Inhibition of human pancreatic and biliary output but not intestinal motility by physiological intraileal lipid loads

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Keller, Jutta, Jens Juul Holst, and Peter Layer. Inhibition of human pancreatic and biliary output but not intestinal motility by physiological intraileal lipid loads. Am J Physiol Gastrointest Liver Physiol 290: G704–G709, 2006. First published December 1, 2005; doi:10.1152/ajpgi.00411.2005.—Lipid perfusion into the distal ileal lumen at supraphysiological loads inhibits pancreatic exocrine secretion and gastrointestinal motility in humans. In the present study, we sought to determine the effects of physiological postprandial intraileal lipid concentrations on endogenously stimulated pancreatobiliary secretion, intestinal motility, and release of regulatory mediators. Eight healthy volunteers were intubated with an oroileal multilumen tube for continuous duodenal perfusion of essential amino acids (450 μmol/min), ileal perfusion of graded doses of lipids (0, 50 and 100 mg/ml, each dose for 90–120 min), aspiration of duodenal and ileal chyme, and intestinal manometry. Venous blood samples were obtained for measurement of GLP-1 and PYY. Ileal lipid perfusion dose dependently decreased endogenously stimulated trypsin (262 ± 59 vs. 154 ± 42 vs. 92 ± 20 U/min (P < 0.05)) and bile acid output (18.6 ± 1.9 vs. 8.4 ± 2.8 vs. 3.0 ± 1.0 μmol/min (P < 0.05)). Duodenal motor activity was not inhibited by either lipid dose. Trypsin and bile acid output correlated inversely with the release of GLP-1 and PYY (absolute value of R > 0.84; P < 0.05), whereas the motility index did not. Physiological postprandial ileal lipid concentrations dose dependently inhibited human digestive pancreatic protease and bile acid output, but not intestinal motor activity. Thus physiological postprandial ileal nutrient exposure may be of importance for the termination of digestive secretory responses. Ileocolic release of GLP-1 and PYY appears to participate in mediating these effects.

INTRAILEAL NUTRIENTS HAVE BEEN SHOWN TO EXERT MARKED HIBERNATORY EFFECTS ON HUMAN GASTROINTESTINAL SECRETORY AND MOTOR FUNCTIONS. HOWEVER, MOST STUDIES HAVE CONCENTRATED ON THE EFFECTS OF ILEAL NUTRIENTS ON GASTROINTESTINAL MOTILITY (9, 20, 30, 31, 34), WHEREAS FEW INVESTIGATORS HAVE STUDIED THE EFFECTS OF ILEAL NUTRIENT EXPOSURE ON GASTROINTESTINAL SECRETORY FUNCTIONS, I.E., THE INHIBITION OF GLP-1 AND PYY (8, 23, 38) AND PANCREATIC EXOCRINE SECRETION (24). AS WE HAVE SHOWN PREVIOUSLY, BOLUS APPLICATION OF HIGH INTRAILEAL CARBOHYDRATE AND LIPID LOADS INDUCES PHASE III MOTILITY AND MARKEDLY INHIBITS PANCREATIC ENZYME SECRETION DURING CONTINUOUS ENDOGENOUS STIMULATION BY DUODENAL PERFUSION OF ESSENTIAL AMINO ACIDS (24). HOWEVER, WE HAVE DEMONSTRATED THAT IN HEALTHY HUMANS, DURATIONS OF BOTH PANCREATIC SECRETORY AND INTESTINAL MOTOR RESPONSES TO A MEAL ARE ASSOCIATED WITH THE LATE POSTPRANDIAL RELATIVE INCREASE IN ILEAL NUTRIENT DELIVERY (19). THEREFORE, ILEAL NUTRIENT EXPOSURE MAY CONTRIBUTE TO THE REGULATION OF THE TRANSITION FROM THE FED TO THE SUBSEQUENT FASTING STATE. FOR FURTHER CLARIFICATION OF THIS HYPOTHESIS, THE EFFECTS OF PROLONGED EXPERIMENTAL ILEAL NUTRIENT PERFUSION WITH NUTRIENT DOSES INDUCING PHYSIOLOGICAL POSTPRANDIAL ILEAL NUTRIENT CONCENTRATIONS ON DIGESTIVE PANCREATIC EXOCRINE FUNCTIONS NEED TO BE TESTED.

