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Sucrose and sodium but not caffeine content influence the retention of beverages in humans under euhydrated conditions

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Abstract

This study systematically examined the influence of carbohydrate (sucrose), sodium and caffeine on the fluid retention potential of beverages under euhydrated conditions, using the beverage hydration index (BHI) method. Three cohorts, each of 12 young, healthy, active men, ingested 1L of beverages containing four different concentrations of a single component (sucrose, sodium or caffeine) in a double blind, crossover manner. Urine output was collected for the subsequent 4-h. Cumulative urine output was lower and net fluid balance were higher after 10% and 20% sucrose beverages than 0% and 5% sucrose beverages (P<0.05), and after 27mmol/L and 52mmol/L sodium beverages than 7mmol/L and 15mmol/L sodium beverages (P<0.05). No difference in urine output or net fluid balance was apparent following ingestion of caffeine at concentrations of 0 - 400 mg/l (P=0.83). Consequently, the calculated BHI was greater in beverages with higher sucrose or sodium content, but caffeine had no effect. No difference was observed in arginine vasopressin or aldosterone between any trials. These data highlight that the key drivers promoting differences in the fluid retention potential of beverages when euhydrated are energy density, likely through slowed fluid delivery to the circulation (carbohydrate content effect), or electrolyte content through improved fluid retention (sodium content effect). These data demonstrate that beverage carbohydrate and sodium content influence fluid delivery and retention in the 4-h after ingestion, but caffeine up to 400mg/L does not. Athletes and others can use this information to guide their daily hydration practices.

Keywords: carbohydrate, diuresis, electrolytes, gastric emptying

Introduction

Several factors are known to affect maintenance or restoration of fluid balance. The volume and composition of ingested fluids are obviously key in meeting daily water needs and in restoration of fluid balance following exercise (Shirreffs & Maughan, 2000). Although the impact of beverage composition on rehydration has been studied widely over the past 25 years, it has been focused around restoration of fluid balance following exercise heat stress-induced dehydration. Responses to fluid intake under euhydrated rested conditions have not been widely explored, though a Beverage Hydration Index (BHI) has recently been proposed to summarise such effects (Maughan et al., 2016) and recently it was demonstrated that body mass and sex do not influence the BHI (Sollanek et al., 2018).

Under resting euhydrated conditions, it appears that the carbohydrate, protein, and electrolyte content of ingested beverages are key to influencing subsequent urine production, and thus fluid retention (Maughan et al., 2016). Ingested fluids with a high-energy content (such as milk and fruit juice), as well as those with high electrolyte content (such as milk, fruit juice, and oral rehydration solution (ORS)) promote longer-term retention of the ingested volume (Maughan et al., 2016). These differences in fluid retention are likely due to mechanisms involving both fluid delivery to the circulation (Calbet & Holst, 2004; Mahe et al., 1992) and effect of electrolytes (particularly sodium) on expansion of blood volume and plasma osmolality (Heer et al., 2000). Energy content and osmolality of beverages are known to influence the rate of gastric emptying (Hunt & Stubbs, 1975; Vist & Maughan, 1994, 1995). In addition, glucose and electrolyte composition and osmolality affect intestinal water transport (Schedl et al., 1994; Gisolfi et al., 1992; Shi et al., 1995). Furthermore, the electrolyte content of drinks also affects the retention of fluid within the extracellular or intracellular fluid compartments (Leiper, 2015). Diuretic agents, such as caffeine and alcohol, have little influence on hydration status and fluid loss/retention if taken in small quantities (Armstrong et al., 2005; Maughan et al., 2016; Roti et al., 2006; Seal et al., 2017; Shirreffs & Maughan, 1997). These outcomes have potentially important implications for guidance to individuals/athletes around the ability to retain fluids for longer; particularly during periods when there may be limited access to beverages and when access to facilities for urination are restricted, e.g. when travelling.

To date, there have been no systematic evaluations of the effect of key beverage components on the retention of beverages during rested euhydrated conditions. For example, the dose of caffeine administered is likely to be key, as doses of caffeine up to 452mg may not induce a significant diuresis vs. matched volumes of water in habitual caffeine users (Armstrong et al., 2005; Killer et al., 2014; Maughan & Griffin, 2003). Recent evidence suggests that only high doses >500mg of caffeine may induce diuresis (Seal et al, 2017) but no systematic evaluation of caffeine dose on fluid balance has been conducted under standardized euhydrated conditions. Furthermore, one study has examined the influence of carbohydrate content of drinks (3% vs 6% carbohydrate) on fluid delivery / retention at rest without prior exercise in mildly dehydrated participants. Over a short follow-up period of only 1-h, no differences were noted for proportion of fluid volume retained between trials (Logan-Sprenger & Spriet, 2013). A recent investigation examined the hydration potential of an amino acid based ORS, a glucose containing ORS and a sports drink and it was demonstrated that the electrolyte content is the primary driver of the fluid retention potential of beverages (Sollanek et al., 2018) . These studies provide some insight but did not systematically examine dose-response effects of different beverage components.

Thus, to date there has been no systematic assessment of key components, such as carbohydrate, caffeine, and sodium content, on the ability to retain fluid of beverages under euhydrated conditions.

Therefore, the objective of the present study was to explore the dose-response effects of individual beverage components (sodium, sucrose and caffeine) on the hydration potential of beverages, expressed as the BHI, when ingested under standardized euhydrated conditions. By characterizing the effects of these individual components, we aimed to provide further insight into the factors that determine the BHI response. We hypothesized that increasing the content of sodium and sucrose would increase the ability to retain fluid of beverages expressed as the BHI, while graded caffeine doses within the range commonly ingested (up to 400 mg) would have little effect.

