Measurements of the gastric emptying rate by use of ultrasonography: studies in humans using bread with added sodium propionate

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ABSTRACT
Background: Foods with a low glycemic index are increasingly being acknowledged as beneficial for individuals with disorders related to the insulin resistance syndrome. The presence of certain salts of organic acids has been shown to lower the glycemic index of bread products and one of the suggested mechanisms is a lowered gastric emptying rate (GER). One obvious pitfall with many of the common techniques for GER measurement is that the food structure, and hence the gastric release of nutrients, may be affected by enclosure of the marker for gastric emptying, eg, paracetamol. Ultrasonography is a noninvasive method for which the above pitfall is to a large extent avoided.

Objective: The main objective was to evaluate the use of ultrasonography to determine whether the lowered glycemic and insulinemic responses to bread ingestion after the addition of sodium propionate are explained by a specific effect of propionate on the GER.

Design: The effect of sodium propionate in bread was evaluated in 9 healthy volunteers. Barley bread products, with or without added sodium propionate, were ingested as breakfast after an overnight fast. The GER was monitored for 2 h by ultrasonography; during this period, capillary blood was withdrawn repeatedly for measurement of blood glucose and insulin.

Results: The GER of the barley bread decreased markedly after the addition of sodium propionate and was accompanied by lowered glycemic and insulinemic responses.

Conclusion: The lowered glycemic response to ingestion of bread with added sodium propionate appears to be related to a lowered GER.

INTRODUCTION
Starchy foods are known to elicit different postprandial blood responses of glucose and insulin. Pasta (1), legumes (2), and products based on whole-cereal grains (3) are slowly digested foods, whereas potatoes (4), most breakfast cereals, and conventional bread products (5) elicit high metabolic responses. The glycemic index (GI) was introduced to classify starchy foods according to their effect on postprandial glycemia (6). The GI is defined as the incremental area under the curve (AUC) for blood glucose after ingestion of a test product as a percentage of the corresponding area for a reference product (glucose or white bread). An insulinemic index can be calculated from the corresponding incremental insulin AUCs.

Accumulating data now suggest that a low-GI diet improves blood glucose control, blood lipid profiles, and fibrinolytic activity (7), suggesting a therapeutic role in the treatment of diseases related to insulin resistance. Epidemiologic studies also imply that such a diet may reduce the risk of type 2 diabetes mellitus (8, 9) and myocardial infarction (10). Consequently, the FAO/WHO strongly advocate increased consumption of low-GI foods (11).

In a study of different bread products, postprandial glucose and insulin responses were lower after ingestion of sourdough bread than after ingestion of a corresponding yeast bread (12). It was suggested that the effect was due to the organic acids formed during sourdough fermentation. In the same study, it was found that a bread with added sodium propionate also lowered blood glucose and insulin concentrations, despite its neutral pH. According to Todesco et al (13), the mechanism whereby sodium propionate affects glucose metabolism is related to the salt acting as an amylase inhibitor. In contrast, in studies by Liljeberg et al (12), there was no effect on the rate of in vitro starch hydrolysis in humans after ingestion of bread containing sodium propionate.

In studies by Liljeberg and Björck (14), the influence of sodium propionate on the gastric emptying rate (GER) was studied by...
using paracetamol as a marker. As judged from a lowered paracetamol concentration in the blood after ingestion of bread with added sodium propionate, it was concluded that the salt reduced the GER (14). Thus, it was suggested that this was the mechanism for the lowered metabolic response to bread with added sodium propionate because there was no evidence of any effects on the rate of starch hydrolysis in in vitro experiments.

An objection to the use of paracetamol as a marker for gastric emptying is that it might affect the properties of the test product. Moreover, the rate of appearance of paracetamol in the blood might also be affected by food factors that interfere with the release or with the rate of absorption of this marker in the upper small intestine. An alternative method for measuring the GER is ultrasonography. The advantages of using this method are that it is noninvasive, it does not affect the product to be studied, and the results obtained are not confounded by other gastrointestinal events.

