The Effect of L-Arginine on Absorption of a Glucose-Electrolyte Solution in Humans

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ABSTRACT

Lambert GP, Lang JA, Welch R, Lanspa SJ. Effect of L-Arginine on Absorption of a Glucose-Electrolyte Solution in Humans. JEPonline 2011;14(4):75-86. The purpose of this study was to determine the effect of a low concentration of L-arginine (Arg) on water absorption from an isotonic glucose-electrolyte solution (GES). Six healthy human subjects (mean ± SD age = 23 ± 2 yr) participated. The GES tested were 222 mM glucose (G), 222 mM glucose + 1.5 mM Arg (G + 1.5 Arg), and 222 mM glucose + 3 mM Arg (G + 3 Arg). To control for the effect of glucose on water absorption, a 222 mM fructose + 1.5 mM Arg (F + 1.5 Arg) solution was also tested. Solutions also contained 20 mEq sodium and 3 mEq potassium (osmolalites = ~270 mOsm/kgH₂O). Water absorption was not different among G, G + 1.5 Arg, and G + 3 Arg. The respective medians (ranges) for those solutions were 5.5 (4.1 to 9.7), 6.6 (2.3 to 7.9), and 5.8 (3.9 to 13.6) ml/h/cm. Each of the glucose-containing solutions promoted faster (P=0.05) absorption than F + 1.5 Arg, which had an absorption rate of 0.8 (-1.7 to 3.0) ml/h/cm. These results indicate water absorption from an isotonic GES in healthy humans is not affected by addition of low concentrations of Arg (1.5 to 3 mM).

Key Words: Intestine, Water, Rehydration
INTRODUCTION

For optimal rehydration, fluid replacement solutions must be absorbed quickly from the intestine. A number of studies have examined intestinal absorption of such solutions to better understand what ingredients may be beneficial in this regard. It is now known that osmolality, substrate concentration, and substrate type are likely the most important factors in healthy humans (9,15,18,19,40,41,44). In general, lower osmolality solutions are absorbed most readily in the small intestine (18,19,44), while hypertonic solutions cause initial net secretion in the proximal small intestine before net absorption can occur more distally (18). The addition of multiple transportable substrates to such solutions also enhances water absorption by increasing solute absorption. Carbohydrates (monosaccharides, disaccharides, and glucose polymers) have been studied extensively in this regard (18,41).

Amino acids such as glycine and alanine have also been studied in humans as potential absorption-enhancing substrates for fluid replacement solutions. For example, Hellier et al. (12) found that glycine and alanine enhanced water and sodium absorption from a saline solution. In contrast, and Shi et al. (41) found no stimulatory effect on water absorption by addition of glycine to solutions containing other transportable carbohydrates.

L-arginine (Arg), which is the substrate for the production of nitric oxide (NO) via nitric oxide synthase (NOS), has been found to have either pro-absorptive or pro-secretory effects depending on the concentration (29). Initial experiments in humans (11,12) found that addition of only 5 to 40 mM Arg to an isotonic saline solution caused net secretion into the intestinal lumen. In contrast, Wapnir et al. (45) found in rats that both water and sodium absorption were enhanced by adding lower concentrations (2 to 10 mM) of Arg to an oral rehydration solution (ORS).

Based on the results of the findings by Wapnir et al. (45), further investigation of the effects of low concentrations of Arg seemed warranted in humans. Thus, the purpose of the present study was to determine if low concentrations of Arg could enhance water and sodium absorption from an isotonic glucose-electrolyte solution (GES) in healthy humans utilizing the segmental perfusion technique. It was hypothesized that a solution containing 1.5 to 3.0 mM Arg (similar to that found be efficacious in the rat model; Wapnir et al. 1997) would have significant pro-absorptive effects.

METHODS

Subjects
Six healthy subjects (4 females and 2 males with a mean ± SD age = 23 ± 2 yr) participated in the study. Written informed consent and HIPAA authorization were obtained from the subjects prior to their participation. All research procedures were approved by the Creighton University Institutional Review Board.