Moreover, there is evidence derived from animal experiments that ileal nutrients inhibit not only pancreatic but also biliary secretion (35). The effects of ileal nutrients on human biliary secretion are unknown. Because duodenal bile acids appear to contribute to the regulation of pancreatic enzyme output (21), the reduction of biliary secretion in response to ileal nutrients might also influence pancreatic exocrine secretion.

Ileal nutrient exposure induces the release of neurohormonal mediators, i.e., glucagon-like peptide (GLP)-1 and peptide YY (PYY), which inhibit gastric acid secretion (23), gastric emptying (27, 30), pancreatic exocrine secretion (12, 17, 39), and small intestinal transit. Whether the release of these mediators also influences biliary secretion is unclear.

Thus our aims in conducting the present study were twofold: 1) to investigate the effects of physiological ileal nutrient concentrations on digestive pancreatobiliary secretion and intestinal motility, and 2) to investigate the release of important regulatory mediators in response to physiological ileal nutrient concentrations and their association with pancreatobiliary and intestinal motor functions. To achieve these goals, we intubated healthy human volunteers with an oroileal multilumen tube to allow duodenal and ileal nutrient and marker perfusion, aspiration of duodenal and ileal chyme, and intestinal manometry. All subjects were administered ileal perfusion of graded lipid doses during continuous endogenous stimulation of digestive responses by duodenal perfusion of essential amino acids. Moreover, plasma levels of potential neurohormonal mediators, i.e., GLP-1 and PYY, were determined.

METHODS

Human subjects. Our study protocol was approved by the Ethical Committee of the Univ. of Essen, Germany, where the experimental part of this study was performed. After providing their written informed consent, eight healthy individuals (4 females; age range, 23–39 yr) participated in this study.

Tubes and motility recordings. Subjects were intubated with a 14-lumen oroileal tube and a double-lumen gastric tube after fasting overnight. The tip of the oroileal tube was placed in the terminal ileum (240 cm from the mouth). In this position, a port for continuous perfusion of a solution containing essential amino acids (150 μmol/ml) and polyethylene glycol (PEG; 15 g/l, 3 ml/min) was located at
the papilla of Vater, and aspiration ports were in the duodenum (proximal to the ligament of Treitz) and the terminal ileum. The port for ileal perfusion of control and test substances was located 35 cm proximal to the ileal aspiration port. The tip of the gastric tube was placed into the antrum. The correct position of both tubes was verified fluoroscopically before the start and after the end of each study. Moreover, continuous recording of antral and small intestinal motility via manometry ports (2) allowed us to identify major dislocation of the oroileal tube during study procedures. A venous catheter was placed into a forearm vein so that we could collect blood samples.

**Experimental protocol.** After ensuring correct tube positioning, complete aspiration of gastric juice and constant duodenal perfusion of a solution containing essential amino acids (450 μmol/min) for stimulation of digestive pancreaticobiliary responses andPEG (45 mg/min, 3 ml/min), a nonabsorbable marker substance, were started. After 1 h of equilibration, the terminal ileum also was perfused with graded lipid doses. To obtain basal values, the ileum was first perfused with 0.9% saline at 1 ml/min for 90 min, followed by ileal lipid perfusion at 50 mg/min (12.5 mg of oleate and 37.5 mg of triglycerides) and then 100 mg/min (25 mg of oleate and 75 mg of triglycerides). Each lipid dose was administered for 2 h. Subsequently, another 2-h ileal saline perfusion was performed. Doses and compositions of the lipid emulsion used were chosen to mimic physiological postprandial ileal lipid exposure (4, 10, 15). All ileal perfusates contained 100 mg/ml phenolsulfonphthalein (PSP) as a marker substance. Throughout experimental procedures, gastric, duodenal, and ileal chyme were collected by aspiration into vials that were immersed in ice at 15-min intervals. Gastric juice was aspirated as thoroughly as possible to prevent acidic inactivation of pancreatic enzyme activities and to exclude salivary amylase and gastric lipase, which otherwise might account for ~15% of duodenal amylase and lipase activity, respectively (18). Venous blood samples were obtained at 60-min intervals for measurement of GLP-1 and PYY.