Methods

General Study Design

Three laboratories (Loughborough, Bangor and Stirling Universities) collaborated to complete this study. At each site, 12 healthy, weight-stable, active men aged 18-35 years were recruited (n=36 total, **Table 1, Figure 1A**). Participants with a history of cardiovascular, renal, musculoskeletal, or metabolic diseases, as determined from a pre-participation health screen questionnaire, were excluded. Using the experimental approach reported previously (Maughan et al., 2016), each site compared the effect of a control beverage and beverages containing three levels of a single component on post-ingestion fluid balance; Loughborough-caffeine, Stirling-sucrose, Bangor-sodium. Briefly, all urine passed over the 4-h post-ingestion period was collected and expressed as a fraction of that on the water trial. Participants recorded their diet including fluid intake (household measures technique; (Marr, 1971)) and any exercise performed in a diary, over the 2-days before the first trial and referred to this diary to replicate this diet/fluid intake and exercise before the three subsequent visits. Participants were asked not to perform any strenuous exercise or consume alcoholic beverages in the 24-h preceding trials. Compliance was verified verbally with the participants on arrival at the laboratory. Approval for the study was obtained from each of the local Ethics Committees, in accordance with the Declaration of Helsinki (2013). All participants provided written informed consent before participation.

Experimental Procedures

Following an overnight fast of ≥ 8-h, participants emptied their bladder upon waking and retained an aliquot. One hour before arriving at the laboratory, volunteers ingested 500ml of still water (Highland Spring™, Perthshire, UK) over the course of 15min. Upon arrival in the laboratory, volunteers remained seated for 20min. A 20G 1.25" cannula (Becton Dickinson Infusion Therapy Systems Inc., USA) was introduced into an antecubital vein and a blood sample was collected. Participants were then asked to void their bladder and bowels before measurement of body mass (underwear only) to the nearest 50g. Participants then steadily ingested 1L divided in 2 aliquots (every 15min) of the assigned test beverage over a period of 30min. At the end of the 30min drinking period, a blood sample was drawn and participants emptied their bladder. This procedure was repeated at hourly intervals, until 4-h post-ingestion. Volunteers remained seated during the drinking period and during the post-ingestion period. Participants stood up when they were asked to empty their bladder or if they needed to void before the collection time point. After the final urine sample was collected, near-nude body mass was recorded again. (**Figure 1B**)

Beverages

The control beverage at all sites consisted of still water (Highland Spring™, Perthshire, UK) with added sugar-free fruit-flavoured concentrate (Tesco Stores, UK). This same beverage, with the addition of three levels of a single beverage component, was administered in a randomized, counter-balanced and double-blind manner; Loughborough 50, 200 and 400mg per L of caffeine (BDH, Leicestershire, UK), Stirling 50, 100 and 200g per L of sucrose (British Sugar Ltd, UK), Bangor 15, 27 and 52mmol/L of Na, as sodium chloride (Glacia Fine 60, British Salt Ltd, UK). The control beverage contained 7mmol/L Na and 0.8 g/L of sugar (due to the addition of fruit squash) and was chosen instead of plain water to blind participants to the control trial. The osmolalities of the four beverages administered at Loughborough were 44 (control, 0mg caffeine/L), 43 (50mg caffeine/L), 44 (200mg caffeine/L) and 44mOsmol/kg (400mg caffeine/L), at Stirling were 46 (control, 0.8g/L sucrose), 205 (50g/L sucrose), 386 (100g/L sucrose) and 808mOsmol/kg (200g/L sucrose); and at Bangor were 33 (control, 7mmol/L Na), 54 (15mmol/L Na), 85 (27mmol/L Na) and 138mOsmol/kg (52mmol/L Na). Test beverages were stored at a standard refrigerated temperature (4-6 °C) until serving.

Urine and blood collection, storage and analysis

Collection, handling, and storage of urine and blood samples were undertaken in accordance with the Human Tissues Act. Stored samples were discarded once analysis was completed.

All urine collected during the study was passed into a 1L plastic container. The volume of each urine pass was determined by measuring the mass on an electronic balance, assuming a specific gravity of 1.00. From each urine pass, a 5ml aliquot was collected and stored at 4˚C. Urine osmolality was measured using freezing-point depression method (Gonotec Osmomat, Germany at Loughborough and Bangor and Roehbling, Camlab, UK at Stirling) within 48-h of collection.

11mL blood samples were drawn into dry syringes and immediately dispensed into a 5mL serum tube, and 1mL and 5mL EDTA tubes. At Stirling, duplicate 100 µL aliquots of whole blood were rapidly deproteinised in Eppendorf tubes containing 1 mL of ice-cold 0.3 N perchloric acid. These samples were centrifuged and the resulting supernatant used to determine blood glucose concentrations (Glucose oxidase method, Instrumentation Laboratory, Italy).

Whole blood in the serum tube was allowed to stand for 1-h at room temperature to clot before centrifugation (10min, 4°C, 2000-3000*g*). Serum was dispensed and stored at 4°C for measurement of osmolality by freezing-point depression and sodium by flame-photometry (Bangor). A further serum aliquot was stored at - 80°C for measurements of aldosterone and arginine vasopressin concentrations by enzyme-linked immunosorbent assay (Enzo Life Sciences, Lausen, Switzerland) and caffeine concentrations by HPLC (Loughborough; Holland et al., 1998)).

Beverage hydration index (BHI) calculation

The beverage hydration index (BHI) (Maughan, et al., 2016) was obtained by dividing the total urine output over a period of time for the control beverage by the total urine output for the same period of time after the test beverage was ingested.

$$BHI = \frac{Total\ urine\ output\ when\ control\ beverage\ ingested\ (L)}{Total\ urine\ output\ when\ test\ \ beverage\ ingested\ (L)}$$

Data and statistical analysis

Participant characteristics at each institution were compared by one-way ANOVA. Pre-drink hydration status, as assessed by body mass, serum and urine osmolality, was compared by repeated-measures ANOVA. For each beverage component studied the cumulative urine mass, net fluid balance and blood parameters were compared each hour and between different beverage doses by 2-way repeated-measures ANOVA. Significant main effects and interactions were further explored by Tukey’s multiple-comparison tests. BHI values were not normally distributed and therefore statistical comparison between beverages was made by Friedman test with significant effects further explored by Dunn’s multiple comparison tests. The meaningfulness of differences observed was calculated using 95% CI of differences between means and Cohen’s d effect size (Cohen, 1988). All statistical analyses were completed with the use of a statistical software package (GraphPad Prism version 6 for Windows). Statistical significance was accepted at $P<0.05$.