The aim of this study was to use ultrasonography to study the potential effects of sodium propionate on the GER of bread in healthy subjects. The results might be an important step toward understanding the mechanisms by which salts of organic acids influence the GI of starchy foods.

SUBJECTS AND METHODS

Subjects

Nine (4 women and 5 men) healthy nonsmoking volunteers aged 17–61 y (± SD: 32 ± 15 y) participated in the study. The subjects had a normal (≤27) body mass index (BMI; in kg/m²; 23.6 ± 2.1), were taking no medications, had no symptoms of gastrointestinal disease, and had no history of gastrointestinal surgery (except for appendectomy). On each study day, any gastrointestinal tract symptoms were noted. Patients reporting temporary abnormal defecation such as constipation or diarrhea (15) were examined on another day when the condition had normalized. All subjects gave informed consent before the study began and knew that they could withdraw from the study at any time. The study was approved by the Ethics Committee of the Faculty of Medicine at the University of Lund, Sweden.

Raw materials

Hull-less, intact barley kernels (no. 8775) were provided by Svalöf Weibull AB (Svalöv, Sweden) and commercial white-wheat flour was obtained from Kungsörnen AB (Järna, Sweden). Before being baked, the barley kernels were milled to pass through a 0.8-mm screen. Whole-meal barley bread products were baked with and without the addition of sodium propionate.

Recipes

A whole-meal barley bread was made by mixing 3280 g water, 2960 g whole-meal barley flour, 740 g white-wheat flour, 200 g yeast, 50 g NaCl, 50 g sucrose, and 37 g monooacylglycerols. The dough was proofed for 50 min, divided into 600-g pieces, reproofed for 20 min (38°C, 75% humidity), and then baked at 200°C for 30 min. An identical bread was baked with the addition of 185 g sodium propionate (SIGMA P-1880; Sigma Chemical Co, St Louis). The bread products were stored overnight at room temperature and then sliced. After the crusts of the breads were removed, 3–4 slices were wrapped in aluminum foil, put in plastic bags, and frozen until used.

Chemical analysis

A portion of each bread was air dried and milled (Cyclotech, Tecator, Sweden) to <1.0 mm before the starch content was measured with use of a modified version of Holm et al’s (16) method. Instead of suspending the sample in distilled water before boiling with Ternaryl 300 L DX (12 kilonovo units/sample; Novo-Nordisk A/S, Copenhagen), a phosphate buffer (0.1 mol/L, pH 6) was used to ensure an optimal pH during incubation. The starch content of both bread products was 32% by wt. In addition, the protein (Kjeldahl method) and fat (17) contents of the bread products were measured and were 5.5% and 2.1% by wt, respectively.

Test meals

The test meals consisted of bread providing 50 g available starch (~155 g bread) that was served with 8.8 g butter and 18.9 g cheese. All test meals contained 50 g carbohydrate, 15 g protein, and 12 g fat. On 2 separate occasions the test products were served in random order as breakfast after the subjects had fasted overnight. Water (250 mL) was served with each meal and was ingested gradually during the meal, which lasted ~13 min. When the meal was finished, the subjects were immediately served 150 mL coffee or tea. The tests were run in the same environment at the same time in the morning and all meals, including coffee or tea, were consumed over 15 min. The study lasted 4 mo.

Glucose and insulin measurements

Finger-prick capillary blood samples were taken before the meal (0 min); 20, 35, 50, 70, 95, and 125 min after the start of the meal for measurement of glucose; and 20, 35, 50, 95, and 125 min after the start of the meal for measurement of insulin. Blood glucose concentrations were measured with use of a glucose oxidase peroxidase reagent. All blood samples used for insulin measurements were frozen and analyzed with an enzyme immunoassay kit (Boehringer Mannheim, Mannheim, Germany).