**Chemical and motility analyses.** Lipid concentrations of ileal chyme as well as pancreatic enzyme activities and bile acid concentrations in duodenal chyme were measured using routine analytical procedures (16, 26, 37). Concentrations of the marker substancesPEG and PSP were measured and used to calculate recovery and intraluminal volume flow rates per minute as described earlier (13).

Mean values of enzyme and bile acid output during the second hour of perfusion of each lipid dose and of the final saline perfusion were used for statistical comparison (unless otherwise stated), because stable intraluminal nutrient concentrations were supposed to be attained during this period. Basal digestive enzyme and bile acid output were defined as output measured during the initial ileal saline perfusion.

The tube included eight motility ports. After correct tube positioning, two recording ports were located in the antrum, one was positioned in the distal duodenum, three were located in the proximal jejunum, and two were placed in the distal ileum. To measure small intestinal motility, all ports were connected to a low-compliance perfusion system and constantly perfused with deionized water (2). The voltage output of each calibrated pressure transducer was amplified and automatically recorded using an eight-channel recorder. Motility recordings allowed visual identification of the fed pattern and amplified and automatically recorded using an eight-channel recorder. Motility recordings allowed visual identification of the fed pattern and were graded at 5-min intervals, and the frequency (F) and mean amplitude (MA) of contractions within each interval were determined. These values were used to calculate a motility index [MI = ln(F/MA5 + 1)] as described earlier (22).

**Results**

**Intraileal lipid concentrations.** Graded ileal lipid perfusion increased intraileal lipid concentrations dose dependently into a range between 3.5 and 7 mg/ml (P < 0.05) (Table 1).

**Pancreaticobiliary secretions.** Duodenal perfusion of essential amino acids induced digestive pancreatic and biliary responses during ileal perfusion of saline. Graded intraluminal lipid perfusion markedly and dose dependently decreased digestive trypsin and bile acid output (P ≤ 0.05) (Table 1 and Fig. 1). The decrease in trypsin and bile acid output was correlated inversely with intraluminal lipid concentrations (Fig. 2). The inhibitory effects of ileal lipids on bile acid output were stronger and lasted longer than their effects on pancreatic enzyme output (Fig. 1); that is, trypsin output was decreased to 46.1 ± 11.6% of basal digestive output in response to the higher lipid dose, whereas bile acid output was decreased to 18.3 ± 7.7% of basal level (P = 0.035 vs. trypsin). During the final ileal saline perfusion, trypsin output returned rapidly to basal levels (81.8 ± 14.8% of basal; P = 0.142 vs. basal). By contrast, bile acid output remained markedly decreased throughout the final ileal saline perfusion period (21.6 ± 4.1% of basal; P < 0.0001 vs. basal) (Fig. 1).

**Gastrointestinal motility.** Duodenal perfusion of essential amino acids induced a fed motor pattern in all subjects during ileal saline perfusion. Ileal lipid perfusion at the lower dose 1-36 and PYY 3-36. Synthetic human PYY 1-36 (Peninsula, Merseyside, UK) was used for standards, and porcine 125I-PYY (no. IM259) was purchased from Amersham Biosciences (Little Chalfont, UK). The detection limit of the assay was <2 pmol/l, and 50% inhibition was obtained with 40 pmol/l PYY. Recovery of PYY added to plasma in concentrations between 5 and 50 pmol/l deviated <15% from expected values. The intraassay coefficient of variation was <5%. The antisera showed no cross reaction with human NPY or human PP in concentrations up to 500 pmol/l. GLP-1 concentrations in plasma were measured using RIA after extraction of plasma with 70% ethanol (vol/vol) final concentration. Carboxy-terminal GLP-1 immunoreactivity was determined using antisera 89390, which has an absolute requirement for the intact amidated carboxy terminus of GLP-1 7-36 amide and cross reacts <0.1% with carboxy-terminally truncated fragments and 89% with GLP-1 9-36 amide. Sensitivity was <5 pmol/l, and intraassay coefficient of variation was <10% (28).