Sample size was based on a minimally important difference using 80% power and a two-tailed alpha level of 0.05. Hypothesized effect size was 0.81, calculated from the difference between estimated mean cumulative urine output (minimally important difference of 168mL) (Maughan, et al., 2016) with a pooled SD of 206ml giving an estimated sample size required of n=12 per site.

Results

Forty participants were recruited: loss to follow-up occurred because of vomiting after beverage ingestion (n=2), or because of voluntary withdrawal from the study (n=2), resulting in n=36 participants, 12 at each site.

Pre-drink ingestion hydration status

On each trial, pre-ingestion hydration status indicated euhydration (**Table 2**). The coefficient of variation (CV) for initial body mass was 0.6%, 0.8% and 0.6% for all sucrose, sodium and caffeine trials, respectively. The CV for initial serum osmolality was 0.7%, 1.0% and 0.7% for all sucrose, sodium and caffeine trials, respectively. The CV for initial urine osmolality was 37%, 39% and 24% for all sucrose, sodium and caffeine trials, respectively.

Blood glucose, serum sodium and plasma caffeine responses

Blood glucose concentration was greater after ingesting beverages containing sucrose (**Figure 2A**, P<0.01). Up to 1-h after beverage ingestion, blood glucose remained higher after the 20% sucrose beverage than the 0% and 5% beverages. Blood glucose was then similar between beverages for the remainder of the 4-h with exception of the 10% sucrose beverage being lower than the 0% and 20% beverages at 2-h. Serum sodium was not changed after ingesting beverages of different sodium contents (**Figure 2B**). Plasma caffeine content increased in a dose-dependent manner (**Figure 2C**, P<0.01).

Urine output and fluid balance responses to sucrose

Immediately after ingesting the different sucrose beverages, urine mass was similar (P=0.12). Cumulative urine output was lower and net fluid balance higher at 1-h, 2-h and 3-h after ingestion of the 10% and 20% sucrose beverages than the 0% and 5% sucrose beverages (**Figures 3A & 3B, P<0.05**). Throughout the 4-h period, cumulative urine output was lower and net fluid balance higher after the 20% sucrose beverage than the 0%, 5% and 10% beverage (P<0.05). The effect sizes at 2-h compared with the 0% beverage were 1.46 for the 20% sucrose beverage and 0.73 for the 10% sucrose beverage. The mean differences in urine output compared with the 0% beverage were 500g (95%CI: 399, 601g) for the 20% sucrose beverage and 189g for the 10% sucrose beverage (95%CI: 87, 290g).

Urine output and fluid balance responses to sodium

One hour after ingesting different sodium beverages urine mass was similar (P = 0.30), but 2-h, 3-h, 4-h after ingestion cumulative urine output was lower and net fluid balance higher after the 27mmol/L and 52mmol/L sodium beverages than the 7mmol/L and 15mmol/L beverages (**Figures 3C & 3D, P<0.05**). The effect sizes at 3-h compared with the 7mmol/L beverage were 1.06 for the 52mmol/L beverage and 0.87 for the 27mmol/L beverage. The mean differences compared with the

7mmol/L beverage were 372g (95%CI: 228, 516g) for the 52mmol/L sodium beverage and 300g (95%CI: 156, 444g) for the 27mmol/L sodium beverage. These differences also exceeded the 3-h cumulative urine output and net fluid balance CV.

Urine output and fluid balance responses to caffeine

Urine mass and net fluid balance were similar throughout the 4-h period on all trials after the ingestion of drinks with different caffeine content (**Figures 3E&3F**, P=0.83).

Beverage Hydration Index

Based on our previous observations, a calculated BHI exceeding twice the CV of the BHI index can be considered as meaningful, representing a better fluid retention (Maughan et al., 2016). BHI was greater in drinks with higher sucrose and sodium content, but was not affected by caffeine content (**Figure 4, P<0.05**). After 1-h, 2-h, 3-h and 4-h, 20% sucrose beverage had higher BHI than control (0% sucrose beverage) and at 2-h and 3-h was higher than 5% sucrose beverage (P<0.05). After 2-h, 3-h and 4-h the 27mmol/L and 52mmol/L sodium beverages had higher BHI than the control trial (**Figure 4A&4B**, all differences P<0.05).

Fluid-regulation and redistribution

Throughout the 4-h period, concentrations of aldosterone and arginine vasopressin were similar irrespective of the sucrose, sodium or caffeine content of beverages (**Table 3**). Immediately after and in the first hour after ingestion of 10% and 20% sucrose content beverages, serum osmolality increased, and was different to control and to 5% sucrose beverage (P<0.05), while it was relatively unchanged and similar after 0% and 5% sucrose beverage ingestion (**Figure 5A**). In contrast, immediately after ingestion of sodium beverages, serum osmolality decreased but to a less extent of 52mmol/L sodium beverage in comparison with the control (**Figure 5B, P<0.05**). Osmolality was not measured in caffeine trials.

Discussion

In the present study cumulative urine output was lower and net fluid balance higher 4-h after the ingestion of the 10% and 20% sucrose beverages than after the ingestion of the 0% and 5% sucrose beverages. A similar response was observed with 27 mmol/L and 52 mmo/L sodium beverages compared to the 7 mmol/L and 15 mmol/L beverages. However, no differences in urine mass or net fluid balance were apparent 4-h following the ingestion of different caffeine contents. These observations are consistent with our initial hypotheses and demonstrate that factors affecting fluid delivery (sucrose content) and retention (sodium content) are dependent upon the dose contained within ingested beverages. These data also demonstrate that caffeine up to 400 mg/L has no impact upon hydration potential or the ability to retain fluid of beverages.

In our previous work (Maughan et al., 2016), we were able to quantify the hydration potential of commercially-available drinks using a beverage hydration index (BHI). The BHI was postulated to be related to energy density and electrolyte composition, both of which can affect fluid delivery and retention. However, combinations of key components (e.g. macronutrients, electrolytes and caffeine) at different doses could influence gastric emptying, intestinal absorption, and fluid retention characteristics. The results of the present study reveal that, in comparison to control beverage, under euhydrated conditions a sucrose content of up to 5%, a caffeine content of up to 400mg/L, and a sodium content of up to 15mmol/L all have no effect on the BHI. However, 10% and 20% sucrose beverages, and beverages containing 27mmol/L and 52mmol/L sodium result in reduced diuresis. Given that these test drinks were examined under euhydrated conditions, the reduced urine output likely occurred due to mechanisms involving a combination of altered gastric emptying (Hunt & Stubbs, 1975) and intestinal absorption (Leiper, 2015). Furthermore, the electrolyte content has potential effects on fluid retention independent of hormonal controls (Schedl & Clifton, 1963).

