



Universiteit
Leiden
The Netherlands

Physiology and pathophysiology of the ileal brake in humans

Vu, M.K.

Citation

Vu, M. K. (2007, September 25). *Physiology and pathophysiology of the ileal brake in humans*. Department Gastroentero-hepatolgy, Medicine / Leiden University Medical Center (LUMC), Leiden University. Retrieved from <https://hdl.handle.net/1887/12350>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/12350>

Note: To cite this publication please use the final published version (if applicable).

PHYSIOLOGY AND PATHOPHYSIOLOGY OF THE ILEAL BRAKE IN HUMANS

Cover: Benjamin Long Allenson
Printed by Mostert & Van Onderen, Leiden

PHYSIOLOGY AND PATHOPHYSIOLOGY OF THE ILEAL BRAKE IN HUMANS

Proefschrift

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden,

op gezag van Rector Magnificus prof.mr. P.F. van der Heijden,

volgens besluit van het College voor Promoties

te verdedigen op dinsdag 25 september 2007

te klokke 13.45 uur

door

My Kieu Vu

geboren te Hanoi

in 1970

Promotiecommissie

Promotores:

Prof. dr. A.A.M. Masclee (Universiteit Maastricht)

Prof. dr. C.B.H.W. Lamers

Referent:

Prof. dr. J.B.M. J. Jansen (Universiteit Nijmegen)

Overige leden:

Prof. dr. D.W. Hommes

Prof. dr. A. van der Laarse

Prof. dr. R.J. Brummer (Universiteit Maastricht)

Prof. dr. J.M. van Laar (Newcastle University, England)

Dr. H.P. Peters (Unilever Food & Health Research Institute)

Dr. I. Biemond

Part of the studies in this thesis were financially supported by a grant from the Maag Darm Lever Stichting, The Netherlands

The printing of this thesis was financially supported by ALTANA Pharma BV, Janssen-Cilag BV, Astra Zeneca BV, Tramedico BV, Novartis Pharma BV, Abbott BV and Schering Plough BV.

For Ben

CONTENTS

Chapter 1:	Introduction, aims and outlines of the thesis	9
-------------------	---	---

PHYSIOLOGY OF THE ILEAL BRAKE

Chapter 2:	Does the intestinal site of fat delivery influence feedback control on gastrointestinal motility in humans ?	29
Chapter 3:	Does jejunal feeding activate exocrine pancreatic secretion ? <i>Eur J Clin Invest. 1999 Dec;29(12):1053-9</i>	55
Chapter 4:	Effect of the ileal brake on satiety and proximal gastric function: is it peptide YY ?	65
Chapter 5:	Medium chain triglycerides activate distal but not proximal gut hormones <i>Clin Nutr. 1999 Dec;18(6):359-63</i>	95
Chapter 6:	The osmotic laxative magnesium sulphate activates the ileal brake <i>Aliment Pharmacol Ther. 2000 May;14(5):587-95</i>	103

PATHOPHYSIOLOGY OF THE ILEAL BRAKE

Chapter 7:	Antroduodenal motility in chronic pancreatitis: are abnormalities related to exocrine insufficiency ? <i>Am J Physiol Gastrointest Liver Physiol. 2000 Mar;278(3):G458-66</i>	115
Chapter 8:	Gastrointestinal motility and peptide secretion in systemic sclerosis	127
Chapter 9:	Gallbladder motility in Crohn disease: influence of disease localization and bowel resection <i>Scand J Gastroenterol. 2000 Nov;35(11):1157-62</i>	151
Chapter 10:	Summary Samenvatting	159

Acknowledgement	185
Publications	187
Curriculum Vitae	191

Chapter 1

INTRODUCTION, AIMS AND OUTLINES OF THE THESIS

M.K. Vu, A.A.M. Masclee

Department of Gastroenterology-Hepatology, Leiden University Medical Center, the Netherlands

INTRODUCTION

Ileal brake history

Digestion and absorption of nutrients are complex processes that involve various functions of the gastrointestinal (GI) tract. This includes the interplay between nutrients, digestive enzymes and gut surface area. Motility, transport of intraluminal content, secretion of enzymes and fluids are regulated by hormonal, neural (enteric and central nervous system) and local regulatory mechanisms. Intraluminal nutrients by themselves have a major role in controlling gastrointestinal transport, digestion and absorption. The presence of nutrients in the small intestine stimulates pancreatic enzyme secretion, gallbladder contraction and converts intestinal motility from the fasted into a fed motility pattern (1). On the other hand, intestinal nutrients also trigger feedback inhibitory mechanisms that will modify gastrointestinal transport, digestion and absorption. For instance, duodenal nutrients inhibit gastric acid secretion and delay gastric emptying. This phenomenon is called the duodenal brake, a negative feedback loop from the proximal gut on gastric functions (1-3).

A nutrient-triggered inhibitory feedback loop from the more distal to the proximal gut was first described by Spiller et al and Read *et al* (4,5). These two groups of researchers showed that transit of a meal through the small intestine was significantly delayed when a lipid emulsion was administered into the ileum. This phenomenon is called the ileal brake. Since then, evidence has increased showing in both humans and animals that intraileal nutrients alter intestinal motility, reduce transit time, delay gastric emptying and inhibit gastric acid and exocrine pancreatic secretion (6-8,15-20,25-28).

One may conclude that non-absorbed nutrients reaching the distal small bowel bring the process of transport, secretion, digestion and absorption to an end.

1. Ileal brake and gastric emptying

Earlier studies in both humans and dogs have demonstrated that infusion of nutrients into the ileum delays gastric emptying of both solid and liquid meals (6,7). These findings have been further extended by Fone *et al*, who showed that ileal fat inhibits antral and duodenal motility while stimulating pyloric contractions. Especially the latter may be responsible for the delayed gastric emptying (8). However, this is the only study demonstrating an association between antropyloroduodenal motility changes and delayed gastric emptying induced by ileal nutrients in humans. Not only the distal stomach, but also the proximal stomach contributes to gastric emptying. The motor function of the proximal stomach is characterised by receptive and adaptive relaxation (9,10). Receptive relaxation is induced by pharyngeal stimulation (swallowing) and distention of the esophagus by the bolus of food. Adaptive relaxation or accommodation is the ability of the proximal stomach to distend in response to an intragastric load with only minimal changes in intragastric pressure (gastric tone) (11). A relationship between proximal gastric tone and gastric emptying has been described previously (12-14). Up till now it is not known whether nutrients in the distal gut affect proximal gastric motor functions.

2. Ileal brake and intestinal motility and transit

The inhibitory effect of ileal nutrients on small intestinal transit has been

confirmed by several studies since the original publications of Spiller *et al* and Read *et al* (4,5,15,16). On the other hand, data on the effect of ileal nutrients on digestive intestinal motility patterns are scarce and the various studies differ in methodology. Welch *et al* demonstrated the early occurrence of phase III after meal ingestion in 3 of 14 healthy subjects by infusion of lipid into the ileum (17). A study by Layer *et al*, using duodenal perfusion of a mixture of essential amino acids instead of a meal to induce a fed-like motor pattern, showed that ileal infusion of fat or carbohydrate induces premature phase III-like activity in 12 of 14 healthy subjects (18). With respect to the effects of ileal nutrients on fasting motor patterns, results are contrasting. Ileal fatty acids in dogs prolong interdigestive cycles and inhibit jejunal motility (19) whereas in humans, ileal perfusion of carbohydrates or fat during interdigestive phase I markedly decreases the duration of phase II motor activity, induces premature phase III motility and shortens the length of the interdigestive cycle (20). Although contradictory, these results nonetheless suggest that fasting motor activities may be modulated by the presence of nutrients in the distal small intestine. However, further research is needed to exactly define the effect of ileal nutrients on intestinal motility patterns in humans.

3. Ileal brake and gastric acid and exocrine pancreatic secretion

It is known that nutrients in the proximal small intestine, especially fat, potentially inhibit gastric acid secretion (21,22). However, there is also evidence suggesting that gastric acid secretory function is regulated by the distal intestine. For instance, colonic perfusion of lipids or protein decreases exogenously stimulated gastric acid secretion in humans and dogs (23, 24). In addition, ileal perfusion of lipids or carbohydrates inhibits both unstimulated and endogenously stimulated

gastric acid secretion in humans (25). The effects of ileal nutrients on the secretory function have been more extensively studied in humans and animals with respect to exocrine pancreatic secretion (26-28, 18, 20). However, the obtained results are contradictory. While in rats and cats, ileal fat decreases pancreatic enzyme secretion this is not the case in dogs (26-28). Layer *et al* showed in humans that ileal perfusion with either carbohydrates or triglycerides inhibits the secretion of all exocrine pancreatic enzymes to an equal extent (18). On the other hand, results presented by Jain *et al* indicate that ileal carbohydrates do not inhibit but increase the release of the pancreatic enzyme amylase when compared to trypsin (29). Thus, the question whether ileal nutrients inhibit exocrine pancreatic secretion still remains to be answered.

4. Ileal brake and satiety

Although the role of the stomach and intraduodenal nutrients in the regulation of food intake and satiety is well established (30-33), effects from the distal gut are poorly defined because of the limited number of studies on this subject up till now. There are two human studies, both by Welch *et al*, who have demonstrated that ileal nutrients significantly reduce the total amount of food intake (34, 35). In addition, one study in rats showed that while ileal infusion of glucose reduces both meal frequency and size, ileal free fatty acids reduce only the latter (36). Although scarce, these consistent data imply that activation of the ileal brake decreases food intake and may induce early satiety. However, further studies are necessary to prove this theory.

Triggers of the ileal brake

The ileal brake is an intraluminal nutrient-triggered feedback control from the distal to the proximal gut. The inhibitory response of upper gastrointestinal motility differs with respect to the type of the nutrients administered into the ileum. In both humans and dogs, infusion of fat into the ileum delays gastric emptying and increases intestinal transit time (4-8; 37, 38). Within the range of different lipid emulsions, free fatty acids and digested triglycerides have been shown to be more potent than neutral triglycerides in eliciting the ileal brake effect (15). Intraileal carbohydrates, on the other hand, delay gastric emptying only at high concentrations (15). Data concerning intraileal proteins and the activation of the ileal brake are contradictory. Read *et al* found that ileal proteins delay small intestinal transit (5) whereas other investigators were not able to demonstrate any inhibitory effects of ileal proteins on gastrointestinal motility in humans (6, 15). These contrasting data may result from methodological differences. Nevertheless, it is generally accepted, based on consistent results from numerous studies, that fat is the most potent trigger of the ileal brake (39). However, it is important to bear in mind that species differences exist concerning triggers for the ileal brake. For instance, free fatty acid, a potent trigger of the ileal brake in humans and dogs, has no effect on the pig (40).

Mediators of the ileal brake

The mechanisms involved in the control of the ileal brake remain to be explored. The ileal brake may be mediated by hormonal and/or neural factors.

Hormonal factors: A number of gut peptides, including glucagon-like peptide 1 (GLP-1), neurotensin and peptide YY (PYY) have been

hypothesized as possible humoral mediators of the ileal brake. Attention has been focused mainly on these peptides because of the localisation of their secretory cells, mainly in the distal gut.

GLP-1 is synthesized within the endocrine L cells in the intestine, primarily in the ileum and colon (41, 42). The release of GLP-1 is stimulated by the direct contact of the L cells to luminal nutrients (25, 43). However, given the rapid release of GLP-1 after meal intake, there is also evidence suggesting that GLP-1 release results from an indirect neural or humoral signals arising from the proximal gut (44, 45). GLP-1 plasma levels have been shown to increase in parallel with the inhibitory effects of the ileal brake on antral motility, gastric acid and exocrine pancreatic secretion (18, 25). However, up to now, there are no studies using a specific GLP-1 antagonist to clearly define the role of endogenous GLP-1 as a hormonal mediator of the ileal brake.

Neurotensin is produced by the mucosal endocrine N cells which are distributed throughout the small intestine, with the highest concentration found in the ileum (46-48). There is only scarce, indirect evidence available suggesting the role of neurotensin as a hormonal mediator of the ileal brake. Spiller *et al* have shown that ileal fat perfusion inhibits jejunal motility and significantly increases plasma neurotensin concentrations (5, 15).

PYY is, like GLP-1, also synthesized and secreted by L cells in the distal ileum and colon (49). The presence of nutrients, especially fats, in the ileum stimulates PYY release (4-8, 15). In contrast to the poorly established role of GLP-1 and neurotensin as hormonal mediators of the ileal brake, associations between plasma PYY concentrations and delayed small intestinal transit and gastric emptying have been shown by numerous investigators (5, 15, 37, 38).

In addition, the role of endogenous PYY as a mediator of the ileal brake has been elucidated using a PYY antagonist. In dogs, administration of PYY antibodies abolishes the prolonged small intestinal transit induced by intraileal fat (38). Although similar studies in humans are lacking, this direct evidence nonetheless suggests that PYY is very likely the humoral mediator of the ileal brake.

Neural factors: Several neural pathways have been suggested to contribute to the regulation of the ileal brake. The role of the extrinsic nervous system, in particular the vagus nerve, has been suggested but evidence is mostly indirect. It has been shown that intraileal fats increase vagal afferent activity in rats (50). In animals, the inhibitory effect of PYY and GLP-1 on meal stimulated gastric acid secretion and gastric motility was significantly reduced or even abolished after vagotomy (51, 52). These results suggest that both the candidate ileal brake hormones PYY and GLP-1 act through vagal innervation but a direct relationship between vagal cholinergic control and the fat induced ileal brake has not yet been proven. On the other hand, direct evidence exists demonstrating the involvement of the sympatho-adrenergic pathway in the inhibitory effect of the ileal brake. In dogs, administration of a combined α - and β -adrenergic blockade totally abolishes the inhibitory action of exogenous PYY on endogenous PYY release by ileal fat on exocrine pancreatic secretion (53). In addition, an adrenoceptor antagonist reverses the delayed intestinal transit induced by intraileal fat (54). Furthermore, evidence exists showing that the intrinsic nervous system (myenteric and submucosal plexus) also plays an important role in the regulation of the ileal brake. The fat-induced ileal brake in dogs is abolished when ondansetron, a 5-HT₃-receptor antagonist was administered into the proximal but not the distal small bowel

(55). Similarly, the prolonged intestinal transit induced by fat in the ileum was blocked when naloxon, an opioid receptor antagonist was infused into the proximal small intestine (56). These findings suggest that both peripheral serotonergic and also opioid pathways are involved in the regulation of the ileal brake. However, it is plausible to assume that all the abovementioned different neural pathways interact and regulate the ileal brake.

Clinical implications of altered ileal brake function

Theoretically, the feedback function of the ileal brake could be impaired due to mucosal defects as in Crohn's ileitis and celiac disease or could be absent following resection of the distal small intestine. It is plausible to hypothesize that when the inhibitory feedback mechanism of the ileal brake is impaired or absent, gastric emptying and small intestinal transit are accelerated, resulting in increased concentrations of undigested and unabsorbed nutrients in the distal gut. This in turn could contribute to the development of symptoms such as diarrhea and malabsorption seen in inflammatory bowel diseases and after small intestine resection.

On the other hand, malabsorption and/or accelerated intestinal transit, irrespective of its cause, may also alter the ileal brake function. Given that PYY is a candidate hormonal mediator of the ileal brake, plasma PYY release could be considered as a marker for the activation of this feedback mechanism. Plasma PYY levels have been found to be increased in chronic pancreatitis and in patients with dumping syndrome after (partial) gastric resection (57-59). These findings suggest that the activation of the ileal brake is enhanced in these disease states due to the increased amount of unabsorbed nutrients reaching the distal small intestine. Based on the above made

assumptions, altered ileal brake function could be a primary defect, thereby giving rise to disease manifestations or secondary, in response to changes caused by the disease.

AIMS AND OUTLINES OF THE THESIS

Given its important role as a nutrient-triggered feedback control mechanism, increasing knowledge and understanding of the ileal brake is relevant for physiology and pathophysiology and may help to develop novel strategies in treating patients with malabsorption and maldigestion. The studies presented in this thesis have been designed to gain further insight into the physiology and pathophysiology of the ileal brake. The following issues were addressed:

Physiology of the ileal brake

- It has been shown in dogs that the ileal brake is a more potent feedback mechanism compared to the more proximal, so called jejunal brake. However, a comparative study between jejunal and ileal brake has not been performed in humans. **Chapter II** was therefore designed to compare the effect of intraileal versus intrajejunal fat on digestive and interdigestive gastrointestinal motor patterns and postprandial gallbladder motility. The latter plays a role in delivering bile acids into the duodenum for the digestion of dietary fats. Furthermore, impaired gallbladder motility contributes to the pathogenesis of sludge and stone formation. Up til now little is known about the effect of the ileal brake on gallbladder motility. Gallbladder volumes were measured by real time ultrasonography and gastrointestinal motility was measured by means of the stationary water perfusion manometry system.

- The study in **Chapter III** was designed to investigate whether pancreatic and biliary secretion will be affected when nutrients are administered more distally into the small intestine than usual during enteral feeding. Duodenal outputs of pancreatic enzymes and bilirubin were measured by aspiration using a recovery marker in healthy volunteers.
- It has been shown in recent years that the distal gut hormone PYY has an important role in satiety and eating behaviour. However, little is known about the effect of the ileal brake on satiety and proximal gastric motor function. **Chapter IV** was undertaken to compare the effects of ileal brake activation with ileal fat (endogenous PYY release) versus exogenous PYY₃₋₃₆ infusion on satiety and on proximal gastric motor function. Two experimental protocols were used. In the first protocol, the effect of ileal fat and subsequent endogenous PYY release was studied and in the second protocol, the dose-response relationship of exogenous PYY was investigated. In both protocols satiety and motor function of the proximal stomach were monitored using Visual Analog Scale (VAS) and an electronic barostat, respectively. Plasma PYY levels were measured by radioimmunoassay.
- Medium chain triglycerides (MCT) are thought to be hydrolysed and absorbed more rapidly and completely compared to long chain triglycerides (LCT). However, patients receiving MCT frequently complain of nausea, cramps, abdominal pain and diarrhoea. We hypothesized that MCT are less rapidly absorbed and cause these gastrointestinal side effects. The release of the distal gut hormone PYY induced by intraduodenal MCT was used as evidence for the hypothesized malabsorption of MCT. Results are described in **Chapter V**.

- **Chapter VI** investigates whether artificially induced malabsorption in healthy volunteers affects gastrointestinal and gallbladder motility through activation of the ileal brake. The osmotic laxative magnesium sulphate was used to induce malabsorption after ingestion of a fatty meal.

Pathophysiology of the ileal brake

- Altered gastrointestinal motility has been observed in patients with chronic pancreatitis with impaired exocrine function. However, the reported results are conflicting. In **Chapter VII** we therefore investigated digestive and interdigestive antroduodenal motility and secretion of several relevant gut hormones in a large group of patients with chronic pancreatitis. Differences in gut motility and hormone secretion were compared between patients with and without exocrine pancreatic insufficiency. Gastrointestinal motility and hormone secretion were also studied after pancreatic enzyme supplementation in order to further elucidate the role of exocrine pancreatic insufficiency and subsequent maldigestion in patient with chronic pancreatitis
- **Chapter VIII** deals with patients with systemic sclerosis. This systemic disorder may give rise to various complications within the gastrointestinal tract. In this chapter we focused on antroduodenojejunal motility and proximal and distal gut hormone release in patients with diffuse and limited type of systemic sclerosis. The obtained data were related to esophageal manometry findings and gastrointestinal symptoms.
- It is known that patients with Crohn's disease have an increased risk of developing gallstones. When considering the possible mechanisms that contribute to gallstone formation in these patients the questions are: 1)

whether gallbladder motility plays a role in the pathogenesis of gallstone formation in Crohn's disease and 2) whether changes in gallbladder motility are explained by altered ileal brake function due to disease localization and bowel resection. These items have been investigated and results are described in **chapter IX**.

