Gastrointestinal permeability during exercise: effects of aspirin and energy-containing beverages

G. PATRICK LAMBERT, LORENZO J. BROUSSARD, BENJAMIN L. MASON, WILLIAM J. MAUERMANN, AND CARL V. GISOLFI†
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Lambert, G. Patrick, Lorenzo J. Broussard, Benjamin L. Mason, William J. Mauermann, and Carl V. Gisolfi. Gastrointestinal permeability during exercise: effects of aspirin and energy-containing beverages. J Appl Physiol 90: 2075–2080, 2001.—The purpose of this study was to determine whether aspirin (A) ingestion combined with prolonged exercise increases gastrointestinal permeability and whether consumption of a carbohydrate-containing (CHO) or a CHO + glutamine-containing (CHO+G) beverage would reduce this effect. Seventeen subjects completed six experiments. They ingested A (1,300 mg) or placebo (P) pills the evening before and before running 60 min at 70% maximal oxygen uptake. Also, before running they ingested a solution containing 5 g lactulose (L), 5 g sucrose (S), and 2 g rhamnose (R). During each trial, either a 6% CHO beverage, a 6% CHO+G (0.6%; 41 mM) beverage, or a water placebo (WP) was consumed. For 4 h after a run, all urine was collected to measure urinary excretion of L, R, and S. S excretion (percentage of dose ingested; measure of gastroduodenal permeability) was significantly greater (P < 0.05) during the A trial while the subjects drank the WP compared with all other trials. Administration of A also significantly increased L/R (measure of intestinal permeability) for the CHO and WP trials compared with all P trials. Ingestion the CHO or CHO+G beverages significantly reduced S excretion and L excretion when A was administered, but it did not reduce L/R. These results indicate that gastroduodenal and intestinal permeability increase after A ingestion during prolonged running and that ingestion of a CHO beverage attenuates the gastroduodenal effect but not the intestinal effect. Furthermore, addition of G to the CHO beverage provided no additional benefit in reducing gastroduodenal or intestinal permeability.

human; nutrition; diet; fluids; nonsteroidal anti-inflammatory drugs; carbohydrate

MANY ATHLETES USE ASPIRIN (A) or other nonsteroidal anti-inflammatory drugs (NSAIDs) for analgesia. Although successful in managing pain, NSAIDs uncouple oxidative phosphorylation and inhibit cyclooxygenase in the gastrointestinal (GI) mucosa, effectively reducing ATP production and prostaglandin synthesis. It has been hypothesized that this interrupts cytoskeletal integrity and intracellular calcium homeostasis, along with promoting oxygen radical generation, proteolysis, osmotic imbalances, and intercellular tight junction damage. Increased intestinal permeability and inflamed mucosa result (19). This effectively increases the risk of passage of harmful substances (i.e., bacteria, xenobiotics, hydrolytic enzymes, and so forth) into the circulation from the GI tract (19). Prolonged and/or intense exercise has also been shown to increase GI permeability (9, 14) and to exacerbate A-induced GI permeability (16). This effect is likely due to reduced splanchic blood flow (15).

Intestinal permeability, defined as permeation by nonmediated diffusion of molecules >150 Da in size (23), is reduced with glutamine (G) administration in animals with endotoxicosis or on total parenteral nutrition (4, 10). G is a nonessential amino acid and is a primary fuel of the epithelial cells of the human GI tract (24, 25). When present in sufficient quantities, glucose also provides a significant amount of energy to the enterocytes (5). Thus the presence of glucose may also be able to reduce GI permeability if observed changes are related to energy-dependent processes.

The purpose of the present investigation was to determine whether the expected increase in GI permeability caused by prolonged exercise and A ingestion (16) could be reduced by consuming either a carbohydrate (CHO)-containing or a CHO+G-containing beverage. The method of differential urinary excretion of lactulose (L) and rhamnose (R) was used to assess intestinal permeability. L is a large nondigestible disaccharide (342 Da) that can only be absorbed paracellularly (between epithelial cells). R (164 Da) is absorbed both transcellularly (through cells) and paracellularly. The ratio of L to R recovery in urine is a clinical measure of intestinal permeability (1, 23). Urinary sucrose (S) excretion was utilized as a measure of gastroduodenal permeability (11, 21).