**Statistical analysis.** ANOVA, paired t-tests, and linear regression analysis were used for statistical analysis as appropriate (5). Data are expressed as means ± SE unless indicated otherwise.

**Table 1. Effects of graded ileal lipid perfusion on pancreaticobiliary secretion, intestinal motility, and release of regulatory mediators**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 mg/min</th>
<th>50 mg/min</th>
<th>100 mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileal lipid concentration (mg/ml)</td>
<td>0.52±0.32</td>
<td>3.46±0.94*</td>
<td>6.96±2.40*</td>
</tr>
<tr>
<td>Trypsin, U/min</td>
<td>262±59</td>
<td>154±42*</td>
<td>92±20*</td>
</tr>
<tr>
<td>Bile acids, μmol/min</td>
<td>18.6±1.9</td>
<td>8.4±2.8*</td>
<td>3.0±1.0*</td>
</tr>
<tr>
<td>Motility index</td>
<td>4.81±0.18</td>
<td>5.39±0.27*</td>
<td>5.11±0.28</td>
</tr>
<tr>
<td>GLP-1, pmol/l</td>
<td>2.6±0.6</td>
<td>11.9±4.3*</td>
<td>12.8±2.7*</td>
</tr>
<tr>
<td>PYY, pmol/l</td>
<td>4.9±1.1</td>
<td>11.2±3.3*</td>
<td>14.0±3.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. 0 mg/ml. †P < 0.10 vs. 0 mg/min. Dose-dependent alterations were observed for intraluminal lipid concentrations, trypsin, and bile acid output. P ≤ 0.05 (ANOVA). GLP-1, glucagon-like peptide-1; PYY, peptide YY.
was associated with phase III activity, i.e., return of the fasting motor pattern in one subject, whereas two subjects showed phase III motility during perfusion of the higher ileal lipid dose. The mean duodenal MI (Fig. 3) was significantly increased during ileal perfusion of the lower lipid dose and was similar to control conditions during perfusion at the higher dose (Fig. 3 and Table 1).

Regulatory hormones. Intraileal lipid perfusion at 50 and 100 mg/min significantly increased the release of GLP-1 and PYY (Table 1). Moreover, plasma concentrations of both hormones remained elevated compared with basal hormone levels throughout the final saline perfusion period (GLP-1, 7.4 ± 0.9 vs. 2.6 ± 0.6 pmol/l, P = 0.004; PYY, 10.8 ± 3.0 vs. 4.9 ± 1.1 pmol/l; P = 0.043). Mean intraileal lipid concentrations were directly correlated with release of PYY and GLP-1 (R ≥ 0.800; P < 0.05). Output of trypsin and bile acids were correlated inversely with plasma levels of GLP-1 and PYY (Fig. 4). By contrast, MI was not associated with the release of these distal intestinal hormones (P > 0.05).

DISCUSSION

Our findings can be summarized as follows. Physiological postprandial ileal lipid concentrations dose dependently and markedly inhibit digestive pancreatic protease and bile acid secretion but do not decrease small intestinal motor activity in healthy humans. The inhibitory effects on bile acid output are more pronounced and last longer than inhibition of enzyme output. This situation leads to a decrease in the bile acid-to-protease ratio. Protease and bile acid output, but not MI, correlate inversely with GLP-1 and PYY plasma levels.

Duodenal nutrients are the most important stimulants of digestive secretory and motor responses (6, 18). Their actions are mediated mainly by cholinergic mechanisms and cholecystokinin (1, 3). On the other hand, it has been shown that intraileal nutrients exert marked inhibitory effects on human gastrointestinal secretory and motor functions. This phenomenon has been called the ileal brake mechanism (31, 34). However, most studies in humans have concentrated on the effects of ileal nutrients on gastrointestinal motility and/or have used supraphysiological ileal nutrient doses (9, 20, 24, 30, 31, 34). To our knowledge, the effect of ileal nutrients on human bile acid secretion has not been tested to date.