REFERENCES

1. Text book of Gastroenterology. Tadataka Yamada, 2003.
2. Parr NJ, Grime JS, Baxter JN, Critchley M, Mackie CR. Small bowel resistances and the gastroduodenal brake. *Gut*. 1987 Aug; 28(8):950-4.
3. Shahidullah M, Kennedy TL, Parks TG. The vagus, the duodenal brake, and gastric emptying. *Gut*. 1975 May;16(5):331-6.
4. Spiller RC, Trotman IF, Higgins BE, Ghatei MA, Grimble GK, Lee YC, Bloom SR, Misiewicz JJ, Silk DB. The ileal brake--inhibition of jejunal motility after ileal fat perfusion in man. *Gut*. 1984 Apr;25(4):365-74.
5. Read NW, McFarlane A, Kinsman RI, Bates TE, Blackhall NW, Farrar GB, Hall JC, Moss G, Morris AP, O'Neill B. Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon.. *Gastroenterology*. 1984 Feb;86(2):274-80.
6. Welch IM, Cunningham KM, Read NW. Regulation of gastric emptying by ileal nutrients in humans. *Gastroenterology*. 1988 Feb;94(2):401-4.
7. Holgate AM, Read NW. Effect of ileal infusion of intralipid on gastrointestinal transit, ileal flow rate, and carbohydrate absorption in humans after ingestion of a liquid meal. *Gastroenterology*. 1985 Apr;88(4):1005-11.
8. Fone DR, Horowitz M, Read NW, Dent J, Maddox A. The effect of terminal

- ileal triglyceride infusion on gastroduodenal motility and the intragastric distribution of a solid meal. *Gastroenterology*. 1990 Mar;98(3):568-75.
9. Jahnberg T, Abrahamsson H, Jansson G, Martinson J. Gastric relaxatory response to feeding before and after vagotomy. *Scand J Gastroenterol*. 1977;12(2):225-28.
 10. Jahnberg T. Gastric adaptive relaxation. Effects of vagal activation and vagotomy. An experimental study in dogs and in man. *Scand J Gastroenterol Suppl*. 1977;46:1-32.
 11. Azpiroz F, Malagelada JR. Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis. *Gastroenterology*. 1987 Apr;92(4):934-43.
 12. Azpiroz F. Control of gastric emptying by gastric tone. *Dig Dis Sci*. 1994 Dec;39(12 Suppl):18S-19S.
 13. Moragas G, Azpiroz F, Pavia J, Malagelada JR. Relations among intragastric pressure, postcibal perception, and gastric emptying. *Am J Physiol*. 1993 Jun;264(6 Pt 1):G1112-7.
 14. Ropert A, des Varannes SB, Bizais Y, Roze C, Galmiche JP. Simultaneous assessment of liquid emptying and proximal gastric tone in humans. *Gastroenterology*. 1993 Sep;105(3):667-74.
 15. Spiller RC, Trotman IF, Adrian TE, Bloom SR, Misiewicz JJ, Silk DB. Further characterisation of the 'ileal brake' reflex in man--effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY. *Gut*. 1988 Aug;29(8):1042-51.
 16. Lin HC, Zhao XT, Wang L. Intestinal transit is more potently inhibited by fat in the distal (ileal brake) than in the proximal (jejunal brake) gut. *Dig Dis*

- Sci. 1997 Jan;42(1):19-25.
17. Welch IM, Davison PA, Worthing J, Read NW. Effect of ileal infusion of lipid on jejunal motor patterns after a nutrient and nonnutrient meal. *Am J Physiol.* 1988 Dec;255:G800-6.
 18. Layer P, Peschel S, Schlesinger T, Goebell H. Human pancreatic secretion and intestinal motility: effects of ileal nutrient perfusion. *Am J Physiol.* 1990;258(2 Pt 1):G196-201.
 19. Wen J, Phillips SF, Sarr MG, Kost LJ, Holst JJ. PYY and GLP-1 contribute to feedback inhibition from the canine ileum and colon. *Am J Physiol.* 1995 Dec;269(6 Pt 1):G945-52.
 20. Layer P, Schlesinger T, Groger G, Goebell H. Modulation of human periodic interdigestive gastrointestinal motor and pancreatic function by the ileum. *Pancreas.* 1993 Jul;8(4):426-32.
 21. Hopert R, Liehr RM, Emde C, Riecken EO. Reduction of 24-hour gastric acidity by different dietary regimens: a randomized controlled study in healthy volunteers. *JPEN J Parenter Enteral Nutr.* 1989 May-Jun;13(3):292-5.
 22. Lloyd KC, Wang J, Solomon TE. Acid inhibition by intestinal nutrients mediated by CCK-A receptors but not plasma CCK. *Am J Physiol Gastrointest Liver Physiol.* 2001 Oct;281(4):G924-30.
 23. Jian R, Besterman HS, Sarson DL, Aymes C, Hostein J, Bloom SR, Rambaud JC. Colonic inhibition of gastric secretion in man. *Dig Dis Sci.* 1981 Mar;26(3):195-201.
 24. Seal AM, Debas HT. Colonic inhibition of gastric acid secretion in the dog. *Gastroenterology.* 1980 Nov;79(5 Pt 1):823-6.
 25. Layer P, Holst JJ, Grandt D, Goebell H. Ileal release of glucagon-like

- peptide-1 (GLP-1). Association with inhibition of gastric acid secretion in humans. *Dig Dis Sci*. 1995 May;40(5):1074-82.
26. Harper AA, Hood AJ, Mushens J, Smy JR. Inhibition of external pancreatic secretion by intracolonic and intraileal infusions in the cat. *J Physiol*. 1979 Jul;292:445-54.
 27. Laugier R, Sarles H. Action of oleic acid on the exocrine pancreatic secretion of the conscious rat: evidence for an anti-cholecystokinin-pancreozymin factor. *J Physiol*. 1977 Sep;271(1):81-92.
 28. Hage G, Tiscornia O, Palasciano G, Sarles H. Inhibition of pancreatic exocrine secretion by intra-colonic oleic acid infusion in the dog. *Biomedicine*. 1974 Jun 10;21(6):263-7.
 29. Jain NK, Boivin M, Zinsmeister AR, DiMagno EP. The ileum and carbohydrate-mediated feedback regulation of postprandial pancreaticobiliary secretion in normal humans. *Pancreas*. 1991 Sep;6(5):495-505.
 30. Deutsch JA. The role of the stomach in eating. *Am J Clin Nutr*. 1985 Nov;42(5 Suppl):1040-3.
 31. Phillips RJ, Powley TL. Gastric volume rather than nutrient content inhibits food intake. *Am J Physiol*. 1996 Sep;271(3 Pt 2):R766-9.
 32. Matzinger D, Gutzwiller JP, Drewe J, Orban A, Engel R, D'Amato M, Rovati L, Beglinger C. Inhibition of food intake in response to intestinal lipid is mediated by cholecystokinin in humans. *Am J Physiol*. 1999 Dec;277(6 Pt 2):R1718-24.
 33. Lieverse RJ, Jansen JB, Masclee AA, Rovati LC, Lamers CB. Effect of a low dose of intraduodenal fat on satiety in humans: studies using the type A cholecystokinin receptor antagonist loxiglumide. *Gut*. 1994 Apr;35(4):501-5.

34. Welch I, Saunders K, Read NW. Effect of ileal and intravenous infusions of fat emulsions on feeding and satiety in human volunteers. *Gastroenterology*. 1985 Dec;89(6):1293-7.
35. Welch IM, Sepple CP, Read NW. Comparisons of the effects on satiety and eating behaviour of infusion of lipid into the different regions of the small intestine. *Gut*. 1988 Mar;29(3):306-11.
36. Woltman T, Reidelberger R. Effects of duodenal and distal ileal infusions of glucose and oleic acid on meal patterns in rats. *Am J Physiol*. 1995 Jul;269(1 Pt 2):R7-14.
37. Pironi L, Stanghellini V, Miglioli M, Corinaldesi R, De Giorgio R, Ruggeri E, Tosetti C, Poggioli G, Morselli Labate AM, Monetti N. Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma levels of peptide YY. *Gastroenterology*. 1993 Sep;105(3):733-9.
38. Lin HC, Zhao XT, Wang L, Wong H. Fat-induced ileal brake in the dog depends on peptide YY. *Gastroenterology*. 1996 May;110(5):1491-5.
39. Van Citters GW, Lin HC. The ileal brake: a fifteen-year progress report. *Curr Gastroenterol Rep*. 1999 Oct;1(5):404-9.
40. Dobson CL, Hinchcliffe M, Davis SS, Chauhan S, Wilding IR. Is the pig a good animal model for studying the human ileal brake?. *J Pharm Sci*. 1998 May;87(5):565-8.
41. Schirra J, Goke B. The physiological role of GLP-1 in human: incretin, ileal brake or more? *Regul Pept*. 2005 Jun 15;128(2):109-15.
42. Eissele R, Goke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Goke B. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest*. 1992 Apr;22(4):283-91.
43. Herrmann-Rinke C, Voge A, Hess M, Goke B. Regulation of glucagon-like

- peptide-1 secretion from rat ileum by neurotransmitters and peptides. *J Endocrinol.* 1995 Oct;147(1):25-31.
44. Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology.* 1999 Apr;140(4):1687-94.
 45. Balks HJ, Holst JJ, von zur Muhlen A, Brabant G. Rapid oscillations in plasma glucagon-like peptide-1 (GLP-1) in humans: cholinergic control of GLP-1 secretion via muscarinic receptors. *J Clin Endocrinol Metab.* 1997 Mar;82(3):786-90.
 46. Bloom SR, Polak JM. Aspects of neurotensin physiology and pathology. *Ann N Y Acad Sci* 1982;400:105-116.
 47. Flaten O, Hanssen LE. Concentration of neurotensin in human plasma after glucose, meals and lipids. *Acta Physiol Scand.* 1982 Feb;114(2):311-3.
 48. Ferris CF, George JK, Eastwood G, Potegal M, Carraway RE. Plasma levels of human neurotensin: methodological and physiological considerations. *Peptides.* 1991 Mar-Apr;12(2):215-20.
 49. Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology.* 1985 Nov;89(5):1070-7.
 50. Randich A, Tyler WJ, Cox JE, Meller ST, Kelm GR, Bharaj SS. Responses of celiac and cervical vagal afferents to infusions of lipids in the jejunum or ileum of the rat. *Am J Physiol Regul Integr Comp Physiol.* 2000 Jan;278(1):R34-43.
 51. Chen CH, Rogers RC. Central inhibitory action of peptide YY on gastric motility in rats. *Am J Physiol.* 1995 Oct;269(4 Pt 2):R787-92.
 52. Lloyd KC, Amirmoazzami S, Friedik F, Heynio A, Solomon TE, Walsh JH.

- Candidate canine enterogastrones: acid inhibition before and after vagotomy. *Am J Physiol*. 1997 May;272(5 Pt 1):G1236-42.
53. Konturek SJ, Bilski J, Pawlik W, Tasler J, Domschke W. Adrenergic pathway in the inhibition of pancreatic secretion by peptide YY in dogs. *Gastroenterology*. 1988 Feb;94(2):266-73.
54. Lin HC, Neevel C, Chen PS, Suh G, Chen JH. Slowing of intestinal transit by fat or peptide YY depends on beta-adrenergic pathway. *Am J Physiol Gastrointest Liver Physiol*. 2003 Dec;285(6):G1310-6.
55. Lin HC, Chen JH. Slowing of intestinal transit by fat depends on an ondansetron - sensitive, efferent serotonergic pathway. *Neurogastroenterol Motil*. 2003 Jun;15(3):317-22.
56. Zhao XT, Wang L, Lin HC. Slowing of intestinal transit by fat depends on naloxone-blockable efferent, opioid pathway. *Am J Physiol Gastrointest Liver Physiol*. 2000 Jun;278(6):G866-70.
57. Adrian TE, Savage AP, Bacarese-Hamilton AJ, Wolfe K, Besterman HS, Bloom SR. Peptide YY abnormalities in gastrointestinal diseases. *Gastroenterology*. 1986 Feb;90(2):379-84.
58. Adrian TE, Long RG, Fuessl HS, Bloom SR. Plasma peptide YY (PYY) in dumping syndrome. *Dig Dis Sci*. 1985 Dec;30(12):1145-8.
59. El-Salhy M, Suhr O, Danielsson A. Peptide YY in gastrointestinal disorders. *Peptides*. 2002 Feb;23(2):397-402.

Chapter 2

DOES THE INTESTINAL SITE OF FAT DELIVERY INFLUENCE FEEDBACK CONTROL ON GASTROINTESTINAL MOTILITY IN HUMANS ?

M.K. Vu, A. Dijkstra, I.C. Schut, I. Biemond, A.A.M. Masclee

Department of Gastroenterology-Hepatology, Leiden University Medical Center,
the Netherlands

Submitted

ABSTRACT

This study was performed to compare in healthy volunteers the effect of intrajejunal versus intraileal fat administration on gastrointestinal and gallbladder motility. Eight healthy volunteers (age 22 ± 5 yr) participated in three experiments, performed in random order with: 1) intrajejunal fat 2) intraileal fat and 3) placebo after oral ingestion of a mixed liquid meal. Antroduodenojejunal motility, gallbladder volumes, and plasma cholecystokinin (CCK) and peptide YY (PYY) were measured. *Results:* 1) both intrajejunal and intraileal fat significantly ($p < 0.01$) prolonged the duration of the fed motor pattern. The duration of the fed pattern correlated significantly with PYY but not with CCK secretion; 2) intrajejunal fat significantly ($p < 0.05$) prolonged MMC cycle length while intraileal fat shortened MMC cycle length; 3) postprandial gallbladder emptying was significantly ($p < 0.01$) reduced with intraileal fat and increased with intrajejunal fat. *Conclusion:* Feedback control mechanisms on gastrointestinal motility and hormone release, evoked by intestinal fat, are qualitatively and quantitatively dependent on the site of fat delivery (jejunum versus ileum).

INTRODUCTION

Feedback control mechanisms triggered by intraluminal nutrients have an important role in the process of digestion and absorption. The responses evoked by intraluminal nutrients differ with respect to the intestinal site. For instance, fat administration in the duodenum stimulates exocrine pancreatic secretion, gallbladder contraction and intestinal transit (1-3) while administration of fat into the ileum inhibits exocrine pancreatic secretion and intestinal transit (4-6). The latter is called the “ileal brake”, a negative feedback loop from the distal to the proximal gut.

In addition to the ileal brake, the existence of an inhibitory feedback control located in the proximal half of the small intestine, the so called “jejunal brake” has also been proposed (7-9). Intrajejunal nutrients inhibit exocrine pancreatic secretion and prolong intestinal transit in both humans and dogs (7-9). It has been shown in a study in dogs that intestinal transit is more potently inhibited by fat delivered in the distal than in the proximal small intestine, suggesting a more potent inhibitory feedback mechanism of the ileal brake compared to the jejunal brake (10). Although in humans both jejunal and ileal brake are operative, they have not been compared quantitatively.

The present study was undertaken to compare in healthy volunteers the effects of intrajejunal versus intraileal fat administration on gastrointestinal and gallbladder motility. The latter plays a role in delivering bile acids into the duodenum for the digestion of dietary fats. It is generally accepted that the proximal gut peptide cholecystokinin (CCK) is the most potent hormonal stimulator of gallbladder contraction (11,12). In addition, there is also evidence for the involvement of the distal gut peptide PYY in the regulation of gallbladder emptying (13,14).

However, little is known about the effect of the ileal brake on gallbladder motility. In the present study, plasma levels of CCK and PYY were measured and related to gastrointestinal and gallbladder motility data.

SUBJECTS

Eight healthy volunteers (2 male, 6 female; mean ($\pm$ SEM) age 22 ± 5 year; mean ($\pm$ SEM) BMI 22 ± 2) participated in this study. None of the subjects had gastrointestinal complaints or history of abdominal surgery. Informed consent was obtained from each person and the study protocol had been approved by the ethical committee of the Leiden University Medical Center.

METHODS

Antroduodenojejunal manometry

Antroduodenojejunal motility was recorded by stationary perfusion manometry using an ileal catheter (outer diameter 4 mm; length 350 cm) consisting of a central perfusion port, 12 side holes, a stainless steel tip weight and a distal inflatable balloon. The 12 side holes are divided in three clusters each consisting of four side hole openings spaced 2,5 cm apart (antrum) and 5 cm apart (duodenum/jejunum). The manometry catheter was connected to a low-compliance pneumohydraulic perfusion system (Arndorfer Medical Systems) and perfused with distilled water at a rate of 0.3 ml/min. Resistance to infusion within the system was detected with pressure transducers (Medex, Hilliard, Ohio, USA). The output of the pressure transducers was translated in a polygraph (PC Polygraph, Metronics, Denmark) and displayed continuously on a monitor. Data were stored on a personal computer for analysis.

Gallbladder measurements

Gallbladder volumes were measured by real time ultrasonography (Toshiba, 3.75 MHz transducer) and calculated by the sum of cylinders method using computerized system (15,16). In this method the longitudinal image of the gallbladder is divided into series of equal height, with diameter perpendicular to the longitudinal axis of the gallbladder image. The uncorrected volume is the sum of volumes of these separate cylinders. To correct for the displacement of the longitudinal image of the gallbladder from the central axis, a correction factor is calculated from the longitudinal and transversal scans of the gallbladder. Gallbladder volume is calculated by multiplication of the uncorrected volume with the square of the correction factor. The mean of two measurements was used for analysis. The assumptions and the mathematical formula used to calculate gallbladder volume have been described and validated previously (15,16).

Study design

Each subject participated in three experiments, performed in random order on three consecutive days in a single blind manner. The experiments started at 7:45 AM.

Day 0: Catheter intubation

Subjects were intubated transnasally with the ileal catheter after an overnight fast. Once the tip of the catheter passed the ligament of Treitz, the distal balloon was inflated with 10 ml air to facilitate further progression of the tube through the small intestine. The progression of the tube was monitored by fluoroscopy. The tip of the catheter was located so that the most distal cluster of four side hole openings was in the jejunum, the second one was in the

duodenum and the most proximal cluster was situated in the antrum. The time required for the tube to reach the distal ileum varied between 10-24 hours. Correct position was verified by fluoroscopy on day 0 and at the start of day 1, 2 and 3. Additionally, the correct position of the tube was fluoroscopically checked at the end of each experiment.

Day 1, day 2, day 3 and day 4: manometry measurements

Measurements were performed in random order with intrajejunal saline, intraileal saline, intrajejunal fat, or intraileal fat.

After the correct position of the catheter was verified, an intravenous cannula was inserted into the antecubital vein of one arm for blood sampling. The spontaneous occurrence of a phase III was defined as the start point for all the experiments. During measurements subjects were lying comfortably in a bed.

At time 0 min (15 min after the occurrence of the spontaneous phase III) the study was started with oral ingestion of 400 ml of a commercially available polymeric liquid meal (Nutrison; Nutricia Zoetermeer, The Netherlands) containing 16 g protein, 48 g carbohydrates and 12 g of saturated and unsaturated triglycerides (400 ml = 400 kcal; osmolality 260 mOsm). At the same time intrajejunal or intraileal infusion with fat emulsion (Intralipid 20%) or saline was started and continued for three hours at a rate of 1 ml/min (2 kcal/min). Intralipid 20% (Pharmacia & Upjohn BV, Woerden) consists of 20 g soybean-oil, 12 g partitionated egg-phospholipids and 22 g glycerol anhydrates per 100 ml.

Gallbladder volume was measured and blood was sampled at regular intervals at : $t = -15, 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 270, 300, 330$, and 360 min. Antroduodenojejunal motility was recorded for at least 6 hr after ingestion of the liquid meal.

Hormone assays

Plasma CCK was measured by a sensitive and specific radioimmunoassay. This antibody binds to all CCK peptides including sulfated CCK octapeptide, but not with gastrin. The detection limit of the assay is 0.1 pmol/l plasma. The intra-assay variation ranges from 4.6 to 11.5% and the inter-assay variation from 11.3 to 26.1% (17). Plasma PYY was measured by radioimmunoassay. PYY antiserum was generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM Biochemica GmbH, Switzerland). PYY was labelled with ¹²⁵Iodine with chloramine T. The assay is highly specific. There is no cross-reactivity with PP or VIP. The detection limit is 10 pmol/l. Both PYY (1-36) and PYY (3-36) bind to the antibody in dilutions up to 250000.

Analysis of manometric data

Motility patterns from antroduodenojunal manometry were analyzed both visually and by computer. Pressure waves with an amplitude ≥ 10 mmHg and duration ≥ 1.5 sec were considered as true contractions. Artifacts due to increments in intra-abdominal pressure or any other reason were excluded from analysis.

The postprandial period was defined as the time interval between the end of the meal and the occurrence of the first small intestinal phase III propagated over at least two channels, independent of the intestinal site of onset. The motility indices (MI) of the postprandial period were calculated semi-automatically for antrum, duodenum and jejunum using the formula $MI = \ln(\Sigma(\text{mmHg} \times \text{sec})/\text{min})$. Phases of the MMC were defined as follows: phase I, no more than 1 contractions per 5 min for at least 5 min and preceded by phase III; phase II: irregular contractile activity at a frequency of more than 2 per 10 min and

amplitude above 10 mmHg; phase III: regular contractile activity at a frequency of 10-12 contractions per min for at least 2 min. Phase III activity had to be propagated over at least 2 recording sites. Antral phase III activity was defined as rhythmic contractile activity at maximum frequency (3 contractions/min) for at least 1 min in temporal relationship with duodenal phase III activity (18). The duration of the MMC cycle was calculated as the interval between the beginning of phase III in the duodenum or jejunum until the beginning of the next phase III cycle.

Data and statistical analysis

Results are expressed as mean $\pm$ SEM. Postprandial gallbladder contraction was calculated as percentage decrement over basal gallbladder volume. Integrated incremental values for plasma hormone secretion and postprandial gallbladder contraction were calculated as the area under the plasma concentration and percentage gallbladder contraction curve respectively after subtraction of the basal value. For all parameters, multiple analysis of variance (MANOVA) was used to test for statistical significance. When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within subjects differ significantly. Coefficient of linear correlation (Spearman) was used to calculate correlations between motility parameters and integrated plasma hormone secretions. The significance level was set at $p < 0.05$.

RESULTS

Gastrointestinal motility

Digestive pattern

After meal ingestion, fed motor patterns were observed in all experiments. However, the duration of the fed motility pattern was significantly ($p<0.01$) prolonged after intrajejunal fat (377 ± 39 min) and intraileal fat administration (326 ± 44 min) compared to control (238 ± 17 min). No significant difference was found in the duration of the fed pattern between the intrajejunal and intraileal fat experiment.

The postprandial duodenal and jejunal MI were significantly ($p<0.05$) decreased in both the intrajejunal and intraileal fat experiment compared to the control (Table 1). The reduction in MI was more pronounced during intraileal fat infusion. The difference in postprandial MI between intraileal and intrajejunal fat was significant ($p<0.05$) from 120-180 min in the duodenum and during the first two postprandial hours in the jejunum (Table 1). In contrast to intestinal MI, postprandial antral MI during intrajejunal and intraileal fat infusion did not differ between the two experiments and controls (Table 1).

