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Article

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

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Running head: Development of a beverage hydration index

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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

Abstract

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

Keywords: fluid balance, dehydration, rehydration

Introduction

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

An adequate daily water intake is defined in the US by the Institute of Medicine (7) at 3.7 L for men and 3.0 L for women, and in Europe by the European Food Safety Authority (8) as 2.5 L for men and 2.0 L for women. The distribution of fluids over the course of the day and their composition may, however, also be important in determining how well an individual is able to maintain an adequate hydration status. The volume and composition of ingested drinks has a strong influence on the rates at which they empty from the stomach and are absorbed in the small intestine, thus affecting their entry into the body water pool (9). Beverage components are also metabolised and excreted on different time scales (9). These various factors are likely to result in different hydration status profiles in the first few hours after ingestion of different beverages. It should therefore be possible to assign a Beverage Hydration Index (BHI) to each drink that will define the hydration response to any particular drink, in much the same way as the glycemic index defines the blood glucose response to ingestion of foods (10). In the case of a BHI, the cumulative volume of urine passed over a fixed period of time is in effect the area under the curve for renal water excretion. The urine volume passed relative to a standard treatment (still water) can therefore be calculated as the BHI of a beverage.

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

Methods

General Study Design

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

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

Pre-Trial Standardization/Exclusion criteria

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

Experimental Procedures

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

Drinks and drink preparation

Each participant consumed still water (Highland Spring™, Perthshire, UK) and three of the following drinks in a randomized, counter-balanced order: sparkling water (Highland Spring™, Perthshire, UK), cola (Coca-Cola®, Uxbridge, UK), diet cola (Diet Coke®, Uxbridge, UK), sports drink (Powerade®, Coca-Cola®, Uxbridge, UK), oral rehydration solution (ORS: Dioralyte™, Sanofi. One, Surrey, UK), orange juice (Tesco Everyday Value, Hertfordshire, UK), Lager beer (Carling®, Staffordshire, UK), hot black coffee (Nescafe® Original, York, UK), hot black tea (PG tips®, Unilever, London, UK), cold black tea (PG tips®, Unilever, London, UK), full fat milk (3.6% fat; Tesco, Hertfordshire, UK) or skimmed milk (0.1% fat; Tesco, Hertfordshire, UK). The nutrient composition of the test drinks is presented in **Table 2**.

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

Urine and Serum Analysis

All urine collected during the study was passed into a 1 L plastic container. The volume of each urine pass was determined by measuring the mass on an electronic balance (to the nearest 0.1 g), with the mass of the empty plastic container subtracted to enable the estimation of urine volume. From each urine sample a 5 ml aliquot was dispensed into a plain screw-capped tube. This was stored at 4˚C for the analysis of urine osmolality, sodium (Na) and potassium (K) concentrations. Urine and serum osmolality was measured in duplicate using freezing-point depression method (either Gonotec Osmomat, Berlin, Germany or Advanced Instruments, MA, USA) within 48 h of collection. Urine Na/K concentrations were measured in duplicate using flame photometry (Corning Flame Photometer, Cambridge, UK) within 5 days of collection. Collection, handling and storage of urine and serum were in accordance with the Human Tissues Act. Stored samples were discarded once satisfied analysis was completed.

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

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

Data and statistical analysis

Participant characteristics, pre-trial participant preparation and urine responses to the still water trial from each institution were initially compared by an ordinary one-way analysis of variance (ANOVA). To confirm that hydration status was similar before each trial, serum and urine osmolality were compared between drinks by repeated measures ANOVA.

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

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

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

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

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

Results

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

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

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

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

Pre-drink ingestion hydration status

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

Urine output and fluid balance

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

Beverage Hydration Index

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

Beverage Hydration Index corrected for water content

The water content of the drinks used in this study varied from 100% to 88% (Table 2), and consequently the amount of water ingested varied between drinks. It might be appropriate therefore to recalculate the BHI to take account of the different volumes of water ingested on the different trials. The BHI values presented in **Figure 3** have been normalized by the drinks' water content to reflect the effect of the drink itself on hydration status excluding the differences in water content. As was the case without the correction for drink water content, the corrected BHI for full fat milk ($P = 0.02$), skimmed milk ($P < 0.01$) and ORS ($P = 0.01$) were higher than that for still water. The effect sizes for corrected BHI data at 2 h were 0.89 for full fat milk, 1.14 for skimmed milk, and 0.98 for ORS (all large effects). The mean differences (95%CI) for corrected 2 h BHI were 0.32 (0.06 to 0.58) for full fat milk, 0.44 (0.16 to 0.72) for skimmed milk, and 0.50 (0.13 to 0.87) for ORS. The BHI for orange juice was, however, no longer different than still water (P = 0.11) with an effect size of 0.60 (a medium effect) and a mean difference (95%CI) of 0.24 (-0.06 to 0.54).

