Thirdly, in a typical day, most people would expect not to have an
358 interval longer than 2 h between drinks, and any subsequent food or fluid ingestion
359 would over-ride the effects of the initial drink. Fourthly, for the drinks used in the
360 present study it made little difference to the calculated BHI whether this was based
361 on the first 2 h or on the whole 4 h collection period.

362

363 Although the results of the present study relate only to the acute effects of a large
364 bolus of fluid over the subsequent four hours, there is evidence to support the
365 suggestion that the results may be extrapolated to a longer time scale. Grandjean et
366 al (24) had subjects consume water or water plus varying combinations of
367 beverages, including carbonated, caffeinated cola and coffee. They observed no
368 significant differences in the effect of various combinations of beverages on 24 h
369 hydration status. In addition, Tucker et al. (25) recently suggested that 24 h hydration
370 status was not different when subjects drank only water or a variety of drinks,
371 including water, cola and fruit juice, provided that an adequate total volume was
372 consumed.

373

374 In summary, the present study describes a novel tool to enable the objective

375 assessment of the effectiveness of beverages to maintain hydration status. The BHI
376 is reproducible and the pattern of response for a range of commonly-consumed
377 beverages is consistent with what is known about the effects of their constituents on
378 water balance. An appreciation of the BHI has relevance to individuals where long
379 term maintenance of fluid balance is important, such as professions where fluid
380 availability is limited (3, 4, 5), as well as in older (2) or incapacitated patients (15).
381 There is also a clear application to industry, where this tool could be employed to
382 label products to indicate the hydration potential of beverages. Due to the complexity
383 of the commercially-available beverages used in this study, it was not possible to
384 directly determine the relative influence of individual drink components on fluid
385 balance (e.g. electrolyte content, energy density). Future studies should apply this
386 model to further examine the significance of these nutrients in isolation, as well as to
387 assign BHI values to a wider range of commercially-available beverages.

388

389

390 **Acknowledgements:** Prof Maughan is Chair of the Scientific Advisory Board for the
391 European Hydration Institute. Dr Watson has received funding in the past 3 years
392 from the European Hydration Institute for other hydration related research. No other
393 authors declare a conflict of interest.

394

395

396 **Authors' contributions to manuscript:** RJM conceived the project, RJM, PW,
397 PAAC, NPW, SJO, NRS and SDRG developed the overall research plan. PW, NPW
398 and SDRG had study oversight. PAAC, AD and NRS conducted the research and
399 analyzed the samples. SJO and NPW performed the statistical analysis. RJM, PW,

400 NPW and SDRG wrote the paper with PAAC, SJO and NRS. RJM had primary
401 responsibility for the final content.

402

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Table 1. Participant physical characteristics and daily water intake at each of the three study sites and for combined data (All sites). Data are mean (SD).

	Bangor	Loughborough	Stirling	All sites	
	(n = 24)	(n = 24)	(n = 24)	(n = 72)	<i>P</i> - value
Age (y)	24 (4)	26 (3)	25 (5)	25 (4)	<i>P</i> = 0.05
Height (cm)	177 (7)	180 (6)	179 (7)	178 (6)	<i>P</i> = 0.48
Body mass (kg)	76.2 (12.3)	77.4 (7.3)	78.3 (9.8)	77.3 (9.9)	<i>P</i> = 0.77
BMI (kg/m ²)	24.2 (3.3)	24.0 (1.6)	24.5 (2.6)	24.2 (2.6)	<i>P</i> = 0.77
Water intake (L/d)	2.0 (0.9)	2.0 (0.6)	2.1 (0.8)	2.0 (0.8)	<i>P</i> = 0.75

NOTE: *P*-values shown were obtained from an ordinary one-way analysis of variance (ANOVA).

Table 2. Drink composition. Drink water, energy and macronutrient content

(carbohydrate (CHO), fat and protein) was obtained from drink labels, whereas osmolality, sodium (Na), potassium (K) and caffeine content were determined by in-house analysis. ORS; oral rehydration solution.

Drink	Water Content (%)	Energy (kcal/L)	CHO (g/100 ml)	Fat (g/100 ml)	Protein (g/100 ml)	Osmolality (mmol/kg)	Na (mmol/L)	K (mmol/L)	Caffeine (mg/L)
Still water	100	0	0	0	0	2	0	0	0
Sparkling water	100	0	0	0	0	7	1	0	0
Cola	89	420	10.6	0	0	432	2	0	96
Diet Cola	100	4	0	0	0	23	2	0	127
Sports drink	96	160	3.9	0	0	297	21	4	0
ORS	97	80	1.8	0.1	0	229	55	20	0
Orange juice	89	470	10.5	0.1	0.5	570	1	33	0
Lager	94	330	2.2	0	0.4	774	1	6	0
Coffee	99	4	0.1	0	0	34	1	7	212
Tea	100	0	0	0	0	16	1	4	179
Cold tea	100	0	0	0	0	18	1	5	179
Full fat milk	88	640	4.7	3.6	3.2	286	18	41	0
Skimmed milk	91	350	5.0	0.1	3.4	282	19	40	0

Table 3. Institutional comparison of pre-trial standardization and urine output response to a standard drink. Data are mean (SD).