Procedures
All subjects underwent a physical examination by a physician and completed a health history questionnaire prior to participation. Subjects were asked to refrain from the use of alcohol and non-steroidal anti-inflammatory medications during the study and to report any illnesses or use of drugs during the study. The subjects were initially screened for the ability to tolerate oral intubation by passing an orogastric tube. It should be noted that a low subject number (i.e., 4 to 6 subjects) (7,8,18,21,22,33) is somewhat common in segmental perfusion studies due to difficulty in recruiting subjects who can tolerate the intubation procedure. In addition, with regard to use of female subjects, there is no evidence that the menstrual cycle affects GI function in healthy, non-pregnant women (1,27).
Experiments were conducted at Creighton University Medical Center. The subjects reported at 7:30 a.m. after an overnight fast on two experimental days. A urine pregnancy test was performed on women subjects upon arrival to eliminate the chance of radiation exposure to a fetus from the fluoroscopy during the intubation procedure. Cetacaine® (topical anesthetic; Cetylite Industries, Inc.) was applied to the throat and a multilumen catheter (Arndorfer, Greendale, WI) was orally inserted into the stomach. The tube was positioned into the duodenojejunum by slow pushing (~1 cm/min) until the infusion port was ~5 to 10 cm distal to the pyloric sphincter. Tube movement and placement were verified intermittently by fluoroscopy. The multilumen catheter contained three lumens with ports either for infusion or sampling of test beverage. One lumen contained the proximal sampling site and was 10 cm distal from the infusion site and the distance from the infusion to proximal sampling sites served as the mixing segment (2). Another lumen contained a distal sampling site that was 40 cm distal to the proximal sampling site. This 40 cm distance served as the test segment for the study, and spanned ~20 cm of the duodenum and ~20 cm of the proximal jejunum (10). The catheter contained a tungsten weight at its distal end to aid in tube placement. A catheter for blood sampling was also placed in an antecubital vein. Infusion (15 mL·min⁻¹) of one of the two test solutions then took place for 70 min with the initial 30 min serving as an equilibration period to achieve steady state absorption rates (46). The order of solutions was randomized and the following four solutions were tested: (a) 222 mM glucose; (b) 222 mM glucose + 1.5 mM Arg; (c) 222 mM glucose + 3.0 mM Arg; and (d) 222 mM fructose + 1.5 mM Arg. The solutions all contained 18.7 ± 1.8 mEq/L sodium, 3.1 ± 0.6 mEq/L potassium, with a mean osmolality of 273 ± 8 mOsm/kgH₂O. These variables did not significantly differ among solutions. Polyethylene glycol 3350 (PEG; Miralax® Brand, Braintree Laboratories Inc.) was also added (1 mg/ml) for determination of intestinal water flux (36). The solution compositions were formulated to be essentially iso-osmotic to reduce the impact that osmolality could have on water absorption. In addition, the fructose-containing solution was used as a control to assess the effects of glucose on water absorption.

During the perfusion period, intestinal samples were aspirated at 10-min intervals from the proximal (1 mL·min⁻¹) and distal (continuous siphoning) ports of the test segment for measurement of PEG, glucose, fructose, osmolality, sodium, and potassium. Calculations of water and solute fluxes were performed based on the formulas of Cooper et al. (2) and as outlined previously [18]. The method of Hyden (16) was used to determine PEG concentrations, high performance liquid chromatography (Dionex Corp., Model DX-500; Sunnyvale, CA) was employed to determine CHO concentrations, freezing point depression (Precision Systems, Model 2430; Natick, MA) was used to determine osmolality, and flame photometry (Instrumentation Labs, Model IL 943; Lexington, MA) was used to determine sodium and potassium concentrations. Nitric oxide concentrations were determined using a Sievers NO analyzer (GE Analytical Instruments, Boulder, CO).

Statistical Analyses
To determine a sample size that would provide adequate statistical power for the current study, a meaningful effect size had to be determined. This was calculated based on what was considered a meaningful difference in water absorption, our primary variable of interest. It was concluded that such a difference in water absorption should be that which would have a significant impact on hydration status over time. For example, in terms of intestinal absorption, an absorption rate difference of 5 ml/h/cm would result in a 250 ml difference in water absorption per hour over the first 50 cm of proximal small intestine. With this in mind, a power analysis was conducted based on data from a previous study in our lab using identical methodology (18) in which a 6% CHO solution was absorbed at a significantly faster rate compared to an 8% CHO solution (i.e., 5.1 ± 4.0 ml/h/cm vs. -1.2 ± 2.4 ml/h/cm, respectively; a difference in absorption rate of 6.3 ml/h/cm; effect size 2.0). The power test
revealed that 6 to 7 subjects would provide 80% power to observe significant differences in water flux, our primary variable of interest.