Interdigestive pattern

After transition from a digestive into an interdigestive motility pattern 17 complete MMC cycles were observed in the control, six complete MMC cycles in the intrajejunal fat and seven complete MMC cycles in the intraileal fat experiment. Complete MMC cycles were found in all eight subjects in the control experiment but only in two subjects in the intrajejunal fat and three subjects in the intraileal fat experiment. The duration of the MMC cycles was significantly ($p<0.05$) prolonged

Table 1. Postprandial antral, duodenal and jejunal motility index ($MI = \ln(\Sigma(mmHg \cdot sec)/min)$) in 60 min periods and for the total fed period, after ingestion of a liquid meal in the intrajejunal fat, intraileal fat and control experiment. * $p < 0.05$ compared to controls; # $p < 0.05$ compared to intrajejunal fat

MI	Control	Intrajejunal fat	Intraileal fat
antrum 0-60 min	5.4±0.5	5.0±0.5	5.3±0.2
antrum 60-120 min	5.3±0.4	5.1±0.4	5.4±0.2
antrum 120-180 min	5.0±0.5	5.0±0.3	5.2±0.1
total fed period	5.1±0.6	5.1±0.3	5.3±0.3
duodenum 0-60 min	5.7±0.3	5.1±0.1	4.4±0.2
duodenum 60-120 min	5.2±0.2	4.2±0.1*	4.0±0.3*
duodenum 120-180 min	5.4±0.3	4.3±0.3*	3.9±0.2*#
total fed period	5.5±0.3	4.8±0.3*	4.3±0.2*
jejunum 0-60 min	5.4±0.2	5.1±0.3	3.8±0.1*#
jejunum 60-120 min	5.2±0.3	4.3±0.4*	3.8±0.2*#
jejunum 120-180 min	5.5±0.2	4.1±0.3*	3.9±0.2*
total fed period	5.5±0.3	4.2±0.4*	3.9±0.2*

in the intrajejunal fat compared to the control experiment. In contrast, the duration of the MMC cycle in the intraileal fat experiment was significantly ($p < 0.05$) shorter compared to the control experiment (Table 2). The differences

in MMC cycle length between the experiments result from significant ($p<0.05$) differences in the duration of phase II (Table 2).

Table 2. Characteristics of MMC cycles (mean $\pm$ SEM; min) during the intrajejunal fat, intraileal fat and the control experiment. * $p<0.05$ compared to the control experiment ; # $p<0.01$ compared to the intrajejunal experiment.

MMC	Control (n=17)	Intrajejunal fat (n=6)	Intraileal fat (n=7)
Cycle length	86 $\pm$ 9	129 $\pm$ 26*	58 $\pm$ 4*#
Phase I	16 $\pm$ 2	18 $\pm$ 3	16 $\pm$ 5
Phase II	65 $\pm$ 10	107 $\pm$ 25*	38 $\pm$ 4*#
Phase III	5 $\pm$ 0.5	4 $\pm$ 1	4 $\pm$ 0.2

Gallbladder volumes

Basal gallbladder volumes were not significantly different between the saline (18.7 $\pm$ 2.4 ml), the intrajejunal fat (18.2 $\pm$ 2.4 ml) and the intraileal fat experiments (18.0 $\pm$ 3.0 ml). After meal ingestion, gallbladder volumes significantly ($p<0.01$) decreased compared to basal volumes in the control and the fat experiments (Figure 1). Postprandial gallbladder volumes in the intrajejunal fat experiment were significantly ($p<0.01$) smaller compared to those in the intraileal fat experiment from 30 to 240 min and to those in the control experiment from 90 to 240 min. Postprandial gallbladder volumes in the intraileal fat experiment were significantly ($p<0.05$) larger compared to control during the period from 30 to 60

min (Figure 1). Integrated gallbladder contraction during the 6 hour postprandial period was significantly ($p<0.05$) increased in the intrajejunal fat ($20949\pm2395\%*360\text{ min}$) compared to the intraileal fat ($12224\pm2943\%*min$) and the control experiment ($12888\pm2559\%*min$). The difference in integrated gallbladder contraction during the 6 hour postprandial period was not significant between the control and the intraileal fat experiment.

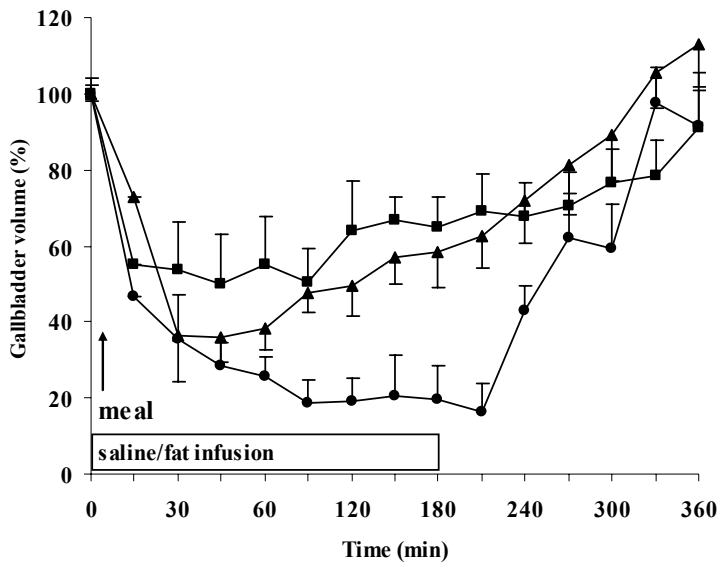


Figure 1. Gallbladder emptying (%; mean $\pm$ SEM) after meal ingestion during perfusion of intrajejunal fat (circles), intraileal fat (squares) and placebo (triangles).

Plasma CCK

Basal plasma CCK levels were not significantly different between the saline, the intrajejunal and the intraileal fat experiment ($0.7\pm0.1\text{ pM}$, $0.9\pm0.2\text{ pM}$ and 0.7 ± 0.2 respectively; Figure 2). After meal ingestion, plasma CCK levels

significantly ($p<0.01$) increased over basal starting from 15 until 210 min in the control experiment and from 15 until 360 min in the intrajejunal and intraileal fat experiment. Postprandial peak increment in plasma CCK levels was significantly lower ($p<0.05$) in the intraileal fat experiment compared to the intrajejunal fat and the control experiment. Integrated plasma CCK secretion during the first three postprandial hours was significantly ($p<0.01$) higher in the intrajejunal fat experiment (599 ± 61 pM*180 min) compared to the control (212 ± 61 pM*180 min) and the intraileal fat experiment (184 ± 26 pM*180 min). Integrated plasma CCK secretion during the 6 hour postprandial period was also significantly ($p<0.001$) higher in the intrajejunal experiment (949 ± 96 pM*360 min) compared to the saline (234 ± 134 pM*360 min) and the intraileal experiment (350 ± 55 pM*360 min). Integrated plasma CCK secretion during the 6 hour postprandial period was not significantly different between the control and the intraileal fat experiment.

Plasma PYY

Basal plasma PYY levels were not significantly different between the control, the intrajejunal and the intraileal fat experiment (18.0 ± 3.0 pM, 19.0 ± 2.3 pM and 18.4 ± 1.8 respectively; Figure 3). Plasma PYY levels increased significantly ($p<0.01$) over basal from 15 min after meal ingestion until 210 min in the control experiment and from 15 min until 360 min in the intrajejunal and intraileal fat experiment (Figure 3). During the first postprandial hour, integrated plasma PYY secretion was significantly ($p<0.05$) higher in the intraileal fat experiment (715 ± 110 pM*60 min) compared to the intrajejunal fat (189 ± 57 pM*60 min) and to the control experiment (217 ± 54 pM*60 min). Integrated plasma PYY secretion during the 6 hour postprandial period was significantly ($p<0.001$)

higher in the intraileal fat experiment (6182 ± 1250 pM*360 min) and in the intrajejunal fat experiment (5452 ± 439 pM*360 min) compared to control (868 ± 216 pM*360 min). Integrated 360 min plasma PYY secretion between intraileal and intrajejunal fat was not significantly different.

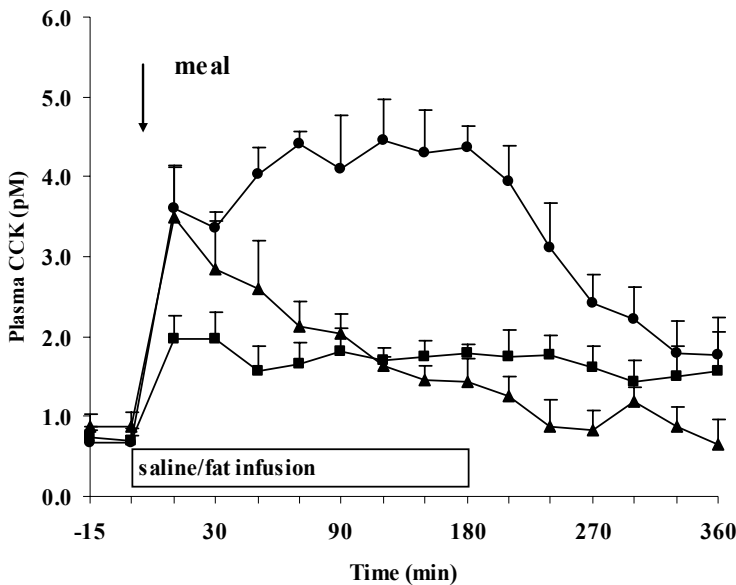


Figure 2. Fasting and postprandial plasma CCK levels (pM; mean $\pm$ SEM) during perfusion of intrajejunal fat (circles), intraileal fat (squares) and placebo (triangles).

Correlations

Gallbladder contraction and hormone secretion

Integrated postprandial gallbladder contraction significantly correlated with integrated plasma CCK secretions during the 6 hour postprandial period ($r=0.62$, $p=0.03$). In contrast, no correlation was found between postprandial gallbladder

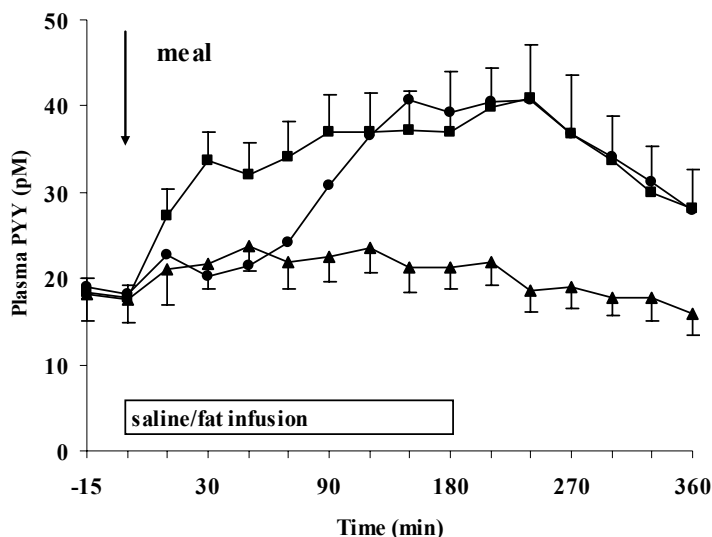


Figure 3. Fasting and postprandial plasma PYY levels (pM; mean±SEM) during perfusion intrajejunal fat (circles), intraileal fat (squares) and placebo (triangles).

contraction and integrated plasma PYY secretions during the postprandial period ($r=0.08$; $p=0.7$).

Gastrointestinal motility and hormone secretion

The duration of the fed pattern was significantly correlated with the integrated incremental plasma PYY secretion during the total postprandial period ($r=0.58$; $p=0.005$). No significant correlation was found between the duration of the fed pattern and the integrated postprandial CCK release ($r=0.35$; $p=0.1$).

Antral MI was not significantly correlated with the integrated plasma CCK or PYY secretion. The duodenal MI strongly correlated with the integrated incremental plasma CCK secretion during the total postprandial period ($r=0.85$;

$p<0.001$) but it did not significantly correlate with the integrated plasma PYY secretion ($r=0.41$; $p=0.13$). In contrast, jejunal MI significantly correlated with the integrated incremental plasma PYY secretion during the total postprandial period ($r=0.54$; $p=0.03$) but did not correlate with the integrated plasma CCK secretion ($r=0.43$; $p=0.10$).

DISCUSSION

Results of the present study show that: 1) intrajejunal and intraileal administration of fat both prolong the duration of the fed pattern induced by an oral liquid meal. The duration of the fed pattern in the fat perfusion experiments correlates significantly with the integrated plasma PYY secretion but not with the integrated plasma CCK secretion; 2) intrajejunal fat prolongs the duration of the MMC length while MMC length was shortened in the intraileal fat experiment; 3) postprandial gallbladder emptying was significantly reduced in the intraileal compared to the intrajejunal fat experiment. Postprandial gallbladder emptying significantly correlates with postprandial plasma CCK but not with plasma PYY release.

We have found in the present study that both continuous intrajejunal and intraileal administration of fat significantly prolonged the duration of the motility fed pattern induced by a liquid meal. The prolonged postprandial motility patterns are likely to result from delayed gastric emptying and increased small intestinal transit time induced by the intestinal feedback. Previous studies in humans have shown that intrajejunal and intraileal nutrients inhibit not only gastric emptying but also delay small intestinal transit time (4,5,7,8). Delayed gastric emptying and increased intestinal transit time result in longer intestinal

nutrient exposure time which may contribute to the prolonged duration of the digestive period. It is interesting to observe that the duration of the fed pattern correlated significantly only with postprandial plasma PYY but not with plasma CCK release. In humans, intraduodenal nutrients and subsequent CCK release play a central role in the transition from interdigestive to digestive motility pattern (19-21). On the other hand, mechanisms regulating the transition from digestive to interdigestive state are unclear. There is, however, evidence that the distal small bowel may be involved in the regulation of the late postprandial period and the transition to the interdigestive state (6,22-24). It has been shown by Keller et al (24) that the duration of the digestive motor pattern does not correlate with duodenal or jejunal nutrients but rather with the late postprandial increase in ileal nutrient concentration. Since peptide YY is released in the distal gut and represents ileal brake activation, our findings are in line with those of Keller et al (24).

It is apparent from the results that the postprandial duodenal and jejunal MI were reduced during intrajejunal and intraileal fat infusion while no significant changes were observed concerning antral MI. Furthermore the reduction in jejunal MI was more pronounced in the intraileal fat experiment. This finding is in line with those of Lin et al demonstrating that in dogs, fat induced ileal brake is more potent than the fat induced jejunal brake (10). Our results further show that duodenal MI correlated more with plasma CCK secretion than with plasma PYY secretion while jejunal MI correlated more with plasma PYY than with plasma CCK secretion. These findings suggest regional small intestinal heterogeneity in responsiveness to gut peptides. An earlier study in rats has shown that intravenous infusion of PYY had less effect on duodenal motility but

almost totally abolished the spiking activity in the jejunum (25). Furthermore, one study in humans has demonstrated that motor responses of the intestine to different gut peptides appear to vary regionally (26). Our results are, on the one hand, consistent with those found by Welch et al demonstrating that fat infusion into the ileum reduces the postprandial contractile activity of the jejunum (27). On the other hand, we also found a decrease in postprandial duodenal motility while in the study by Welch et al. duodenal motility was not affected. Differences in study design such as a shorter duration of intraileal fat infusion (20 min by Welch et al versus 180 min in the present study) may account for this difference in results. It is conceivable that the degree of activation of the ileal brake also determines the extent of the inhibitory action.

Delayed gastric emptying induced by intrajejunal and intraileal fat has repeatedly been demonstrated. Although a reduced antral MI would be expected during ileal or jejunal fat perfusion, we did not observe differences in antral MI between the intrajejunal, intraileal fat and the control experiment. The reason underlying this finding is not obvious. However, delayed gastric emptying may result from factors other than impaired antral motor activity such as increased pyloric tone, disturbed antro-pyloro-duodenal coordination. In animals, activation of the ileal brake through infusion of PYY inhibits interdigestive but not postprandial antral motility (28,29). It has been shown in pigs that ileal fat infusion increases pyloric tone (30). In humans, the effect of the ileal brake on pyloric motility has not been studied .

In contrast to the prolonged MMC cycle length induced by intrajejunal fat, intraileal intralipid significantly shortened MMC cycle length by reducing the duration of phase II. The mechanism underlying this difference is not apparent.

To date, we confirm the results of Layer et al reporting a shorter duration of the MMC cycle with shorter phase II during intraileal fat infusion (6). Moreover, similar changes in MMC cycle length have been observed in patients with malabsorption disorders with increased ileal fat delivery (31,32). These data consistently point to a shortened MMC cycle length during ileal brake activation. The prolonged MMC cycle length induced by intrajejunal fat results from prolonged duration of phase II. This finding is difficult to explain. One possibility is that the plasma CCK levels that remained elevated after the transition from fed to a fasting motor pattern in the intrajejunal fat experiment, have contributed to the prolongation of phase II. This is plausible since CCK is known to induce fed like motor activity (20,21) and the irregular contractions observed during phase II resemble the fed intestinal motor pattern. This concept is in line with the results found by Defilippi et al showing that in dogs, MMC cycle length was prolonged with an increased duration of phase II during intraduodenal infusion of nutrients of low caloric load (33). The study by Defilippi et al also demonstrated that the number of MMC cycles further decreased and was completely suppressed when higher caloric loads were administered in the duodenum. Although plasma CCK levels were not measured in that study, the stepwise increasing inhibitory effect on the MMC cycle induced by intraduodenal caloric loads may well have been mediated by CCK. It is well known that the number of CCK producing cells is highest in the duodenum (34).

Ingestion of a liquid meal significantly decreased gallbladder volumes which gradually returned to basal at the end of the postprandial period. Fat in the jejunum significantly increased postprandial gallbladder emptying compared to

the intraileal fat experiment and to control especially in the early postprandial period. A similar pattern was observed for the proximal gut hormone CCK. Since gallbladder contraction is merely dependent on CCK secretion (11,12) these results indicate that increased postprandial gallbladder emptying results from increased plasma CCK release. Indeed, postprandial gallbladder emptying strongly correlated with postprandial plasma CCK levels. Moreover, the significantly higher plasma CCK levels during intrajejunal fat infusion indicate that CCK is not only released in the duodenum but also in the more distal small intestine.

No significant correlations were found between gallbladder emptying and PYY secretion neither in the early nor in the late postprandial period. This finding is in line with previous studies in humans and dogs showing that PYY did not inhibit gallbladder contraction stimulated by exogenous or endogenous CCK (13, 35). However, it is worth noticing the delayed peak plasma PYY level obtained in the intrajejunal intralipid experiment compared to the intraileal experiment. This time-related difference reflects the time needed for intralipid to reach the ileum and stimulate PYY release.

In conclusion, the data obtained in the present study demonstrate that intestinal feedback control mechanisms evoked by the fat induced ileal brake on proximal small intestine, postprandial gallbladder motility and hormone release differ qualitatively and quantitatively from those evoked by the fat induced jejunal brake.

REFERENCES

1. Ledebor M, Masclee AA, Coenraad M, Vecht J, Biemond I, Lamers CB.

- Antroduodenal motility and small bowel transit during continuous intraduodenal or intragastric administration of enteral nutrition. *Eur J Clin Invest* 29:615-623, 1999
2. Ledeboer M, Masclee AA, Biemond I, Lamers CB. Effect of intragastric or intraduodenal administration of a polymeric diet on gallbladder motility, small-bowel transit time, and hormone release. *Am J Gastroenterol* 93:2089-2096, 1998
 3. Kerstens PJ, Lamers CB, Jansen JB, de Jong AJ, Hessels M, Hafkenschied JC. Physiological plasma concentrations of cholecystikinin stimulate pancreatic enzyme secretion and gallbladder contraction in man. *Life Sci* 36:565-569, 1985
 4. Pironi L., V. Stanghellini, M. Miglioli, R. Corinaldesi, R. De Giorgio, E. Ruggeri, G. Tosetti, G. Poggioli, A. M. M. Labate, N. Monetti, G. Gozetti, L. Barbara, and V. L. W. Go. Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma level of peptide YY. *Gastroenterology* 105:733-739, 1993
 5. Read N. W., A. MacFarlane, and R. Kinsman. Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon in man. *Gastroenterology* 86:274-280, 1984
 6. Leyer P, Schlesinger T, Groger G, Goebell H. Modulation of human periodic interdigestive gastrointestinal motor and pancreatic function by the ileum. *Pancreas* 8:426-432, 1993
 7. Vidon N, Pfeiffer A, Chayvialle JA, Merite F, Maurel M, Franchisseur C, Huchet B, Bernier JJ. Effect of jejunal infusion of nutrients on gastrointestinal transit and hormonal response in man. *Gastroenterol Clin*

Biol 13:1042-1049, 1989

8. Vidon N, Chaussade S, Merite F, Huchet B, Franchisseur C, Bernier JJ. Inhibitory effect of high caloric load of carbohydrates or lipids on human pancreatic secretions: a jejunal brake. *Am J Clin Nutr* 50:231-236, 1989
9. Lin HC, Zhao XT, Wang L. Jejunal brake: inhibition of intestinal transit by fat in the proximal small intestine. *Dig Dis Sci* 41:326-329, 1996
10. Lin HC, Zhao XT, Wang L. Intestinal transit is more potently inhibited by fat in the distal (ileal brake) than in the proximal (jejunal brake) gut. *Dig Dis Sci* 42:19-25, 1997
11. Niederau C, Heintges T, Rovati L, Strohmeyer G. Effects of loxiglumide on gallbladder emptying in healthy volunteers. *Gastroenterology* 97:1331-1336, 1989
12. Jebbink MC, Masclee AA, van der Kleij FG, Schipper J, Rovati LC, Jansen JB, Lamers CB. Effect of loxiglumide and atropine on erythromycin-induced reduction in gallbladder volume in human subjects. *Hepatology* 16(4): 937-42, 1992
13. Hoentjen F, Hopman WP, Jansen JB. Effect of circulating peptide YY on gallbladder emptying in humans. Hoentjen F, Hopman WP, Jansen JB. *Scand J Gastroenterol* 36:1086-1091, 2001
14. Conter RL, Roslyn JJ, Taylor IL. Effects of peptide YY on gallbladder motility. *Am J Physiol* 252: G 736-G741, 1987
15. Everson GT, Braverman DZ, Johnson ML, Kern F, Jr. A critical evaluation of real-time ultrasonography for the study of gallbladder volume and contraction. *Gastroenterology* 79:40-46, 1980
16. Hopman WPM, Brouwer WFM, Rosenbusch G, Jansen JBMJ, Lamers

