Nutrition and Exercise

COMBINED EFFECTS OF GLUCOSE AND FRUCTOSE ON FLUID ABSORPTION FROM HYPERTONIC CARBOHYDRATE-ELECTROLYTE BEVERAGES

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ABSTRACT

Lambert, GP, Lanspa, SJ, Welch R, Shi, X. Combined Effects of Glucose and Fructose on Fluid Absorption from Hypertonic Carbohydrate-Electrolyte Solutions. JEPonline 2008;11(2):46-55. This study examined the effect of glucose and fructose, compared to glucose alone on water absorption from hypertonic carbohydrate-electrolyte solutions (CES) in the small intestine. Six solutions were perfused into the duodenojejunum and water flux was determined using the segmental perfusion technique. The solutions were: 1) 6% glucose, 2) 3% glucose + 3% fructose, 3) 8% glucose, 4) 4% glucose + 4% fructose, 5) 10% glucose, or 6) 5% glucose + 5% fructose. All solutions also contained 20 mEq sodium, 3 mEq potassium and flavoring/coloring to simulate commonly ingested CES. Water flux was related to osmolality (r = 0.79) and the 6% glucose, 3% glucose + 3% fructose, and 4% glucose + 4% fructose solutions promoted a greater (P < 0.05) water absorption rate than the 8% glucose, 10% glucose and 5% glucose + 5% fructose solutions. These results indicate increasing osmolality negatively affects fluid absorption, and this is attenuated in a moderately hypertonic 8% CES by using both fructose and glucose compared to glucose alone.

Key Words: Intestine, Water, Rehydration
INTRODUCTION

Intestinal fluid absorption rate is a limiting factor to rapid oral rehydration. It has been determined that the primary factors affecting the rate of fluid absorption are the osmolality and substrate content of the rehydration solution (1, 2). Many commonly ingested sport drinks are hypertonic, ranging from 300-600 mOsm (3) with sodas being even higher (i.e., 600-700 mOsm) (4). Such hyperosmolality is mainly a reflection of the carbohydrate (CHO) concentrations used (i.e., usually 6-10% range). These amounts of CHO improve palatability and, in the case of sports drinks, provide working muscles with more exogenous energy than solutions of lower CHO content. However, the rate of fluid absorption from hypertonic CHO-electrolyte solutions (CES) is not normally as great as from isotonic or hypotonic solutions (1), and may result in gastrointestinal symptoms (5), which may be exacerbated with dehydration (6).

Inclusion of two or more transportable substrates (e.g., glucose and fructose) rather than one (i.e., glucose) in isotonic or slightly hypertonic solutions appears to enhance fluid absorption rate (1). This follows what is known regarding the coupling of water absorption to solute absorption in the intestine (7, 8). In terms of sports drinks, the combination of glucose and fructose also appears to enhance carbohydrate oxidation (9). It is not known however whether the enhancement of fluid absorption would hold true for solutions of even greater osmolality, as discussed above. Using indirect measures of water absorption, Jentjens et al. (9) have shown this may be possible during exercise in the heat. Based on this, and the fact that many individuals use hypertonic beverages to rehydrate, the purpose of the present study was to directly determine whether the presence of two monosaccharides, glucose and fructose, would promote a greater water absorption rate, compared to glucose alone, under conditions of increasing CHO concentration and osmolality. We hypothesized that adding fructose, in an equimolar amount to glucose, would produce a greater rate of water absorption from moderately hypertonic solutions compared to solutions only containing glucose.

METHODS

Subjects

Subjects provided written informed consent prior to participation in the study, and both male and female subjects were recruited. Because the segmental perfusion technique involves oral intubation with a multilumen catheter, all subjects were screened for their ability to be intubated comfortably. In addition, a health history and physical examination were conducted by a physician to rule out any contraindications to participation. All procedures and protocols were approved by the Creighton University Institutional Review Board.