Data were analyzed for main effects with repeated measures ANOVA (parametric data) or the Friedman test (nonparametric data). Significant differences were identified using Tukey’s post-hoc test (parametric data) or the Wilcoxon test (nonparametric data). The level of significance was set at P=0.05.

RESULTS

Intestinal water flux results are shown in Figure 1. Solute flux results for the different solutions are shown in Table 1. Flux data from only five of the six subjects was obtained for the 222 mM glucose and 222 mM glucose + 1.5 mM Arg solutions, due to technical problems in one subject for these two experiments. The mean substitution method was used to correct for the two missing data points.

Figure 1. Intestinal water flux for the four solutions in the test segment. Values are medians (ranges). Asterisk (*) indicates significant (P < 0.05) difference from all other sources.
The glucose-containing solutions all promoted significantly (P=0.05) greater water absorption than the fructose-containing solution. The addition of Arg at 1.5 and 3.0 mM to the glucose-containing solutions did not significantly enhance water absorption compared to the glucose solution without Arg. There were no differences among solutions in solute fluxes.

Table 1. Glucose, fructose, sodium and potassium fluxes in the intestinal test segment.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Glucose Flux (mmol/h/cm)</th>
<th>Fructose Flux (mmol/h/cm)</th>
<th>Sodium Flux (mEq/h/cm)</th>
<th>Potassium Flux (mEq/h/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>222 mM Glucose</td>
<td>6.2 ± 0.8</td>
<td>---</td>
<td>0.8 ± 0.2</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>222 mM Glucose + 1.5 mM Arg</td>
<td>4.7 ± 1.7</td>
<td>---</td>
<td>0.8 ± 0.1</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>222 mM Glucose + 3.0 mM Arg</td>
<td>5.6 ± 0.7</td>
<td>---</td>
<td>0.9 ± 0.2</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>222 mM Fructose + 1.5 mM Arg</td>
<td>---</td>
<td>4.1 ± 0.8</td>
<td>0.8 ± 0.1</td>
<td>0.08 ± 0.01</td>
</tr>
</tbody>
</table>

N = 6. All values indicate absorption and are mean ±SD.

The mean concentrations of sodium and potassium and the osmolality of the solution in the test segment are presented in Table 2. No differences were noted among solutions for these variables except for a lower mean test segment potassium concentration during experiments with the fructose-containing solution. Test segment osmolality (mean = 279 ± 6 mOsm/kgH₂O for all solutions) increased significantly compared to the original solutions (273 ± 8 mOsm/kgH₂O) as did sodium concentration (45.0 ± 6.8 mEq/L) compared to the original solutions (18.7 ± 1.8 mEq/L) and potassium concentration (4.7 ± 0.7 mEq/L) compared to the original solutions (3.1 ± 0.6 mEq/L).

The results for plasma osmolality, sodium, and potassium are found in Table 3. There were no differences observed in any plasma variables among the solutions. Nitric oxide concentrations in the distal perfusates from the intestinal test segment and in the plasma at the end of each experiment are found in Table 4. The addition of Arg increased NO concentration in the intestinal samples. This effect was significant between the glucose-only solution to the glucose solution containing 3.0 mM Arg and between the glucose-only solution and the fructose solution containing 1.5 mM Arg. No significant differences were found between solutions for plasma NO.
Table 2. Intestinal test segment osmolality, sodium, and potassium concentrations.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Test Segment Osmolality (mOsm/kgH₂O)</th>
<th>Test Segment Sodium (mEq/L)</th>
<th>Test Segment Potassium (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>222 mM Glucose</td>
<td>276 ± 4</td>
<td>43.2 ± 6.3</td>
<td>5.0 ± 0.9</td>
</tr>
<tr>
<td>222 mM Glucose + 1.5 mM Arg</td>
<td>280 ± 6</td>
<td>45.9 ± 7.4</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>222 mM Glucose + 3.0 mM Arg</td>
<td>276 ± 5</td>
<td>44.1 ± 6.2</td>
<td>5.2 ± 1.0</td>
</tr>
<tr>
<td>222 mM Fructose + 1.5 mM Arg</td>
<td>283 ± 10</td>
<td>46.9 ± 7.4</td>
<td>3.4 ± 0.4*</td>
</tr>
</tbody>
</table>

N = 6. All values are mean ± SD. Asterisk (*) indicates significant (P < 0.05) difference from other solutions.