Urinary electrolyte excretion and balance

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

Discussion

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

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

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

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

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

The known diuretic effects of caffeine and alcohol, because of their action in inhibiting the release of arginine vasopressin (AVP; 20, 21), would influence the response to ingested drinks that contain caffeine or alcohol. An acute dose of less than 250-300 mg of caffeine is unlikely to have a measurable effect on urine output, though such an effect is likely to be seen when the dose exceeds about 300 mg (22). In line with these observations, we did not observe an impact of moderate caffeine intake (96-212mg) on net fluid balance in the present study. Furthermore, the alcohol content of the lager did not increase diuresis over other drinks, but the alcohol may have countered the hypothesized positive influence of energy density on the BHI. Perhaps surprisingly, only one study has examined fluid balance responses to alcohol in a euhydrated state (23). These authors reported a 12% greater diuresis following the ingestion of 1L of lager beer containing 4% alcohol, compared to the ingestion of the same volume of a non-alcholic control beer.

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

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

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

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Authors’ contributions to manuscript: RJM conceived the project, RJM, PW, PAAC, NPW, SJO, NRS and SDRG developed the overall research plan. PW, NPW and SDRG had study oversight. PAAC, AD and NRS conducted the research and analyzed the samples. SJO and NPW performed the statistical analysis. RJM, PW, NPW and SDRG wrote the paper with PAAC, SJO and NRS. RJM had primary responsibility for the final content.

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

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

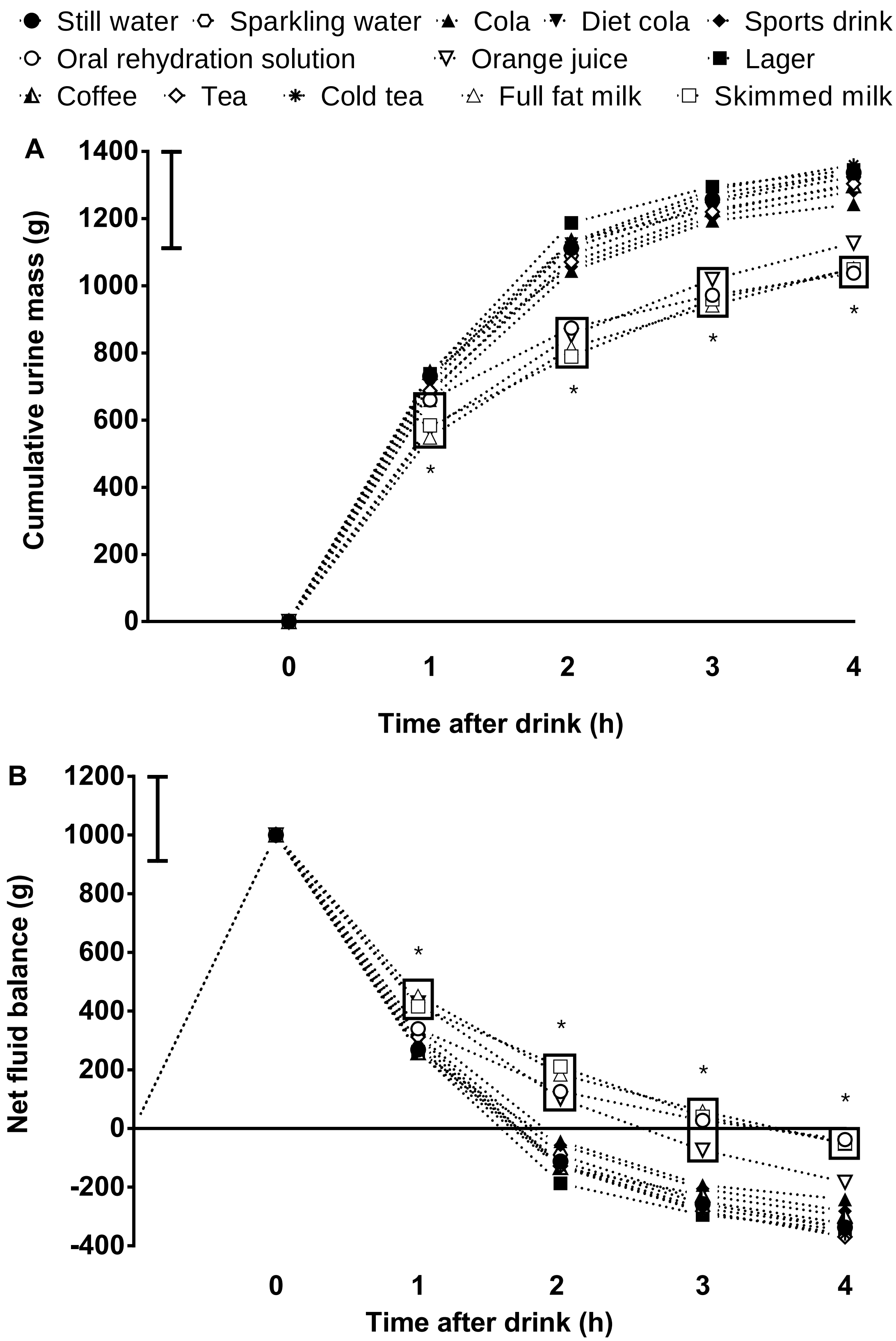
Table 3. Institutional comparison of pre-trial standardization and urine output response to a standard drink. Data are mean (SD).