- CBHW. A computerized method for rapid quantification of gallbladder volume from real-time sonograms. *Radiology* 154:236-237, 1985
17. Jansen J B M J, Lamers C B H W. Radioimmunoassay of cholecystokinin in human tissue and plasma. *Clin Chim Acta* 131:305-316, 1983
 18. Kellow J. E., J. F. Borody, S. F. Phillips, R. L. Tucker, and A. C. Haddad. Human interdigestive motility: variations in patterns from esophagus to colon. *Gastroenterology* 91:386-395, 1986
 19. Behrns KE, Sarr MG. Duodenal nutrients inhibit canine jejunal fasting motor patterns through a hormonal mechanism. *Dig Dis Sci* 39:1665-1671, 1994
 20. Schmidt WE, Creutzfeldt W, Schleser A, Choudhury AR, Nustede R, Hocker M, Nitsche R, Sostmann H, Rovati LC, Folsch UR. Role of CCK in regulation of pancreaticobiliary functions and GI motility in humans: effects of loxiglumide. *Am J Physiol* 260:G197-G206, 1991
 21. Thor P, Laskiewicz J, Konturek P, Konturek SJ. Cholecystokinin in the regulation of intestinal motility and pancreatic secretion in dogs. *Am J Physiol* 255:G498-G504, 1988
 22. Holgate AM, Read NW. Effect of ileal infusion of intralipid on gastrointestinal transit, ileal flow rate, and carbohydrate absorption in humans after ingestion of a liquid meal. *Gastroenterology* 88:1005-1011, 1985
 23. Spiller RC, Trotman IF, Adrian TE, Bloom SR, Misiewicz JJ, Silk DB. Further characterisation of the 'ileal brake' reflex in man--effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptideYY. *Gut* 29:1042-1051, 1988

24. Keller J, Runzi M, Goebell H, Layer P. Duodenal and ileal nutrient deliveries regulate human intestinal motor and pancreatic responses to a meal. *Am J Physiol* 272:G632-G637, 1997
25. Al-Saffar A, Hellstrom PM, Nylander G. Correlation between peptide YY-induced myoelectric activity and transit of small-intestinal contents in rats. *Scand J Gastroenterol* 20:577-82, 1985
26. Kellow JE, Miller LJ, Phillips SF, Haddad AC, Zinsmeister AR, Charboneau JW. Sensitivities of human jejunum, ileum, proximal colon, and gallbladder to cholecystokinin octapeptide. *Am J Physiol* 252:G345-G356, 1987
27. Welch IM, Davison PA, Worthing J, Read NW. Effect of ileal infusion of lipid on jejunal motor patterns after a nutrient and nonnutrient meal. *Am J Physiol* 255:G800-G806, 1988
28. Zai H, Haga N, Fujino MA, Itoh Z. Effect of peptide YY on gastric motor and secretory activity in vagally innervated and denervated corpus pouch dogs. *Regul Pept* 61:181-188, 1996
29. Suzuki T, Nakaya M, Itoh Z, Tatemoto K, Mutt V. Inhibition of interdigestive contractile activity in the stomach by peptide YY in Heidenhain pouch dogs. *Gastroenterology* 85:114-121, 1983
30. Cuhe G, Malbert CH. Ileal short-chain fatty acids inhibit transpyloric flow in pigs. *Scand J Gastroenterol* 34:149-55, 1999
31. Vu MK, Vecht J, Eddes EH, Biemond I, Lamers CB, Masclee AA. Antroduodenal motility in chronic pancreatitis: are abnormalities related to exocrine insufficiency?. *Am J Physiol Gastrointest Liver Physiol* 278:G458-G466, 2000
32. Layer P, von der Ohe MR, Holst JJ, Jansen JB, Grandt D, Holtmann G,

- Goebell H. Altered postprandial motility in chronic pancreatitis: role of malabsorption. *Gastroenterology* 112:1624-1634, 1997
33. Defilippi C. Canine small bowel motor activity in response to intraduodenal infusion of nutrient mixtures of increasing caloric load in dogs. *Dig Dis Sci* 48:1482-1486, 2003
34. Larsson LI, Rehfeld JF. Distribution of gastrin and CCK cells in the rat gastrointestinal tract. Evidence for the occurrence of three distinct cell types storing COOH-terminal gastrin immunoreactivity. *Histochemistry* 58:23-31, 1978
35. Lluís F, Fujimura M, Lonovics J, Guo Y, Gomez G, Greeley GH, Townsend CM, Thompson JC. Peptide YY and gallbladder contraction. Studies in vivo and in vitro. *Gastroenterology* 94:1441-1446, 1988

Chapter 3

DOES JEJUNAL FEEDING ACTIVATE EXOCRINE PANCREATIC SECRETION ?

My K. Vu¹, Patrick P. J. van der Veek¹, Marijke Frölich², John H.M. Souverijn², Izak Biemond¹, Cornelis B.H.W. Lamers¹, Ad A.M. Masclee¹
Departments of Gastroenterology-Hepatology¹ and Clinical Chemistry²,
Leiden University Medical Center, the Netherlands

Does jejunal feeding activate exocrine pancreatic secretion?

M. K. Vu, P. P. J. van der Veek, M. Frölich, J. H. M. Souverijn, I. Biemond, C. B. H. W. Lamers and A. A. M. Masclee

Leiden University Medical Center, Leiden, the Netherlands

Abstract

Background The upper small bowel is of pivotal importance for the stimulation of exocrine pancreatic secretion in response to a meal. We hypothesize that more distal delivery of nutrients into the small intestine will result in less activation of pancreatic secretion.

Materials and methods Eight healthy subjects (3 male, 5 female; age 23 ± 1 years) participated in two experiments, performed in random order. Subjects were intubated with a 4-lumen tube. Duodenal outputs of pancreatic enzymes and bilirubin were measured by aspiration using a recovery marker. The distal opening was used for continuous administration of a mixed liquid meal and located at either the ligament of Treitz or 60 cm further distally. Gallbladder volume was measured and blood samples were drawn for determination of gastrointestinal hormones. The duration of each experiment was 4 h; with 1 h fasting and 3 h continuous administration of nutrients.

Results During proximal jejunal feeding, pancreatic enzyme output increased significantly over basal levels. No significant increase over basal levels was observed during distal jejunal feeding. Bilirubin output and gallbladder contraction were significantly ($P < 0.05$) reduced during distal compared to proximal jejunal feeding. No significant differences were found in plasma levels of CCK, PYY and neurotensin between proximal and distal jejunal feeding.

Conclusion Continuous feeding in the distal jejunum does not stimulate exocrine pancreatic secretion but maintains gallbladder contraction, although to a lesser extent. These effects are not related to hormonal changes but probably reduced activation of the enteropancreatic reflexes.

Keywords Cholecystokinin, exocrine pancreatic secretion, gallbladder contraction, jejunal feeding, neurotensin, pancreatic polypeptide, peptide YY.

Eur J Clin Invest 1999; 29 (12): 1053–1059

Introduction

Within the last decade enteral nutrition has been accepted as an alternative for the parenteral route in the nutritional support of patients with gastrointestinal disorders. Recently, several studies have yielded promising results concerning the clinical benefits of early enteral nutrition

in patients with acute pancreatitis [1–3]. However, in parallel with preserving the integrity of the gut, intraluminal nutrients stimulate exocrine pancreatic secretion, which may negatively influence the course of pancreatitis.

Concerning the physiology of pancreatic secretion the intestinal phase of digestion accounts for the majority of the postprandial exocrine pancreatic secretory output. It is well established that the duodenum is the most important site of stimulation where the two classical pancreatic stimulatory hormones, CCK and secretin, are released and enteropancreatic reflexes are situated [4–6]. Not only the proximal but also the distal gut may participate in the regulation of exocrine pancreatic secretion. Several studies in both humans and animals have shown that infusion of nutrients into the distal ileum or colon inhibits exocrine pancreatic enzyme output [7,8].

Little is known about the influence of the site of nutrient delivery on pancreatic secretion. Such information is of

Departments of Gastroenterology-Hepatology (M. K. Vu, P. P. J. van der Veek, I. Biemond, C. B. H. W. Lamers and A. A. M. Masclee) and Clinical Chemistry (M. Frölich, J. H. M. Souverijn), Leiden University Medical Center, Leiden, the Netherlands.

Correspondence to: Dr A. A. M. Masclee, Department of Gastroenterology-Hepatology, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands. Tel.: +31 71 5261846; fax: +31 71 5248115.

Received 12 April 1999; accepted 22 August 1999

physiological relevance for further clinical studies on site-specific nutrient delivery of enteral nutrition. We hypothesize that the more distally nutrients are delivered into the jejunum, bypassing the duodenum, the less activation of the exocrine pancreatic secretion will be found. This study was therefore performed to compare, in healthy volunteers, the effect of a commercially available nutrient solution on exocrine pancreatic secretion, gallbladder contraction and subsequent release of proximal and distal gut hormones when administered either in the proximal or in the distal jejunum.

Subjects and methods

Subjects

Eight healthy subjects (3 male, 5 female; age 23 ± 1 years) without a history of gastrointestinal symptoms or abdominal surgery participated in this study. None of them were taking any medication. Informed consent was obtained from each individual and the protocol had been approved by the ethics committee of the Leiden University Medical Center.

Study design

Each subject participated in two experiments, performed in random order on separate days with an interval of at least one week. The experiments started at 07.45 h. After an overnight fast, subjects were intubated transnasally with a polyvinyl 4-lumen tube consisting of two perfusion and two aspiration ports. One perfusion port was located at the papilla of Vater for continuous perfusion of the nonabsorbable marker polyethyleneglycol (PEG 4000; 15 mg mL^{-1}). The second perfusion port was for continuous administration of a liquid meal (Nutrison standard, Nutricia, the Netherlands) containing 4 g emulsified fat, 12 g carbohydrate and 4 g protein per 100 mL (1 kcal mL^{-1}) at a rate of 100 mL hour^{-1} . This perfusion port was either located just proximal to the ligament of Treitz or 60 cm further distally. Aspiration ports were located in the antrum and in the duodenum, 15 cm distal to the papilla of Vater. After the correct position of the tube was checked under fluoroscopy, a cannula was inserted into the antecubital vein of one arm for blood sampling. The PEG infusion was then started ($t = -60 \text{ min}$) and continued until the end of the experiment ($t = 180 \text{ min}$) at a rate of 3 mL min^{-1} . At $t = 0 \text{ min}$, either proximal or distal intrajejunal infusion of Nutrison was started at a rate of 100 mL h^{-1} and continued until the end of the experiment ($t = 180 \text{ min}$). Gastric contents were aspirated continuously. Duodenal contents were aspirated in 15 min portions under basal and stimulated conditions for determination of trypsin, lipase, amylase, bilirubin and PEG concentration.

Assays of duodenal contents

Duodenal samples were collected in ice-chilled tubes

during the experiment. Enzymatic activity of amylase and lipase was determined as described previously [9,10]. Enzymatic activity of trypsin was determined according to Hummel [11]. Concentrations of PEG in the aspirated samples were determined turbidimetrically by a modified method of Hydén [12] and were used to calculate the outputs of amylase, trypsin, lipase and bilirubin.

Measurements of gallbladder volumes

Gallbladder volumes measured by real time ultrasonography (Toshiba, 3.75 MHz transducer) were calculated by the sum of cylinders method using a computerized system [13,14]. In this method the longitudinal image of the gallbladder is divided into series of equal height, with diameter perpendicular to the longitudinal axis of the gallbladder image. The uncorrected volume is the sum of volumes of these separate cylinders. To correct for the displacement of the longitudinal image of the gallbladder from the central axis, a correction factor is calculated from the longitudinal and transversal scans of the gallbladder. Gallbladder volume is calculated by multiplication of the uncorrected volume with the square of the correction factor. The mean of two measurements was used for analysis. The assumptions and the mathematical formula used to calculate gallbladder volume have been described and validated previously [13,14].

Hormone assays

Blood samples for measurement of plasma pancreatic polypeptide (PP), cholecystokinin (CCK) and peptide YY (PYY) were drawn at $t = -15, 0, 15, 30, 45, 60, 90, 120, 150$ and 180 min during each experiment. The blood samples were collected in EDTA containing ice-chilled tubes. The samples were centrifuged at a rate of 3000 r.p.m. for 10 min at a temperature of 4°C . Plasma CCK was measured by a sensitive and specific radioimmunoassay [15]. This antibody binds to all CCK peptides including sulfated CCK octapeptide, but not gastrin. The detection limit of the assay is 0.1 pM plasma. Plasma PYY was measured by radioimmunoassay. PYY antiserum was generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM AG, Bubendorf, Switzerland). PYY was labelled with ^{125}I iodine using chloramine T. There is no cross-reactivity with PP or VIP. The detection limit is 10 pM plasma. Both PYY [1–36] and PYY [3–36] bind to the antibody in dilutions up to 25 000. Plasma neurotensin was measured by a specific radioimmunoassay with a sensitivity of 4 pmol L^{-1} of plasma. Neurotensin antiserum was generated in rabbits by multiple intradermal injections of synthetic human neurotensin (BACHEM AG, Bubendorf, Switzerland). Neurotensin was labelled by a modification of the convention chloramine T. The antiserum was directed towards the amino terminal region of neurotensin. Plasma PP was determined by radioimmunoassay as described previously [16].

Statistical analysis

Basal and stimulated outputs of amylase, lipase, trypsin and bilirubin were calculated in a 60-min period by summing up outputs of 15-min periods. Fasting gallbladder volumes were expressed in mL. Gallbladder emptying was calculated as percentage of fasting gallbladder volume. Integrated incremental values for plasma hormone secretion were calculated as the area under the plasma concentration curve after subtraction of the basal value at time $t=0$. For all parameters, multiple analysis of variance (MANOVA) was used to test for statistical significance. When this indicates a probability of less than 0.05 for the null hypothesis, Newman-Keuls student t -test analyses were performed to determine which values, between or within subjects, differ significantly. The significant level was set at $P<0.05$.

Results

Pancreatico-biliary secretion

Basal outputs of pancreatic enzymes and bilirubin were not significantly different between the two experiments (Table 1). Outputs of lipase and trypsin increased significantly ($P<0.01$) and remained increased over basal during 3 h continuous proximal feeding, while no significant increases over basal values were found when nutrients were administered in the distal jejunum. Lipase and trypsin outputs during proximal feeding were significantly ($P<0.01$) higher compared to those during distal feeding (Fig. 1). Outputs of amylase increased significantly ($P<0.05$) over basal during continuous feeding in the proximal jejunum but not during distal jejunal feeding, and were significantly higher in the second and the third hour of nutrition compared to the distal experiment. Bilirubin outputs increased significantly ($P<0.05$) over basal during proximal jejunal feeding. Nutrients in the distal jejunum induced a significant rise ($P<0.05$) of

bilirubin output over basal level during the first hour of nutrition. No significant increases were found during the second and the third hour of nutrition (Fig. 1).

Gallbladder volumes

Basal gallbladder volumes were not significantly different between the two experiments (18.4 ± 2.3 mL in the proximal experiment vs. 18.4 ± 1.9 mL in the distal experiment). Continuous enteral feeding either in the proximal or distal jejunum induced a significant ($P<0.01$) reduction in gallbladder volumes starting at 15 min until the end of the experiment at 180 min. Gallbladder contraction from 90 min until 180 min was significantly ($P<0.01$) reduced during distal feeding compared to proximal feeding (Fig. 2). This finding is in agreement with the reduced bilirubin output found with distal vs. proximal nutrient administration.

Plasma PP

Basal plasma PP concentrations were not significantly different between proximal feeding (21 ± 4 pM) and distal feeding (20 ± 2 pM) (Fig. 3a). Proximal feeding induced a significant ($P<0.05$) rise in plasma PP levels throughout the 3 h stimulated period, while nutrients in the distal jejunum induced a significant ($P<0.05$) rise of plasma PP over basal only in the last 90 min of the stimulated period. The integrated incremental PP secretion during proximal feeding (3944 ± 806 pM $\times$ 180 min) was significantly ($P<0.05$) higher compared to that during distal feeding (2427 ± 755 pM $\times$ 180 min).

Plasma CCK

Basal plasma CCK concentrations were not significantly different between the proximal (0.2 ± 0 pM) and the distal experiment (0.1 ± 0 pM). Both proximal and distal enteral

Table 1 Basal and stimulated pancreatic enzyme and bilirubin secretion during the first, second and third hour of proximal and distal jejunal feeding in 8 healthy volunteers

	Basal	1 h	2 h	3 h
Proximal infusion				
Lipase (kU h ⁻¹)	130 $\pm$ 30	261 $\pm$ 50*†	270 $\pm$ 32*†	274 $\pm$ 30*†
Trypsin (U h ⁻¹)	65 $\pm$ 18	272 $\pm$ 38*†	322 $\pm$ 44*†	267 $\pm$ 35*†
Amylase (kU h ⁻¹)	17 $\pm$ 5	34 $\pm$ 5*	42 $\pm$ 5*†	38 $\pm$ 7*†
Bilirubin (μ mol h ⁻¹)	13 $\pm$ 3	86 $\pm$ 23*	48 $\pm$ 8*	37 $\pm$ 9*
Distal infusion				
Lipase (kU h ⁻¹)	93 $\pm$ 31	110 $\pm$ 31	77 $\pm$ 24	78 $\pm$ 34
Trypsin (U h ⁻¹)	93 $\pm$ 28	142 $\pm$ 31	108 $\pm$ 30	99 $\pm$ 37
Amylase (kU h ⁻¹)	20 $\pm$ 5	26 $\pm$ 6	24 $\pm$ 6	26 $\pm$ 6
Bilirubin (μ mol h ⁻¹)	16 $\pm$ 2	60 $\pm$ 18*	28 $\pm$ 6	21 $\pm$ 3

* $P<0.05$ compared to basal; † $P<0.05$ compared to distal infusion.

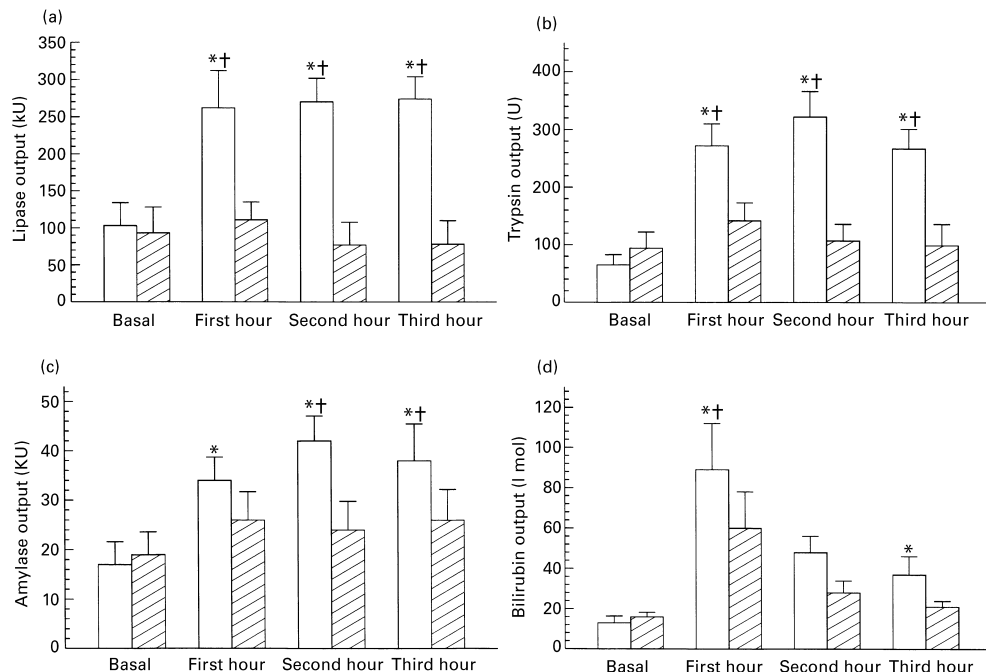


Figure 1 Basal and stimulated lipase (a), trypsin (b), amylase (c) and bilirubin (d) outputs in the first, second and third hour during continuous proximal (open bars) and distal (shaded bars) jejunal feeding in 8 healthy subjects. * $P < 0.01$ compared to basal and † $P < 0.05$ compared to distal jejunal feeding.

feeding induced a significant ($P < 0.05$) rise in plasma CCK levels throughout the 3 h stimulated period with a peak at $t = 15$ min (Fig. 3b). The integrated incremental CCK release during proximal feeding (154 ± 35 pM $\times$ 180 min) was not significantly different from that when nutrients were infused distally (185 ± 88 pM $\times$ 180 min).

Plasma PYY

Basal plasma PYY concentrations were not significantly different between proximal (20 ± 1 pM) and distal feeding (21 ± 1 pM). Neither proximal nor distal feeding significantly increased plasma PYY over basal levels (Fig. 4a).

Plasma neurotensin

Basal plasma neurotensin concentrations were not significantly different between the proximal experiment (15 ± 1 pM) and the distal experiment (15 ± 1 pM). Neither proximal nor distal jejunal feeding significantly increased plasma neurotensin over basal levels (Fig. 4b).

Discussion

We have demonstrated in the present study that continuous administration of a mixed liquid meal in the distal jejunum did not stimulate pancreatic enzyme secretion. Gallbladder contraction, on the other hand, was induced by distal

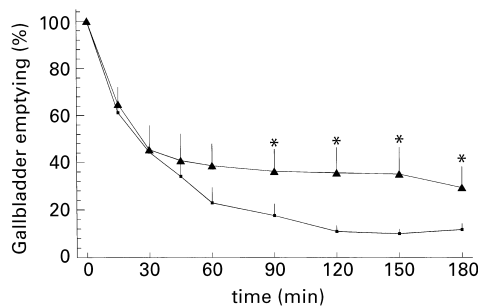


Figure 2 Percentage of gallbladder emptying during continuous proximal (squares) and distal jejunal feeding (triangles) in 8 healthy subjects. * $P < 0.05$ proximal vs. distal jejunal feeding.