Gastric emptying, intestinal absorption and renal excretion of fluids

Early studies demonstrated that the addition of sodium to test drinks with low glucose content increased the rate of gastric emptying (Hunt & Pathak, 1960) and intestinal absorption (Phillips & Summerskill, 1967). Other studies demonstrated that glucose at >4% solution content reduced the rate of gastric emptying compared to water, that warm/hot fluids reduced gastric emptying compared to cold beverages, and that faster initial emptying rates were reached with higher bolus volumes (Costill & Saltin, 1974; Hunt & Macdonald, 1954; Vist & Maughan, 1994, 1995). Applying these observations to the current study it can be proposed that gastric emptying rate would be increased with an increasing sodium content of beverages (above 33 mmol/L), reduced with an increasing energy/carbohydrate content (above 4-5% carbohydrate), and likely remain unchanged by increasing caffeine content (up to 269 mg). Indeed, these largely reflect the reported observations in the present study.

Intestinal perfusion studies reveal that hypertonic solutions (>300mOsm/kg) result in transient net water secretion into the intestinal lumen whereas hypotonic solutions (<260mOsm/kg) stimulate net water absorption (Hunt et al., 1992). High carbohydrate solutions with high osmolality will therefore delay gastric emptying, slow delivery of fluid to the intestine, and cause net water secretion into the intestinal lumen. Water absorption appears to be independent of carbohydrate at concentrations up to 6% (Gisolfi et al., 1992). Applying these observations to the present study would suggest that more concentrated sucrose solutions (≥10%) would likely slow gastric emptying result in transient net water secretion into the intestinal lumen. The effect of increasing the sodium content upon the ability to retain fluid of beverages suggests an initial fast gastric emptying inducing increase in intestinal water and sodium transport, and subsequently greater retention of the fluid in the body water pool. The decrease in serum osmolality observed following beverage ingestion supports these assertions.

The principal determinant of permeability, and consequently of water reabsorption, in the collecting ducts of the kidneys is arginine vasopressin (AVP) (Bourque, 2010). Aldosterone, produced by the adrenal cortex, also stimulates sodium reabsorption in the cortical collecting ducts (Stanhewicz & Kenney, 2015). In the present study, the responses of aldosterone and AVP to fluid ingestion were similar regardless of the content of sucrose, sodium or caffeine within the beverages. AVP and aldosterone also did not change over time during the ingestion or follow-up period. Thus, in the present work it can be concluded that differences in urine output between sucrose beverages and between sodium-containing beverages are not influenced by differences in renal water or sodium excretion. Thus, by studying participants in a euhydrated state we have been able to isolate effects on fluid delivery/retention while removing potential interaction of hormonal controls. The differences in 2-h cumulative urine output and in net fluid balance observed in the sucrose and in the sodium trials can be considered meaningful as they exceeded the CV calculated previously (Maughan et al., 2016) and the minimally important difference of 168mL calculated a priori.

Caffeinated beverages and hydration

Caffeine is an adenosine receptor antagonist reducing fractional sodium reabsorption in the proximal tubule and in the distal nephron (Shirley et al., 2002) which could lead to increased renal water loss. Previous studies exploring the effect of administering different doses of caffeine have observed increased urine volume only when participants ingested 360 mg of caffeine (Passmore et al., 1987), 6 mg/kg of caffeine (Seal et al., 2017) or 624 mg (Neuhauser et al., 1997). In the present study, no difference in urine volume was noted following any of the doses of caffeine administered. This suggests that sodium excretion was not influenced by caffeine in our participants. Unfortunately, sodium excretion in urine was not determined in our trials to enable confirmation of this proposal. The lack of effect of all the caffeine doses studied in the present study supports and adds to earlier observations on caffeine dose. Thus, caffeinated beverages (containing up to 400mg of caffeine) can contribute to daily total fluid intake targets without negative effects on fluid balance.

Practical Perspectives / Study Limitations

This study provides further evidence that the sodium content of a beverage is likely to be a main driver for improved fluid delivery and retention, while high carbohydrate content likely delays fluid delivery and increases the serum osmolality, and caffeine up to 400mg has no impact on diuresis 4-h after the beverage ingestion. These mechanistic observations can provide useful information for athletes as their teams can develop a fluid intake strategy for when there is limited access to fluid or when the access to facilities to urinate is restricted (e.g. when the athletes are travelling) The outcomes of the present study require further exploration in other groups such as older adults who have a reduced ability to alter renal water excretion. Future studies also should examine the effects of other macro- and micro-nutrients on the hydration potential of beverages.