	Bangor	Loughborough	Stirling	*P* - value
	(n = 24)	(n = 24)	(n = 24)	
	Pre still-water ingestion			
Body mass (kg)	76.1 (12.3)	76.7 (7.3)	78.2 (9.7)	*P* = 0.76
Serum Osmolality (mmol/kg)	293 (8)	291 (4)	295 (3)	*P* = 0.14
Urine Osmolality (mmol/kg)	564 (243)	607 (302)	538 (176)	*P* = 0.62
	Post still-water ingestion			
Urine mass (g)	1341 (360)	1337 (352)	1333 (288)	*P* = 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
A
1400
1200
1000
800
600
400
200
0
Cumulative urine mass (g)
*
0
1
2
3
4
Time after drink (h)
B
1200
1000
800
600
400
200
0
-200
-400
Net fluid balance (g)
*
0
1
2
3
4
Time after drink (h)

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).

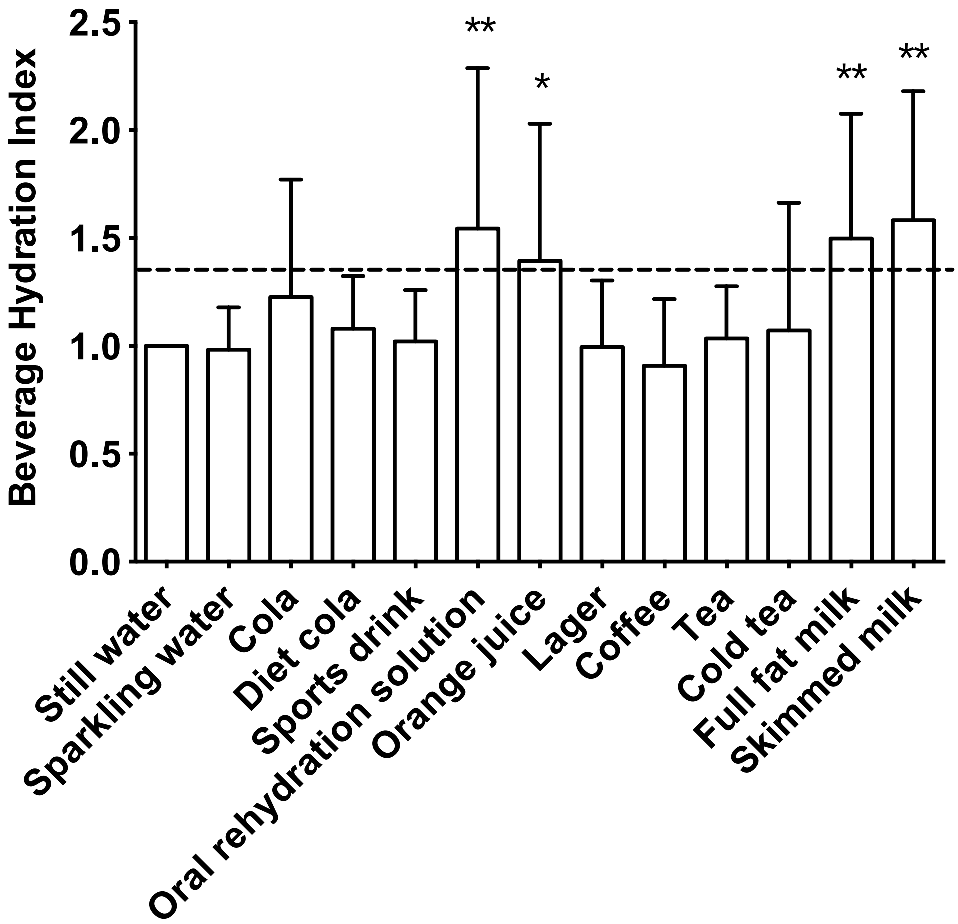