Following the screening process, eight healthy individuals (6 males: age 26 ± 2 y; body mass 78.3 ± 2.7 kg; 2 females: age 22 ± 0 y; body mass 66.0 ± 5.9 kg) participated. Two subjects withdrew in the middle of the study after experiencing gastrointestinal symptoms (i.e., nausea, diarrhea) following testing of the 10% CHO solutions (see beverage compositions below). These solutions were subsequently excluded from further study. One subject also withdrew in the middle of the study due to throat discomfort. Overall, 4-6 subjects were tested for each solution. A power analysis from a previous study (10) indicated that four subjects would provide 80% probability for detecting significant effects in water flux, which was our primary variable of interest, so no further subjects were recruited. It should be noted that studies employing the segmental perfusion technique many times must rely on relatively low subject numbers (i.e., 4-6 subjects) (11-15) due to difficulty in recruiting subjects able to comfortably tolerate intubation.
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**Procedures**

Subjects reported to the Creighton University Medical Center at 7:30 a.m. on two experimental days following an overnight fast. Women subjects had a urine pregnancy test performed upon arrival to eliminate the risk of possible radiation exposure from fluoroscopy to a fetus. On a given experimental day, Cetacaine® (topical anesthetic; Cetylite Industries, Inc.) was administered to the throat and a multilumen catheter (Arndorfer, Greendale, WI) was orally passed into the duodenojejunalum, under fluoroscopic guidance, with the infusion port placed ~10 cm distal to the pyloric sphincter. The catheter was constructed with three lumens each terminating with ports either for infusion or sampling of test solution. The proximal sampling site was 10 cm distal from the infusion site and this distance served as the mixing segment (16). The distal sampling site was 40 cm distal from the proximal sampling site and this distance served as the test segment for the study (10), essentially encompassing ~20 cm of duodenum and ~20 cm of jejunum. The tube was weighted at the distal end with tungsten. After tube placement, the first of either two or three test solutions for that day was infused at 15 ml/min for 70 min into the duodenojejunalum via the infusion port of the multilumen tube. The first 30 min served as an equilibration period to achieve steady state conditions (17). Solution order was randomized among subjects. The following six solutions were tested: 1) 6% glucose (333 mM), 2) 3% glucose (167 mM) + 3% fructose (167 mM), 3) 8% glucose (444 mM), 4) 4% glucose (222 mM) + 4% fructose (222 mM), 5) 10% glucose (555 mM), and 6) 5% glucose (278 mM) + 5% fructose (278 mM). All solutions also contained 20 mEq sodium (Na\(^{+}\)), 3 mEq potassium (K\(^{+}\)), flavoring and coloring, and 1 mg/ml polyethylene glycol (PEG; 3350 Da; Miralax®, Braintree Laboratories Inc.; for determination of intestinal water flux). All solutions had a pH of 3.0.

Following a 30-min equilibration period, and at the end of the total 70 min of perfusion for each beverage, a blood sample (10 ml) was collected from an antecubital vein, centrifuged, and the plasma was frozen at -20°C for subsequent measurement of plasma osmolality, Na\(^{+}\), and K\(^{+}\). During the perfusion period, intestinal samples were aspirated at 10-min intervals from the proximal (1 ml/min) and distal (continuous siphoning) ports of the test segment. These samples were also frozen at -20°C and subsequently assayed for PEG, glucose, fructose, osmolality, Na\(^{+}\), and K\(^{+}\). Calculations of water flux were performed based on the formulas of Cooper et al. (16):

\[
\begin{align*}
Q_\text{E} &= I \cdot [\text{PEG}]_i/[\text{PEG}]_p - S_p \\
Q_\text{L} &= Q_\text{E} \cdot [\text{PEG}]_p/[\text{PEG}]_d \\
Q_\text{N} &= Q_\text{L} - Q_\text{E}
\end{align*}
\]

where I is infusion rate (15 ml/min); \(Q_\text{E}\) is entering flow rate; \(Q_\text{L}\) is leaving flow rate; \(Q_\text{N}\) is net movement of water into or out of the intestinal lumen; \(S_p\) is the sampling rate from the proximal sampling site; and \([\text{PEG}]_i\), \([\text{PEG}]_p\), and \([\text{PEG}]_d\) are the PEG concentrations in the infused beverage, at the proximal sampling site, and at the distal sampling site, respectively. Solute flux was determined from PEG concentrations along with the concentration of each solute of interest (i.e., glucose, fructose, Na\(^{+}\), K\(^{+}\)) by the following formula:

\[
S_\text{N} = [\text{solute}]_d \cdot Q_\text{L} - [\text{solute}]_p \cdot Q_\text{E}
\]

where \(S_\text{N}\) is the net movement of solute into or out of the intestinal lumen, \([\text{solute}]_d\) is the solute concentration at the distal sampling site and \([\text{solute}]_p\) is the solute concentration at the proximal sampling site. To determine total electrolyte fluxes, Na\(^{+}\) and K\(^{+}\) fluxes were doubled to account for concurrent anion transport.

PEG concentrations were determined by the turbidometric method (18), CHO concentrations were determined by high performance liquid chromatography (Dionex Corp., Model DX-500; Sunnyvale
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CA), osmolality was determined by freezing point depression (Precision Systems, Model 2430; Natick, MA), and Na\(^+\)/K\(^+\) were determined by flame photometry (Instrumentation Labs, Model IL 943; Lexington, MA).

**Statistical Analyses**
The following dependent variables were analyzed: water flux, CHO flux, Na\(^+\) flux, K\(^+\) flux, total solute flux, solution osmolality, test segment osmolality, plasma osmolality, and plasma Na\(^+\) and K\(^+\) concentrations. Data were analyzed with simple ANOVA and significant differences were identified with Fisher’s Protected Least Significant Difference test. The Pearson \(r\) was computed for determining correlations. The level of significance for all comparisons was set at \(p < 0.05\).

**RESULTS**
Water flux differed significantly \((p < 0.05)\) among solutions (Table 1) and was highly correlated \((r = 0.79)\) to the osmolality in the test segment (Figure 1). Water absorption was significantly increased by the presence of fructose when comparing the 8% CHO solutions. Solutions below \(~370\ \text{mOsm in}\)

<table>
<thead>
<tr>
<th>Solution</th>
<th>Solution Osmolality (mosm/kg H(_2)O)</th>
<th>Test Segment Osmolality (mosm/kg H(_2)O)</th>
<th>Water Flux (ml/h/cm)</th>
<th>CHO Flux (mmol/h/cm)</th>
<th>Total Solute Flux (mmol/h/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% glucose</td>
<td>414 ± 7(^{c,d,e,f})</td>
<td>333 ± 21(^{c,e,f})</td>
<td>-3.2 ± 2.9(^{c,e,f})</td>
<td>-1.8 ± 1.1</td>
<td>-1.5 ± 1.5</td>
</tr>
<tr>
<td>3% glucose + 3% fructose</td>
<td>413 ± 12(^{c,d,e,f})</td>
<td>326 ± 25(^{c,e,f})</td>
<td>-5.1 ± 4.0(^{c,e,f})</td>
<td>-2.1 ± 1.3</td>
<td>-2.0 ± 1.7</td>
</tr>
<tr>
<td>8% glucose</td>
<td>541 ± 61(^{a,b,e,f})</td>
<td>394 ± 12(^{a,b,e})</td>
<td>1.2 ± 2.4(^{a,b,d})</td>
<td>-2.0 ± 3.2</td>
<td>-1.5 ± 3.4</td>
</tr>
<tr>
<td>4% glucose + 4% fructose</td>
<td>540 ± 6(^{a,b,e,f})</td>
<td>362 ± 28(^{a,f})</td>
<td>-2.4 ± 2.9(^{c,e,f})</td>
<td>-3.4 ± 2.4</td>
<td>-3.2 ± 2.4</td>
</tr>
<tr>
<td>10% glucose</td>
<td>633 ± 75(^{a,b,c,d})</td>
<td>445 ± 29(^{a,b,c,d})</td>
<td>4.3 ± 2.5(^{a,b,d})</td>
<td>-3.1 ± 4.8</td>
<td>-2.4 ± 5.0</td>
</tr>
<tr>
<td>5% glucose + 5% fructose</td>
<td>667 ± 21(^{a,b,c,d})</td>
<td>433 ± 58(^{a,b,d})</td>
<td>2.8 ± 0.9(^{a,b,d})</td>
<td>-4.6 ± 5.1</td>
<td>-3.9 ± 5.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Negative values indicate absorption. Superscripts denote significant \((P < 0.05)\) differences: \(a = \) different from 6% glucose beverage; \(b = \) different from 3% glucose/3% fructose beverage; \(c = \) different from 8% glucose beverage; \(d = \) different from 4% glucose/4% fructose beverage; \(e = \) different from 10% glucose beverage; \(f = \) different from 5% glucose/5% fructose beverage.
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the test segment (6% glucose, 3% glucose + 3% fructose, and 4% glucose + 4% fructose) promoted net water absorption and were significantly (p < 0.05) different from those with test segment osmolalities of ~390 mOsm or greater (8% glucose, 10% glucose and 5% glucose + 5% fructose) which promoted net water secretion. This is highlighted in Figure 2.