DISCUSSION

Both proabsorptive and prosecretory effects have been attributed to NO production in the intestine (29,37). In animal models, it has been shown that basal NO production is necessary for water and electrolyte absorption (24,38,43). Furthermore, administration of low concentrations of Arg to the intestinal lumen enhances absorption. This was shown in rats by Wapnir et al. (45) at Arg concentrations of 1 to 2 mM, and by Maher et al. (24) in dogs at an Arg concentration of 0.5 mM. In contrast, high levels of NO production caused by disease (43) or administration of higher Arg concentrations (5 to 40 mM) in animals or humans (11,12,28,43) results in secretion. In the present study, we hypothesized that addition of a low concentration (i.e., 1.5 mM) of Arg, similar to that used by Wapnir et al. (45), would enhance water and sodium absorption from a dilute GES in healthy humans. We also studied a slightly higher concentration (3.0 mM) to determine whether any further benefit could be observed, although we did not want to use too high of a concentration as it was previously found that 5 mM Arg induces secretion and GI symptoms (11).

In the present study, we did not observe enhanced water or sodium absorption compared to other studies using low concentrations of Arg. There are several possible reasons for this that may be due to the following experimental differences between studies. For example, previous studies that found an enhancement in absorption with Arg were done in animal models (24,45) whereas our study was conducted with humans. Several studies examined either only the jejunum (45) or ileum (24); whereas, the present study examined the duodenum and proximal jejunum. Previous studies used perfusion solutions dramatically different from the current study (i.e., different glucose concentrations,
osmolalities, and Arg concentrations) (24,45). There is also the concern regarding the lack of statistical power to detect slight differences in absorption rates.

### Table 3. Plasma osmolality, sodium and potassium concentrations.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Plasma Osmolality (mOsm)</th>
<th>Plasma Sodium (mEq)</th>
<th>Plasma Potassium (mEq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>222 mM Glucose</td>
<td>Pre: 287 ± 4 Post: 285 ± 6</td>
<td>Pre: 137 ± 2 Post: 137 ± 3</td>
<td>Pre: 4.3 ± 0.9 Post: 4.1 ± 0.7</td>
</tr>
<tr>
<td>222 mM Glucose + 1.5 mM Arg</td>
<td>Pre: 290 ± 12 Post: 289 ± 11</td>
<td>Pre: 137 ± 3 Post: 137 ± 3</td>
<td>Pre: 4.5 ± 0.9 Post: 4.2 ± 0.6</td>
</tr>
<tr>
<td>222 mM Glucose + 3.0 mM Arg</td>
<td>Pre: 290 ± 8 Post: 291 ± 8</td>
<td>Pre: 139 ± 2 Post: 138 ± 3</td>
<td>Pre: 4.3 ± 1.0 Post: 4.1 ± 0.7</td>
</tr>
<tr>
<td>222 mM Fructose + 1.5 mM Arg</td>
<td>Pre: 289 ± 2 Post: 286 ± 4</td>
<td>Pre: 138 ± 2 Post: 138 ± 2</td>
<td>Pre: 4.4 ± 0.5 Post: 4.3 ± 0.3</td>
</tr>
</tbody>
</table>

N = 6. Values are mean ± SD.

The obvious species difference may have played a large role in the discrepancy in the current findings and those of others. It is unclear whether humans produce NO at similar rates as other animals when Arg is present (i.e., there may be differences in NOS concentrations between species). Although we observed a significant increase in NO concentrations between the glucose solution without Arg and the glucose solution containing 3.0 mM Arg (and the fructose solution containing 1.5 mM Arg) in the intestinal test segment, this did not influence water or sodium absorption rates. Differences in plasma NO did not reach significance, which was likely due to large variability in the data (refer to Table 4). Interestingly, Wapnir et al. (45) also found no significant increase in intestinal NO production with Arg administration up to 20 mM, but they did observe changes in water and sodium absorption.

With regard to the differences among studies in the intestinal segment examined, it is possible that different areas of the small intestine contain different amounts of NOS and, therefore, produce
different amounts of NO when exposed to Arg. This in turn would appear to account for differences in absorption rates between studies.

