Colonic Fermentation Influences Lower Esophageal Sphincter Function in Gastroesophageal Reflux Disease

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Background & Aims: **Colonic fermentation of carbohydrates is known to influence gastric and esophageal motility in healthy subjects. This study investigated the effects of colonic fermentation induced by oral administration of fructooligosaccharides (FOS) in patients with gastroesophageal reflux disease (GERD).** ***Methods:*** **In the cross-over design used in the study, 9 patients with symptomatic GERD were administered a low-residue diet (i.e., 10 g fiber/day) during 2, 7-day periods, receiving either 6.6 g of FOS or placebo 3 times daily after meals. Each period was separated by a wash out of at least 3 weeks. On day 7, esophageal motility and pH were recorded in fasting conditions and after a test meal containing 6.6 g of FOS or placebo. Breath hydrogen concentrations (reflecting colonic fermentation) and plasma concentrations of glucagon-like peptide 1 (GLP-1), peptide YY, and cholecystokinin were monitored.** ***Results:*** **Compared with placebo, FOS led to a significant increase in the number of transient lower esophageal sphincter relaxations (TLESRs) and reflux episodes, esophageal acid exposure, and the symptom score for GERD. The integrated plasma response of GLP-1 was significantly higher after FOS than placebo.** ***Conclusions:*** **Colonic fermentation of indigestible carbohydrates increases the rate of TLESRs, the number of acid reflux episodes, and the symptoms of GERD. Although different mechanisms are likely to be involved, excess release of GLP-1 may account, at least in part, for these effects.**

The role of dietary and lifestyle factors in the pathogenesis of gastroesophageal reflux disease (GERD) has been a controversial issue for decades. Most studies have focused on the role of energy and lipids, but the results do not indicate that dietary factors play a major role in GERD.[1–4] Surprisingly, the role of carbohydrates has not often been investigated, despite the fact that 2%–20% of carbohydrates escape digestion in the human small intestine.[5] Unabsorbed carbohydrates that reach the cecum are mostly metabolized by the microflora into short-chain fatty acids (SCFAs) and hydrogen. Our previous works concerning healthy volunteers showed that the exposure of the proximal colon to SCFAs contributes to the regulation of gastric motility[6] and lower esophageal sphincter (LES) function.[7] Colonic fermentation of ingested lactulose as well as direct colonic infusion of a mixture of SCFAs, resulted in marked dose-dependent relaxation of the proximal stomach,[6] which in turn triggered transient relaxations of the LES (TLESRs).[8,9] In healthy volunteers, colonic infusion of lactose (a disaccharide frequently malabsorbed in the small intestine) increases the occurrence of postprandial TLESRs, an effect that can be reproduced by colonic infusion of a mixture of SCFAs.

The mechanisms by which colonic fermentation and SCFAs influence LES and gastric motility are unknown, although any of various neuroendocrine peptides could induce a feedback effect. Glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are colocalized in endocrine L cells of the distal intestine and released in plasma after oral ingestion of food or intraintestinal administration of nutrients. Several studies have shown that both GLP-1 and PYY possess inhibitory effects on gastrointestinal motility.[10–14] However, our previous studies found that endogenous release of these peptides was not specifically related to colonic fermentation-induced changes of gastric tone[6] or LES motility.[7] Cholecystokinin (CCK) may also been involved because it plays a major role in the occurrence of meal-induced TLESRs[15] and has been related to the inhibitory effect of GLP-1 on gastric emptying induced by ingested acarbose, a well known α glucosidase inhibitor.[16]

It is still unclear whether LES motility is affected by exposure of the colon to unabsorbed carbohydrates in

Abbreviations used in this paper: CCK, cholecystokinin; FOS, fructooligosaccharides; GERD, gastroesophageal reflux disease; GLP-1, glucagon-like peptide 1; H_2, hydrogen; LES, lower esophageal sphincter; PYY, peptide YY; SCFAs, short chain fatty acids; TLESR, transient lower esophageal sphincter relaxation.

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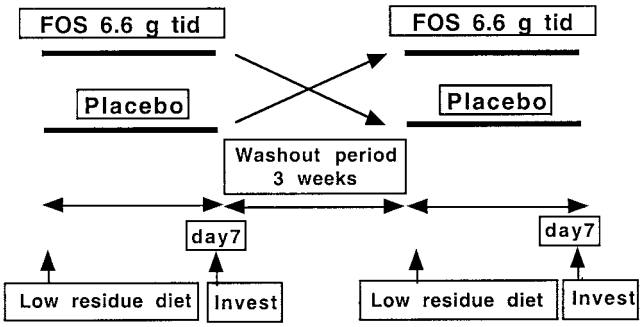

Figure 1. Study design. Invest: investigations (i.e., Dent sleeve manometry and pH monitoring, before and following test meal).

patients with GERD. Fructooligosaccharides (FOS) are a mixture of oligosaccharides consisting of glucose linked to 2, 3, or 4 fructose units with β (1-2) bonds. FOS constitute an interesting undigested carbohydrate model because they are completely fermented in the colon[17] and frequently used in many processed dietary products.