Measurement of gastric emptying rate

The GER was assessed by using real-time ultrasonography (18). The patients were examined with an EBU 400 ultrasonicograph (Hitachi, Tarrytown, NY) with a 3.5-MHz abdominal transducer while in a supine position. Between examinations, the subjects rested seated in a chair. The same ultrasound technician assessed all 9 subjects and the study was double blind. Gastric emptying was monitored indirectly by determining the longitudinal (D₁) and anteroposterior (D₂) cross-sectional diameters of a single section of the gastric antrum. The intragastric volume was assumed to be directly proportional to the cross-sectional area of the gastric antrum (19), which was measured at the longitudinal scan at the level of the abdominal aorta and the left lobe of the liver.

At each observation, 3 measurements were taken using the mean values of the longitudinal (D₁mean) and anteroposterior (D₂mean) diameters to calculate the gastric antral area. At this level, the scan showed the stomach shaped as either a circle or an ellipse; therefore, the gastric antral cross-sectional area (A) was calculated in all subjects by using the formula:

\[ A = \pi \times \text{longitudinal radius} \times \text{anteroposterior radius} \]

\[ A = \pi \times (D_1\text{mean}/2) \times (D_2\text{mean}/2) \]

\[ A = \pi \times (D_1\text{mean} \times D_2\text{mean}/4) \quad (1) \]

Measurements were taken before the meal (0 min) and 15, 30, 75, 105, and 120 min after the meal. The maximum increase in
gastric antral cross-sectional area from fasting to the end of the meal (AA\textsubscript{max}) was calculated as follows:

\[ AA\textsubscript{max} = A\textsubscript{max} - A\textsubscript{f} \]  

(2)

where \( A\textsubscript{max} \) is the antral area computed at the end of the meal (15 min) and \( A\textsubscript{f} \) is the antral area computed at fasting (0 min). The difference between the value observed at each observation (\( A\textsubscript{n} \)) and the one computed during fasting (\( A\textsubscript{f} \)) was then calculated and called AA\textsubscript{n} (AA\textsubscript{n} = A\textsubscript{n} - A\textsubscript{f}). The GER was estimated assuming that AA\textsubscript{max} was 100% (AA\textsubscript{max}/AA\textsubscript{max} \times 100) and then transforming all the values calculated from each observation as a percentage decrease in AA\textsubscript{max} as follows: AA\textsubscript{n}/AA\textsubscript{max} \times 100. The time to half-emptying (\( t_{1/2} \)) was defined as the time when the GER had reached 50%.

Calculations and statistical methods

Postprandial blood glucose and insulin responses

For each subject and type of bread, the glucose and insulin AUCs were calculated by using PRISM (version 2.0; Graph Pad Software, San Diego). The AUCs and mean concentrations of glucose and insulin were analyzed at each time point. Values are presented as means ± SEMs and all statistical calculations were performed by using MINITAB Statistical Software (release 12 for WINDOWS; Minitab, Inc, State College, PA). Significant differences were evaluated with analysis of variance and Student’s \( t \) test. \( P \) values <0.05 were considered significant.

**RESULTS**

Postprandial blood glucose and insulin responses

Ingestion of the bread with added sodium propionate resulted in a significantly lower blood glucose response in the initial postprandial phase (20 min) than did the reference bread without propionate (Figure 1). The AUC (0–20 min) for glucose was significantly lower after ingestion of the bread with sodium propionate than after the reference bread (Table 1). However, the AUCs at 0–35, 0–50, 0–70, and 0–95 min did not differ significantly between the 2 groups. Glucose concentrations peaked 35 min after ingestion of the reference bread and markedly later (after 70 min) after ingestion of the bread with added sodium propionate. Also, comparisons of changes at the intervals 20–50, 50–70, and 70–95 min did not change the results.

Differences in postprandial insulin responses between the bread products were more pronounced (Figure 2). Insulin concentrations were significantly lower 20, 35, and 95 min after ingestion of the bread with added sodium propionate than after ingestion of the reference bread. The incremental insulin AUCs were significantly lower 0–20, 0–35, and 0–50 min after ingestion of the bread with added sodium propionate than after ingestion of the reference bread (Table 2). Insulin concentrations peaked 35 min after ingestion of the reference bread and 95 min after ingestion of the bread with added sodium propionate.