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“Sucrose and Sodium But Not Caffeine Content Influence the Retention of Beverages in Humans Under Euhydrated Conditions” by Maughan RJ et al.
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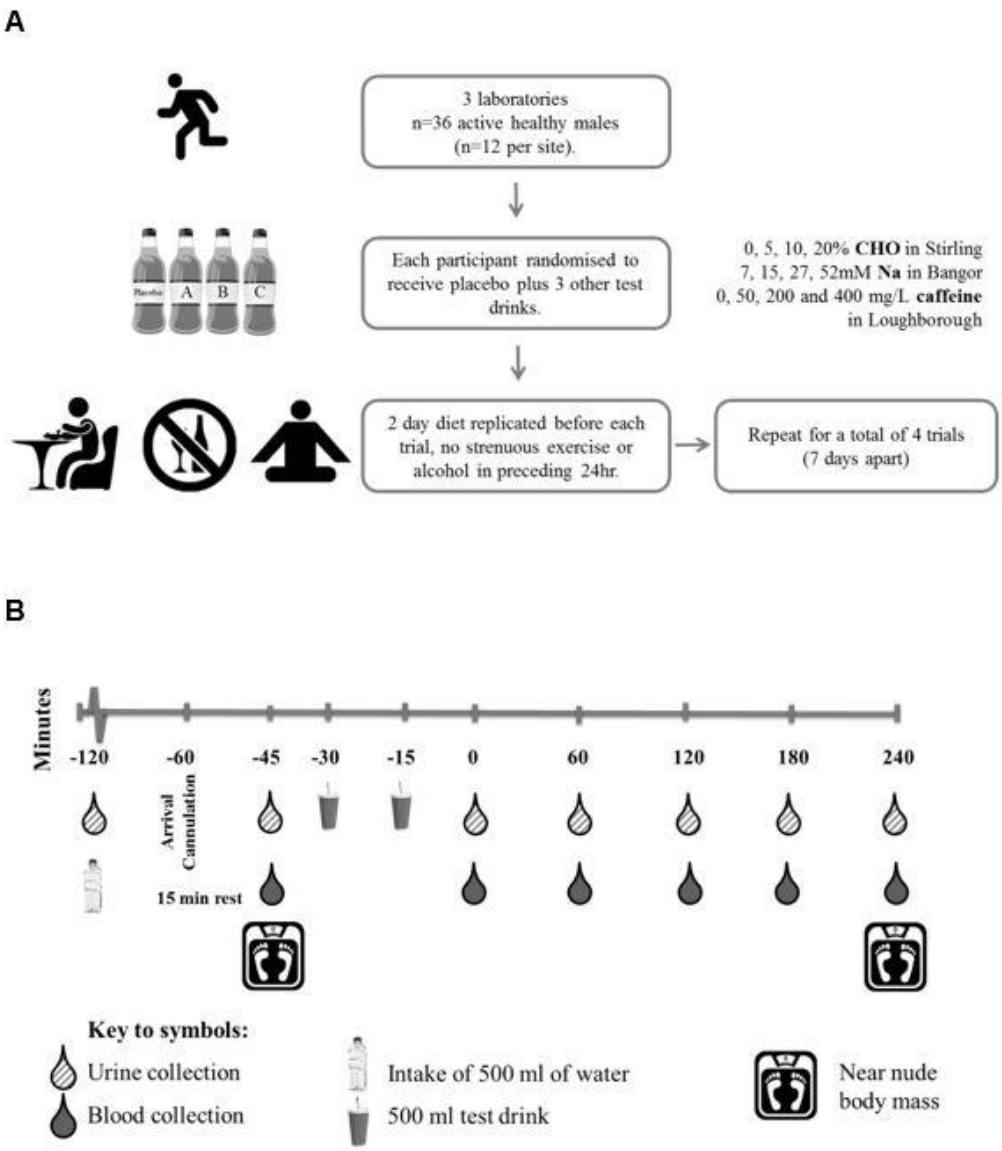

FIGURE 1. Experimental design of the study (A) and schematic of experimental protocol (B). CHO = carbohydrate (sucrose), Na = sodium.