It was hypothesized that sustained activation of colonic fermentation after repeated oral administration of FOS could influence LES motility in patients with symptomatic GERD. The present controlled double-blind cross-over study was designed to investigate LES function (TLESRs), esophageal pH, and the symptoms of patients with GERD in response to activation of colonic fermentation after chronic ingestion of FOS and to explore more thoroughly the role of several intestinal peptides, including GLP-1, PYY, and CCK.

Patients and Methods

Patients

Nine patients (5 men 45.6 ± 5.5 years of age and 4 women 57.7 ± 9.4 years of age) with proven GERD (abnormal esophageal pH monitoring) with (n = 2) or without (n = 7) mucosal breaks at endoscopy were studied on 2 separate occasions. The subjects complained about typical GERD symptoms (e.g., heartburn and/or regurgitation) and were not known to be FOS intolerant. None had hiatal hernia or was taking any medication known to alter esophageal motor function or gastric emptying. All were asymptomatic while on proton pump inhibitors (PPIs) and were studied after discontinuation of drug therapy for at least 10 days prior to the experiments. Written informed consent was obtained from all subjects after the protocol was approved by the local Research Ethics Committee (Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale Numéro 2, Région des Pays de Loire).

Study Design

In the cross-over design used in the study (Figure 1), patients were administered a low-residue diet (i.e., <10 g fibers per day) during 2, 7-day periods, receiving either 6.6 g of FOS or placebo 3 times daily after meals. The 2 periods were separated by a wash out of at least 3 weeks. FOS and placebo were packaged into small sachets to allow easy dispersion in water. FOS (Raftilose P95, ORAFTI, Belgium) consisted of a mixture of oligosaccharides containing mainly oligofructose produced by partial enzymatic hydrolysis of chicory inulin. Placebo consisted exclusively of sucrose with an appropriate formulation and packaging. The subjects were completely blind to FOS or placebo as was one investigator who analyzed LES and pH recordings. Subjects were not informed of the kind of potential side effects that may results from FOS consumption. To check for compliance, paracetamol (250 mg) was added in each FOS and placebo sachet for rapid detection in the plasma of volunteers (Abbott immunoassay). On the last day of each period (day 7), patients were hospitalized after fasting overnight. They then responded to a questionnaire evaluating GERD symptoms during the previous 6 days. A nutritional diary was kept during both periods, and food intake was estimated by a dietitian. Patients previously treated with proton pumps inhibitors had to stop acid suppressors at least 10 days before the experiments.

Subjects were studied in a semirecumbent position after positioning of an antecubital venous catheter for blood sampling. Esophageal motility (Dent sleeve) and pH were recorded simultaneously for 2 hours in fasting conditions and for 5 hours following a standard nonfermentable meal supplemented with 6.6 g of FOS or placebo. The test meal was composed of an egg, 10 g of butter, 2 slices of toast, 1 slice of ham, 100 mL of orange juice, and 100 mL of water supplemented with FOS or placebo. The subjects were asked to eat the meal over a 15-minute period. The total energy content of the test meal was 324 kcal, and it provided 26 g of carbohydrates, 16 g of lipids, and 19 g of proteins. A previous study showed that this meal triggers TLESRs in healthy volunteers.[7] Breath hydrogen (H_2) was monitored to evaluate colonic fermentation. Blood samples for GLP-1, PYY, and CCK were collected before and after the test meal at 15-minute intervals during the first hour and then every 30 minutes for 3 hours (11 samples).

Assessments

LES function. A standard motility catheter fitted with a 6-cm Dent sleeve (Arndorfer Medical Specialties Inc., Milwaukee, WI) was used to monitor esophageal pressures. The assembly was introduced through a nostril and positioned so that pressures could be recorded from the LES (sleeve), fundus (2 cm below the sleeve), esophageal body (side holes 3, 9, and 15 cm proximal to the sleeve), and pharynx (side hole 29 cm proximal to the sleeve to detect swallows). A side hole sensor was used at the proximal margin of the sleeve (3 cm) to monitor distal displacement of the sleeve out of the LES during investigations. The sleeve and side holes (except for the pharynx) were perfused at 0.5 mL/min with distilled water through a low-compliance hydraulic capillary infusion system (Arndorfer Medical Specialties Inc.) driven by a pressure head of nitrogen. The side hole corresponding to the pharynx (e.g.,

swallow) was perfused at 0.15 mL/min to avoid excessive swallowing during experiments. The infusion system was connected to a computer. A software program (version 5.06, Polygram Digestif, Medtronic Synectics, Stockholm, Sweden) served to monitor esophageal pH and LES pressure simultaneously.

