Neither body mass nor sex influences beverage hydration index outcomes during randomized trial when comparing 3 commercial beverages

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ABSTRACT

Background: The beverage hydration index (BHI) assesses the hydration potential of any consumable fluid relative to water. The BHI is a relatively new metric, and the impact of body mass, sex, and reproducibility has yet to be investigated.

Objectives: To assess the independent impact of body mass and sex on BHI using beverages not previously assessed, including an amino acid–based oral rehydration solution (AA-ORS), a glucose-containing ORS (G-ORS), and a sports drink (SpD), compared with water (control). The reproducibility of the results was examined using statistical modeling (bootstrap analysis).

Design: Using a repeated-measures design, 40 euhydrated and fasted subjects (17 male, 23 female; urine specific gravity < 1.025) were studied on 4 separate occasions. During each trial, subjects ingested 1 L of each beverage, and urine output was measured immediately postingestion and at 1-h intervals for the next 4 h. The BHI was calculated as a ratio of each individual’s cumulative urine output after drinking 1 L of water over their cumulative urine output after drinking each of the test beverages.

Results: The calculated mean ± SD BHIs of the beverages were as follows: water (1.0 ± 0.0), AA-ORS (1.15 ± 0.28), G-ORS (1.21 ± 0.28), and SpD (1.09 ± 0.26). The BHI for both AA-ORS and G-ORS was greater than that for water (P < 0.05). Despite overall differences in body mass, neither body mass nor sex independently affected BHI. Based upon statistical modeling, our results demonstrate excellent reproducibility of outcomes and external validity.

Conclusions: Our results suggest that the BHI may be used and interpreted with confidence independently of body mass or sex. Furthermore, a novel carbohydrate-free AA-ORS and a traditional commercially available G-ORS were superior to water in optimizing hydration, whereas SpD was not. This trial was registered at clinicaltrials.gov as NCT03262597.

INTRODUCTION

Recently, this journal published a metric called the beverage hydration index (BHI), which assessed the short-term hydration potential of different beverages when ingested in the euhydrated state (1). Although this metric serves as a tool to assess the hydration potential of beverages, unanswered questions exist regarding its utility and application. For example, the BHI is obtained by administering a fixed volume of test beverage (i.e., 1 L), which can result in dramatically different relative fluid loads (i.e., mL/kg), depending upon the body mass of the participants. In principle, large differences in relative (to body mass) dosing could produce differences in fluid regulatory responses. Indeed, body mass is commonly used to scale clinical fluid prescriptions (2) and is a fundamental consideration for water deficit therapy (3, 4). Thus, further investigation into the influence of body mass on the BHI is warranted.

The original BHI investigation included only male volunteers, yet there is evidence to suggest that sex impacts fluid regulation. For example, sex differences in fluid handling related to sex hormones have been reported by some (5), but not all (6), investigators. Further, adequate water intakes for men and women differ markedly, as defined by both US and European guidelines (7, 8). Therefore, investigation into the role that sex plays on BHI is also warranted.

Lastly, the repeatability and variability of the BHI has yet to be determined. The original investigation made some assessments of variability (1). However, the variability of this metric can be further honed by increasing the number of trials that participants...
undertake and/or by increasing the sample size. Additionally, the repeatability of the BHI results can be examined by using a very practical, powerful and cost-effective statistical modeling approach, such as bootstrapping (9), which allows numerous iterations of the results to be run from a given sample to assess repeatability more robustly.

Therefore, in the current experiments, we explored the utility of the new BHI metric by examining the independent effects of body size and sex. We then tested the repeatability of our BHI results using statistical bootstrap modeling. We accomplished this by measuring the BHI of 3 previously unmeasured, commercially available beverages, including a novel amino acid–based oral rehydration solution (AA-ORS) that may confer additional health benefits to various clinical populations because of its ability to improve intestinal health (10, 11).

SUBJECTS AND METHODS

Study overview

The Sonoma State University Institutional Review Board approved these procedures and the informed consent document that all subjects signed prior to the start of the study. During the study, participants came to the laboratory on 4 separate occasions to consume 1 of the 3 test beverages or the control beverage (water) in a randomized order. Thereafter, participants had their urine output collected and recorded during the subsequent 4 h to assess the BHI of the beverages to model the original BHI investigation (1).