	Bangor	Loughborough	Stirling	<i>P</i> - value
	(n = 24)	(n = 24)	(n = 24)	
<i>Pre still-water ingestion</i>				
Body mass (kg)	76.1 (12.3)	76.7 (7.3)	78.2 (9.7)	<i>P</i> = 0.76
Serum Osmolality (mmol/kg)	293 (8)	291 (4)	295 (3)	<i>P</i> = 0.14
Urine Osmolality (mmol/kg)	564 (243)	607 (302)	538 (176)	<i>P</i> = 0.62
<i>Post still-water ingestion</i>				
Urine mass (g)	1341 (360)	1337 (352)	1333 (288)	<i>P</i> = 0.99

NOTE: *P*-values shown were obtained from a one-way repeated measures analysis of variance (ANOVA). No differences were observed between institutions for body mass, serum osmolality or urine osmolality immediately before still water ingestion or four-hour cumulative urine mass after 1 L still water ingestion, suggesting at each institution that participants' preparation for trials was similar and that participants in the three institutions had similar fluid regulation.

Figure legends

Figure 1 Cumulative urine mass (A) and net fluid balance (B) after ingestion of 1 L of various commonly-consumed and commercially available drinks (n=17 observations on each test drink, with exception to orange juice and diet cola (n=16), and tea (n=15). Drinks with different responses to still water were identified by paired t test analysis at each time point and highlighted in rectangular boxes with asterisks. * equals $P < 0.05$. The vertical error bar in top left corner represents the overall mean SD for all drinks during the 4-hour collection.

- Still water ◊ Sparkling water ▲ Cola ▼ Diet cola ◆ Sports drink
- Oral rehydration solution ▽ Orange juice ■ Lager
- ▲ Coffee ◇ Tea * Cold tea △ Full fat milk □ Skimmed milk