TLESRs were defined according to Holloway et al.[18] as (1) a LES relaxation occurring in the absence of a pharyngeal swallow signal for 4 seconds before and 2 seconds after the onset of the LES relaxation, (2) a decrease in LES pressure (LESP) of $\geq$1 mm Hg/s, (3) a time from onset to complete relaxation of 10 seconds, (4) a nadir pressure of 2 mm Hg and, (5) a LESP decrease to 2 mm Hg for >10 seconds (excluding multiple rapid swallows).

pH monitoring. Esophageal pH was monitored using a unipolar antimony electrode (Medtronic Synectics, Stockholm, Sweden) positioned 5 cm above the proximal margin of the sleeve. The electrode was calibrated with pH 1 and pH 7 buffers before and at the end of each session. Signals from the pH electrode were synchronized with pressure signals through a computer for analysis.

Acid reflux episodes were defined as previously published.[7,19] Briefly, reflux episodes were defined as an abrupt decrease of at least 2 pH units for at least 5 seconds or, if pH was already below 4, a further abrupt decrease of at least 1 pH unit for at least 5 seconds. Esophageal acid exposure was defined as the percentage of time below pH 4. Slow downward drifts of pH during several minutes were not scored as reflux episodes or counted in the evaluation of esophageal acid exposure.

The motor mechanisms underlying all reflux episodes were determined in each patient by visual analysis of pH and manometric recordings and then classified into 5 categories: (1) TLESRs, (2) swallow-induced relaxations of the LES, (3) strain, (4) absent LESP, and (5) nonidentified.

H_2 breath test. End expiratory breath samples were collected through a mouthpiece into 2 bags connected with a 3-way valve according to a previously published technique.[20] H_2 concentrations in breath samples were determined with a microlyser gas chromatograph (Quintron Instrument CO. Inc., Milwaukee, WI). Samples were collected at 30-minute intervals for 2 hours and then at 15-minute intervals for 5 hours. Results are expressed as part per million (ppm) of H_2 in exhaled air (1 ppm = approximately 0.05 μmol/L). An increment of 10 ppm of H_2 above the basal excretion sustained was considered as indicative of the arrival of FOS in the cecum and the activation of colonic fermentation.

Hormonal assays. Blood samples were collected into glass tubes containing ethylenediaminetetraacetic acid (EDTA) plus aprotinin, centrifuged at 1200*g* for 6 minutes at 4°C within 10 minutes of venipuncture and then stored at −30°C until assay. For GLP-1 analysis, blood samples were collected into chilled tubes containing EDTA (7.4 mmol/L, final concentration), aprotinin (500 kallikrein inhibitory equivalents/mL blood; NovoNordisk, Bagsvaerd, Denmark), and diprotin A (0.1 mmol/L/final concentration; Bachem, Budendorf, Switzerland) for hormone analysis. Carboxy-terminal GLP-1 immunoreactivity was determined using antiserum 89390,[21] which has an absolute requirement for the intact amidated carboxy terminus of GLP-7 36 amide and cross reacts less than 0.01% with carboxy terminally truncated fragments and 89% with GLP-1 9-36 amide. The intraassay coefficient of variation was <6 %. Plasma samples were extracted with 70% ethanol (vol/vol, final concentration) before assay yielding recoveries of 75%.[22]

The radioimmunoassay of PYY in plasma was performed using antiserum code No. 84122II (a gift from R. Häkanson, Department of Pharmacology, University of Lund, Sweden) raised in synthetic porcine PYY 136 (Peninsula Europe, Merseyside, United Kingdom) as previously described[23] but without conjugation to carrier protein.[24] Preliminary antiserum assays indicated that the antibodies were directed against the N terminus of PYY. The antiserum cross-reacts 100% with human PYY and showed no cross-reactions with human NPY. The detection limit of the assay was below 1 pmol/L, and 50% inhibition was obtained with 23 pmol/L PYY. Recovery of the PYY added to plasma in concentrations of 5 and 50 pmol/L varied less than 15% from expected values. The intraassay coefficient of variation was below 5%.