As indicated in the Methods, the 6%, 8%, and 10% solutions differing in total CHO concentration also differed in initial osmolality. There were no differences in osmolality, however, among solutions with the same CHO concentration. The mean test segment osmolality data (Table 1) indicate that the 6% CHO solutions were significantly different from both 10% CHO solutions and the 8% glucose solution, but were not different from the 4% glucose + 4% fructose solution. This latter solution was also different from both 10% CHO solutions in the test segment.

There were no significant differences among the solutions for CHO or total solute flux (Table 1). Na⁺ flux ranged from 0.3 ± 0.2 to 1.0 ± 0.2 mEq/L (net secretion) and K⁺ flux ranged from 0.21 ± 0.01 to 0.27 ± 0.07 mEq/L (net absorption) among the solutions and there were no significant differences. Figure 3 highlights the results for total solute flux as a function of the CHO concentration of the original solution, and whether glucose or glucose + fructose was present in the solution.

Plasma osmolality remained essentially unchanged during the experiments, ranging from 288-291 mOsm (Table 2). Plasma sodium and potassium values also did not change within or between experiments (Na⁺ range = 138-141 mEq; K⁺ range = 3.9 – 4.5 mEq; Table 2).

DISCUSSION

The primary findings were that: 1) the replacement of 4% glucose with 4% fructose in a hypertonic 8% glucose solution enhanced its absorption, 2) solutions with test segment osmolalities below ~370 mOsm were absorbed faster than those above ~390 mOsm (which produced net water secretion), and 3) water absorption among the solutions tested was highly related to test segment osmolality. These findings follow what is known about the coupling of water absorption to glucose, fructose, and electrolyte absorption across the small intestinal epithelium. As solutes are absorbed, water follows based on the osmotic gradient produced. If greater solute
absorption can be achieved by adding more transportable substrates to a solution, water absorption should also increase, as long as luminal osmolality is not overly compromised. However, the net flux of water is also highly dependent on the luminal osmolality. Specifically, if the luminal osmolality in the test segment exceeds ~400 mOsm, as observed in the present study (Table 1), net secretion will take place in an effort to achieve a favorable osmotic gradient for subsequent water absorption. If luminal osmolality is closer to isotonic (or hypotonic), net water absorption occurs due to solute absorption and a favorable osmotic gradient. In addition, glucose alone enhances water absorption by activating SGLT1 which can act as a water pump, with an estimation of 260 transported water molecules for each glucose molecule absorbed (i.e., 26 grams of water transported for every gram of glucose transported) (19). SGLT1 activation has also been shown to promote water and solute transport via the paracellular pathway by opening tight junctions (20, 21). Thus, glucose alone can stimulate significant water absorption. Since the solutions in the present study all had glucose concentrations (167-555 mM) much greater than the Km for SGLT1 (Km = 28 mM) (20), the addition of fructose, which is transported by a separate mechanism (GLUT-5) could enhance water transport by creating an even greater osmotic gradient. The following discussion will address the effects of osmolality and solute absorption on net water flux in the present study.