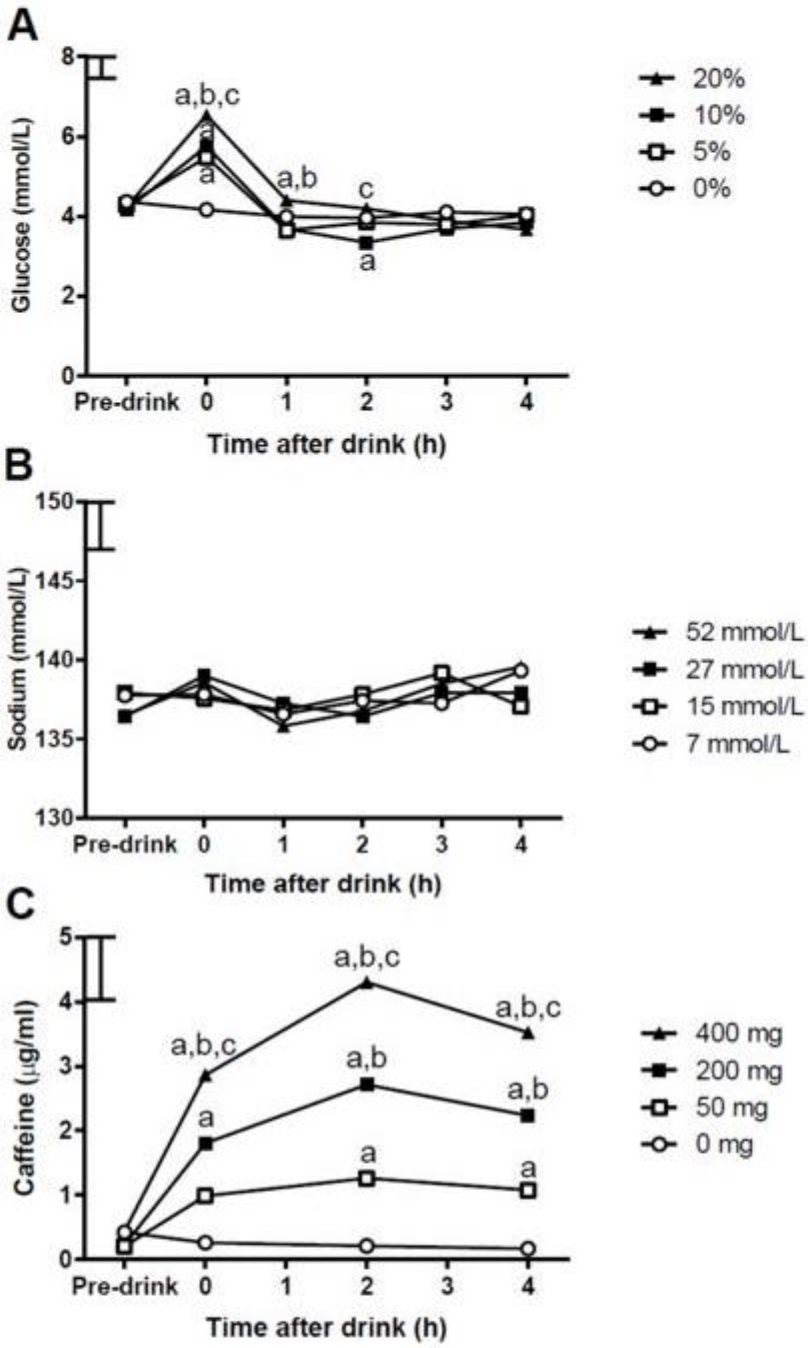

FIGURE 2. Blood glucose (A), serum sodium (B) and plasma caffeine responses (C) after the ingestion of 1 L of various sucrose (A), sodium (B) and caffeine (C) content beverages vs. control. n = 12 observation on each beverage. Beverages with different responses are identified by Tukey's multiple comparison test: a, indicates difference to 0% sucrose (control) or 0 mg caffeine (control) beverage, b, indicates difference to 5% or 50 mg caffeine, c, indicates difference to 10% or 200 mg caffeine. Statistical significance was accepted at $P<0.05$. The vertical error bar in the top left corner represents the overall mean SD during the 4-h collection.

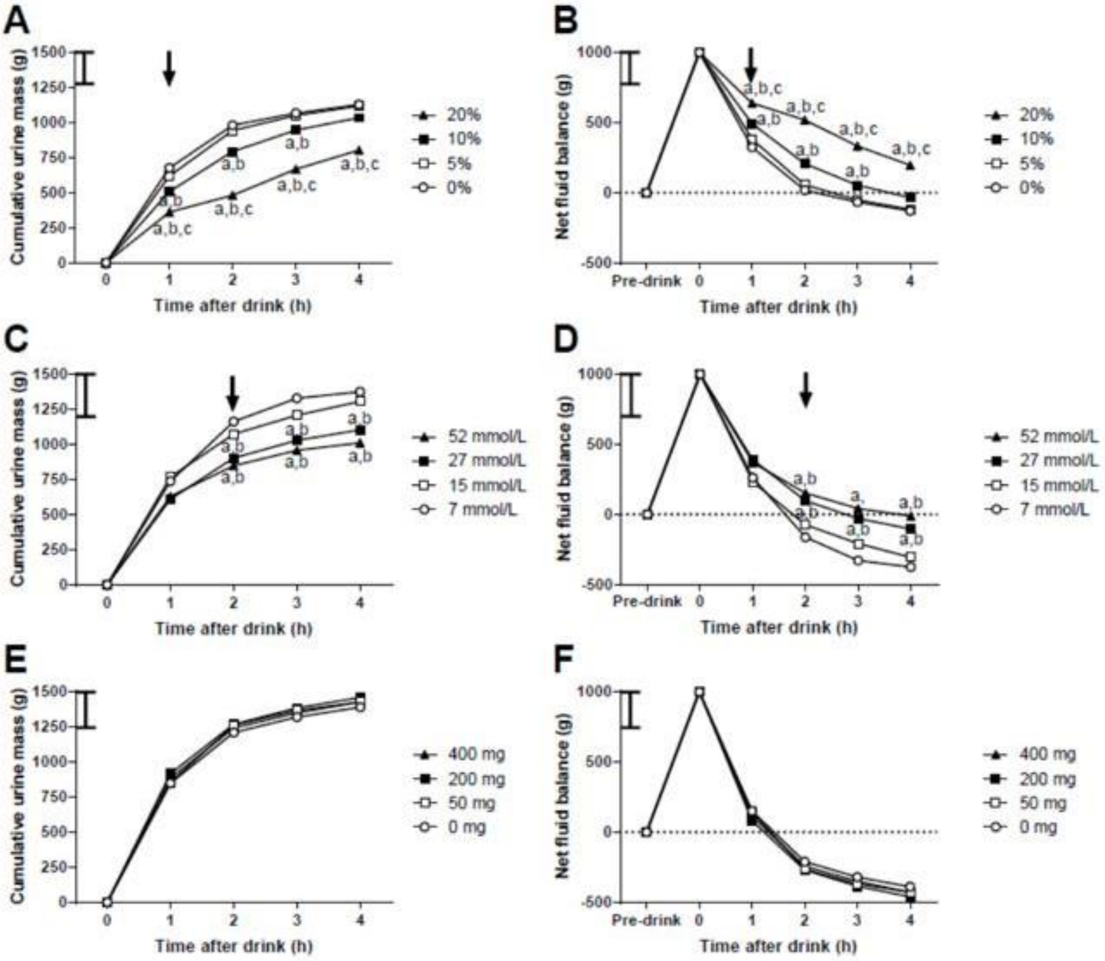

FIGURE 3. Cumulative urine output and net fluid balance after the ingestion of 1 L of various sucrose (A & B), sodium (C & D) and caffeine (E & F) content beverages. n = 12 observation on each beverage. Beverages with different responses are identified by Tukey's multiple comparison test: a, indicates difference to 0% sucrose (control) or 7 mmol/L sodium (control) beverage; b, indicates difference to 5% sucrose or 15 mmol/L sodium beverage; c, indicates difference to 10% sucrose beverage. Downward arrows indicate the first time when statistical differences were detected between beverages. Statistical significance was accepted at $P<0.05$. The vertical error bar in the top left corner represents the mean SD during the 4-h collection.