METHODS

Subjects. Thirteen men and four women (age 27 ± 1 yr; body mass 71.3 ± 1.8 kg; maximal oxygen uptake (VO2 max) 62.2 ± 2.1 ml·kg−1·min−1) volunteered to participate. All subjects provided signed informed consent before the study.

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and were healthy, recreational runners and cyclists with no history of GI disease and no regular use of NSAIDs. The subjects were asked to refrain from alcohol or NSAID use during the study and to report any illness or use of drugs during this time. All procedures were approved by the University of Iowa Human Use Committee.

Preliminary testing. Before the experiments, $\dot{V}O_{2\text{max}}$ was determined on each subject by using a progressive protocol on a motor-driven treadmill. Before maximal testing, oxygen uptake was determined at three submaximal intensities to allow subsequent determination (linear regression) of the speed or workload required for the experimental trials (70% $\dot{V}O_{2\text{max}}$). Expired gases and ventilation were analyzed with a Q-Plex I metabolic system (Quinton Instruments, Seattle, WA).

Protocol and design. A balanced double-blind design was used to administer pills and solutions for six experiments. All subjects completed all experiments. Either four A (325 mg each; 1,300 mg total) or four placebo (P) tablets were ingested both the night before and immediately before the experiment then followed by running on an exercise treadmill. In addition, one of three determined fluid replacement solutions was consumed during an experiment. The three solutions were 1) CHO [117 mM (4%) sucrose, 111 mM (2%) glucose, 18 meq Na+, and 3 meq K+], 2) CHO+G [formulated exactly the same as solution 1 but containing 41 mM (0.6%) L-glutamine (L-glutamine was added to this mixture <3 h before an experiment to ensure stability in solution)], and 3) WP (a deionized water placebo containing lemon-lime flavoring and aspartame to match the taste of solutions 1 and 2). L-Glutamine did not affect the taste of the CHO beverages in which it was added. The night before each experiment (~12 h prior), subjects ingested the assigned pills with food and then fasted (except water) until the experiment the next morning. In the morning, subjects reported to the laboratory and immediately completed a questionnaire. This consisted of visual analog scales (100-mm lines) to assess GI symptoms such as heartburn, nausea, side ache, diarrhea, and so forth. The subject was asked to place a mark on a line pertaining to their perception, and these were quantitated in terms of percent full scale (i.e., 0% = none, 100% = severe). The subject then voided, a sample was collected, and the volume was recorded. Urine specific gravity was immediately determined by refractometry to verify euhydration (~1.025). The remainder of the sample was frozen at ~20°C for subsequent analysis. A nude body weight and rectal temperature ($T_{\mathrm{rect}}$, clinical thermometer) were obtained, and the subject ingested 6 ml/kg body wt of the assigned fluid replacement beverage. Fifteen minutes after ingestion of the initial bolus, a heart rate (HR) was obtained (Polar Vantage/Favor HR monitors, Polar USA, Stamford, CT) and the probe to test GI permeability was ingested. This probe consisted of 5 g S, and 5 g L plus 2 g R in a 50-ml solution (osmolality ~1,200 mosmol/kgH2O). Immediately on ingestion of the probe, the subject began running on the treadmill for 60 min at the speed calculated to elicit 70% $\dot{V}O_{2\text{max}}$. During the run the subject ingested 3 ml/kg of the fluid replacement beverage every 10 min. Average temperature of the beverages was 5°C. The average ambient conditions in the environmental chamber were 22.4°C and 48.0% relative humidity. At 15-min intervals, the subject reported perceptions of perceived exertion (6–20 Borg scale) and thermal sensation (1–7 scale) and a HR was obtained. If the subject had to urinate during a run, he or she did so into a urine collection bottle which was subsequently used for the collection period after the run. On completion of the 60-min run, $T_{\mathrm{rect}}$ was immediately obtained, a nude body weight obtained, and another GI questionnaire was administered. The subject ingested water to match any weight lost during the run. Over the next 4 h, subjects were asked to drink plenty of water, eat only nonsucrose foods, and void only into the urine container. The urine containers were kept cool during this period by placing ice packs around them in a carrying case or by placing them in a refrigerator. After the 4-h postrun period, a final GI questionnaire was obtained, urine volume was recorded, and samples were frozen at ~20°C for subsequent analysis of S, L, and R.