TABLE 1

<table>
<thead>
<tr>
<th>Area under the curve</th>
<th>Bread without sodium propionate</th>
<th>Bread with sodium propionate</th>
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<tbody>
<tr>
<td>mmol·min/L</td>
<td></td>
<td></td>
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<tr>
<td>0–20 min</td>
<td>12.1 ± 2.9</td>
<td>3.8 ± 1.6(^{2})</td>
</tr>
<tr>
<td>0–35 min</td>
<td>37.4 ± 7.3</td>
<td>17.5 ± 6.0</td>
</tr>
<tr>
<td>0–50 min</td>
<td>67.0 ± 11.8</td>
<td>42.3 ± 12.3</td>
</tr>
<tr>
<td>0–70 min</td>
<td>100.8 ± 16.9</td>
<td>80.4 ± 18.5</td>
</tr>
<tr>
<td>0–95 min</td>
<td>131.8 ± 20.9</td>
<td>123.3 ± 23.2</td>
</tr>
</tbody>
</table>

\(^{1} t \pm \) SEM; \( n = 9 \).

\(^{2}\) Significantly different from bread without sodium propionate, \( P < 0.05 \).
TABLE 2

<table>
<thead>
<tr>
<th>Area under the curve</th>
<th>Bread without sodium propionate mmol·min⁻¹/L</th>
<th>Bread with sodium propionate mmol·min⁻¹/L</th>
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<tbody>
<tr>
<td>0–20 min</td>
<td>1.8 ± 0.4</td>
<td>0.6 ± 0.3*</td>
</tr>
<tr>
<td>0–35 min</td>
<td>5.2 ± 0.9</td>
<td>2.1 ± 0.8*</td>
</tr>
<tr>
<td>0–50 min</td>
<td>8.9 ± 1.0</td>
<td>4.7 ± 1.4*</td>
</tr>
<tr>
<td>0–95 min</td>
<td>16.5 ± 1.3</td>
<td>16.1 ± 2.7</td>
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*Significantly different from bread without sodium propionate, P < 0.05.

Gastric emptying rate

The median cross-sectional areas of the gastric antrum were similar before (0 min) and immediately after (15 min) ingestion of the reference bread [257 mm² (range: 235–377 mm²) and 973 mm² (945–1156 mm²), respectively] and the bread with added sodium propionate [259 mm² (198–427 mm²) and 995 mm² (914–1204 mm²), respectively] (Figure 3). However, the median gastric antral cross-sectional areas were significantly larger after ingestion of the bread with added sodium propionate than after ingestion of the reference bread at 30 min [872 mm² (835–1000 mm²) and 766 mm² (652–855 mm²)], 105 min [500 mm² (279–696 mm²) and 355 mm² (276–446 mm²)], and 120 min [424 mm² (220–692 mm²) and 340 mm² (258–361 mm²)]. There were no significant differences between gastric antral cross-sectional areas at 75 min.

The GERs after ingestion of both breads differed significantly. Median AUCs were 47.78%/min (range: 40.05–55.58%/min) after ingestion of the bread with added sodium propionate than after ingestion of the reference bread (276–446 mm²), 105 min [500 mm² (279–696 mm²) and 355 mm² (276–446 mm²)], and 120 min [424 mm² (220–692 mm²) and 340 mm² (258–361 mm²)]. There were no significant differences between gastric antral cross-sectional areas at 75 min.

The GERs after ingestion of both breads differed significantly. Median AUCs were 47.78%/min (range: 40.05–55.58%/min) after ingestion of the reference bread and 60.90%/min (56.33–8978%/min) after ingestion of the bread with added sodium propionate (Figure 4). Also, t₁/₂ values were significantly longer after ingestion of the bread with added sodium propionate (median: 70 min; range: 49–107 min) than after ingestion of the reference bread (54 min; 45–63 min).