Participants

The healthy men and women, aged between 18 and 35 y, who took part in the study were recruited from Sonoma State University via word-of-mouth and e-mail advertisements. Applicants were excluded if they had a history of cardiovascular, renal, musculoskeletal, or metabolic diseases, as were those currently undertaking an energy-restricted diet. No female volunteer was or became pregnant during the course of the study. The menstrual cycle phase could not be standardized, so differences in female sex hormones between or within female subjects was accepted as a study limitation but had no effect on the ecological validity.

During the study, participants were asked to record their diet, fluid intake (household measures technique), and any exercise undertaken and/or by increasing the sample size. Additionally, the repeatability of the BHI results can be examined by using a very practical, powerful and cost-effective statistical modeling approach, such as bootstrapping (9), which allows numerous iterations of the results to be run from a given sample to assess repeatability more robustly.

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Test beverages

The investigators assigned each participant to consume in a random order, using a generalized Latin square arrangement, distilled water (Poland Spring) and the following 3 drinks: an AA-ORS (enterade, Entrinsic Health Solutions, Inc.), a glucose-based ORS (G-ORS; Pedialyte, Abbott Laboratories), and a glucose-based sports drink (SpD; Gatorade, PepsiCo). The nutrient composition of each of the test drinks is presented in Table 1. All drinks were stored at a standard refrigeration temperature (4–6°C) until serving.

Pilot study procedures

In order to establish the practical importance of any statistically significant outcomes, we studied the variation associated with the BHI formula numerator (water) by repeating the water-only trial 3 times with 7 subjects separately from the main study [3 men, 4 women; mean ± SD age, 21 ± 3 y].

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Comparison of the nutrient composition in 1 L of each beverage tested1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloric content (kcal/L)</td>
<td>Carbohydrate (g/L)</td>
</tr>
<tr>
<td>AA-ORS</td>
<td>21</td>
</tr>
<tr>
<td>G-ORS</td>
<td>105</td>
</tr>
<tr>
<td>SpD</td>
<td>237</td>
</tr>
</tbody>
</table>

1Data derived from the drink labels. AA-ORS, amino acid–based oral rehydration solution; G-ORS, glucose-based oral rehydration solution; SpD, sports drink.
Data and statistical analysis

The main outcome measure was cumulative urine output after ingestion of each beverage. This was also expressed as the BHI (metric of fluid retention), which is a ratio of each individual’s cumulative urine output after drinking 1 L of water over their cumulative urine output after drinking each of the test beverages (1). Thus, a larger BHI (>1.0) indicates that a given test beverage was retained more than an equal amount of water. A fixed model PROC MIXED procedure was used to compare the BHI among repeated-measures treatments and examined the potential effects of body mass and sex, and any interaction effect between body mass and sex. Tukey’s post hoc test was used to test all possible pairwise comparisons. As stated above, to assess the practical importance of the BHI, we determined the variation in urine output after drinking 1 L of water during 3 separate trials. Our results demonstrated that the variation in urine output was ±130 mL (11% CV). Therefore, to assess a beverage’s practical importance and demonstrate its superiority to water, the beverage’s BHI value had to exceed 1.13. Because neither covariate influenced the outcomes (see Results), the individual hourly cumulative urine output was compared with the net fluid balance by conventional two-way (beverage × time) repeated-measures ANOVA with Tukey’s post hoc test. Descriptive group differences between men and women were compared by Student’s t test.

Lastly, the empirical BHI performance of the 3 beverages was further examined by using bootstrap analysis. Briefly, the 40 observed BHI values for each beverage were resampled (with replacement) 1000 times to produce a universe of 40,000 individual BHI values. The fidelity with which AA-ORS, G-ORS, and SpO might perform upon repeated testing of the study sample, or in a larger parent sample of like-volunteers, was inferred by equivalence to bootstrap results when the bias (mean BHI – mean BHIbootstrap) was <0.25 SE of the mean BHI (9).