**Effect of Osmolality on Water Absorption**

Figure 1 illustrates that test segment osmolality was strongly correlated to water absorption rate in this study. Table 1 and Figure 2 further indicate that solutions with test segment osmolalities below ~370 mOsm (i.e., 6% glucose, 3% glucose + 3% fructose, and 4% glucose + 4% fructose) had a significantly greater water absorption rate than those with osmolalities above ~390 mOsm (i.e., 8% glucose, 10% glucose, and 5% glucose + 5% fructose) which resulted in net water secretion. In addition, those below ~370 mOsm were not different from each other, and those above ~390 mOsm were not different from each other.

The present results are in agreement with most other studies examining absorption in the duodenojejunum. In studies of 6% CHO solutions with test segment osmolalities ranging between 250-350 mOsm, no differences were observed in water absorption (12, 22, 23). In addition, no effect of osmolality on jejunal water absorption was observed when only hypotonic (<290 mOsm) solutions were tested (24). However, as was also shown in the present study, when test segment osmolality is markedly hypertonic, net water secretion occurs (25, 26).

In contrast to the present findings, Ryan et al. (27) observed that an 8% CHO beverage containing glucose, fructose, and maltodextrin, and a mean test segment osmolality of 328 mOsm was not absorbed as rapidly as a 6% CHO solution containing glucose and sucrose and a mean test segment osmolality of 284 mOsm. The reason for the discrepancy between studies is likely due to Na⁺ fluxes.
In the study by Ryan et al. (27), the 6% CHO solution contained 20 mEq Na\(^+\) and promoted sodium absorption whereas the 8% CHO solution had only 5 mEq Na\(^+\) and caused sodium secretion. This secretion of sodium likely caused water secretion and resulted in a lower net water absorption compared to the 6% CHO solution. This discrepancy in findings points out that a number of factors must be considered when comparing water absorption rates of from different studies.

**Table 2. Plasma osmolality, sodium, and potassium concentrations.**

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Plasma Osmolality (mosm/kg H(_2)O)</th>
<th>Plasma Sodium (mEq/L)</th>
<th>Plasma Potassium (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>6% glucose (n = 5)</td>
<td>290 ± 2</td>
<td>288 ± 4</td>
<td>138 ± 4</td>
</tr>
<tr>
<td>3% glucose + 3% fructose (n = 6)</td>
<td>289 ± 3</td>
<td>289 ± 3</td>
<td>139 ± 3</td>
</tr>
<tr>
<td>8% glucose (n = 6)</td>
<td>290 ± 6</td>
<td>289 ± 5</td>
<td>138 ± 2</td>
</tr>
<tr>
<td>4% glucose + 4% fructose (n = 6)</td>
<td>291 ± 6</td>
<td>288 ± 7</td>
<td>139 ± 3</td>
</tr>
<tr>
<td>10% glucose (n = 5)</td>
<td>288 ± 4</td>
<td>288 ± 6</td>
<td>141 ± 2</td>
</tr>
<tr>
<td>5% glucose + 5% fructose (n = 4)</td>
<td>289 ± 8</td>
<td>291 ± 10</td>
<td>140 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SD