The radioimmunoassay of CCK was performed as previously described.[25] Briefly, antiserum 67H was obtained in a rabbit after repeated injection of CCK 33 coupled to albumin through 1-ethyl-3 (3-dimethyl-amino-propyl) carbodimide. CCK 10 was radiolabeled with Bolton-Hunter reagent and purified by HPLC. This antiserum was equally sensitive for the displacement by CCK 33, CCK 39, and CCK 9. The cross-reactivity of CCK 8 was 30%, whereas that of nonsulfated CCK 8 or gastrin was less than 1%. An ethanol-extracted plasma system was used for the assay. The integrated response of each peptide was calculated by the area under the curve (AUC).

GERD symptoms. Heartburn and regurgitation were evaluated by a questionnaire previously used by authors in pharmacologic studies but not validated for clinical trials. Each symptom was scored in frequency (0: absent, 1: occasional, 2: often, 3: every day), time of occurrence (0: absent, 1: day, 1: night, 2: night and day), intensity (0: absent, 1: mild alteration of activities, 2: moderate alteration of activities, 3: severe alteration of activities), and duration (0: absent, 1: several minutes, 2: longer than 1 hour). The maximum score for both heartburn and regurgitation was 10.

Statistical Analysis

Results are expressed as means ± SEM. The number of TLESRs and reflux episodes were compared by Student *t* test. Postprandial variations of plasma GLP-1, PYY, CCK, and H_2 concentrations were compared by ANOVA for repeated measurements and integrated hormonal response by Mann Whitney *U* test. Percentages were compared using a contingency table. Statistical analysis was performed with Statview (software package v 4.01, Brain Power Inc., Calabasas, CA). A P value < 0.05 was considered significant.

Results

Compliance and Tolerance

All 9 patients completed the 2 periods of the crossover study. Five patients received placebo first and 4 FOS first without any apparent carryover effect. One complained of mild diarrhea while on FOS, and one experienced bloating. Energy and nutrient intakes (i.e., lipids, carbohydrates, and proteins) did not differ significantly between the 2 periods, although there was a slight nonsignificant trend toward higher energy and lipid consumption during the FOS period. Moreover, paracetamol was detected in the plasma of all subjects enrolled in the study, confirming the good compliance of these GERD patients with respect to FOS or placebo consumption.

TLESRs

FOS, as compared with placebo, produced a significant increase in the total number of TLESRs (16.2 ± 2.3 vs. 25.6 ± 3.2, respectively, $P = 0.006$), and this increase was observed for each individual patient (Figure 2). The rate of TLESRs per hour is shown in Figure 3*A*. In fasting conditions (2 hours), the rate of TLESRs was greater when subjects received FOS (5.6 ± 0.9 vs. 3.4 ± 0.8, respectively, $P = 0.06$), and the difference was statistically significant during the first hour of recording (3.4 ± 0.7 vs. 1.6 ± 0.5, respectively, $P = 0.005$). As expected, the test meal increased the rate of TLESRs, which was significantly higher with FOS than placebo during the first hour after the test meal (5.8 ± 0.6 vs. 3.3 ± 0.4, respectively, $P = 0.02$). The rate of TLESRs then decreased but always remained higher on FOS than placebo. During the fifth hour after the test meal, a significant increase in the rate of TLESRs was observed again after FOS (but not placebo) administration (3.2 ± 0.6 vs. 1.8 ± 0.3, respectively, $P = 0.05$).

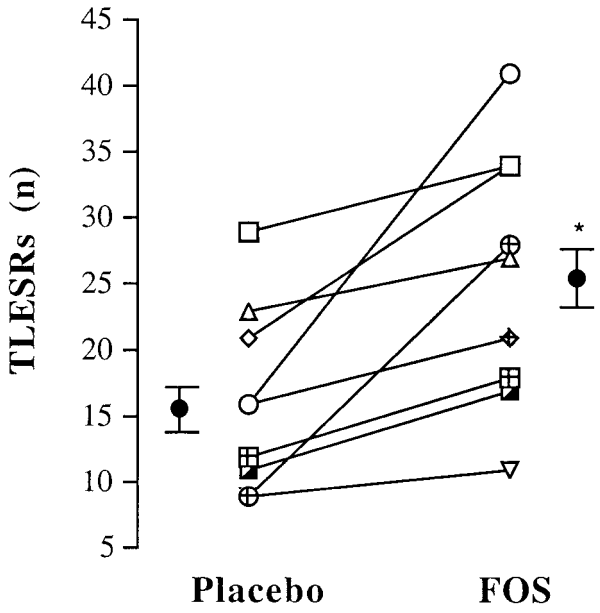

Figure 2. Effect of chronic ingestion of FOS (or placebo) on TLESRs. Values are number and means ± SEM. *$P < 0.05$.