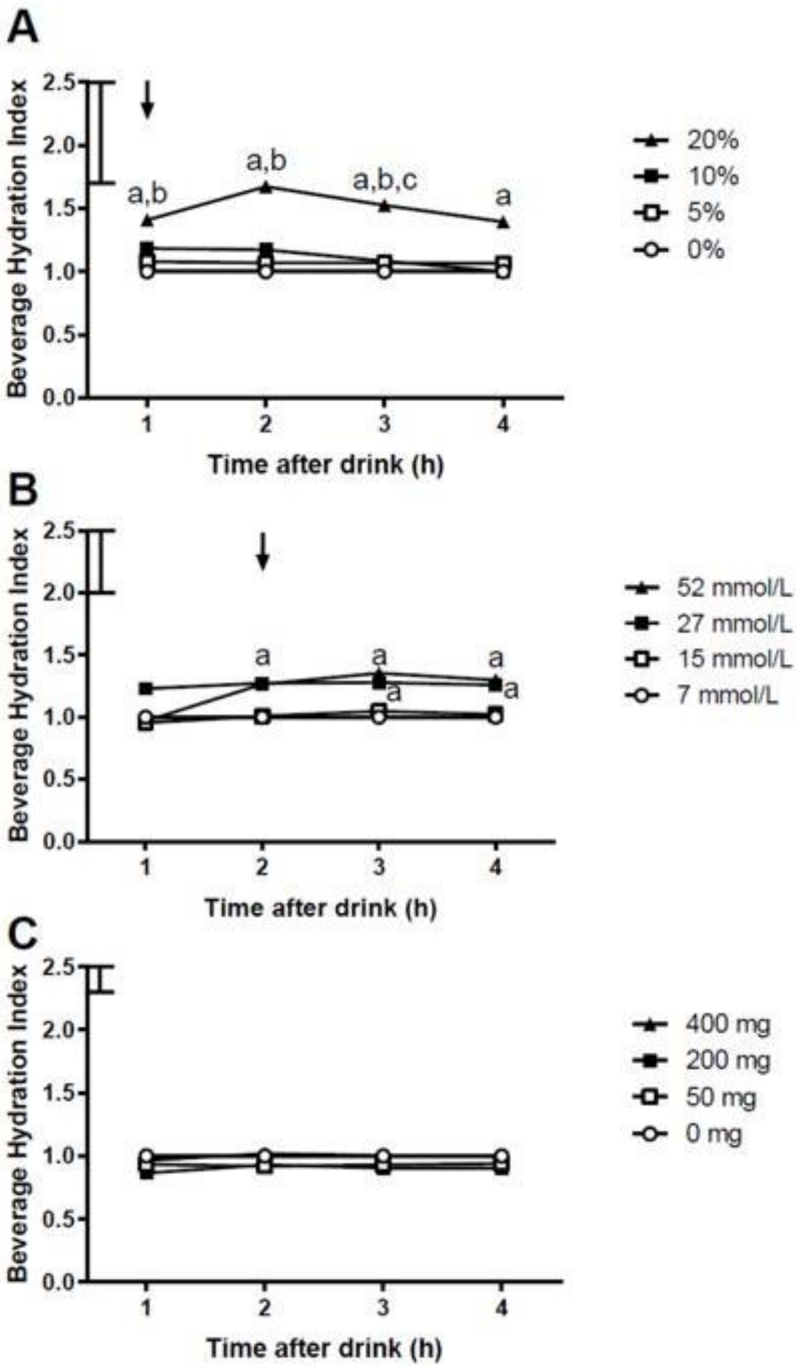

FIGURE 4. Beverage hydration index for various sucrose (A), sodium (B) and caffeine (C) content beverages. n = 12 observation on each beverage. Beverages with different responses are identified by Dunn's multiple comparison test: a, indicates difference to 0% sucrose (control) or 7 mmol/L sodium (control) beverage; b, indicates difference to 5% sucrose beverage; c, indicates difference to 10% sucrose beverage. Statistical significance was accepted at P<0.05. These are median data with the mean IQR during the 4-h collection represented by the vertical error bar in the top left corner. Downward arrows indicate the first time when statistical differences were detected between beverages.

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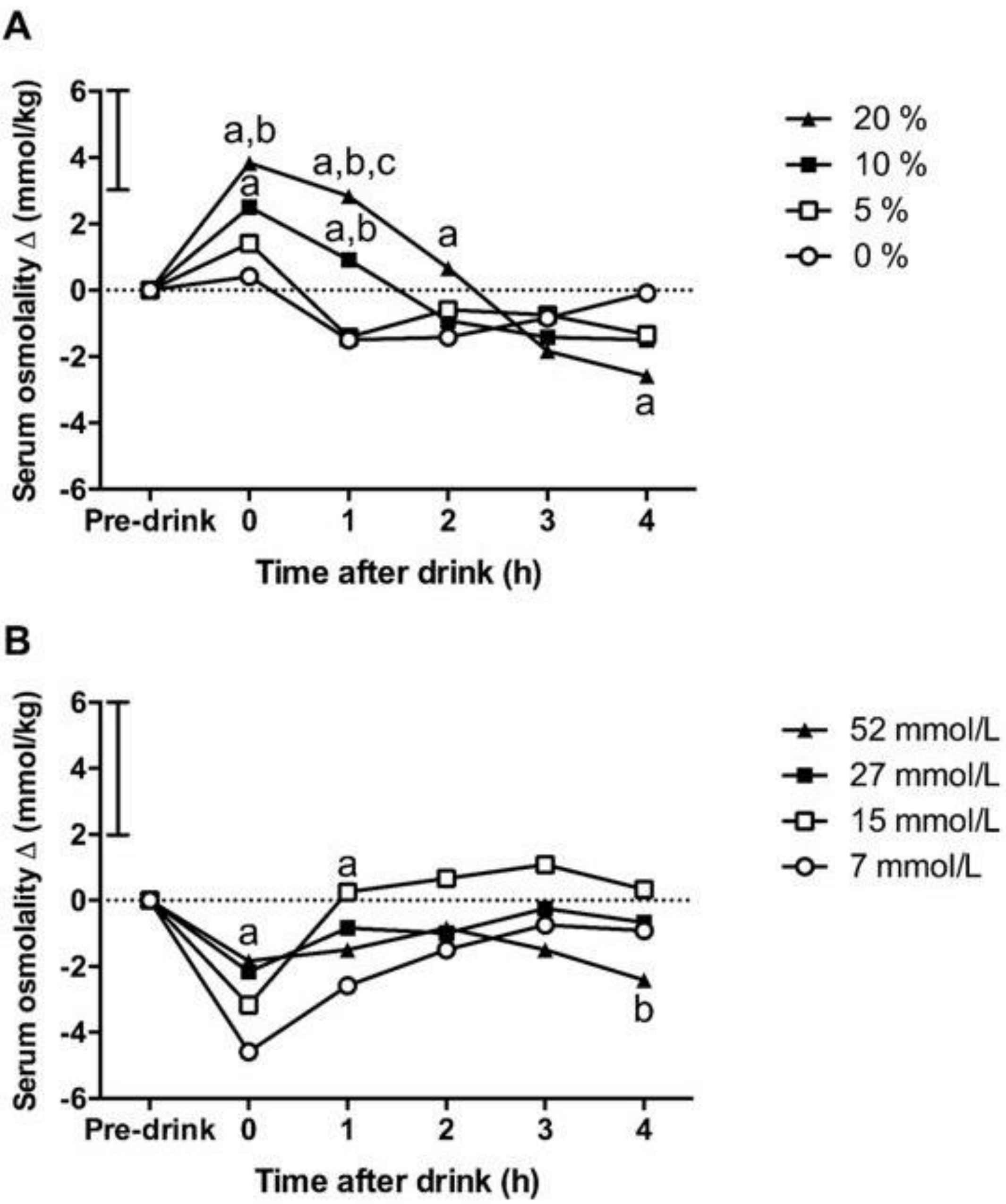