Interestingly, some (1, 12, 22, 23, 28-30), but not all (24), human studies have also shown that solutions which are isotonic are absorbed as rapidly (1, 22, 23, 27, 29) or faster (10, 26, 30) than hypotonic solutions or water. Discrepancies among studies examining the effect of osmolality on human intestinal water absorption may reflect the types of CHO used, electrolyte concentrations (as noted above), or the intestinal segment studied. For example, solutions containing oligo- or polysaccharides (i.e., maltodextrins) will have low osmolality prior to ingestion or infusion; however, once the CHO is hydrolyzed in the intestine, solution osmolality increases markedly and can inhibit water absorption (23). Another factor that can make comparing the current results to others is the intestinal test segment studied. In many studies, including the current one, the test segment has included both the duodenum and proximal jejunum (1, 10, 12, 22, 23, 27-29, 31-35), whereas in others only the jejunum has been studied (24, 30). The duodenum is more permeable than the jejunum, so including it in the test segment normally enhances water absorption compared to testing only the jejunum (29). Furthermore, its inclusion is more practical in terms of simulating what would occur if the solution was ingested. Accordingly, it was previously found that water absorption in the duodenum is similar whether an individual drinks the solution or has it infused at a constant rate (33).
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The mechanism allowing for greater water absorption from lower osmolality solutions is related to the osmotic gradient created between the intestinal lumen fluid and the small intestinal villous tissue. Hallback et al. (36, 37) has determined that when the intestinal mucosa is exposed to solutions with different osmolalities, the villous tissue osmolality increases to create a favorable osmotic gradient (up to a point) for water absorption. For example, when exposed to an a glucose-electrolyte solution (320 mOsm), the average human villous tip osmolality is ~700 mOsm (36). This relatively high tissue osmolality is created by an intestinal countercurrent multiplier, and allows for passive water absorption. An osmotic gradient can even be created under hypertonic luminal conditions, such as in the present study. This allows moderately hypertonic solutions to be absorbed. Under the conditions of the present study, it appears that absorption occurs up to a luminal osmolality of ~390 mOsm at which point net secretion then predominates.

Effect of Solute Absorption on Water Absorption

It was hypothesized that the presence of glucose and fructose, rather than glucose alone, would enhance water absorption from a moderately hypertonic CES (e.g., 8% CES). This hypothesis was based on previous direct (segmental perfusion method) (1) and indirect (deuterium oxide method) (9) evidence indicating increased solute absorption, due to the presence of multiple transportable substrates, improves water absorption. Our hypothesis was supported; however, no differences were observed in CHO or total solute flux and because of this the mechanism for this effect is not clear. The likely reason for the lack of significance in CHO flux is due to insufficient statistical power and relatively large variances among solutions. However, we do not feel the variability among subjects was due to gender or age differences as no studies have shown differences between males and females in absorption, and there was no significant difference in ages between the males and females. The power analysis that was conducted for determining sample size in this study was based on water flux and not CHO flux. So, while water flux differences were observed with 4-6 subjects per solution, supporting our hypothesis, the subject number and large variation among subjects precluded observing differences in CHO flux. Based on the present data in CHO flux, 30 subjects would need to be tested to see significant differences. Nevertheless, it appears that a moderately hypertonic CES (i.e., 8% CHO) can benefit from the presence of a second transportable substrate to enhance water absorption.

Net sodium secretion occurred with all solutions in this study, whereas, net potassium absorption concurrently took place. The observed sodium secretion has been reported previously with other hypertonic solutions (1, 27), and likely reflects either: 1) an attempt to bring the hypertonic solution to isotonicity by secretion of chloride and bicarbonate ions which causes electrical drag of sodium, and, thus, water into the lumen (38), or 2) the large plasma-to-lumen concentration gradient for sodium. For example, with regard to the latter case, when a higher concentration of sodium (60 mEq) is perfused, along with a relatively high glucose concentration (140 mM), net sodium absorption (rather than secretion) is observed (39).

Effect of Water Flux Differences on Plasma Variables

Even though both of the 6% CHO solutions and the 4% glucose + 4% fructose solution were absorbed more rapidly in the proximal intestine than the other solutions, this was not detected by changes in plasma measurements (e.g., osmolality). These findings are not surprising as plasma variables are tightly regulated by the kidneys and any deviations from normal are rapidly corrected by appropriate renal responses (e.g., increased free water clearance).
Conclusions
In summary, this study indicates that in healthy adults, water absorption in the duodenojejunum from moderately hypertonic CES (i.e., 8% CHO) can be enhanced by the presence of glucose and fructose compared to glucose alone. Furthermore, CES that produce osmolalities below ~390 mOsm in the proximal intestine are absorbed faster than those above ~390 mOsm, which promote net secretion. From a practical standpoint, individuals wishing to rehydrate rapidly would likely benefit the most from a CES with < 8% CHO that contains both glucose and fructose compared to glucose alone.

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