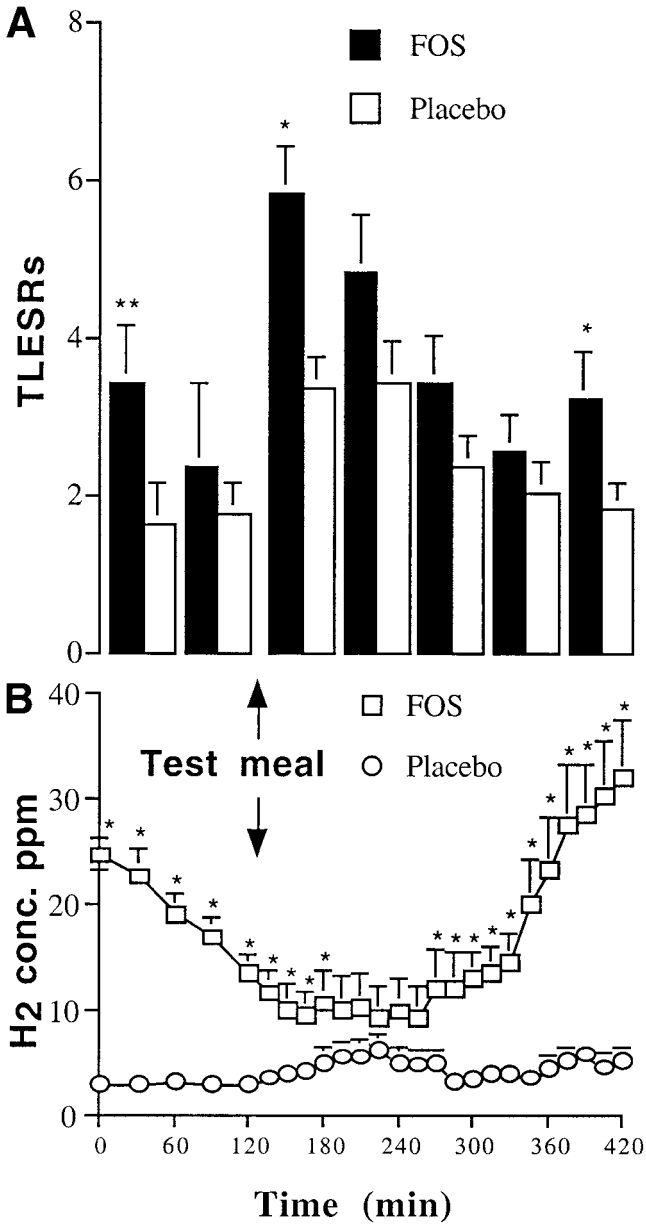

Figure 3. Effect of chronic ingestion of FOS (or placebo) on (*A*) TLESRs per hour and (*B*) hydrogen concentrations in exhaled air. Values are means ± SEM. *$P < 0.05$, **$P < 0.01$.

Figure 3 shows the temporal relation between the rate of TLESRs per hour (Figure 3*A*) and colonic fermentation (as reflected by elevated H_2 concentrations in exhaled air; Figure 3*B*). In fasting conditions, chronic ingestion of FOS (but not placebo) led to a significant increase of H_2 concentrations. H_2 concentrations in-

Table 1. Effect of FOS and Placebo on the Number of Reflux Episodes and Esophageal Acid Exposure

	Placebo (n = 9)	FOS (n = 9)
Number of reflux episodes		
Baseline fasting (per 2 h)	3.3 ± 0.8	4.7 ± 1.1
Postprandial (per 5 h)	23.5 ± 2.2	35.3 ± 5.9[a]
Esophageal acid exposure (% time with pH <4)		
Baseline fasting (per 2 h)	1.1 ± 0.3	1.1 ± 0.6
Postprandial (per 5 h)	5.4 ± 1.3	9.4 ± 2.5[a]

NOTE. Data are means ± SEM.
[a]$P < 0.05$ (FOS vs. placebo).

creased 2 hours after the test meal containing FOS, becoming maximal at the fifth postprandial hour in conjunction with a rise in the TLESRs rate. During the placebo period, H_2 concentrations remained at a low level (i.e., under 10 ppm) throughout the experiments.

GER Episodes and Esophageal Acid Exposure

As expected, most GER episodes occurred during the postprandial period (Table 1). The test meal was followed by an increased number of GER episodes, which were significantly more frequent after FOS than placebo administration (35.3 ± 5.9 vs. 23.5 ± 2.2, respectively, $P = 0.03$). The number of GER episodes was higher in all but one patient during the FOS period (Figure 4). The postprandial esophageal acid exposure was significantly increased when patients received FOS as compared with placebo (9.4% ± 2.5% vs. 5.4% ± 1.3%, $P = 0.04$).