The sample size was estimated for the primary BHI repeated-measures ANOVA. Based upon the observed repeatability during pilot testing, an effect size approximately half of what was reported by Maughan et al. (1) was anticipated (15), and between 29 and 55 subjects were calculated to be necessary to see significant differences >0.6SD (16) using conventional alpha (0.05) and beta (0.20) values. All statistical analyses were completed with the use of computerized statistical software packages (SAS 9.4, GraphPad Prism version 6 for Windows). Statistical significance was accepted at P ≤ 0.05. Data are presented as means ± SDs.

RESULTS

A total of 46 subjects were recruited and started the study (January–April), with 3 subjects not completing the study owing to external factors (n = 3). This sample size exceeded those in previous investigations (1). All subjects’ initial hydration status was assessed based on the USG of their first morning void collected before laboratory arrival. A subject’s data were only included if the USG met the euhydration criterion value of ≤1.025 (12, 17). In ≥1 trial, some subjects’ first morning void USG exceeded 1.025, so their data were excluded from the analysis (n = 3). Therefore, the total sample size included in the final analysis was 40 (17 men, 23 women; Table 2).

BHI mixed modeling

In order to assess the potential independent effects of body mass and sex on BHI, we used fixed effects PROC MIXED modeling with special provisions for repeated measures. Seventeen men and 23 women were used in the analysis (Table 2). Body mass was different between men and women (Table 2), ranging from 59 to 103 kg for the men and from 46 to 82 kg for the women. Relative BHI water dose was also different between men and women (Table 2), ranging from 9.8 to 17.0 mL/kg for the men and from 12.3 to 22.1 mL/kg for the women. Despite the numbers of men and women and the ranges of body mass studied, neither covariate [body mass (P = 0.927) or sex (P = 0.862)] was significantly related to the BHI main effects. When both covariates were held constant, post hoc analysis indicated that the BHI for both AA-ORS and G-ORS was greater than water (P < 0.05) (Figure 1). Moreover, there was no sex × body mass interaction for BHI (Figure 2). All data were subsequently analyzed without concern for the influence of body mass or sex.

Urine output and fluid balance

The urine output did not differ among treatments both immediately and 1 h after ingestion of the beverages. However, 2 h after ingestion, the cumulative urine output was lower and the net fluid balance higher for G-ORS and AA-ORS compared with

![Table 2: Participant physical characteristics](https://academic.oup.com/ajcn/article-abstract/107/4/544/4964651)

*Significant difference between men and women, P < 0.05.
FIGURE 1  BHI calculated from the cumulative urine masses at 2 h. The dotted line is positioned at a BHI value of 1.13, which represents the urine variation observed after drinking just water (±130 mL). This serves as the threshold for establishing practical importance. Values are means ± SDs. A fixed model PROC MIXED procedure was used. *Significantly different from water (n = 40), P < 0.05. AA-ORS, amino acid–based oral rehydration solution; BHI, beverage hydration index; G-ORS, glucose-based oral rehydration solution; SpD, sports drink.

FIGURE 2  Sex comparison of the BHI. The BHI was calculated from the cumulative urine masses at 2 h. The dotted line is positioned at a BHI value of 1.13, which represents the urine variation observed after drinking just water (±130 mL). This serves as the threshold for establishing practical importance. Values are expressed as means ± SDs. A fixed model PROC MIXED procedure was used. There was no difference between sexes. AA-ORS, amino acid–based oral rehydration solution; BHI, beverage hydration index; G-ORS, glucose-based oral rehydration solution; SpD, sports drink.

FIGURE 3  Cumulative urine mass (A) and net fluid balance (B) following ingestion of water, G-ORS, SpD, and AA-ORS. Values are means ± SDs. Drinks with different responses to water were identified by conventional two-way repeated-measures ANOVA with Tukey’s post hoc test at each time point and highlighted in rectangular boxes. *Significant difference for AA-ORS and G-ORS compared with water (n = 40), P < 0.05. AA-ORS, amino acid–based oral rehydration solution; G-ORS, glucose-containing oral rehydration solution; SpD, sports drink.

VALIDATION OF BHI: ROLE OF SEX AND MASS

water. These findings held consistent throughout the 4 h time frame within which urine was collected (Figure 3A and B).