DISCUSSION

Because of the increasing incidence of type 2 diabetes and the number of individuals with other metabolic diseases, it is of great importance to identify food factors that have low GIs and insulinemic indexes. One of the major aims of therapy for diabetes is to normalize the blood glucose profile, which can be difficult despite intensified dietary and pharmacologic treatments. Meals containing low-GI products could optimize dietary treatments by provoking a low glycemic response, thereby producing less fluctuation in blood glucose concentrations.

In the current study, postprandial glucose and insulin responses were lower after ingestion of the bread with added sodium propionate than after ingestion of the reference bread. These findings agree with those of previous studies; however, different mechanisms for these metabolic responses to sodium propionate have been postulated. Todescu et al (13) suggested that sodium propionate may act as an amylase inhibitor. Liljeberg et al (12) reported lower GERs after ingestion of bread with added sodium propionate with paracetamol used as a marker.

Scintigraphy is still considered the gold standard for clinical measurement of gastric emptying (20, 21); however, real-time ultrasonography is another well-documented, and in clinical research widely used, method for dynamic imaging of gastric peristaltic activity and its effect on stomach emptying. Good correlation between the results of ultrasonography and scintigraphy were shown in simultaneous studies of gastric emptying (18). Sonographic measurement of gastric antral volume is reproducible and linearly correlated with the amount of ingested or administered liquids; therefore, this measurement effectively represents the entire gastric contents (19). An even easier way to determine gastric emptying time indirectly is by using single-scan measurements of the changes in the cross-sectional area or dimensions of the gastric antral region at one selected level (18).

In the current study, the ability of sodium propionate to lower GERs was confirmed by use of a standardized ultrasound technique that uses changes in the gastric antral cross-sectional area to estimate GERs. One of the most important advantages of ultrasonography is that it is noninvasive. Moreover, this procedure does not involve the use of markers such as paracetamol (14) or radionuclide-labeled technetium (22). Paracetamol mainly reflects the GER of soluble gastric contents. In addition, the rate of appearance of paracetamol in the blood may be affected by the...
presence of viscous dietary fiber or other meal components that interfere with the absorptive process in the upper small intestine. Thus, ultrasonography is a suitable method for measuring GERs and should be evaluated further in nutrition research.

It is known that GERs are influenced by the volume, energy content, and density of the meal and by the particle size of the gastric contents (23); however, other meal factors are likely to influence the luminal receptors of the small intestine, which control gastric emptying. Lower GERs were found in studies in which propionic acid rather than the sodium salt was given to humans (24) and animals (25). It can be assumed that sodium propionate is converted to propionic acid in the human stomach. Similarly to propionic acid, acetic acid was also shown to reduce the GERs of bread when paracetamol was used as the marker (26).

As previously discussed by Liljeberg and Björck (14), different theories have been presented regarding the effects of salts of organic acids on gastric emptying. According to Lin et al (27), a nonspecific acid or pH receptor situated in the proximal part of the small intestine is responsible for the inhibition of gastric emptying. The mechanisms by which sodium propionate affects gastric emptying were not clarified by our study and should be the focus of another study. However, an influence of sodium propionate on gastric secretion is one possible mechanism.

In a recent study by Liljeberg and Björck (14), satiety lasted longer after ingestion of bread with added sodium propionate than after ingestion of a reference bread without sodium propionate. Prolonged postmeal satiety usually occurs concomitant with low GERs because the extension of the stomach is probably one factor that promotes a feeling of satiety. Even though no measurements of satiety were made in the current study, many of the subjects described a feeling of fullness after ingesting the test product, and the trend toward prolonged satiety was also reported by those who ingested bread with added sodium propionate. We conclude that ingestion of bread with added sodium propionate results in lower postprandial glucose and insulin responses than does ingestion of bread without sodium propionate because of the resultant reduction in the GER. The method of adding salts of organic acids to foods could be exploited to produce nonacidic low-GI foods.

REFERENCES