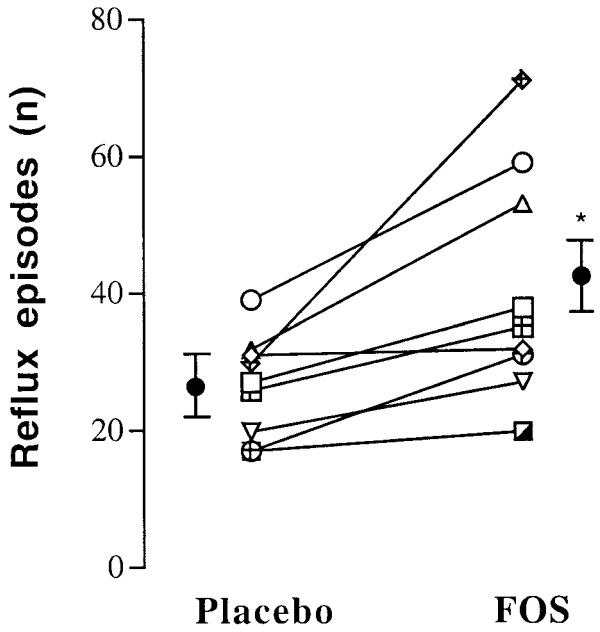

Figure 4. Effect of chronic ingestion of FOS (or placebo) on acid reflux episodes. Individual values and means ± SEM. *$P < 0.05$.

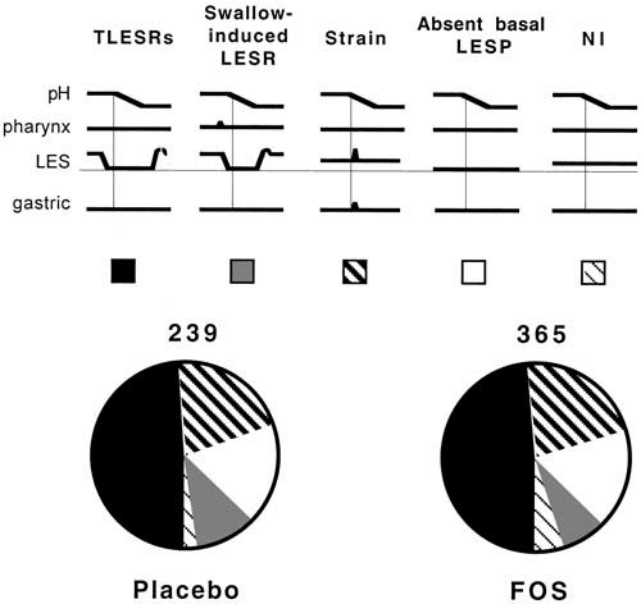

Figure 5. Motor mechanisms associated with the occurrence of reflux episodes. The different patterns of motor events are presented at the top. *Fine horizontal line* in the LES tracing indicates gastric pressure, whereas *vertical lines* indicate the onset of gastroesophageal reflux. Pie chart shows the proportion of reflux episodes associated with each pattern of LES motor event. *Numbers* above each pie represent the total numbers of reflux episodes in all individual patients. NI, not identified.

The proportions of reflux episodes associated with each pattern of LES motor function during placebo and FOS periods are detailed in Figure 5. The motor events associated with reflux in individual patients were not different between FOS and placebo periods. A large proportion of reflux events occurred during TLESRs (48.9% vs. 48.4% after placebo and FOS, respectively, NS), with most of the remainder occurring during absent basal LESP (20.5% vs. 21.6% after placebo and FOS, respectively, NS) and straining (17.5% vs. 17.2% after placebo and FOS, respectively, NS).

GER Symptoms

FOS, as compared with placebo, produced a significant increase in heartburn and regurgitation scores (Figure 6).

GLP-1, CCK, and PYY Plasma Concentrations

Compared with baseline level, GLP-1 concentrations increased postprandially during both the FOS and placebo periods (Figure 7). However, the integrated response for GLP-1 was significantly higher after the meal containing FOS than after that containing placebo. GLP-1 concentrations reached their maximum plateau