Table 4. Final intestinal and plasma nitric oxide (NO) concentrations.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Intestinal NO (nM)</th>
<th>Plasma NO (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>222 mM Glucose</td>
<td>1781 (1556-11008)</td>
<td>2434 (1758-11594)</td>
</tr>
<tr>
<td>222 mM Glucose + 1.5 mM Arg</td>
<td>1987 (1863-5456)</td>
<td>4538 (1833-48891)</td>
</tr>
<tr>
<td>222 mM Glucose + 3.0 mM Arg</td>
<td>2171 (2036-25392)*</td>
<td>6059 (1818-48891)</td>
</tr>
<tr>
<td>222 mM Fructose + 1.5 mM Arg</td>
<td>2201 (1796-19749)*</td>
<td>3005 (1758-15683)</td>
</tr>
</tbody>
</table>

N = 6. Values are medians (ranges). Asterisk indicates significant (P=0.05) difference from 222 mM glucose solution.

In regards to the solution differences between the present study and other studies, the solutions may have masked the potential beneficial effects of Arg. The GES was essentially isotonic and contained a higher glucose and lower sodium concentration than the solutions used in previous investigations (24,45). Because glucose enhances water and sodium absorption, the subjects may have been at their near maximal absorption rates for such solutions in humans. However, the results for water absorption (average = ~6.0 ml/h/cm) from the glucose-containing solutions are similar to that observed in other human segmental perfusion studies using glucose-electrolyte solutions with similar perfusion rates (15 ml/min). As an example, Hunt et al. (15) observed an absorption rates of ~5 ml/h/cm from the British National Formulary ORS that contained 200 mM glucose and 35 mM sodium (osmolality = 310 mOsmol/kg H2O) and the WHO-ORS that contained 111 mM glucose and 90 mEq/L sodium (osmolality = 331 mOsmol/kg H2O). The same lab also reported water absorption rates of ~4 to 7 ml/h/cm from solutions varying in glucose concentration between 90 to 111 mM, sodium concentrations between 45 to 90 mEq/L and osmolalities between 210 to 331 mOsmol/kg H2O (14).

With reference to the statistical power of the present study, 6 subjects should have provided greater than 80% power for observing significant differences among solutions when employing a meaningful effect size. The slight differences observed in the present study (e.g., 5.5 vs. 6.6 ml/h/cm between 222 mM glucose and 222 mM glucose + 1.5 mM Arg solutions, respectively) would only amount to an
extra 44 ml of water absorption for the latter solution over the course of an hour in the 40 cm test
segment studied (i.e., the proximal small intestine). Such a difference would likely have very little
physiological significance in most rehydration situations, thus it is reasonable to conclude that the
study has sufficient power to detect meaningful differences.

Water absorption in the present study was significantly lower for the fructose-containing solution.
This agrees with previous findings that fructose does not stimulate water transport as effectively as
glucose (5). The transport of glucose through the sodium-glucose cotransporter (SGLT1) aids water
absorption in two ways that fructose cannot. First, it does so via the “water pump” mechanism (23), by
which water flows through SGLT1 as glucose and sodium are co-transported into the enterocyte.
Second, it opens tight junctions allowing for water absorption via the paracellular pathway (30). Thus,
the use of fructose alone as a carbohydrate in fluid replacement solutions does not appear to be
beneficial for fluid absorption.

The segmental perfusion technique has been used extensively under different conditions by different
investigators to study water and solute absorption in humans (2-4,6,10-12,15,17,20,25,26,31,32,34,
39,41,42,46). It is considered the “gold standard” method for determining net water and solute fluxes
in the human intestine (35). In a reply to a Letter to the Editor in 1997 (13), Wapnir et al. encouraged
further studies on the effects of addition of low concentrations of Arg to oral rehydration solutions in
humans. In the present study, using the segmental perfusion technique in healthy humans, we have
observed that low concentrations of Arg (1 to 3 mM) do not significantly enhance water or sodium
absorption from an isotonic GES.

CONCLUSIONS

The results of the present study indicate that the addition of low concentrations of Arg do not promote
faster water absorption from an isotonic GES compared to solutions not containing Arg. In addition,
this study provides further evidence that fructose does not stimulate water absorption as well as
glucose in an isotonic solution. These findings enhance the knowledge-base with regard to
formulation of fluid replacement solutions for humans during exercise.

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