Bootstrap

In the case of each non-water beverage tested (AA-ORS, G-ORS, SpD), the BHI bias was well below 0.25 SE, suggesting that the empirical study results (n = 40) are representative of a larger virtual population and that extrapolation of the results is therefore justified (Table 3).

DISCUSSION

The present study demonstrates that there was no differential response in the overall BHI values of participants of varying body mass or sex. Moreover, using statistical modeling to extrapolate our results, the current BHI values are indicative of a larger population, suggesting high repeatability of BHI outcomes when testing large-enough study samples. Lastly, AA-ORS and G-ORS have a higher BHI than water, while SpD does not.

The first question we investigated was whether or not, given the same absolute volume of fluid, body mass would have an effect on the overall BHI value. The original BHI methodologies used a standard fluid bolus (1 L) for all subjects, which can lead to quite large differences in the relative fluid bolus (mL/kg) if the subjects vary greatly in body mass. Moreover, women typically have smaller body masses than men and may also handle fluid loads differently based on sex hormone differences (5, 6). Our large sample size (17 men, 23 women) and considerable range in body masses allowed us to examine the potential independent influences of body mass and sex on BHI via ANCOVA. Our results demonstrated that although, on average, the females were lighter than the males (Table 2) and took in a larger relative fluid bolus during the testing (mL/kg), there were no differences in BHI values between the sexes (Figure 2), nor were there any differences due to body mass per se within the ranges tested. Therefore, there was no independent impact of body mass or sex on the BHI.

We also sought to determine the repeatability of our large-sample BHI results. The original BHI investigation tested 12 different beverages in addition to distilled water (control) and
each beverage was tested by 15–17 subjects (1). Although certainly a robust sample, large variations in the BHI values were still observed within many of the tested beverages. Given the reported variations in the BHI, we wanted to know if our results would hold true in a larger population. We feel confident that they would for a number of reasons. First, our final data set comprised 40 subjects who completed all 4 beverage trials, thus allowing us to use a repeated-measures design. Second, we used statistical modeling (bootstrapping) to determine if our results would hold when extrapolated to a larger population with similar demographics. The bootstrap results (Table 3) add strong support to the study conclusions, particularly as we included a sufficient sample size (40 > 10 sample observations) and resampling rate (1000 > 200) for model bias comparisons (9, 18). Lastly, in the original BHI investigation, the practical significance threshold was set at twice the obtained 18% CV, which required ~360 mL greater retention to reach the practical significance threshold. The choice of practical threshold must be a thoughtful one, and should include such considerations as the population under study, the test conditions, and the analysis to be performed. Our choice of 1% CV (11% ∼ 130 mL) was in anticipation of smaller effect sizes because of the highly correlated samples stemming from our repeated-measures design (15). Had we set our null value to 11% as an inferential tical threshold must be a thoughtful one, and should include such considerations as the population under study, the test conditions, and the analysis to be performed. Our choice of 1% CV (11% ∼ 130 mL) was in anticipation of smaller effect sizes because of the highly correlated samples stemming from our repeated-measures design (15). Had we set our null value to 11% as an inferential tical threshold must be a thoughtful one, and should include such considerations as the population under study, the test conditions, and the analysis to be performed. Our choice of 1% CV (11% ∼ 130 mL) was in anticipation of smaller effect sizes because of the highly correlated samples stemming from our repeated-measures design (15). Had we set our null value to 11% as an inferential tical threshold must be a thoughtful one, and should include such considerations as the population under study, the test conditions, and the analysis to be performed. Our choice of 1% CV (11% ∼ 130 mL) was in anticipation of smaller effect sizes because of the highly correlated samples stemming from our repeated-measures design (15). Had we set our null value to 11% as an inferential.