Figure 3 Beverage hydration index for 13 commonly-consumed and commercially available drinks after correction for water content of drink ingested. Drinks with different responses to still water were identified by paired t test analysis and highlighted by asterisks.* equals *P* < 0.05, ** equals *P* < 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).

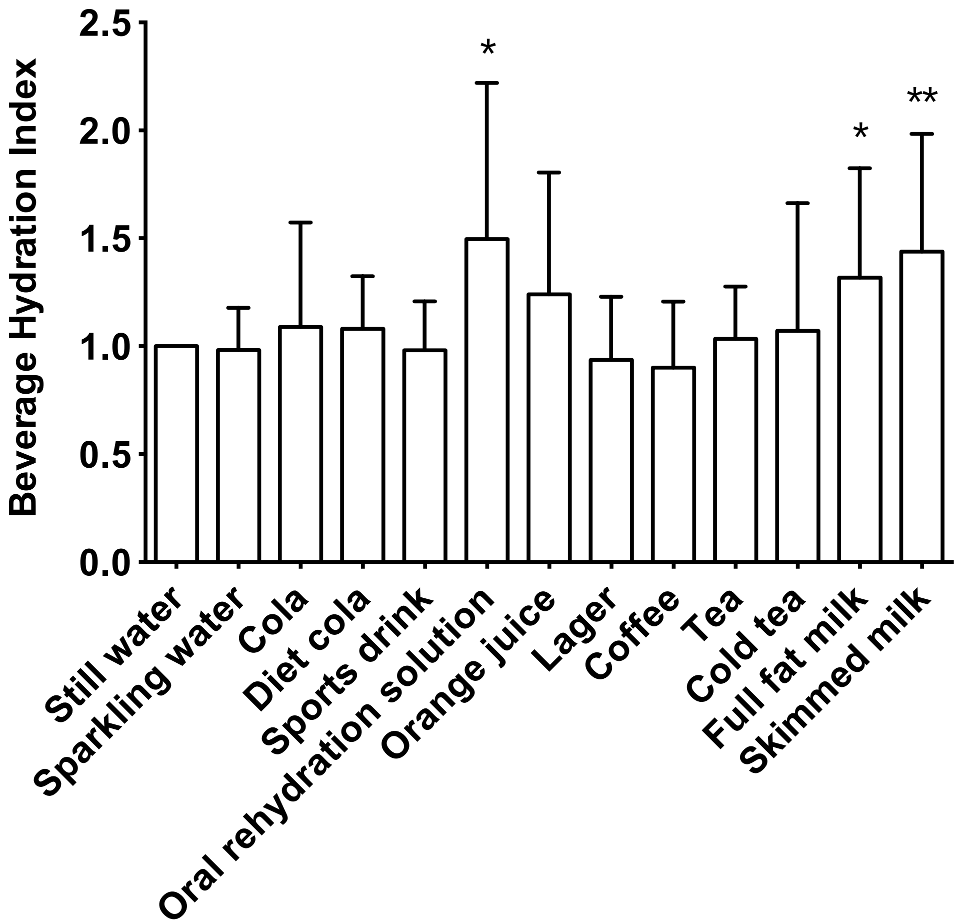


Figure 4 Sodium (A) and potassium (B) net balances 2 hours after ingestion of 1 L of various commonly-consumed and commercially available drinks. Drinks with different responses to still water were identified by paired t test analysis and highlighted by asterisks, * equals $P < 0.05$, ** equals $P < 0.01$. 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).