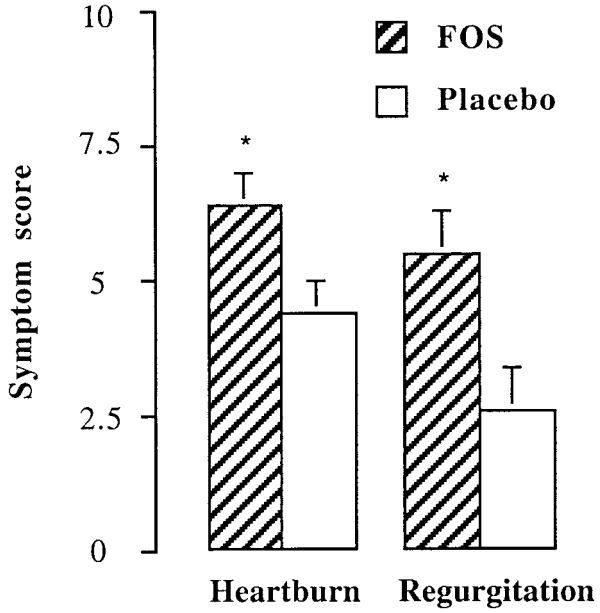

Figure 6. Effect of chronic ingestion of FOS (or placebo) on the symptom score of heartburn and regurgitation. Values are means ± SEM. $*P < 0.05$, $**P < 0.01$.

level during the third hour after the meal and then returned to placebo levels.

No significant changes in plasma PYY concentrations were observed after the test meal, and there was no difference in PYY concentrations between the 2 periods. Plasma concentrations of CCK were significantly increased after the test meal, but the response was not statistically different between FOS and placebo (data not shown).

Discussion

This study performed in patients with symptomatic GERD shows that sustained activation of colonic fermentation induced by chronic ingestion of FOS is accompanied by an increased rate of TLESRs. This effect is associated with a significant increase of acid reflux episodes, esophageal acid exposure, and GERD symptoms.

First, some methodologic issues deserve consideration. In this study, our primary goal was to determine the effects of colonic fermentation on LES motility, especially TLESRs, in patients with symptomatic GERD. However, an attempt was made to select patients with relatively mild disease (i.e., mild esophagitis and no large hiatus hernia). Indeed, it has recently been shown that excess reflux in GERD patients with hiatal hernia is caused by other mechanisms than TLESRs.[26] Similarly, the proportion of spontaneous reflux related to low resting LESP was found to be correlated with the severity of esophagitis.[27]

FOS were regarded as a suitable physiologic model for testing the influence of chronic ingestion of an unabsorbed oligosaccharide on LES function. In fact, FOS are found in a large variety of food products, including onions, garlic, salsify, leek, and asparagus root. They are also produced in a commercial scale either by hydrolysis of inulin or by enzymatic synthesis from sucrose or lactose. Because of their physicochemical properties and sweetening power, FOS are mainly consumed in pastry, confectionery, and diary products. Our previous work showed that FOS are completely fermented in the human colon[17] and do not modify glucose plasma levels after oral ingestion.[28] Breath H_2 studies in humans have also shown that the same doses of FOS and lactulose result in similar H_2 concentrations when given orally.[29] In the present study, a dose of 20 g of FOS was chosen with reference to our previous studies in which ingestion of 20 g of lactulose led to a significant decrease of gastric tone, an effect that was reproduced by colonic infusion of the same dose of lactose or a mixture of SCFAs (90 mmol).[6] A significant increase of the rate of TLESRs was also observed postprandially after the colonic infusion of SCFAs in the colon of healthy volunteers.[7] Moreover, FOS at this dose were usually well tolerated in healthy subjects, who showed no gastrointestinal symptoms after an 11-day oral load.[17] Thus, it was assumed that chronic administration of 20 g of FOS per day would be physiologically relevant and well tolerated by subjects in the present study. Actually, only 2 subjects experienced mild

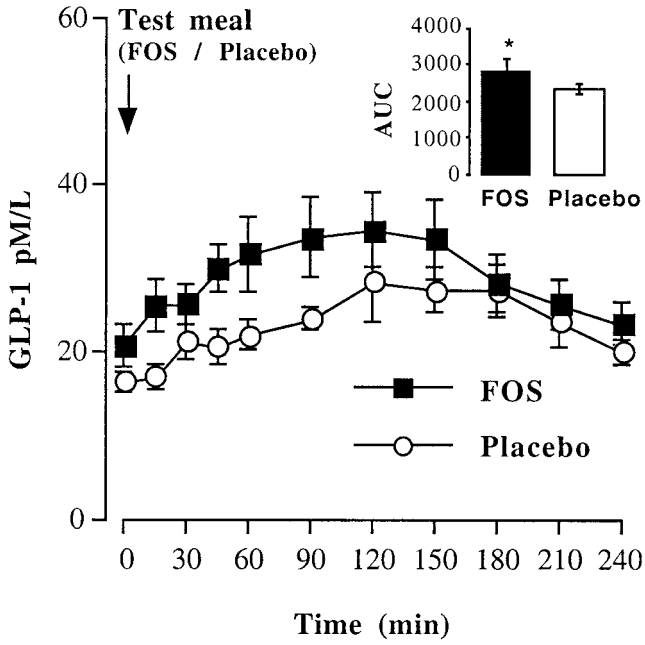

Figure 7. Effects of chronic ingestion of FOS (or placebo) on GLP-1 plasma levels (mean values ± SEM) and integrated plasma response (AUC, pM/L × min). $*P = 0.04$.

diarrhea and bloating while on FOS. Excluding these 2 subjects from statistical analysis did not change the result because TLESRs increased in all individual patients after FOS consumption (Figure 2).