After mixed meals, physiological intraileal lipid concentrations between 3.6 and 10 mg/ml have been measured previously (4, 10, 15). Ileal lipid perfusion at 50 and 100 mg/min performed in our study mimicked these physiological intraileal lipid concentrations, i.e., 3.5 and 7 mg/ml, respectively. Moreover, digestive secretory and motor responses were constantly stimulated by continuous duodenal perfusion of essential amino acids (18). Thus our experimental setting allowed for investigation of the effects of physiological ileal nutrient concentrations on endogenously stimulated small intestinal motility and pancreaticobiliary secretions in healthy humans.

Pancreaticobiliary secretions. Graded intraileal lipid perfusion decreased digestive trypsin and bile acid output markedly (Table 1 and Fig. 1). The decreases in both pancreatic enzyme and bile acid outputs correlated inversely with intraileal lipid concentrations (Fig. 2) and were dose dependent. However, the inhibitory effects of ileal lipids on bile acid output were...
stronger and lasted longer than their effects on pancreatic enzyme output. After termination of ileal lipid perfusion, protease output returned to basal levels within ~30 min, whereas bile acid output remained significantly suppressed throughout the final 2-h saline perfusion period (Fig. 1). In dogs, Tohno et al. (35) observed decreased duodenal bile acid delivery in response to ileal carbohydrates. By contrast, to our knowledge, the present study is the first to report inhibition of human biliary secretion by ileal nutrient exposure.

Motility. Endogenously stimulated small intestinal motility was not inhibited by physiological ileal lipid concentrations, i.e., the fed pattern was not regularly converted to the fasting motility pattern as observed with bolus application of high ileal nutrient loads (24), and the MI did not decrease (Fig. 3 and Table 1). These data apparently are in contrast to previous findings published by Read et al. (31) and Spiller et al. (34), who established the concept of an ileal brake mechanism that decreases jejunal motility and slows small intestinal transit in

Fig. 3. Duodenal motility index (MI), calculated as ln (MA × F/5 + 1), with MA = mean amplitude and F = frequency of contractions during 5-min intervals, during endogenous stimulation with duodenally perfused essential amino acids and ileal perfusion of saline (A), lipids at a dose of 50 mg/min (B), and lipids at a dose of 100 mg/min (C). MI during the last 30 min of each perfusion period were compared in 8 healthy subjects. Data are means ± SE. Broken line indicates mean MI for the last 30 min of each perfusion period. This was significantly increased in response to the lower lipid dose. *P < 0.05 vs. saline.

Fig. 4. Association between release of GLP-1 and peptide YY (PYY) and output of trypsin and bile acids in response to graded ileal lipid perfusion during endogenous stimulation of digestive responses. Data are mean values (n = 8) for hormone plasma concentrations measured at baseline and at 60-min intervals thereafter until the conclusion of lipid perfusion and corresponding mean trypsin and bile acid output. Open squares, trypsin; filled circles, bile acids.
response to ileal fat exposure in humans and with respective findings in dogs (25). However, most of these studies measured not intestinal motor activity but transit times (25, 31). Because the MI obtained in our study estimated only the frequency and amplitude of contractions and did not provide any information about propulsive efficacy, prolongation of transit time does not necessarily correlate with a decrease in the MI. This hypothesis is supported by our own preliminary data showing that physiological and supraphysiological GLP-1 doses strongly increase duodenoccecal transit time but only weakly affect small intestinal MI in humans (11). Moreover, Spiller et al. (34) reported a decrease in jejunal MI in response to ileal lipid doses that exceeded our higher dose by >200% (333 vs. 100 mg/min). Thus discrepant findings might be due to region-specific effects (duodenal vs. jejunal motility) or might depend on different dosing regimens. On the other hand, it has been shown that fat malabsorption in the low pathological range (as observed in experimental diarrhea; see Ref. 14) does not delay gastrointestinal transit. However, these findings have been obtained in patients with carcinoid syndrome, in whom the motility effects of excessively released serotonin may interfere with the ileal brake mechanism (32). Altogether, digestive small intestinal motor activity appears to be less susceptible to inhibition by ileal lipids than digestive pancreaticobiliary secretions.