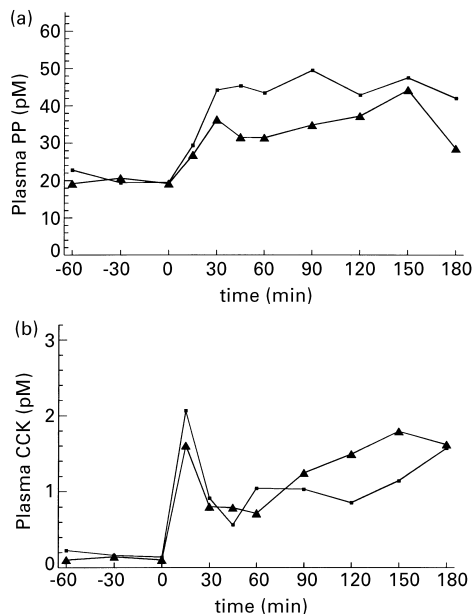


Figure 3 Continuous enteral nutrition: plasma PP levels during continuous proximal (squares) and distal jejunal feeding (triangles) in 8 healthy subjects (a); plasma CCK levels during continuous proximal (squares) and distal jejunal feeding (triangles) in 8 healthy subjects (b).

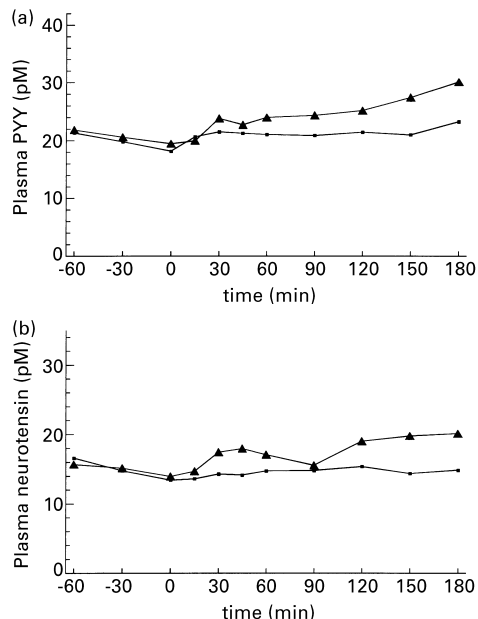


Figure 4 Continuous enteral nutrition: plasma PYY levels during continuous proximal (squares) and distal jejunal feeding (triangles) in 8 healthy subjects (a); plasma neurotensin levels during continuous proximal (squares) and distal jejunal feeding (triangles) in 8 healthy subjects (b).

jejunal nutrients, though to a lesser extent compared to when nutrients were given in the proximal jejunum.

Within the last decade there is growing interest for enteral feeding as a route for nutritional support in patients with various gastrointestinal disorders. The clinical benefits of enteral feeding have been demonstrated, for instance, in acute pancreatitis [1–3]. Recent studies suggested that early enteral feeding is effective in severe acute pancreatitis to prevent infections and other complications [1–3]. It is questioned whether delivery of nutrients more distally into the small intestine is more in line with the concept of ‘the pancreas at rest’. Results on the effect of jejunal feeding on basal exocrine pancreatic secretion are contradictory. In dogs, jejunal infusion of elemental nutrients did not stimulate exocrine pancreatic secretion in one study while a significant increase in exocrine pancreatic output was found in another study [17,18]. Vidon *et al.* have shown that intrajejunal infusion of nutrients stimulates pancreatic secretion in healthy volunteers [19]. This finding was not supported by another study which showed that exocrine pancreatic secretion during jejunal infusion was not significantly different from fasting [20]. An explanation for these contrasting results is not readily available. Differences in study results may be related to the type of nutrients and the length of jejunum exposed to nutrients.

In contrast to proximal jejunal feeding, a significant rise in pancreatic enzyme output over basal level was not observed during distal jejunal feeding. Several mechanisms may be involved such as [1]: a decreased release of stimulatory hormones [2]; reduced activation enteropancreatic reflexes as a result of distal administration of nutrients [3]; and activation of an inhibitory feedback from the distal small intestine. The proximal gut hormone CCK is generally accepted as a major hormonal regulator during the intestinal phase of postprandial pancreatic secretion in man [4,21]. CCK receptor antagonists reduce meal-stimulated pancreatic enzyme response by over 60% [21]. The present results show that plasma CCK levels during distal infusion of nutrients were not significantly different compared to those during proximal infusion of nutrients. These findings exclude a role for CCK in the impaired activation of exocrine pancreatic secretion during distal nutrient administration. PYY and neurotensin are distal gut hormones [22,23]. PYY is considered one of the most potent hormonal mediators of the so-called ‘ileal brake’, a negative feedback loop from the distal to the proximal digestive tract [7,8,24]. Studies in both humans and animals have demonstrated that exogenous as well as endogenous PYY inhibits exocrine pancreatic secretion [25,26]. Based on the observations that large meals and intrail infusion of

nutrients activate PYY release, we hypothesized that compared to proximal nutrients delivery, more distal delivery would result in higher plasma PYY levels and in turn inhibit exocrine pancreatic secretion. However, results of plasma PYY levels during proximal and distal jejunal infusion of nutrients did not support this hypothesis. The presence of emulsified triglycerides in the test meal and the continuous administration of nutrients may explain why the ileal brake was not activated. Emulsified fats are more easily dispersed and more readily and completely absorbed. As a result, even during distal jejunal nutrient delivery the amount of undigested nutrients reaching the ileum is probably too small to activate distal gut hormone release. This concept was further substantiated by the finding that plasma neurotensin levels did not significantly increase during distal jejunal infusion of nutrients. Neurotensin is released in response to a fat rich meal and is found in highest concentrations in the ileal mucosa [23]. The physiological role of neurotensin in the regulation of pancreatic secretion is not yet fully established. Exogenous neurotensin stimulates pancreatic secretion in man [27] but there is also evidence suggesting an inhibitory effect of neurotensin on secretin and cerulein stimulated pancreatic secretion [28]. Plasma neurotensin remained at basal levels during proximal infusion of nutrients when pancreatic enzyme secretion was significantly increased. These observations are in agreement with earlier study demonstrating that intraduodenal infusion of a mixed meal at a rate of 1 kcal min^{-1} did not stimulate neurotensin release [29].

Although hormonal pathways have long been considered the predominant mechanism controlling pancreatic secretion, different studies have provided evidence that pancreatic response to intestinal stimulants is to a large extent mediated by cholinergic enteropancreatic reflexes [6,30]. When nutrients are given more distally into the small intestine, duodenal induced neurally mediated exocrine pancreatic responses will not be evoked. This may explain the absence for activation of pancreatic enzyme output during distal jejunal nutrient infusion. The reduced secretion of plasma PP, a pancreatic hormone under vagal control further supports this idea. In dogs, selective removal of the duodenum or infusion of nutrients into the distal small intestine decreased postprandial PP release [31].

In contrast to pancreatic enzyme output, nutrients in the distal jejunum induced a significant gallbladder contraction. Gallbladder emptying during the last 2 h of distal feeding was significantly decreased compared to proximal feeding. This finding is in line with intraduodenal bilirubin output during distal feeding. It is not obvious which mechanism(s) are responsible for the differences in emptying response between proximal and distal feeding. The fact that gallbladder volumes differed only during the last 90 min of nutrient infusion does not favour a neurally mediated pathway, which is responsible for the initial phase of gallbladder emptying. With regard to a hormonally mediated pathway there are several factors that should be taken into consideration. First, plasma levels of CCK, the major stimulator of postprandial gallbladder

contraction, were not significantly different between proximal and distal nutrients. Second, plasma levels of PP, which enhance gallbladder relaxation, were even lower during distal jejunal infusion of nutrients [32]. Thus, differences in gallbladder volumes between proximal and distal feeding can not be explained based on plasma levels of CCK and PP. With respect to the two distal gut hormones PYY and neurotensin, the slightly, although not significantly, higher plasma levels of PYY and neurotensin may contribute to the larger gallbladder volumes during distal jejunal feeding. It has been shown previously that infusion of PYY induces gallbladder relaxation after CCK stimulated gallbladder contraction [33].

The findings from the present study on site specific activation of exocrine pancreatic secretion put forward a concept of 'a pancreas at rest' that may be relevant in certain clinical conditions. Further clinical evaluation in acute pancreatitis may be hampered by the presence of paralytic ileum that may impede positioning of a feeding tube in the distal jejunum.

In summary, we have shown that a standard mixed liquid meal when administered into the distal jejunum does not activate exocrine pancreatic secretion. This finding is not because of changes in hormonal responses but probably as a result of reduced activation of the enteropancreatic reflexes. On the other hand, gallbladder contraction was maintained, although to a lesser extent during distal jejunal feeding. Thus, distal jejunal delivery of nutrients does not activate exocrine pancreatic secretion while gallbladder motility is preserved.

References

- McClave SA, Greene LM, Snider HL *et al.* Comparison of the safety of early enteral vs parenteral nutrition in mild acute pancreatitis. *JPEN* 1997;21:14–20.
- Windsor AC, Kanwar S, Li AG *et al.* Compared with parenteral nutrition, enteral feeding attenuates the acute phase response and improves disease severity in acute pancreatitis [see comments]. *Gut* 1998;42:431–5.
- Nakad A, Piesseaux H, Marot JC *et al.* Is early enteral nutrition in acute pancreatitis dangerous? About 20 patients fed by an endoscopically placed nasogastrojejunal tube. *Pancreas* 1998;17(2):187–93.
- Adler G, Beglinger C. Hormones as regulators of pancreatic secretion in man. *Eur J Clin Invest* 1990;20 (Suppl. 1): S27–S32.
- Chey WY, Lee YH, Hendricks JG, Rhodes RA, Tal HH. Plasma secretin concentrations in fasting and postprandial state in man. *Am J Dig Dis* 1978;23:981–8.
- Dooley CP, Valenzuela JE. Duodenal Volume and osmoreceptors in the stimulation of human pancreatic secretion. *Gastroenterology* 1984;86:23–7.
- Owyang C, Green L, Rader D. Colonic inhibition of pancreatic and biliary secretion. *Gastroenterology* 1984;84:470–5.
- Layer P, Peschel S, Schlesinger T, Goebell H. Human pancreatic secretion and intestinal motility: effects of ileal nutrient perfusion. *Am J Physiol* 1990;258:G196–G201.

- 9 Kruse-Jarres JD, KaiSeries C, Hafkenschied JC *et al.* Evaluation of a new alpha-amylase assay using 4,6-ethylidene-(G7)-1-4-nitrophenyl-(G1)-alpha-D-maltoheptaosid as substrate. *J Clin Chem Clin Biochem* 1989;27(2):103-13.
- 10 Lott JA, Patel ST, Sawhney AK, Kazmierczak SC, Love JE. Assays of serum lipase: analytical and clinical considerations. *Clin Chem* 1986;32(7):1290-302.
- 11 Hummel BC. A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. *Can J Biochem* 1955;37:1393-7.
- 12 Hydén S. A turbidimetric method for the determination of higher polyethylene glycols in biological materials. *Kungl Lantbrukshögsk Ann* 1956;22:139-45.
- 13 Everson GT, Braverman DZ, Johnson ML, Kern F Jr. A critical evaluation of real-time ultrasonography for the study of gallbladder Volume and contraction. *Gastroenterology* 1980;79:40-6.
- 14 Hopman WPM, Brouwer WFM, Rosenbusch G, Jansen JBMJ, Lamers CBHW. A computerized method for rapid quantification of gallbladder Volume from real-time sonograms. *Radiology* 1985;154:236-7.
- 15 Jansen JBMJ, Lamers CBHW. Radioimmunoassay of cholecystokinin in human tissue and plasma. *Clin Chim Acta* 1983;131:305-16.
- 16 Lamers CBHW, Diemel CM, Van Leer E, Van Leusen R, Peetoom JJ. Mechanism of elevated serum pancreatic polypeptide concentrations in chronic renal failure. *J Clin Endocrinol Metab* 1982;55:922-6.
- 17 Ragins H, Levenson SM, Signer R, Stamford W, Seifter E. Intrajejunal administration of an elemental diet at neurotral pH avoids pancreatic stimulation. *Am J Surg* 1973;126:606-14.
- 18 Cassim MM, Allardyce DB. Pancreatic secretion in response to jejunal feeding of elemental diet. *Ann Surg* 1974;180:228-31.
- 19 Vidon N, Hecketsweiler P, Butel J, Bernier JJ. Effect of continuous jejunal perfusion of elemental and complex nutritional solutions on pancreatic enzyme secretion in human subjects. *Gut* 1978;19:194-8.
- 20 Keith RG. Effect of a low fat elemental diet on pancreatic secretion during pancreatitis. *Surg Gynecol Obstet* 1980;151(3):337-43.
- 21 Hildebrand P, Beglinger C, Gyr K *et al.* Effects of a cholecystokinin receptor antagonist on intestinal phase of pancreatic and biliary responses in man. *J Clin Invest* 1990;85:640-6.
- 22 Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 1985;89:1070-7.
- 23 Bloom SR, Polak JM. Aspects of neurotensin physiology and pathology. *Ann N Y Acad Sci* 1982;400:105-16.
- 24 Pironi L, Stanghellini V, Miglioli M *et al.* Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma level of peptide YY. *Gastroenterology* 1993;105:733-9.
- 25 Guan D, Maouyo D, Taylor IL, Gettys TW, Greeley GH Jr, Morisset J. Peptide-YY, a new partner in the negative feedback control of pancreatic secretion. *Endocrinology* 1991;128:911-6.
- 26 Lluís F, Gomez G, Fujimura M, Greeley GH Jr, Thompson JC. Peptide YY inhibits nutrient-, hormonal-, and vagally-stimulated pancreatic exocrine secretion. *Pancreas* 1987;2:454-62.
- 27 Feurle GE, Hofmann G, Carraway R, Baca I. Reproduction of postprandial neurotensin plasma levels by intravenous neurotensin and the effect of neurotensin on exocrine pancreatic secretion in humans. *Pancreas* 1986;1(4): 329-34.
- 28 Fletchett DR, Blackburn AM, Adrian TE, Chadwick VS, Bloom SR. Effect of neurotensin on pancreas secretion in man. *Life Sci* 1981;29:2157-61.
- 29 Pfeiffer A, Vidon N, Feurle GE, Chayvialle JA, Bernier JJ. Effect of jejunal infusion of different caloric loads on pancreatic enzyme secretion and gastro-intestinal hormone response in man. *Eur J Clin Invest* 1993;23:57-62.
- 30 Valenzuela JE, Lamers CB, Modlin IM, Walsh JH. Cholinergic component in the human pancreatic secretory response to intraintestinal oleate. *Gut* 1983;24:807-11.
- 31 Malfertheiner P, Sarr MG, Nelson DK, DiMagno EP. Role of the duodenum in postprandial release of pancreatic and gastrointestinal hormones. *Pancreas* 1994;1(9):13-9.
- 32 Conter RL, Roslyn JJ, DenBesten L, Taylor IL. Pancreatic polipeptide enhances postcontractile gallbladder filling in the pairie dog. *Gastroenterology* 1987;92:771-6.
- 33 Conter RL, Roslyn JJ, Taylor IL. Effects of peptide YY on gallbladder motility. *Am J Physiol* 1987;252:G736-G741.

Chapter 4

EFFECT OF THE ILEAL BRAKE ON SATIETY AND PROXIMAL GASTRIC FUNCTION: IS IT PEPTIDE YY?

M. K. Vu, P.W.J. Maljaars, B. Mearadji, I. Biemond, A.A.M. Masclee

Department of Gastroenterology-Hepatology, Leiden University Medical Center, the Netherlands

Submitted

ABSTRACT

Recent studies indicate that peptide YY (PYY) influences satiety, but the role of PYY as a physiological regulator of satiety is under debate. This study was designed to compare the effects of ileal brake activation through ileal fat perfusion (endogenous PYY) versus exogenous PYY infusion on satiety and proximal gastric motor function. Two protocols were performed in both fasting and postprandial states. Protocol 1: ileal fat perfusion versus saline (control). Protocol 2: intravenous PYY₃₋₃₆ infusion at low and high doses of 15 and 30 pM/kg/hr vs. placebo. Plasma PYY (RIA), satiety scores and proximal gastric motility (barostat) were measured. *Results:* Ileal fat significantly ($p<0.05$) increased plasma PYY from 15 ± 1 to 26 ± 2 pM. During PYY infusion plasma levels of 28 ± 3 pM (low dose) and 55 ± 10 pM (high dose) were reached. Both in the fasting and postprandial state ileal fat induced satiety ($p<0.05$) in contrast to PYY₃₋₃₆. Fasting gastric volume (barostat) increased significantly ($p<0.01$) in response to ileal fat (from 150 ± 14 ml to 433 ± 54 ml) but not in response to PYY₃₋₃₆ infusion. In all experiments meal ingestion resulted in identical increments in proximal gastric volume. Only ileal fat, not PYY₃₋₃₆ significantly ($p<0.01$) enhanced postprandial gastric relaxation. *Conclusions:* Ileal fat induces satiety and results in proximal gastric relaxation, in contrast to exogenous PYY₃₋₃₆ at identical plasma levels. These data do not support a role for PYY as a physiological mediator in ileal brake induced satiety or ileal brake induced proximal gastric relaxation.

INTRODUCTION

Feelings of hunger and satiety are associated with gastrointestinal signals. Stimulation of gastric mechanoreceptors through balloon distension results in relaxation of the proximal stomach and is associated with feelings of fullness and a reduction in hunger and wish to eat (1-3). After meal ingestion the proximal stomach is able to accommodate a large volume meal. In patients with early satiety type dyspepsia the accommodation response of the proximal stomach is impaired (4). Not only mechanical but also chemical stimulation results in gastric relaxation and induces satiety. For instance, duodenal fat induces relaxation of the proximal stomach and results in feelings of fullness and satiation (5).

In the last decade evidence has become available indicating that not only the proximal gut but also the distal gut participates in the regulation of gastrointestinal motor and sensory functions and satiety. Perfusion of the ileum with nutrients delays gastric emptying, prolongs small intestinal transit time and inhibits pancreatico biliary secretion (6-8). This phenomenon, called the “ileal brake”, is a negative feedback loop from the distal to the proximal gut. There is evidence suggesting that the ileal brake is mediated through hormonal factors. Peptide YY (PYY) is considered an important mediator of the ileal brake (6,9). This 36-amino acid peptide first isolated from pig intestine, is localized in the endocrine cells of the ileal, colonic and rectal mucosa (10,11). The number of PYY secretory cells increases going more distally in the gut (11). PYY is released into the circulation in response to meals. The postprandial increase in plasma PYY levels is proportional to meal size with a peak response about an hour after food ingestion (11). In

both animals and humans intravenous infusion of PYY delays gastric emptying and inhibits pancreatico biliary secretion (12,13). Recently evidence has become available suggesting that PYY has an important role in satiety and eating behaviour. Batterham *et al* demonstrated that PYY₃₋₃₆, when infused intravenously reduced food intake in both humans and rodents (14,15). More recently Degen *et al* also found that intravenous infusion of PYY₃₋₃₆ reduced food intake but only at supraphysiological plasma PYY levels (16). The physiological role of PYY in the regulation of satiety therefore remains to be defined. Furthermore, up till now, little is known about the effect of ileal brake activation and PYY release on proximal gastric motor function

The present study was therefore performed to compare the effects of ileal brake activation with ileal fat (endogenous PYY release) versus exogenous PYY₃₋₃₆ infusion on satiety and on proximal gastric motor function. We therefore used two experimental protocols. In the first protocol we studied the effect of ileal fat and subsequent endogenous PYY release and in the second protocol we investigated the dose-response relationship of exogenous PYY. In both protocols satiety and motor and sensory function of the proximal stomach were monitored. PYY was infused at doses reaching plasma PYY levels comparable to those obtained during ileal fat perfusion.

SUBJECTS AND METHODS

Subjects

Fourteen healthy subjects (6 male; 8 female; mean ($\pm$ SEM) age 29 ± 3 year; mean ($\pm$ SEM) BMI 22 ± 3) without a history of gastrointestinal symptoms or

abdominal surgery participated in the study. For each experiment 8 subjects were studied. Two subjects participated in both experiments. None of them were taking any medication. Informed consent was obtained from each individual and the protocol had been approved by the ethics committee of the Leiden University Medical Center.

Gastric barostat

An electronic barostat (Medtronic Visceral Stimulator; Medtronic, Skovlunde, Denmark) was used to distend the stomach. A polyethylene bag (1000 mL maximum capacity) was tied to the end of a multilumen tube (16 French). This catheter was connected to the barostat. The barostat keeps the pressure in the intragastric bag at a preselected level. When the stomach relaxes, the system injects air. When the stomach contracts, the system aspirates air. Thus, the barostat measures gastric motor activity as changes in intragastric volume at a constant intragastric pressure (17).

Pressure (mmHg) and volume (ml) are constantly monitored and recorded on a personal computer connected to the barostat. On the day of the experiments, subjects were intubated with the barostat catheter through the mouth into the fundus. To unfold the bag, air was inflated to a volume of 200 ml and the catheter was carefully pulled back until its passage was restricted by the oesophageal sphincter. Then the tube was introduced another 2 cm. The correct position was checked under fluoroscopy at the start and the end of each experiment.

Study design

Experiment 1: ileal fat

Two experiments were performed in random order on two consecutive days in a double blind manner. The experiments started at 7:45 AM.

Day 0: Catheter intubation

In the morning subjects were intubated transnasally with an ileal catheter. The catheter (outer diameter 4 mm; length 350 cm) consists of a central perfusion port, a stainless steel tip weight and a distal inflatable balloon. Once the tip had passed the ligament of Treitz, the distal balloon was inflated with 10 ml air to facilitate further progression of the tube through the small intestine. Progression of the tube through the gut was monitored by fluoroscopy. The tip of the tube with the central perfusion port was located in the ileum. The time required for the tube to reach the distal ileum varied between 8-22 hours. Correct position was verified by fluoroscopy on day 0 and at the start and end of day 1 and 2.