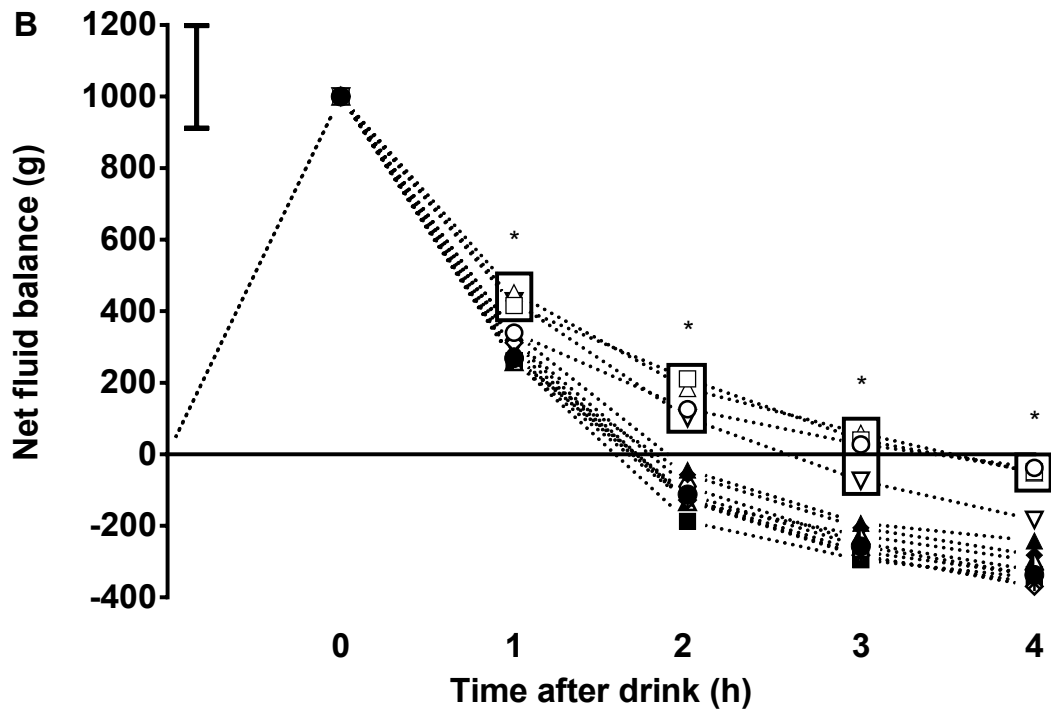
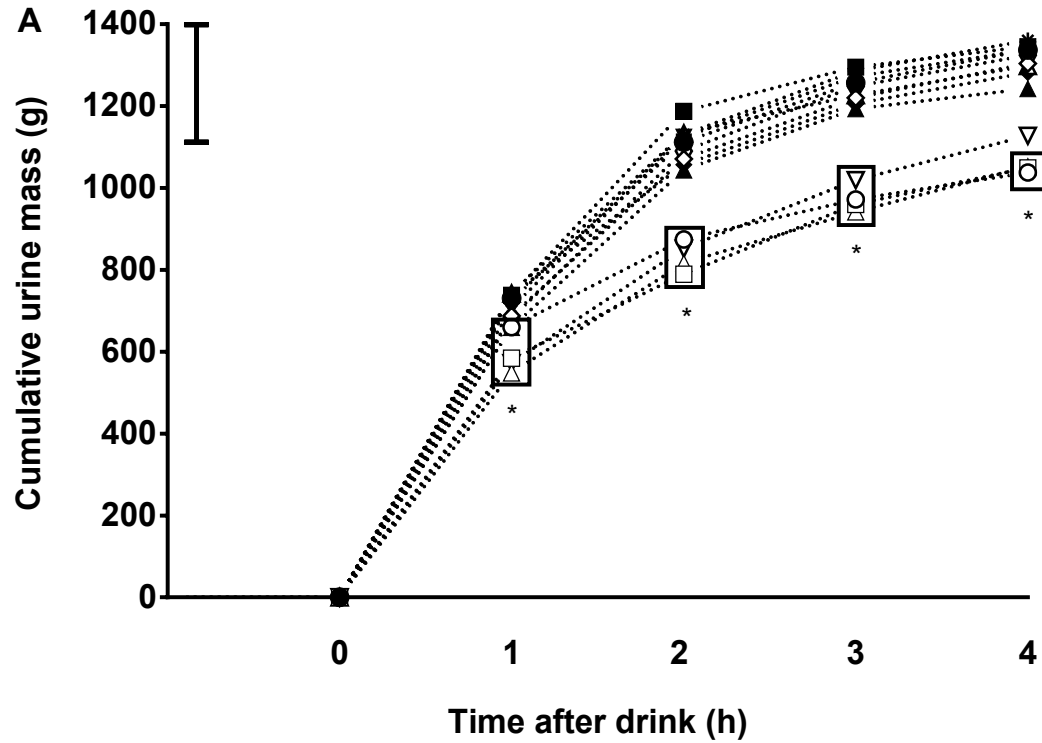


Figure 2 Beverage hydration index for 13 commonly-consumed and commercially available drinks. Drinks with different responses to still water were identified by paired t test analysis and highlighted by asterisks. * equals $P < 0.05$, ** equals $P < 0.01$. The dashed line represents twice the CV of the BHI measure. Values are mean (SD) of $n=17$ observations on each test drink, with exception to orange juice and diet cola ($n=16$), and tea ($n=15$).