FIGURE 5. Serum osmolality change after the ingestion of 1 L of various sucrose (A) and sodium (B) beverages. n = 12 observation on each beverage. Beverages with different responses are identified by Tukey's multiple comparison test: a, indicates difference to 0% sucrose (control) or 7 mmol/L sodium (control) beverage; b, indicates difference to 5% sucrose beverage or 15 mmol/L sodium beverage; c, indicates difference to 10% sucrose beverage. Statistical significance was accepted at P<0.05. The vertical error bar in the top left corner represents the mean SD during the 4-h collection.

Table 1. Participant physical characteristics, measured during the pre-screening consultation, estimated daily water, alcohol and caffeine intake from the food diaries at each of the three study sites and for combined data (all sites).

	Stirling - Sucrose (n = 12)	**Bangor - Sodium (n = 12)**	**Loughborough - Caffeine (n = 12)**	**All (n = 36)**	***P***
Age (y)	26 ± 6	25 ± 4	27 ± 2	26 ± 4	0.53
Height (cm)	181 ± 7	179 ± 7	178 ± 7	179 ± 7	0.67
Mass (kg)	77.6 ± 9.3	78.2 ± 7.8	77.1 ± 8.9	77.6 ± 8.5	0.95
BMI (kg/m^2)	23.9 ± 2.7	24.6 ± 2.2	24.2 ± 1.5	24.2 ± 2.1	0.75
Water intake (L/d)	1.9 ± 0.3	2.2 ± 0.9	1.9 ± 0.5	2.0 ± 0.6	0.42
Caffeine intake (mg/d)	210 ± 142	180 ± 123	206 ± 176	199 ± 145	0.87
Alcohol intake (g/d)	5 ± 6	4 ± 4	3 ± 2	4 ± 4	0.55

Notes: Data are Mean ± Standard Deviation. Water intake represent fluid from beverages only. Alcohol intake includes all forms of alcoholic beverages. BMI = Body Mass Index.

Table 2. Pre-ingestion hydration status at each of the three study sites.

	Stirling – Sucrose (n = 12)				
	0%	**5%**	**10%**	**20%**	***P***
Body mass (kg)	77.5 ± 9.2	77.5 ± 9.4	77.7 ± 9.1	77.5 ± 9.5	0.70
Serum osmolality* (mmol/kg)	295 ± 3	296 ± 2	296 ± 2	295 ± 2	0.77
Urine osmolality (mmol/kg)	524 ± 323	557 ± 209	488 ± 290	664 ± 332	0.38
	Bangor – Sodium (n = 12)				
	7 mmol/L	**15 mmol/L**	**27 mmol/L**	**52 mmol/L**	***P***
Body mass (kg)	78.2 ± 7.8	78.4 ± 8.1	78.5 ± 7.8	78.1 ± 8.2	0.50
Serum osmolality (mmol/kg)	289 ± 3	290 ± 3	291 ± 4	292 ± 4	0.17
Urine osmolality (mmol/kg)†	520 ± 215	544 ± 232	475 ± 201	513 ± 300	0.82
	Loughborough – Caffeine (n = 12)				
	0 mg	**50 mg**	**100 mg**	**400 mg**	***P***
Body mass (kg)	77.3 ± 10.1	77.5 ± 10.1	77.7 ± 10.1	77.3 ± 10.1	0.26
Serum osmolality (mmol/kg)	287 ± 4	289 ± 5	289 ± 6	290 ± 5	0.05
Urine osmolality (mmol/kg)	441 ± 179	486 ± 144	478 ± 163	519 ± 168	0.48

Notes: Data are presented as Mean ± Standard Deviation.

*osmolality assessment of an identical control solution (mean 292 mmol/kg) at each site indicated that the Roehbling osmometer (Stirling) consistently reported a +4 mmol/kg bias compared with the Gonotec osmometer (Loughborough and Bangor). † n = 11 for Bangor urine osmolality analysis.

Table 3. Mean plasma aldosterone and plasma arginine vasopressin (AVP) responses over the 4-h follow-up period following each test drink ingestion, at each study site.

	Stirling – Sucrose (n = 12)				
	0%	**5%**	**10%**	**20%**	***P***
Aldosterone (pg/ml)	103 ± 31	113 ± 27	100 ± 30	106 ± 34	0.47
AVP (pg/ml)	3.5 ± 0.6	3.4 ± 0.6	3.6 ± 0.6	3.7 ± 0.7	0.50
	Bangor – Sodium (n = 12)				
	7 mmol/L	**15 mmol/L**	**27 mmol/L**	**52 mmol/L**	***P***
Aldosterone (pg/ml)	109 ± 41	126 ± 67	150 ± 59	100 ± 62	0.16
AVP (pg/ml)	3.7 ± 0.7	3.6 ± 0.9	3.8 ± 1.2	3.9 ± 0.8	0.79
	Loughborough – Caffeine (n = 12)				
	0 mg	**50 mg**	**200 mg**	**400 mg**	***P***
Aldosterone (pg/ml)	90 ± 73	99 ± 64	72 ± 64	87 ± 108	0.60
AVP (pg/ml)	3.5 ± 1.4	3.5 ± 1.1	2.9 ± 0.9	3.8 ± 0.9	0.22

Note: Data are presented as Mean ± Standard Deviation.