Analytical procedures. Urine samples were diluted (200 ml urine in 800 µl deionized H2O) and mixed with ion-exchange resin (300 mg Amberlite IRA-400 with 200 mg Amberlite IR-120; ICN Biomedicals, Aurora, OH) for desalting. After centrifugation (5 min at 1,000 g) of the mixture, the supernatant was analyzed for L, R, and S by HPLC (Dionex-500 System, Dionex, Sunnyvale, CA). Percent excretion of R, L, and S (%R excretion, %L excretion, and %S excretion, respectively) in the urine samples was calculated as a percentage of the dose administered (i.e., recovery) by converting the concentration of each sugar in the urine (µmol/l) to a mass (g) (i.e., concentration × volume of urine produced) and then calculating the percentage excreted. The ratio of %L excretion to %R excretion (LR) was then determined. The S content of the CHO beverages was accounted for in calculating S excretion. Percent recovery from our analytical technique was determined by spiking control urine samples with known amounts of each sugar to achieve a concentration similar to that found in our experimental samples (i.e., 25 µM for L and S and 200 µM for R). Mean recovery values were 101, 94, and 124% for R, L, and S, respectively, providing a measure of the accuracy of the HPLC assay.

Statistical analysis. All data were tested for normality by using the Shapiro-Wilk test. Data not normally distributed were transformed (square-root transformations, normal score transformations, and/or rankings) to a normal distribution. Because subjects completed all of the different experiments in each study, it was necessary to account for the correlation among repeated observations on the same subject. The mixed-model ANOVA was used as implemented in the Proc Mixed in SAS (SAS/STAT software). We assumed a constant correlation among observations on the same subject (compound symmetry). The P values for all pairwise comparisons among means were adjusted using the Tukey-Kramer method implemented in Proc Mixed in SAS. All pairwise comparisons are reported for the 5% level of significance. Parallel analyses were run for the raw data and the transformed data. The results were similar; thus the raw data are presented in RESULTS. The GI symptom severity data were analyzed by using the Friedman nonparametric test with the level of significance set at $P < 0.05$.

RESULTS

Percent body weight changes, sweat rates, $T_{\mathrm{rect}}$, and HRs are shown in Table 1. These variables were unaffected by A ingestion or by addition of G to the fluid replacement solutions. More importantly, significant differences ($P < 0.05$) were found among trials in GI permeability. There were no differences between trials in %R excretion in the urine (Fig. 1), indicating GI permeation to small molecules was not affected by the interventions. In contrast, %L excretion (Fig. 1), %S excretion (Fig. 2), and LR (Fig. 2) increased significantly in the WP/A trials compared with all P experiments. The significant increase in LR indicates GI permeation to large molecules increased with A ingestion. Furthermore, the CHO/A and CHO+G/A trials
produced significantly lower percent S and percent L excretion compared with WP/A trial. CHO and CHO\textsubscript{1}G ingestion also reduced (\textasciitilde 30\%) the greater L/R ratio found with A ingestion while the subjects drank the WP, although this effect was not significant (Fig. 2). There were no differences between CHO and CHO\textsubscript{1}G trials for urinary excretion measures in any trials. The visual analog data for GI symptoms were transformed to positive (symptom present) or negative (no symptom) responses for each trial to examine occurrence of each symptom. Pooled data for GI symptom severity and percent occurrence are reported in Table 2. When individual trials were compared, significant differences in GI symptom severity were observed from pre- to postrun for “urge to defecate” in the WP/P and WP/A trials. The severity of nausea and heartburn was significantly greater post-run during the WP/A trial compared with all other trials.

DISCUSSION

The present results indicate that acute A ingestion before prolonged exercise increases gastroduodenal and intestinal permeability as assessed by %S excretion and L/R, respectively. The gastroduodenal effect, but not the intestinal effect, was significantly reduced by ingestion of the CHO-containing beverages (with or without L-glutamine added).