Day 1 and day 2: ileal saline or fat

After an overnight fast subjects were intubated with the barostat catheter with bag through the mouth into the fundus, as described previously. An intravenous cannula was inserted into the antecubital vein of one arm for blood sampling. During measurements subjects were seated in a comfortable lying chair in a semi-recumbent position with the lower extremities just above abdominal level.

A commercially available fat emulsion (Intralipid 20%; Pharmacia & Upjohn BV, Woerden, The Netherlands) was used to perfuse the ileum. Intralipid

20% consists of 20 g soybean oil, 1.2 g purified egg phospholipids, and 2.2 g glycerol anhydrous per 100 ml. The perfusion rate was 1 ml/min (2 kcal/min). In random order either Intralipid 20% or placebo (saline 0.9%), was given into the ileum on day 1 and day 2.

A 200 ml liquid meal (Nutridrink; Nutricia Zoetermeer, Holland) containing 10g protein, 36g carbohydrates and 13g fat was used as test meal (300 kcal).

The following procedures with the barostat were performed:

- 1- **Minimal distending pressure (MDP)** was determined by stepwise increasing pressures in steps of 1 mmHg every 90 sec from 0 mmHg until. MDP was defined as the first pressure level at which the intragastric bag volume was more than 30 ml.
- 2- **Barostat procedure:** The barostat was set at a pressure of MDP+2 mmHg. The basal intragastric bag volumes were measured during the first 15 min. Then ileal infusion of either saline or fat was started. The intragastric bag volumes were continuously measured for 60 min after the start of the ileal perfusion.
- 3- **Recovery period** of two hours. During this period the ileum was perfused with saline at a rate of 1ml/min.
- 4- **Isobaric distension:** Stepwise increasing bag pressures in steps of 1 mmHg every 90 sec from 0 mmHg to a maximum of 14 mmHg or when a maximum bag volume of 750 ml was reached. Thereafter the intragastric bag was deflated.
- 5- **Recovery period** of three hours. During this period the ileum was perfused with saline at a rate of 1ml/min
- 6- **Meal:** The barostat was set at a pressure of MDP+2 mmHg. After 15 min recording under fasting conditions, the liquid test meal was ingested

within 3 min. At the start of meal ingestion ileal infusion of either saline (1 ml/min) or fat was also started. Measurements were continued for 90 min after the start of the ileal perfusion and meal ingestion.

Experiment 2: PYY₃₋₃₆ infusion

The three experiments were performed in double-blind randomized order. The experiments were separated by intervals of at least 7 days. Each subject received an intravenous infusion of: A) saline, B) PYY₃₋₃₆ at a dose of 15 pM/kg/hour and C) PYY₃₋₃₆ at a dose of 30 pM/kg/hour. PYY₃₋₃₆ was purchased from Clinalfa, Switzerland. The doses of PYY₃₋₃₆ we choose were based on results of previous studies (11,18,19). The low dose results in plasma PYY levels seen after ingestion of a regular meal, whereas the high dose results in plasma PYY levels that have been observed after meal ingestion in patients with malabsorptive disorders. On the day of the experiments, after an overnight fast, subjects were intubated with the barostat catheter with bag through the mouth into the fundus as described previously. A 200 ml liquid meal (Nutridrink; Nutricia Zoetermeer, Holland) containing 10g protein, 36g carbohydrates and 13g fat was used as test meal (300 kcal). The following procedures with the barostat were performed (for details see experimental protocol 1):

- 1- **Minimal distending pressure (MDP).**
- 2- **Barostat procedure:** The barostat was set at a pressure of MDP+2 mmHg. The basal intragastric bag volumes were measured during the first 15 min. Then intravenous infusion with either saline or PYY₃₋₃₆ was started. The intragastric bag volumes were continuously measured for 60 min.

3- **Recovery period (15 min)**

4- **Isobaric distention:** Stepwise increasing pressures in steps of 1 mmHg every 90 sec from 0 mmHg to a maximum of 14 mmHg or when a maximum bag volume of 750 ml was reached.

5- **Recovery period (15 min)**

6- **Meal:** The barostat procedure was started at a pressure of MDP+2 mmHg. After 15 min recording under fasting conditions, the test meal was ingested within 3 min. At the start of meal ingestion intravenous infusion with either saline or PYY₃₋₃₆ was also started. Measurements were continued for 90 min after the start of intravenous PYY infusion and meal ingestion.

Perception scores

Subjective feelings of fullness, hunger, desire to eat and nausea were scored at 15 min intervals throughout experiment 1 and 2 using 100 mm visual analogue scales (VAS).

Plasma PYY

Blood samples for measurement PYY were drawn at time t=-15, 0, 15, 30, 45, 60 min during the fasting barostat procedure and at time t=-15, 0, 15, 30, 45, 60, 75 and 90 min after meal ingestion. Plasma PYY was measured by radioimmunoassay. PYY antiserum was generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM Biochemica GmbH, Switzerland). PYY was labeled with ¹²⁵Iodine with chloramine T. The assay is highly specific. There is no cross-reactivity with PP or VIP. The detection limit is 10 pM/l. Both PYY₍₁₋₃₆₎ and PYY₍₃₋₃₆₎ bind to the antibody in dilutions up to

250000.

Data analysis

Intragastric volumes measured while pressure was set at MDP+2 mmHg are given as average values over 5 min periods. Intragastric volumes during the isobaric distension were determined as mean volumes during the last 60 sec of each pressure step. Perception scores during volume measurements at set pressure (MDP+2 mmHg) were calculated relative to the perception scores obtained at 0 min, immediately before the onset of the infusion. Perception scores obtained prior to the meal were used as zero reference points for the calculation of postprandial perception scores.

Statistical analysis

Results are expressed as mean $\pm$ SEM. All data between and within groups were analysed for statistical significance using multiple analysis of variance (MANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman Keuls analyses were performed to determine which values between or within groups differed significantly. Coefficient of linear correlation (Spearman) was used to calculate correlations between intragastric volumes, plasma PYY levels and perception scores. The significance level was set at $p < 0.05$.

RESULTS

Experiment 1: ileal fat

Barostat, fasting

The MDP was not significantly different between the ileal saline (6.0 ± 0.4 mmHg) and the ileal fat (6.1 ± 0.9 mmHg) experiment. Intragastric volumes at MDP+2 mmHg before the start of ileal infusion at time 0 min were similar between the ileal saline (149 ± 14 ml) and the ileal fat experiment (148 ± 23 ml). Intragastric volumes increased significantly ($p < 0.01$) during ileal fat perfusion compared to basal and compared to the ileal saline experiment from time $t = 10$ min until the end of the procedure at time $t = 60$ min (Figure 1, left panel).

Stepwise isobaric distension resulted in progressive increments in intragastric bag volume in both the saline and the ileal fat experiments. Intragastric volumes during pressure distension from level 6-11 mmHg were significantly ($p < 0.01$) higher in the ileal fat compared to the ileal saline experiment (Figure 2; left panel).

Barostat, postprandial

Basal intragastric volumes at MDP+2 mmHg were not significantly different between the ileal saline (142 ± 10 ml) and the ileal fat experiment (146 ± 13 ml). After meal ingestion, intragastric volumes increased significantly ($p < 0.01$) in both the experiments (Figure 3, left panel). In the ileal saline experiment, intragastric volumes reached a maximal volume at 15 min after meal ingestion and gradually returned to basal value at the end of the postprandial

period. On the other hand, intragastric volumes in the ileal fat experiment remained significantly ($p<0.01$) increased over basal during the 90 min postprandial period. Furthermore, intragastric volumes in the ileal fat experiment were significantly ($p<0.01$) higher compared to those in the ileal saline experiment during the period from 15 min until 90 min postprandially.

Satiety, fasting

During ileal fat perfusion, fullness increased significantly ($p<0.05$) over basal starting from 15 min while no significant changes were observed in the ileal saline experiment (Figure 4, upper left panel). Scores of hunger (Figure 4, lower left panel) and desire to eat (data not shown) were significantly ($p<0.05$) decreased in the ileal fat compared to the ileal saline experiment. Nausea scores were not affected.

Satiety, postprandial

In both the ileal saline and fat experiment, scores of fullness increased significantly ($p<0.01$) over basal value starting from 15 min after meal ingestion (Figure 5, upper left panel). In the saline experiment the perception of fullness gradually returned to basal values. The perception of fullness during ileal fat perfusion, on the other hand, remained significantly ($p<0.01$) increased compared to the basal value and compared to the saline experiment. Perception scores of hunger (Figure 5, lower left panel) and desire to eat (data not shown) were significantly ($p<0.05$) decreased in the ileal fat experiment compared to the saline experiment and compared to baseline values. Nausea was not affected (data not shown).

Plasma PYY, fasting

Basal plasma PYY levels were not significantly different between the ileal saline (13 ± 1 pM) and the ileal fat experiment (15 ± 1 pM). Plasma PYY levels gradually increased during ileal fat perfusion and were significantly ($p < 0.05$) increased over basal from 30-60 min after the start of ileal fat perfusion (26 ± 2 pM at 60 min). No significant changes compared to basal were observed in the ileal saline experiment (Figure 6, left panel).

Plasma PYY, postprandial

Plasma PYY levels before meal ingestion were not significantly different between the ileal saline (15 ± 2 pM) and the ileal fat experiment (16 ± 1 pM). Plasma PYY levels in response to meal ingestion were significantly ($p < 0.05$) increased over basal from 15 min to 45 min in the ileal saline experiment. Ileal fat infusion resulted in significantly ($p < 0.01$) higher plasma PYY levels compared to those in the ileal saline experiment during the period from 15 to 90 min (Figure 7, left panel).

Correlations

Perception of fullness at $t = 60$ min during fasting correlated significantly with gastric bag volume ($r = 0.5$; $p = 0.05$). In addition, postprandial fullness at $t = 90$ min also correlated significantly with postprandial gastric bag volume ($r = 0.4$; $p = 0.05$). Postprandial perception of hunger was significantly inversely correlated with gastric volume ($r = -0.6$; $p = 0.04$). No significant correlations were found between satiety scores and plasma PYY levels neither in the fasting nor in the fed state. The correlation between plasma PYY and postprandial gastric volume ($r = 0.4$; $p = 0.09$) was not statistically significant.

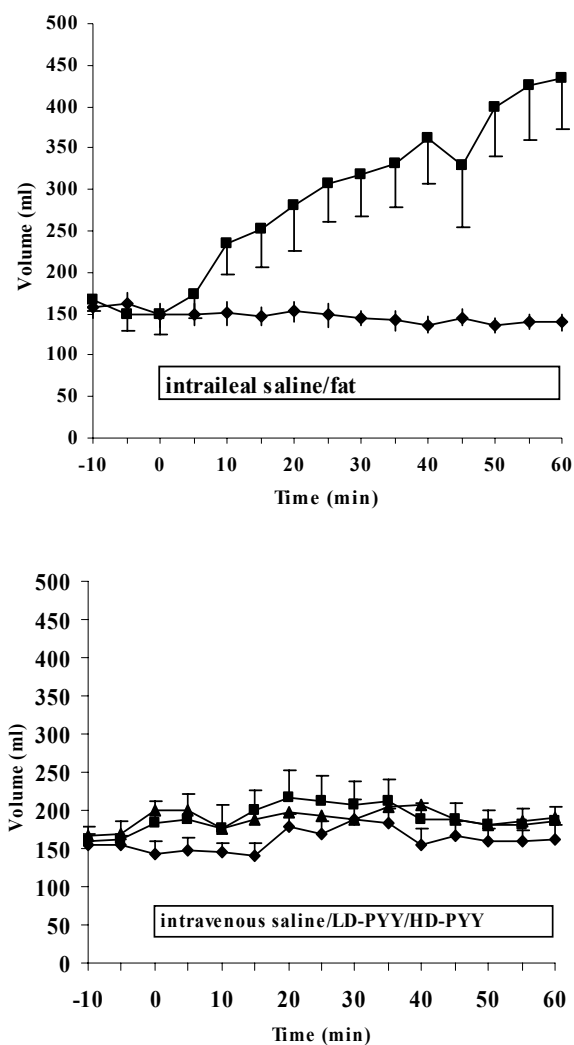


Figure 1: Intragastric bag volume at MDP+2 mmHg. Upper panel: during ileal perfusion of saline (diamonds) and fat (squares). Lower panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean $\pm$ SEM.

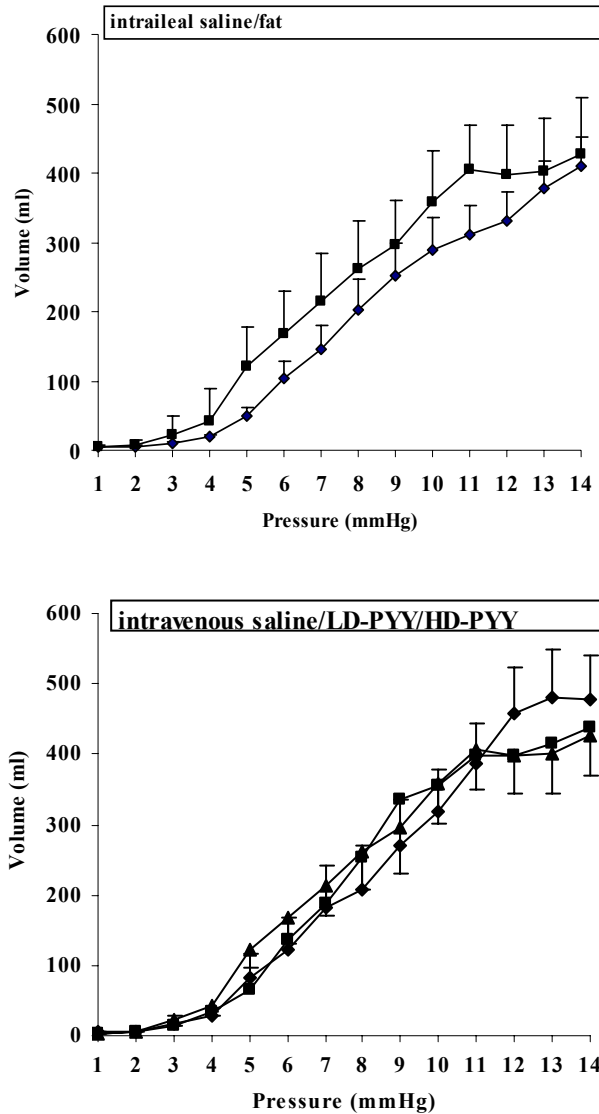


Figure 2: Intragastric bag volume in response to stepwise pressure distension. Upper panel: during ileal perfusion of saline (diamonds) and fat (squares). Lower panel during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean $\pm$ SEM.

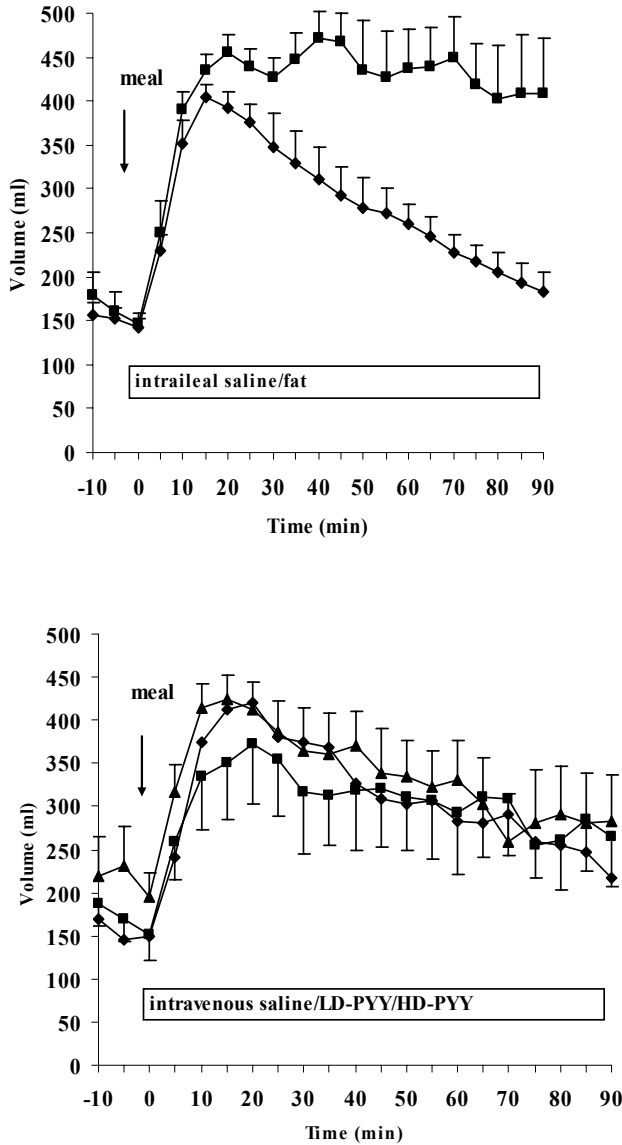


Figure 3: Intragastric volume at MDP+2 mmHg in response to meal ingestion. Upper panel: during ileal perfusion of saline (diamonds) and fat (squares). Lower panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean $\pm$ SEM.

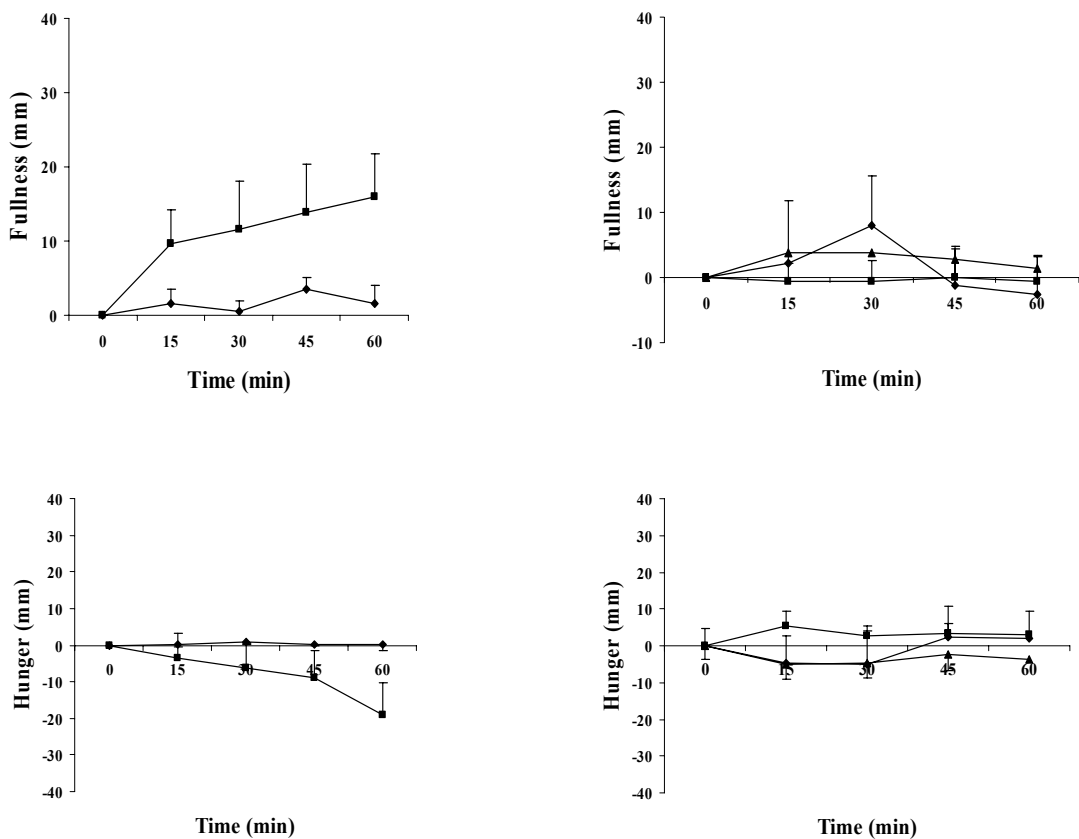


Figure 4: Satiety score of fullness and hunger during fasting. Left panel: during ileal perfusion of saline (diamonds) and fat (squares). Right panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean $\pm$ SEM.

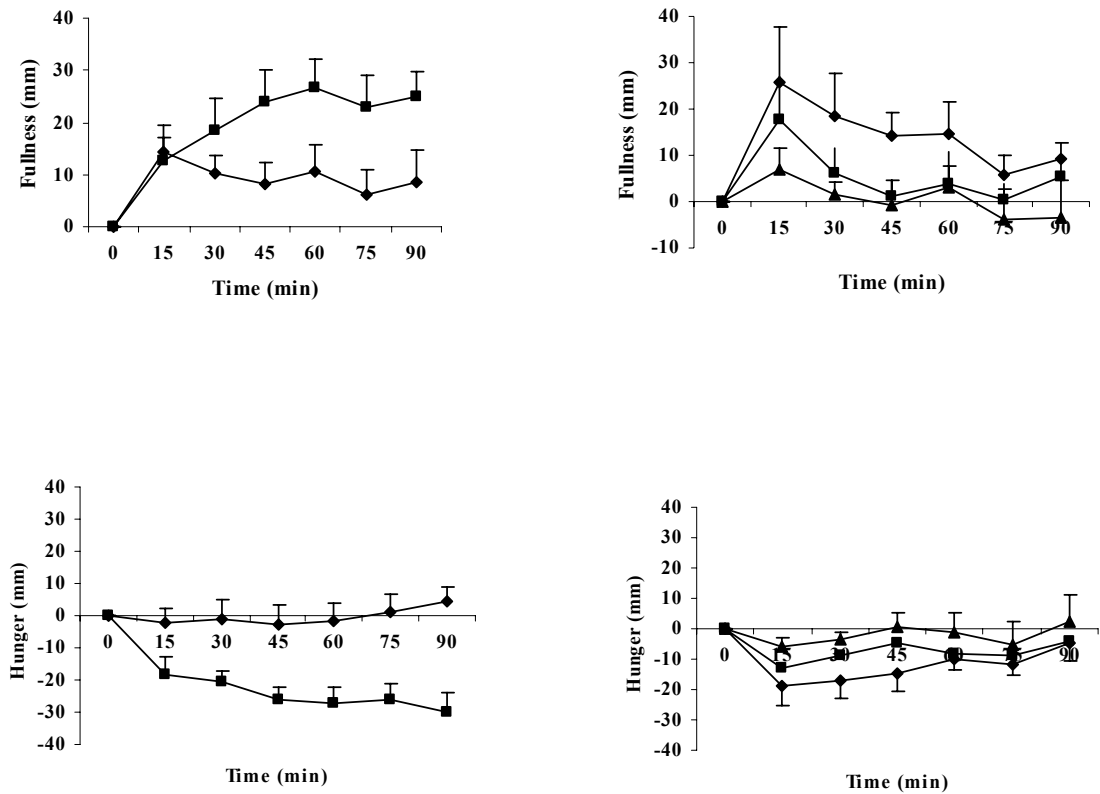


Figure 5: Satiety score of fullness and hunger in response to meal ingestion. Left panel: during ileal perfusion of saline (diamonds) and fat (squares). Right panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean $\pm$ SEM.