The pioneering BHI investigation observed that electrolyte and macronutrient composition played a major role in the fluid-retaining abilities of a given beverage (1). Thus, beverages with the highest electrolyte and/or macronutrient composition demonstrated the highest BHI values (e.g., ORS and full-fat or skim milk). Our data are consistent with the original investigation, where beverages containing greater electrolyte concentrations (AA-ORS and G-ORS) had greater BHI values compared with beverages with lower electrolyte concentrations (SpD; Table 1). Note that the original BHI investigation measured the BHI of a different SpD (Powerade, Coca-Cola) (1). Powerade has a very similar carbohydrate and electrolyte composition to the SpD used in the present investigation (Gatorade). Importantly, our study corroborates the original study’s results, demonstrating that the BHI of an SpD is not different from that of water.

The novel beverage tested in the current investigation was an AA-ORS. Amino acids are absorbed from the small intestine by multiple transport systems, including passive transport (diffusion), sodium-independent transporters, and sodium-dependent cotransporters (19). The benefit of adding a small amount of protein, or amino acids, to a rehydration beverage in order to enhance sodium and water absorption from the intestine has been well researched in a variety of clinical models (20–24). Moreover, the addition of protein, dipeptides, or amino acids has demonstrated improved rehydration compared with water or an SpD following exercise-induced water losses (i.e., sweat) (25–27). Of note, a recent investigation demonstrated that the AA-ORS beverage outperformed a traditional SpD in rehydrating individuals after a diuretic dehydration trial, which is used to model fluid losses in various clinical situations, and after a sweat loss trial (27). It is important to note that while AA-ORS and G-ORS both performed better than water in the current investigation, AA-ORS accomplished this without glucose-sodium cotransport and with a lower caloric content (kcal/L). Therefore, this beverage may have high utility as a hydration beverage in various clinical situations, such as when glucose-mediated intestinal chloride secretion is undesirable (10, 11).

The BHI is a useful tool for assessing the hydrating potential of various beverages in euhydrated individuals. We examined the utility of the BHI, demonstrating that, while body mass and sex do not influence the BHI, it appears that electrolyte content may be its primary driver. Moreover, the bootstrap results strongly suggest that our conclusions are repeatable and therefore valid even in a much larger sample of subjects when tested under similar conditions. Lastly, our results demonstrate that a novel AA-ORS and a traditional G-ORS hydrate better than water, while a traditional SpD does not. Importantly, the AA-ORS contains no sugar and minimal calories, which gives this beverage potential robust application for a number of clinical populations.

We thank Upendra Bhattacharai for his help with the statistical modeling. We sincerely acknowledge and thank the numerous volunteers who participated as subjects in this study, in addition to those who have helped with the ongoing data collection process.

The authors’ responsibilities were as follows—KJS, RWK, and SNC: designed the research and wrote the manuscript; KJS and MT: conducted research; SV: provided essential materials; KJS and SNC: analyzed data; KJS: had primary responsibility for final content; and all authors: read and approved the final manuscript. SV is the Chief Science Officer for Entrinsic Health, Inc. The remaining authors declare no conflicts of interest. The opinions or assertions contained herein are the private views of the authors and should not be construed as official or reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations. Approved for public release: distribution unlimited.

### TABLE 3

BHI values collected during the current investigation and the results from the bootstrap statistical analysis, which resampled the data set 1000 times.

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Original values (n = 40)</th>
<th>Values after 1000 iterations (n = 40,000)</th>
<th>Bias (mean BHI – mean BHIboot)</th>
<th>0.25 SE of BHI mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-ORS</td>
<td>1.1513 ± 0.0439</td>
<td>1.1504 ± 0.0014</td>
<td>0.0009</td>
<td>0.0110</td>
</tr>
<tr>
<td>G-ORS</td>
<td>1.2105 ± 0.0451</td>
<td>1.2099 ± 0.0014</td>
<td>0.0006</td>
<td>0.0113</td>
</tr>
<tr>
<td>SpD</td>
<td>1.0888 ± 0.0416</td>
<td>1.0874 ± 0.0013</td>
<td>0.0014</td>
<td>0.0104</td>
</tr>
</tbody>
</table>

Values are means ± SEs. Decimal extended to 4 places to appreciate the bias threshold (i.e., <0.25 SE). AA-ORS, amino acid–based oral rehydration solution; BHI, beverage hydration index; G-ORS, glucose-based oral rehydration solution; SpD, sports drink.
REFERENCES