In evaluating these results, it is important to discuss the usefulness of urinary excretion of ingested CHO “probes” for evaluating GI permeability. These probes have been found to be 90\% reliable for the detection of Crohn’s disease (1). The principle of “differential urinary excretion” of test substances (e.g., L and R) was introduced to reduce the effect of other premucosal or postmucosal factors (1), such as gastric emptying, intestinal transit, or renal clearance (13), on urinary excretion results. The usefulness of S as a single gastric permeability probe was found to be reliable in studies by Meddings et al. (11) and Sutherland et al. (21). A possible confounding variable in the present study is that L and R (along with S) may have significantly permeated the gastric mucosa. This could influence the validity of L/R as an exclusive measure of intestinal permeability. It is possible that L/R values actually reflect both gastric and intestinal permeability when barrier function is compromised in both areas simultaneously.

In reference to the clinical relevance of the present findings, it is important to note that this study observed significant increases in GI permeability on an acute basis of combining A with exercise. The A effect during exercise was similar to that seen in a previous study from our laboratory and can cause more than a doubling in intestinal permeability compared with ingestion of A at rest (16). Prolonged use of NSAIDs also results in increased permeability (18), which is likely

<table>
<thead>
<tr>
<th>Condition</th>
<th>Body Mass Change, %</th>
<th>Sweat Rate, l/h</th>
<th>Rectal Temperature, °C</th>
<th>Final Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO/A</td>
<td>0.15±0.08</td>
<td>1.4±0.11</td>
<td>38.4±0.1</td>
<td>152±3</td>
</tr>
<tr>
<td>CHO+G/A</td>
<td>0.08±0.11</td>
<td>1.4±0.09</td>
<td>38.4±0.1</td>
<td>152±3</td>
</tr>
<tr>
<td>WP/A</td>
<td>0.05±0.11</td>
<td>1.4±0.10</td>
<td>38.3±0.1</td>
<td>147±3</td>
</tr>
<tr>
<td>CHO/P</td>
<td>0.03±0.12</td>
<td>1.5±0.09</td>
<td>38.4±0.2</td>
<td>154±3</td>
</tr>
<tr>
<td>CHO+G/P</td>
<td>0.19±0.10</td>
<td>1.3±0.11</td>
<td>38.4±0.1</td>
<td>153±3</td>
</tr>
<tr>
<td>WP/P</td>
<td>0.05±0.13</td>
<td>1.5±0.12</td>
<td>38.3±0.1</td>
<td>151±3</td>
</tr>
</tbody>
</table>

Values are means ± SE; body mass changes all represent gains. CHO, carbohydrate; G, glutamine; WP, water placebo; A, aspirin; P, aspirin placebo; \textsubscript{VO2}\textsubscript{max}, maximal oxygen uptake.
exacerbated by regular exercise (16). Chronic increases in GI permeability could result in an immune response and further GI dysfunction (17). It is feasible that, of the GI complaints of athletes (3, 6, 12), some are related to a chronic combination of NSAID use and exercise. As noted in Table 2, symptoms were reported in a fairly low percentage of the trials, except for “urge to defecate” after the run, in which the symptom was reported in 40% of the trials. Interestingly, severity of nausea and heartburn was greatest (P < 0.05) immediately postrun in the WP/A trial compared with the other trials. This finding corresponds to the significant increase observed in gastric permeability for this condition. As previously stated, this is an acute study on the effect of A ingestion combined with exercise. More long-term studies on GI function and symptoms in athletes who chronically use NSAIDs are warranted.

Intake of A combined with moderate-intensity running (16), intense, prolonged running (14), and extremely prolonged exercise (9) are known to promote increased GI permeability. It has been hypothesized by Ryan et al. (16) that increased GI permeability may be related to the etiology of exertional heat stroke in endurance athletes. The cause for increased GI permeability during prolonged running may be different from that caused by A ingestion, but the effects are likely additive (16). It has long been known that splanchnic blood flow is reduced during strenuous exercise (15), which may result in GI tract ischemia. Splanchnic vasoconstriction has been shown in thermally injured rats, resulting in ischemia and significant bacterial translocation (7). It is believed that ischemia-reperfusion stimulates free radical production, resulting in mucosal damage and increased permeability (20). A, on the other hand, likely damages the GI tract through local biochemical disturbances such as uncoupling of oxidative phosphorylation and electron transport. This results in reduced ATP synthesis, mitochondrial calcium leakage, reactive oxygen species production, altered Na\(^+\)-K\(^+\) and osmotic balance, dilation of intercellular tight junctions, and lower ATP/ADP and ATP/AMP ratios (resulting in compromised protection from free radical damage due to less reducing equivalents). A also reduces prostaglandin synthesis through inhibition of cyclooxygenase. This may reduce reparative capacity (19).