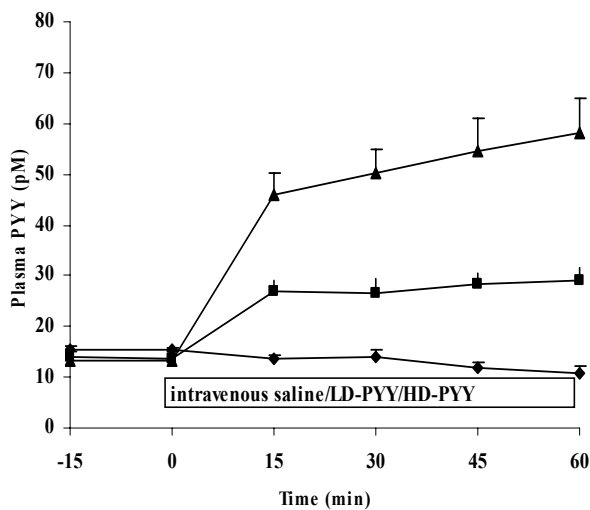
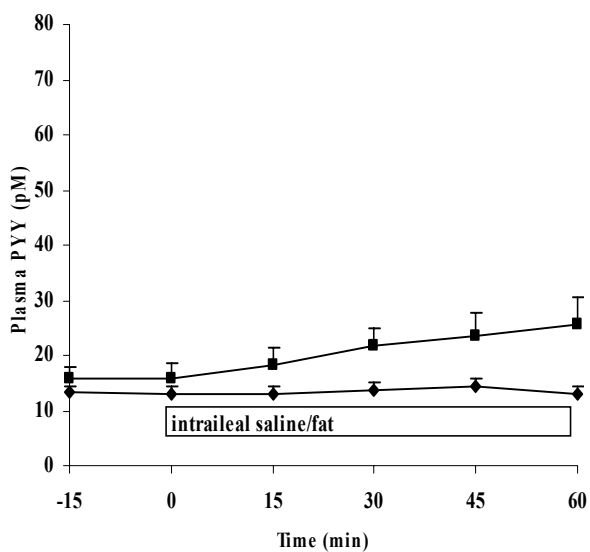


Figure 6: Plasma PYY level during fasting. Upper panel: during ileal perfusion of saline (diamonds) and fat (squares). Lower panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean $\pm$ SEM.

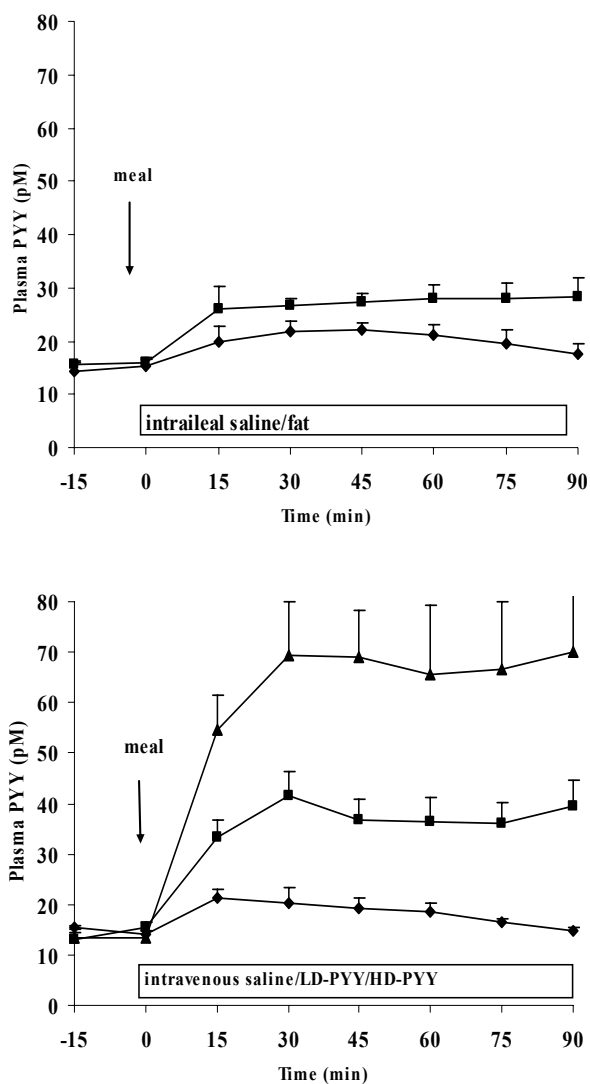


Figure 7: Plasma PYY level in response to meal ingestion. Left panel: during ileal perfusion of saline (diamonds) and fat (squares). Right panel: during intravenous infusion of saline (diamonds), low dose PYY (squares) and high dose PYY (triangles). Results are expressed as mean $\pm$ SEM.

Experiment 2: PYY₃₋₃₆ infusion

Barostat, fasting

The MDP was not significantly different between the saline (6.4 ± 0.6 mmHg), the low dose PYY (6.8 ± 0.5 mmHg) and the high dose PYY experiment (6.5 ± 0.7 mmHg). Intra gastric volumes at MDP+2 mmHg before the start of the infusion were similar between the saline (155 ± 14 ml), the low dose PYY (159 ± 18 ml) and the high dose PYY experiment (167 ± 22 ml). During PYY infusion intra gastric volume did not change significantly (Figure 1, right panel). Stepwise isobaric distension resulted in progressive increments in intra gastric bag volume in all experiments. No significant differences in intra gastric bag volume were observed between the saline, the low dose and the high dose PYY experiment (Figure 2, right panel).

Barostat, postprandial

Basal intra gastric volumes at MDP+2 mmHg were not significantly different between the saline (148 ± 24 ml), the low dose PYY (151 ± 30 ml) and the high dose PYY experiment (195 ± 27 ml). After meal ingestion, intra gastric volumes increased significantly ($p < 0.01$) in all experiments. No significant differences in intra gastric bag volume were observed between the saline, the low dose PYY and the high dose PYY experiment (Figure 3, right panel).

Satiety, fasting

During PYY infusion scores of fullness, hunger, desire to eat and nausea did not change significantly over basal. No significant differences in perception scores were observed between the three experiments (Figure 4, right upper and lower panel).

Satiety, postprandial

After meal ingestion scores of fullness, hunger, desire to eat changed significantly ($p<0.05$) at time 15 min compared to basal. Nausea was not affected. No significant differences in fullness, hunger, desire to eat or nausea were observed between the three experiments (Figure 5, right upper and lower panel).

Plasma PYY, fasting

Basal plasma PYY levels before the start of infusion were not significantly different between the three experiments, respectively 16 ± 1 pM in the saline experiment, 14 ± 1 pM in the low dose PYY experiment and 13 ± 2 pM in the high dose PYY experiment. During infusion of PYY, plasma PYY levels increased significantly ($p<0.01$) over basal and compared to the saline experiment (Figure 6, right panel). Note that the plasma PYY levels obtained during low dose PYY infusion are in the range of those reached with ileal fat (25-30 pM). The plasma levels obtained during high dose PYY infusion are in the range of those reached after meal ingestion in patients with maldigestion that is 50-60 pM.

Plasma PYY, postprandial

Meal ingestion resulted in significantly ($p<0.05$) increases in plasma PYY levels during saline infusion from 15 to 60 min (Figure 7, right panel). Plasma PYY levels during intravenous infusion of both the low dose PYY and high dose PYY were significantly higher compared to the control experiment. Plasma PYY levels of the high dose PYY experiment were significantly ($p<0.01$) higher compared to those of the low dose PYY experiment.

DISCUSSION

Recently much attention has been given to PYY as potential anorexogenic substance. PYY is mainly present in the ileocolonic region expressed by endocrine L cells and secreted in response to nutrient ingestion (11). PYY immunoreactivity has also been reported to be present in the central nervous system. Animal studies indicate that PYY can transmit signals via central Y2 receptors to which PYY binds with high affinity (20). Several animal experiments have shown that truncated PYY₃₋₃₆ reduces food intake and impairs a gain in body weight (14,21). Recently, human studies with PYY infusion have obtained similar results: infusion of PYY₃₋₃₆ reduced food intake and appetite not only lean but also in obese subjects (15). Subsequent studies failed to reproduce the anorexogenic effect of PYY₃₋₃₆ (22). More recently, Degen et al again attempted to solve the issue by performing classical experiments with graded doses of PYY infusion reaching plasma levels in the physiological range (16). These authors clearly observed an anorexogenic effect of PYY because food intake was decreased by 30%. It should be noted that this effect was present only at supraphysiological plasma PYY levels.

The design we have chosen was based on the following research question: compare effects of exogenous PYY infusion and of ileal brake activation (endogenous PYY) by aiming at comparable plasma PYY levels with satiety (fasted, fed) and proximal gastric motor function as parameters. In doing so we have shown that in healthy volunteers: 1) ileal fat induces satiety in the fasting state and increases satiety in the fed state 2) ileal fat significantly increases proximal gastric volume and enhances postprandial proximal gastric relaxation and 3) exogenous PYY₃₋₃₆ infusion did not affect satiety nor

proximal gastric motor and sensory function.

Our findings that ileal fat significantly increased gastric compliance and fasting gastric volume are in agreement with a previous study in dogs demonstrating that nutrients in the distal small bowel elicit a gastric relaxatory response (23). Several studies have shown that ileal fat activates the so called ileal brake with subsequent inhibition of gastric emptying and delay in intestinal transit (6,7). An increased postprandial fundic relaxation may contribute to the delay in gastric emptying induced by ileal fat since the proximal stomach accommodates food after meal ingestion and regulates the transfer of food to the distal stomach. It is not clear by which mechanism(s) the ileal fat induced gastric relaxatory response is mediated. Several distal gut hormones such as PYY, GLP-1 and enteroglucagon may be involved. PYY is considered to be an important hormonal mediator of the ileal brake. In humans, i.v. administration of exogenous PYY delays gastric emptying (12). In response to ileal fat, endogenous PYY is released resulting in higher plasma levels (6). In the present study infusion of PYY₃₋₃₆, to plasma levels reached during ileal brake activation did not result in changes in gastric volume neither in the fasting nor in the fed state. These findings do not support a role for PYY as mediator of ileal fat induced gastric relaxation and accommodation. Gastric emptying results from coordinated motor activities of different parts of the stomach, pylorus and duodenum. It is therefore conceivable that other factors apart from proximal gastric tone may contribute to delayed gastric emptying induced by PYY. To date, it is not known whether PYY affects antral, pyloric or duodenal motility. Not only hormonal but also neural factors may be involved in the gastric relaxatory response to ileal fat, for instance vagal afferent neural reflexes triggered by luminal osmo-

and chemosensitive receptors (24,25).

We have demonstrated that ileal fat induces satiety. We did not assess food intake, but quantified sensations related to eating behaviour such as fullness, hunger and desire to eat in both the fasting and the fed state. The finding of ileal fat induced satiety can be explained in a number of ways. First, satiety may have been induced by ileal fat through an increase in gastric volume. It is known that afferent fibers of the vagus nerve express mechanoreceptors (stretch receptors) which are sensitive to volume and to luminal pressure (26). The activated vagus then in turn activates centers in the brainstem, eliciting reflexes that control satiety and eating behaviour. In support of this concept is the significant correlation we observed between sensations of fullness, hunger and intragastric bag volumes. In addition, previous studies have shown that gastric distension is one of the most powerful triggers that decrease hunger (27,28). Penagini *et al* found a significant inverse correlation between postprandial hunger and proximal gastric volume and the increase in sensation of fullness paralleled the increase in gastric volume in patients with reflux disease (29). Second, satiety induced by ileal fat can be mediated by gut peptides through endocrine, paracrine or neurocrine routes. Activation of the ileal brake stimulates release of PYY and Glucagon-like peptide-1 (GLP-1) from the endocrine L cells located primarily in the ileum and colon (6,30). Of these peptides, especially PYY is of interest because PYY reduces food intake and elicits satiety in humans and animals (14-16).

Concerning PYY and satiety, the satiating effect of ileal fat perfusion could not be reproduced during infusion of PYY (15 pM/kg/hr) which resulted in similar plasma PYY levels ranging from 25 pM to 30 pM. Neither did the high dose of PYY (30 pM/kg/hr) affect satiety. With this dose PYY levels

were reached in the range of 50-60 pM, comparable to postprandial levels in patients with malabsorption (18,19). In line with this observation is the finding that infusion of PYY neither at low nor at high doses did affect proximal gastric motor function.

The mechanisms that regulate gut-brain signalling are poorly understood. CCK, but also PYY₃₋₃₆ may act as a neurocrine rather than as an endocrine substance. Animal studies indicate that gut PYY can transmit signals to the CNS but only via an intact vagus nerve (31). Previously several authors have clearly demonstrated the satiating effect of PYY with a marked reduction in food intake (14-16). An explanation for the discrepancy in results of studies may be related, for instance, to the doses of PYY given. Degen *et al* recently showed that exogenous PYY₃₋₃₆ only at a high, pharmacological doses of 48 pM/kg/hr significantly reduced feelings of hunger and decreased food intake. These authors could not demonstrate any effect of PYY when infused at doses of 12 or 24 pM/kg/hour (16). In the studies of Batterham *et al*, a reduction in food intake was observed after PYY infusion at doses of above 35 pM/kg/hr (14,15). Taken together, we suggest that the doses of PYY required to produce a significant effect on satiety and food intake are in the supraphysiological range.

In conclusion, we have shown that ileal fat induces satiety and results in proximal gastric relaxation, in contrast to exogenous PYY at identical plasma levels. These data do not support a role for PYY as physiological mediator in ileal brake induced proximal gastric relaxation.

REFERENCES

1. Ahluwalia NK, Thompson DG, Barlow J, Troncon LE, Hollis S. Relaxation responses of the human proximal stomach to distension during fasting and after food. *Am J Physiol* 1994;267:G166-172.
2. Notivol R, Coffin B, Azpiroz F, Mearin F, Serra J, Malagelada JR. Gastric tone determines the sensitivity of the stomach to distention. *Gastroenterology* 1995;108:330-336.
3. Carmagnola S, Cantu P, Penagini R. Mechanoreceptors of the proximal stomach and perception of gastric distension. *Am J Gastroenterol* 2005;100:1704-1710.
4. Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. *Gastroenterology* 1998;115:1346-1352.
5. Feinle C, Grundy D, Read NW. Effects of duodenal nutrients on sensory and motor responses of the human stomach to distension. *Am J Physiol* 1997;273:G721-726.
6. Pironi L., V. Stanghellini, M. Miglioli, R. Corinaldesi, R. De Giorgio, E. Ruggeri, G. Tosetti, G. Poggioli, A. M. M. Labate, N. Monetti, G. Gozetti , L. Barbara, and V. L. W. Go. Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma level of peptide YY. *Gastroenterology* 1993;105:733-739.
7. Read N. W., A. MacFarlane, and R. Kinsman. Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon in man. *Gastroenterology* 1984;86:274-280.

8. Layer P, Schlesinger T, Groger G, Goebell H. Modulation of human periodic interdigestive gastrointestinal motor and pancreatic function by the ileum. *Pancreas* 1993;8:426-432.
9. Lin HC, Zhao XT, Wang L, Wong H. Fat-induced ileal brake in the dog depends on peptide YY. *Gastroenterology* 1996;110:1491-1495.
10. Tatemoto K. Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. *Proc Natl Acad Sci USA* 1982;79:2514-2518.
11. Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 1985;89:1070-1077.
12. Savage AP, Adrian TE, Carolan G, Chatterjee VK, Bloom SR. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut* 1987;28:166-170.
13. Symersky T, Biemond I, Frolich M, Masclee AA. Effect of peptide YY on pancreatobiliary secretion in humans. *Scand J Gastroenterol* 2005;40:944-949.
14. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 2002;418:650-654.
15. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR. Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med* 2003;349:941-948.
16. Degen L, Oesch S, Casanova M, Graf S, Ketterer S, Drewe J, Beglinger C. Effect of peptide YY3-36 on food intake in humans. *Gastroenterology*

- 2005;129:1430-1436.
17. Azpiroz F, Malagelada JR. Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis. *Gastroenterology* 1987;92:934-943.
 18. Wahab PJ, Hopman WP, Jansen JB. Basal and fat-stimulated plasma peptide YY levels in celiac disease. *Dig Dis Sci* 2001;46:2504-2509.
 19. Adrian TE, Long RG, Fuessl HS, Bloom SR. Plasma peptide YY (PYY) in dumping syndrome. *Dig Dis Sci* 1985;30:1145-1148.
 20. Wynne K, Stanley S, Bloom S. The gut and regulation of body weight. *J Clin Endocrinol Metab* 2004;89:2576-2582.
 21. Babu M, Purhonen AK, Bansiewicz T, Makela K, Walkowiak J, Miettinen P, Herzig KH. Effect of total colectomy and PYY infusion on food intake and body weight in rats. *Regul Pept* 2005;131:29-33.
 22. Gura T. Labs fail to reproduce protein's appetite-suppressing effects. *Science* 2004;305:158-159.
 23. Azpiroz F, Malagelada JR. Intestinal control of gastric tone. *Am J Physiol* 1985;249:G501-509.
 24. Garnier L, Mei N, Melone J. Further data on the inhibitory enterogastric reflex triggered by intestinal osmotic changes in cats. *J Auton Nerv Syst* 1986;16:171-180.
 25. Mei N, Garnier L. Osmosensitive vagal receptors in the small intestine of the cat. *J Auton Nerv Syst* 1986;16:159-170.
 26. Andrews PL, Grundy D, Scratcherd T. Vagal afferent discharge from mechanoreceptors in different regions of the ferret stomach. *J Physiol* 1980;298:513-524.
 27. Bergmann JF, Chassany O, Petit A, Triki R, Caulin C, Segrestaa JM.

Correlation between echographic gastric emptying and appetite: influence of psyllium. *Gut* 1992;33:1042-1043.

28. Horowitz M, Jones K, Edelbroek MA, Smout AJ, Read NW. The effect of posture on gastric emptying and intragastric distribution of oil and aqueous meal components and appetite. *Gastroenterology* 1993;105:382-390.
29. Penagini R, Hebbard G, Horowitz M, Dent J, Bermingham H, Jones K, Holloway RH. Motor function of the proximal stomach and visceral perception in gastro-oesophageal reflux disease. *Gut* 1998;42:251-257.
30. Meier JJ, Nauck MA. Glucagon-like peptide 1(GLP-1) in biology and pathology. *Diabetes Metab Res Rev* 2005;21:91-117.
31. Konturek SJ, Konturek JW, Pawlik T, Brzozowski T. Brain-gut axis and its role in the control of food intake. *J Physiol Pharmacol* 2004;55:137-154.

Chapter 5

MEDIUM CHAIN TRIGLYCERIDES ACTIVATE DISTAL BUT NOT PROXIMAL GUT HORMONES

My K. Vu, Marco Verkijk, Evelien S.M. Muller, Izak Biemond, Cornelis B.H.W. Lamers, Ad A.M. Masclee

Department of Gastroenterology-Hepatology, Leiden University Medical Center, the Netherlands

Medium chain triglycerides activate distal but not proximal gut hormones

M. K. VU, M. VERKIJK, E. S. M. MULLER, I. BIEMOND, C. B. H. W. LAMERS, A. A. M. MASCLEE

Department of Gastroenterology–Hepatology, Leiden University Medical Center, the Netherlands (Correspondence to: AAMM, Department of Gastroenterology–Hepatology, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands.)

Abstract—Background and aims: Compared to long chain triglycerides (LCT), medium chain triglycerides (MCT) are considered an attractive caloric source in malabsorptive diseases because of their favorable physico-chemical characteristics. The use of MCT is, however, limited by the occurrence of gastrointestinal symptoms such as diarrhoea. We have, therefore, investigated the effects of MCT and LCT on proximal (cholecystokinin; CCK) and distal (peptide YY; PYY) gut hormone secretion.

Methods: Eight healthy volunteers participated in four experiments performed in random order during continuous intraduodenal administration for 360 min of a) saline (control); b) LCT 15 mmol/h; c) MCT 15 mmol/h (equimolar); d) MCT 30 mmol/h (equicaloric). Plasma CCK and PYY were determined at regular intervals (radioimmunoassay). Duodenocecal transit (DCTT) was measured by lactulose H₂ breath test.

Results: DCTT during LCT (105 ± 11 min) was not significantly different from saline (111 ± 10 min). Both low dose MCT (54 ± 5 min) and high dose MCT (61 ± 6 min) significantly accelerated DCTT ($P < 0.05$). Plasma CCK increased significantly ($P < 0.05$) during LCT but not during MCT or saline. PYY increased significantly ($P < 0.05$) not only during LCT, but also during low and high dose MCT but not during saline.