Nutrients in the small bowel can regulate gastric tone and LES function by mechanisms that are partly nutrient specific. For instance, fat in the duodenum exerts an inhibitory effect on gastric tone,[30,31] whereas proteins have little influence and carbohydrates have no effect.[30] Fatty foods may also affect LES motility[32,33] and the perception of heartburn,[34] although controversial results have been reported in this respect. Surprisingly, there appear to be no data in the literature concerning the effects of indigestible carbohydrates on LES motility in GERD patients.

The design of the present study involved 2, 7-day periods during which patients were instructed to eat a low-residue diet (i.e., $<$10 g fiber/day) to allow standardization of the regimen between the 2 periods (the only difference related to FOS supplementation). The main result was that FOS-induced colonic fermentation was accompanied by an increased rate of TLESRs both in fasting conditions and postprandially. This increased rate was observed in association with an increased number of reflux episodes, longer acid exposure, and more severe or frequent GERD symptoms. In contrast, the different motor mechanisms associated with reflux episodes were not affected by FOS-induced colonic fermentation (Figure 5). The temporal relation between H_2 concentrations and TLESRs was not perfect but only indicative of a causal effect between FOS fermentation and changes in LES motility. This is not really surprising because H_2 is unlikely to be the direct stimulus of motility changes. Although H_2 breath test was primarily used to document the colonic fermentation of FOS, the change in the rate of TLESRs is highly consistent with our previous results obtained in healthy volunteers and reproduced by direct colonic infusion of lactose or SCFAs, the main end products of colonic fermentation.[7]

Although the mechanisms through which colonic fermentation influences LES motility remain unclear, our data suggest that humoral pathways are involved in the observed changes in LES motility. Some regulatory peptides such as GLP-1 and PYY, which are colocalized in the endocrine L cell of the distal intestine, have been proposed as ileocolonic mediators of upper gastrointestinal inhibition under several conditions.[10,35–38] However, conflicting results have been published concerning the effects of distal gut hormones on upper gastrointestinal function. In our previous studies performed on healthy subjects, no significant relation was found between the endogenous release of these peptides in response to colonic infusions of SCFAs and the regulation of gastric tone[6] or LES function.[7] However, the present study performed in GERD patients showed that a marked elevation of GLP-1 occurred after the test meal, and this response was significantly more pronounced after FOS than placebo. It is also noteworthy that an oral meal is a more physiologic stimulus than colonic infusions of nutrients to release GLP-1 from the gut. The effect of GLP-1 on LES motility could be either direct or indirect, especially through an effect on proximal stomach (i.e., gastric relaxation). Indeed, GLP-1 is a physiologic inhibitory regulator of gastric emptying and fundus motility,[13,16] which in turn plays a major role in triggering TLESRs.[8,9] Although CCK can also influence LES function through the fundic relaxation induced by a meal,[8] our data suggest that this peptide has no role in regulating LES motility in response to the activation of colonic fermentation. After the test meal, CCK concentrations were similarly elevated during the FOS and placebo periods, and the same was true for PYY, confirming our previous data.[6,7] Some data also suggest that a neural pathway cannot be excluded. It is indeed well established that fibers contained in the vagus nerves are involved both in the occurrence of TLESRs[39] and in the gastric relaxation induced by intestinal nutrients[40] or distention.[41] Finally, it is possible to speculate that the sustained fermentation induced by FOS might have influenced the responsiveness of neurons to other physiologic stimuli (e.g., the gastric distention induced by the meal or the early release of GLP-1; Figure 7), an interpretation more in agreement with the kinetics of H_2 concentrations (Figure 3*B*).

In summary, in patients with symptomatic GERD, sustained activation of colonic fermentation by chronic ingestion of FOS increases significantly the rate of TLESRs. This effect is associated with an increased number of acid reflux episodes and symptoms. Although different mechanisms are likely to be involved in these effects, excess release of GLP-1 in patients with GERD may account at least in part for this effect. The therapeutic relevance of these physiologic observations remains to be assessed by clinical trials.

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