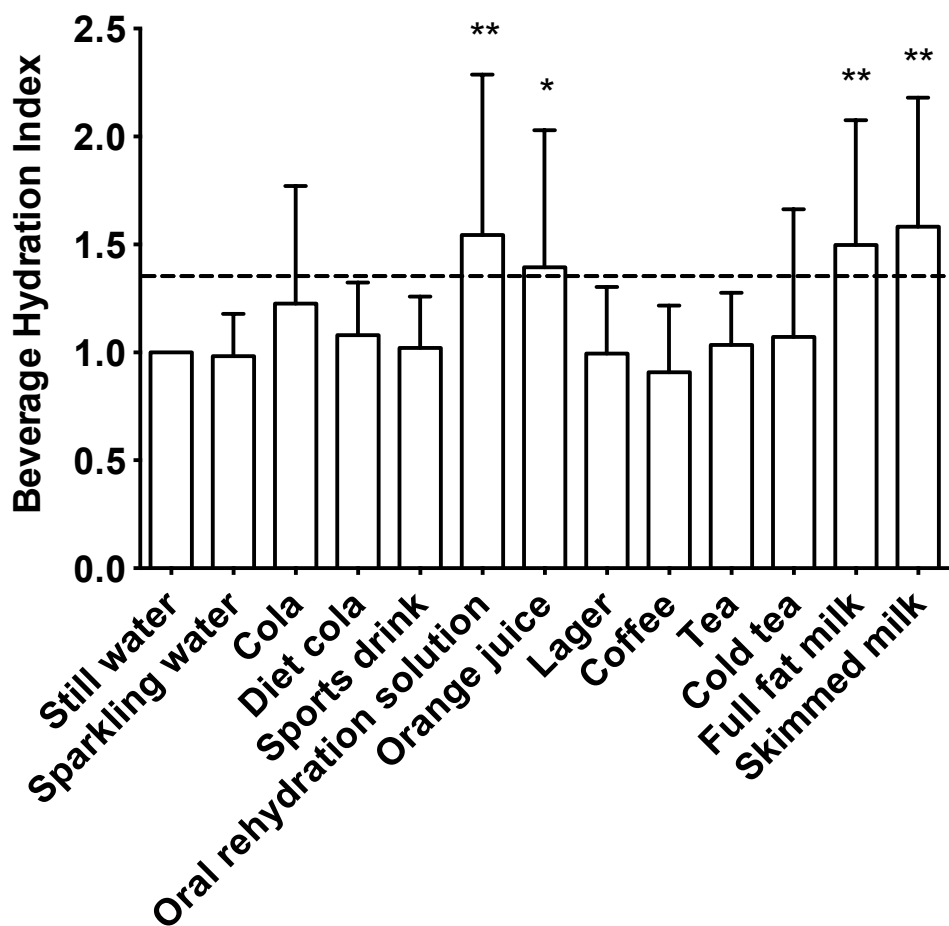


Figure 3 Beverage hydration index for 13 commonly-consumed and commercially available drinks after correction for water content of drink ingested. Drinks with different responses to still water were identified by paired t test analysis and highlighted by asterisks. * equals $P < 0.05$, ** equals $P < 0.01$. Values are mean (SD) of n=17 observations on each test drink, with exception to orange juice and diet cola (n=16), and tea (n=15).

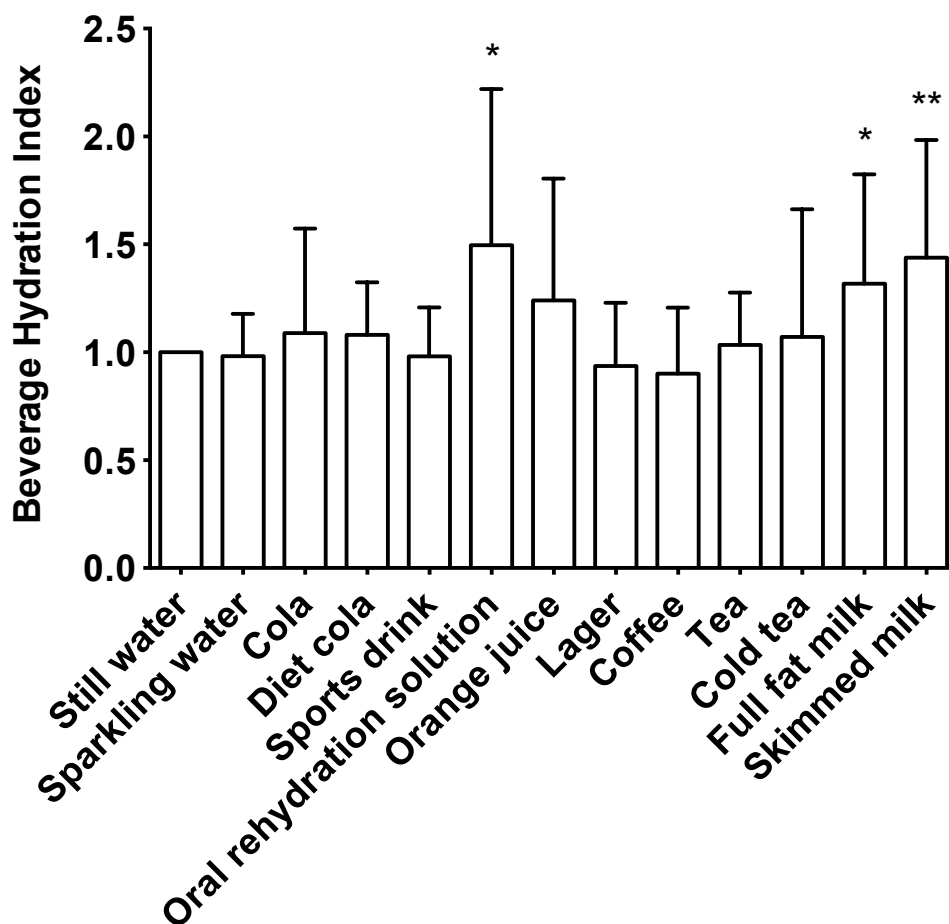


Figure 4 Sodium (A) and potassium (B) net balances 2 hours after ingestion of 1 L of various commonly-consumed and commercially available drinks. Drinks with different responses to still water were identified by paired t test analysis and highlighted by asterisks, * equals $P < 0.05$, ** equals $P < 0.01$. Values are mean (SD) of n=17 observations on each test drink, with exception to orange juice and diet cola (n=16), and tea (n=15).