We hypothesized that the addition of L-glutamine to a fluid replacement beverage would reduce GI permeability caused by A ingestion and prolonged exercise. The intestinal epithelial cells rely on G for a large portion of their metabolic energy and obtain it from both the intestinal lumen and the blood. G is a nonessential amino acid that is endogenously obtained through protein metabolism but that is also ingested in the normal diet (24, 25). In the presence of factors known to produce intestinal mucosal damage and in-

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**Table 2. Pooled GI symptom severity and occurrence data**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Prerun</th>
<th>Immediately Postrun</th>
<th>4 h Postrun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side ache</td>
<td>0.4 ± 0.3(2%)</td>
<td>0.4 ± 0.2(5%)</td>
<td>0.2 ± 0.2(1%)</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>1.6 ± 0.9(5%)</td>
<td>2.9 ± 1.3(9%)</td>
<td>2.0 ± 0.7(11%)</td>
</tr>
<tr>
<td>Heartburn</td>
<td>0.6 ± 0.3(3%)</td>
<td>1.5 ± 0.7(4%)</td>
<td>0.9 ± 0.5(4%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>2.6 ± 1.2(6%)</td>
<td>2.0 ± 0.9(6%)</td>
<td>1.4 ± 0.7(5%)</td>
</tr>
<tr>
<td>Urge to defecate</td>
<td>2.8 ± 0.7(12%)</td>
<td>18.0 ± 2.9*(40%)</td>
<td>6.0 ± 1.6(20%)</td>
</tr>
</tbody>
</table>

Severity data are means ± SE; percent occurrence data for all trials are in parentheses. *Significantly different from pre- and 4 h postrun values, P < 0.05.
creased intestinal permeability in animal models, G has been shown to be a beneficial preventative measure (4, 10, 22). Only one study has examined the effects of G on A-induced damage (22). In that study, pylorus-ligated rats were administered high doses of both A and G (100 and 1,000 mg/kg, respectively). Gastric lesions were induced by A but to a lesser extent when A was given with G. The dosages used were much higher than those in the present study (35–40 mg/kg A; ~130 mg/kg G), accounting for both the mucosal lesions produced and the protective effect of G. In the present study we have found that a CHO beverage alone is as protective as a CHO+G beverage against A- and exercise-induced GI permeability. The reason for this may be metabolically based.

As noted above, A interrupts ATP production from oxidative phosphorylation and electron transport. G would require this pathway for oxidation. It was previously believed that G was the major fuel source for enterocytes and that glucose contributed <10% of the energy (24, 25), but more recent evidence indicates that glucose provides 50–60% of the net ATP production when glucose (5 mM) and G (5 mM) are both present (5). NSAIDs are not believed to inhibit glycolysis (8); thus sufficient ATP could be derived anaerobically from glucose during NSAID-induced uncoupling of aerobic metabolism to maintain enterocyte viability. When glucose is present in higher concentrations than G (5 vs. 2 mM, respectively), glucose provides 75% of total ATP production (26). This was possibly the case in the present study. Bjarnason et al. (2) have also shown that indomethacin-induced increases in intestinal permeability can be reduced by ingesting glucose and citrate with the indomethacin. They suggest that when both substrates are present, citrate may inhibit phosphofructokinase and “funnel” glucose into the hexose monophosphate pathway, which would provide reducing equivalents for defense against oxygen radical damage. They alternately propose that these substrates may reverse the inhibitory action of indomethacin, allowing continued ATP production, presumably via a greater mass action effect. Interestingly, these investigators did not see any independent effects of either glucose or citrate, whereas we found a significant benefit in the stomach from the ingestion of only a CHO-containing beverage. This difference is likely due to greater glucose ingestion in our study (~90 g after hydrolysis of sucrose) compared with that of Bjarnason et al. (2), who administered only 750 or 1,125 mg glucose with 50 or 75 mg indomethacin, respectively.

In summary, both gastroduodenal and intestinal permeability were greater during running at 70% VO2 max when A was ingested while the subjects drank the WP. This effect was significantly reduced in the gastric region by consumption of a CHO-containing beverage. Furthermore, consumption of a CHO drink containing G provided no greater benefit than consumption of the CHO beverage alone.

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