Regulatory hormones. GLP-1 and PYY are released in response to nutrient exposure by mucosal L cells, which are most prevalent in the human ileum, and these gut peptides are regarded as the most important hormonal mediators of the ileal brake mechanism in humans. Neureotensin, which is also released in response to ileal nutrients (31), stimulates rather than inhibits pancreatic exocrine secretion (7). Therefore, we concentrated on GLP-1 and PYY as potential mediators of the ileal brake mechanism. In humans, digestive products of carbohydrates and lipids have been shown to increase plasma levels of both hormones, whereas proteins and their digestive products are ineffective (23). Thus, in our study, the increase in GLP-1 and PYY release can be attributed solely to ileal lipids and not to an overspill of duodenally perfused essential amino acids. Our present data show that the release of GLP-1 and PYY is strongly correlated with intraileal lipid concentrations and thus is dose dependent. Remarkably, plasma concentrations of both hormones remained elevated during the final 2-h saline perfusion period (Fig. 1). Because of the short plasma half-life of both hormones (29, 36), this finding suggests ongoing hormone release, despite the absence of ileal nutrients, probably from colonic sources.

Enhanced susceptibility of biliary secretion to GLP-1 and/or PYY may cause or contribute to the stronger and prolonged decrease in bile acid output in response to ileal lipids. In addition, there is evidence that duodenal bile acid depletion increases pancreatic enzyme output in humans (21). Consequently, partial disinhibition of pancreatic secretion by reduced intraduodenal bile acid loads may partly explain divergent effects of ileal nutrient exposure on bile acids and pancreatic enzymes.

In contrast to pancreaticobiliary secretion, intestinal motor activity was not correlated with GLP-1 or PYY release. This finding is in line with our own preliminary data showing that intravenous infusion of GLP-1 or PYY at physiological postprandial doses hardly influences the small intestinal MI (11, 17), although prolongation of small bowel transit time was shown in these studies as well as in previous studies (25, 31, 34).

It must be kept in mind, however, that correlations do not prove causal relationships and need to be interpreted cautiously. To prove the importance of endogenously released GLP-1 and PYY for the inhibition of gastrointestinal secretory and motor functions, it is necessary to test specific antagonists that are not yet readily available for human use. Thus this remains an area for future research.

Another important area for future studies may be the association between proximal and distal small intestinal nutrient exposure, the release of regulatory mediators, and gastrointestinal secretory and motor functions in diseases such as inflammatory bowel disease and irritable bowel syndrome.

Physiological role of the ileal brake mechanism. It has been speculated that the ileal brake mechanism is operative in states of maldigestion and malabsorption (31, 34), rather than under physiological circumstances, and serves to slow gastrointestinal transit to increase small intestinal absorptive capacity. However, concomitant inhibition of digestive secretions, which we have demonstrated in this study as well as in previous studies (24) and which likely impairs luminal digestion of nutrients, does not fit this pathophysiological concept. Moreover, our present study has demonstrated marked inhibition of digestive pancreaticobiliary secretion in response to physiological, postprandial, intralkeletal lipid concentrations. Thus it appears likely that the ileal brake mechanism is of physiological importance. Although under the experimental conditions chosen for the present study phase III motility was induced by ileal lipid perfusion in a minority of subjects, our previous findings suggest that the physiological role of the ileal brake mechanism may consist of termination of digestive responses after a meal (19).

In conclusion, physiological ileal lipid concentrations differentially regulate human digestive pancreaticobiliary secretions and intestinal motility. Both pancreatic protease and bile acid output are markedly and dose dependently inhibited, but inhibition of bile acid secretion is more pronounced and prolonged. By contrast, small intestinal motor activity is not reduced, although phase III activity is induced in single subjects. These differential effects may be due to the varying sensitivity of pancreaticobiliary secretions and intestinal motility to ileal mediators, because only protease and bile acid outputs are correlated inversely with GLP-1 and PYY release.

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