Conclusions: Intraduodenal MCTs a) accelerate intestinal transit; b) do not stimulate CCK release; c) but stimulate release of the distal gut hormone PYY. These results suggest that MCTs are not rapidly absorbed in the proximal gut but probably reach the ileocolonic region and stimulate PYY release.

© 1999 Harcourt Publishers Ltd.

Key words: medium chain triglycerides; small intestinal transit; cholecystokinin; peptide YY

Introduction

Medium chain triglycerides (MCT) contain fatty acids with a chain length of 6 to 12 carbon atoms. Compared to long chain triglycerides (LCT), MCT may present favorable physicochemical and metabolic properties. The intraluminal hydrolysis and absorption of MCT occur independently of micellar formation and is thought to be more rapid and complete compared to LCT (1–3). Because of these unique characteristics, MCT have been applied in the nutritional support of patients with malabsorption (4). The use of MCT is, however, limited by the occurrence of gastrointestinal side effects. Patients receiving MCT frequently complain of nausea, borborygmi, cramps, abdominal pain and diarrhoea (1, 4). The mechanisms underlying these MCT associated side effects are still poorly understood.

In contrast with LCT, MCT do not stimulate the release of the proximal gut hormone cholecystokinin (CCK) or subsequent gallbladder contraction (5–7). CCK is released from the proximal gut by digested intraluminal fat (8, 9). The occurrence of abdominal symptoms together with findings of recent studies that MCT accelerate small intestinal transit

and stimulate motility (10, 11) contrast with the concept of rapid hydrolysis and absorption of MCT. In a case of accelerated intestinal transit secretion of distal gut hormones such as peptide YY (PYY) may be activated. PYY is released from the ileo-colonic region by intraluminal non-absorbed nutrients (12–14). We have evaluated the effect of MCT on proximal and distal gut hormone secretion, in search of evidence that MCT are less rapidly-absorbed and cause gastrointestinal side-effects.

Subjects and methods

Subjects

Eight healthy volunteers (5 men, 3 women; mean age 26 years, range 21–30 years), without a history of gastrointestinal symptoms or surgery, participated in the study. None of the subjects were taking any medication. Informed consent was obtained from each individual and the protocol had been approved by the ethics committee of the Leiden University Medical Center.

Study design

All subjects were studied at random order on four separate occasions with an interval of at least 7 days. After an over-

night fast, subjects were intubated transnasally with a polyurethane feeding tube. The tip of the catheter was positioned in the horizontal part of the duodenum. The correct position was checked under fluoroscopy. A cannula was then inserted into the antecubital vein of one arm for bloodsampling. At time $t=0$ min, a solution of lactulose was administered into the duodenum to determine small bowel transit time. Immediately thereafter, continuous intraduodenal instillation was started with either long chain triglycerides (LCT, 313 mOsm/kg; Reddy sunflower oil, NV Vandermoortele, Oudimbosch, The Netherlands), medium chain triglycerides (MCT, 616 mOsm/kg; Ceres-MCT dietary oil, Union Deutsche Lebensmittelwerke, Hamburg, Germany), or saline solution (control, 15 ml/h, 300 mOsm/kg) for 360 min. LCT was administered in a dose of 15 mmol/h (125 kcal/h), whereas MCT was administered in both equimolar (15 mmol/h; 56 kcal/h) and equicaloric (30 mmol/h; 113 kcal/h) amounts compared to LCT. The fatty acid composition of the sunflower oil was palmitic acid (C16:0) 6.5%, stearic acid (C18:0) 5%, oleic acid (C18:1) 23%, and linoleic acid (C18:2) 63%. MCT oil contained 60% octanoic acid (C8:0) and 40% decanoic acid (C10:0).

Small bowel transit time

Small bowel transit time was determined by the lactulose hydrogen breath test (15) after intraduodenal instillation of 6 gram lactulose (Legendal, Amersfoort, The Netherlands), dissolved in 60 ml of distilled water as described previously (11). End expiratory breath samples were first collected under fasting conditions at time $t=-15$ and 0 min and every 10 min thereafter and were analyzed immediately in a hydrogen breath test unit (Lactoscreen, Hoekloos, The Netherlands). Duodenocecal transit time was defined as the time between administration of lactulose and a sustained rise in breath H_2 -concentration of at least 10 parts per million (ppm) above basal levels. At our department the mean coefficient of variation for duodenocecal transit using the lactulose hydrogen breath test with 6 g lactulose is $12 \pm 5\%$ (11).

Hormone assays

Blood samples for measurement of plasma CCK and PYY were withdrawn at time $t=-15, 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300$ and 360 min during each experiment. The blood samples were collected in EDTA containing ice-chilled tubes. The samples were centrifuged at a rate of 3000 rpm for 10 min at a temperature of 4°C. Plasma CCK was measured by a sensitive and specific radioimmunoassay (16, 17). This antibody binds to all CCK peptides, including sulfated CCK octapeptide, but not gastrin. The detection limit of the assay is 0.3 pM plasma. Plasma PYY was measured in our laboratory by radioimmunoassay. PYY antiserum was generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM Biochemica GmbH, Switzerland). PYY was labelled with 125 Iodine using chloramine T. There is no cross-reactivity with PP or VIP. The

detection limit is 10 pM plasma. Both PYY (1–36) and PYY (3–36) bind to the antibody in dilutions up to 25 000.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Integrated incremental values for plasma CCK and PYY secretion were calculated as the area under the plasma concentration curve after subtraction of the basal value at time $t=0$. Plasma CCK and PYY secretion in time and between experiment arms were analysed by multiple analysis of variance (MANOVA). Duodenocecal transit time and integrated plasma values were analysed by means of Student paired t -tests with Bonferroni's correction for multiple comparisons. The significance level was set at $P < 0.05$.

Results

Intraduodenal administration of the high dose MCT (30 mmol/h) resulted in abdominal symptoms such as cramps and diarrhoea in all subjects. Diarrhoea was present not only during the experiment but persisted until the day after the experiment. After the administration of the low dose of MCT (15 mmol/h), abdominal symptoms and diarrhoea were present in six of the eight subjects. Abdominal symptoms were, however, milder and diarrhoea occurred only during the day of the experiment. No abdominal symptoms were reported during intraduodenal administration of LCT or saline.

Duodenocecal transit time

Individual data of duodenocecal transit time (DCTT) are depicted in Figure 1. DCTT was significantly ($P < 0.05$) accelerated during intraduodenal administration of both the low dose (54 ± 5 min) and of the high dose (61 ± 6 min) of MCT compared to saline (111 ± 10 min), while LCT did not affect DCTT (105 ± 11 min).

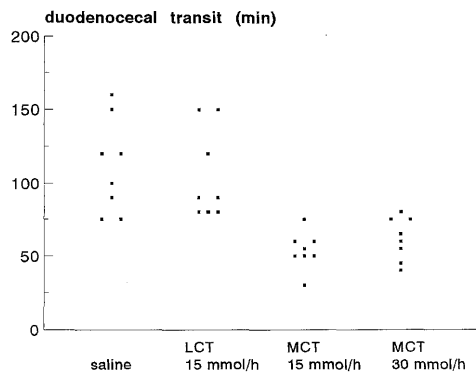


Fig. 1 Individual data of duodenocecal transit time (min) in eight healthy subjects during 360 min of continuous intraduodenal administration of saline (control), MCT 15 mmol/h, MCT 30 mmol/h and LCT 15 mmol/h.

Plasma CCK

Basal plasma CCK levels were not significantly different between the four experiments: saline, 0.7 ± 0.2 pM; low dose MCT, 0.6 ± 0.2 pM; high dose MCT, 0.6 ± 0.2 pM; and LCT, 0.7 ± 0.2 pM. Plasma CCK levels increased significantly ($P < 0.05$) over basal, starting from time $t=60$ min until time $t=360$ min during intraduodenal administration of LCT. No significant alterations in plasma CCK levels over basal were observed during administration of both low and high dose MCT and saline (Fig. 2). Integrated plasma CCK during 360 min LCT administration (315 ± 60 pM) was significantly increased compared to that of low dose MCT (-8 ± 51 pM), high dose MCT (-5 ± 33 pM) and saline (-2 ± 32 pM) (Table 1).

Plasma PYY

Basal plasma PYY levels were not significantly different between the four experiments: saline, 17.1 ± 1.3 pM; low dose MCT, 17.9 ± 1.0 pM; high dose MCT, 17.8 ± 1.7 pM; and LCT, 18.1 ± 1.3 pM. Plasma PYY levels increased

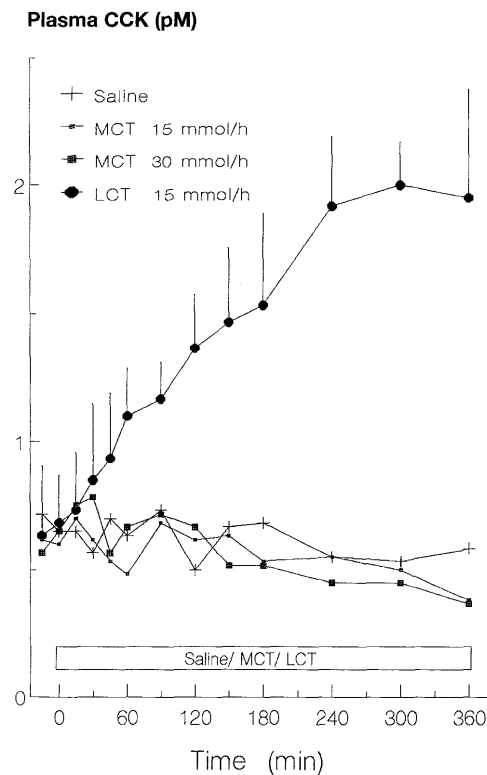


Fig. 2 Plasma CCK levels (pM, mean $\pm$ SEM) before and during 360 min of continuous intraduodenal administration of saline (control, crosses), MCT 15 mmol/h (small squares), MCT 30 mmol/h (big squares) and LCT 15 mmol/h (circles).

Plasma PYY (pM)

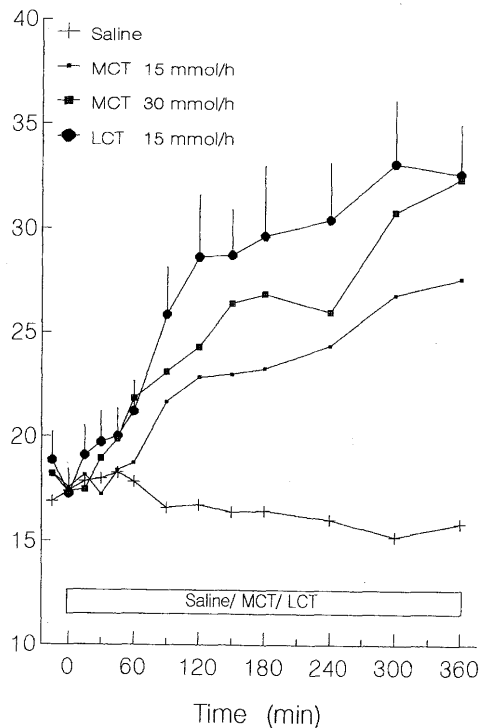


Fig. 3 Plasma PYY levels (pM, mean $\pm$ SEM) before and during 360 min of continuous intraduodenal administration of saline (control, crosses), MCT 15 mmol/h (small squares), MCT 30 mmol/h (big squares) and LCT 15 mmol/h (circles).

significantly ($P < 0.05$) over basal, starting between time 60 and 90 min, until 360 min during intraduodenal administration of both low and high dose MCT and LCT compared to saline (Fig. 3). Integrated plasma PYY during 360 min administration of low dose MCT (2006 ± 452 pM) was significantly ($P < 0.05$) lower compared to that of LCT (3896 ± 524 pM) but was not significantly different from that of high dose MCT (3009 ± 644 pM). No significant difference was found in integrated plasma PYY values between LCT and high dose MCT (Table 1).

Table 1 Integrated plasma CCK and PYY secretion during 360 min intraduodenal infusion of either saline (control), LCT (15 mmol/h), low dose MCT (15 mmol/h) or high dose MCT (30 mmol/h)

	CCK (pM* 360 min)	PYY (pM*360 min)
Saline	-2 ± 32	333 ± 91
LCT 15 mmol/h	$315 \pm 60^*$	$3896 \pm 524^*$
MCT 15 mmol/h	5 ± 33	$2006 \pm 542^{\#}$
MCT 30 mmol/h	-8 ± 51	$3009 \pm 644^*$

Results are expressed as mean $\pm$ SEM in pM*360 min. * $P < 0.01$ compared to saline; $\# P < 0.05$ compared to LCT.

Discussion

The present study confirms that MCT significantly accelerate small intestinal transit compared to saline and LCT. Furthermore, MCT do not stimulate the release of the proximal gut hormone CCK. On the contrary, intraduodenally administered MCT induce a significant increase in the plasma levels of the distal gut hormone PYY.

The absence of CCK secretion in response to MCT found in this study is in line with previous observations (5–7). CCK is released after digestion of triglycerides for fatty acids. CCK is an important hormonal regulator for pancreatic secretion and gallbladder contraction (8, 9, 18). The reason for this lack of CCK release by MCT is not yet clear. However, differences in CCK release induced by dietary fats have been documented (19, 20). It has been shown in animal studies that only fatty acids with chain length of nine or more carbon atoms stimulate CCK release (20). Furthermore, rapid hydrolysis and absorption of MCT may result in low intraluminal concentrations of MCT and medium chain free fatty acids in the duodenum that are not sufficient to stimulate CCK release.

If the concept of rapid hydrolysis and absorption of MCT is true, it is remarkable that patients receiving MCT supplements often complain about gastrointestinal symptoms such as abdominal cramps and diarrhoea (1, 4). Indeed, high dose MCT (30 mmol/h) as used in the present study induced cramps and diarrhoea in all eight healthy subjects. These symptoms were milder at the low dose MCT (15 mmol/h) but still present in 75% of the subjects. The underlying mechanism of diarrhoea induced by MCT is not yet clear. Although different in caloric content, both low and high dose MCT have the same osmolality which is twice as high as LCT and saline. It has been suggested earlier that enteral solutions, with higher osmolality are more likely to cause osmotic diarrhoea (21). In addition, the rapid hydrolysis of MCT, resulting in even higher intraluminal osmolality, may possibly potentiate this effect (4). We have recently demonstrated that alterations in the intestinal motility pattern may contribute to the accelerated small intestinal transit time induced by MCT (10). It seems unlikely that the higher osmolality of MCT solutions is responsible for this finding since intravenous MCT induces similar changes in small intestinal motor pattern (22). However, we can not exclude the possibility of an increased intestinal secretion due to the osmotically higher concentration of MCT to be an indirect cause of the accelerated small intestinal transit. Rapid transport of intraluminal content from the proximal to the distal gut may limit absorption by reducing the contact-time of nutrients with the intestinal epithelium and thus may result in diarrhoea. In line with these findings, intraduodenal MCT, even at the low dose, significantly increase PYY release. PYY is a distal gut hormone and the highest concentration of PYY producing cells are found in the distal ileum, colon and rectum (23). The release of PYY is stimulated by the presence of unabsorbed nutrients in the distal gut (12–14). There is evidence suggesting that PYY is one of the hormonal mediators of the so-called ileal brake: a nega-

tive feedback from the distal to the proximal gut. In humans, intraileal infusion of lipids increases plasma level of PYY which is correlated with delayed gastric emptying (24). In dogs, fat-induced ileal brake slows intestinal transit which is accelerated with PYY immunoneutralization (25). However, a recent study in rats has shown that inhibition of gastric emptying induced by fat in the distal gut is independent of PYY (26). Plasma PYY concentrations are markedly increased in diseases associated with malabsorption such as chronic pancreatic insufficiency, coeliac disease and short bowel syndrome (27, 28).

The elevated plasma levels of PYY after intraduodenal administration of MCT at both low and high dose suggest that MCT are not completely absorbed but reach the distal gut and stimulate PYY release. At this point, one could argue that PYY release could be activated not only by intraluminal nutrients but also directly from the proximal gut via neural and/or hormonal pathways or systemically via circulating free fatty acids. It has been suggested that CCK may be one of the proximal gut factors that participates in the regulation of PYY release (29). Several studies in dogs have demonstrated that exogenous CCK stimulates PYY release (30, 31). PYY release during LCT administration may result from endogenous CCK secretion. In contrast, PYY release induced by MCT cannot be mediated by CCK since MCT did not stimulate CCK release. In addition, the concept of MCT reaching the distal gut and stimulating PYY release is supported by an earlier study demonstrating that intraileal infusion of MCT markedly stimulated PYY release (12). Moreover, one should keep in mind that most of the data supporting the concept of rapid hydrolysis and absorption of MCT are derived from animal experiments (32, 33). Based on these findings, one might argue that accelerated small intestinal transit induced by MCT may have detrimental effects on absorption since small intestinal transit is often already accelerated in disorders associated with malabsorption (34).

In conclusion, results of the present study raise questions about the concept of rapid hydrolysis and absorption of MCT. We have shown that MCT accelerate small bowel transit; MCT are possibly incompletely absorbed, reach the distal gut and stimulate PYY release.

References

1. Timmermann F. Medium chain tryglycerides. The unconventional oil. *Int Food Ingredients* 1993; 3: 18–11
2. Bach A C, Babayan V K. Medium chain triglycerides: an update. *Am J Clin Nutr* 1982; 36: 950–962
3. Guillot E, Vaugelade P, Lemarchal P, Rerat A. Intestinal absorption and liver uptake of medium chain fatty acids in non-anaesthetized pigs. *Br J Nutr* 1993; 69: 431–432
4. Ruppin D C, Middleton W R J. Clinical use of medium chain triglycerides. *Drugs* 1980; 20: 216–224
5. Ladas S D, Isaacs P E T, Murphy G M, Sladen G E. Comparison of the effects of medium and long chain triglyceride containing liquid meals on gallbladder and small intestinal function in normal man. *Gut* 1984; 25: 405–411
6. Hopman W P M, Jansen J B M W, Rosenbusch G, Lamers C B H W. Effect of equimolar amounts of long chain triglycerides and medium chain triglycerides on plasma cholecystokinin and gallbladder contraction. *Am J Clin Nutr* 1984; 39: 356–359

7. Isaacs P E T, Ladas S, Forgacs I C, Dowling R H, Ellam S V, Adrian T E, Bloom S R. Comparison of effects of ingested medium and long chain triglyceride on gallbladder volume and release of cholecystokinin and other gut peptide. *Dig Dis Sci* 1987; 32: 481–486
8. Guimbaud R, Moreau J A, Bouisson M, Durand S, Escourrou J, Vaysses N, Frexinos J. Intraduodenal free fatty acids rather than triglycerides are responsible for the release of CCK in humans. *Pancreas* 1997; 14: 76–82
9. Hildebrand P, Petrig C, Burckhardt et al. Hydrolysis of dietary fat by pancreatic lipase stimulates cholecystokinin release. *Gastroenterology* 1998; 114: 123–129
10. Verkijk M, Vecht J, Gielkens H A J, Lamers C B H W, Masclee A A M. Effects of medium chain and long chain triglycerides on antroduodenal motility and small bowel transit time in man. *Dig Dis Sci* 1997; 42: 1933–1939
11. Ledebor M, Masclee A A M, Jansen J B M J, Lamers C B H W. Effect of equimolar amounts of long chain triglycerides and medium chain triglycerides on small bowel transit time in human. *JPEN* 1995; 19: 5–8
12. Spiller R C, Trotman I F, Adrian T E, Bloom S R, Misiewicz J J, Silk D B A. Further characterisation of the ileal brake reflex in man – effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY. *Gut* 1984; 29: 1042–1051
13. Pironi L, Stanghellini V, Miglioli M et al. Fat induced ileal brake in humans: a dose dependent phenomenon correlated to the plasma levels of peptide YY. *Gastroenterology* 1993; 105: 733–739
14. Fone D R, Horowitz M, Read N W, Dent J, Maddox A. The effect of terminal ileal triglyceride infusion on gastroduodenal motility and intragastric distribution of a solid meal. *Gastroenterology* 1990; 98: 568–575
15. Bond J H, Levitt M D. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H₂) measurements. *J Lab Clin Med* 1975; 85: 546–555.
16. Jansen J B M J, Lamers C B H W. Molecular forms of cholecystokinin in plasma from normal and gastrectomized human subjects following a fat meal. *Peptides* 1987; 8: 801–805
17. Jansen J B M J, Lamers C B H W. Radioimmunoassay of cholecystokinin in human tissue and plasma. *Clin Chim Acta* 1983; 131: 305–316.
18. Solomon T E. Control of exocrine pancreatic secretion. In: Johnson L H, ed. *Physiology of the gastrointestinal tract*. Vol. 2, 3rd edn. New York: Raven, 1994: 1499–1529
19. Beardshall K, Morarji Y, Bloom S R, Frost G, Domin J, Calam J. Saturation of fat and cholecystokinin release: implications for pancreatic carcinogenesis. *Lancet* 1989; 1008–1010
20. Meyer J H, Jones R S. Canine pancreatic responses to intestinally perfused fat and products of fat digestion. *Am J Physiol* 1974; 226: 1178–1187
21. Keohane P P, Attrill H, Love M, Frost P, Silk D B. Relation between osmolality of diet and gastrointestinal side effects in enteral nutrition. *Br Med J* 1984; 288: 678–80
22. Hebuterne X, Ducrotte P, Denis P, Guedon C, Colin R, Lerebours E. Effects of intraduodenal and intravenous fat infusion on small bowel motility in humans. A comparative study of medium- and long- chain triglycerides. *Eur J Gastroenterol Hepatol* 1993; 5: 351–56
23. Adrian T E, Ferri G-L, Bacarese-Hamilton A J, Fuessl H S, Polak J M, Bloom S R. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 1985; 89: 1070–1077
24. Pironi L, Stanghellini V, Miglioli M et al. Fat-induced ileal brake in humans: a dosedependent phenomenon correlated to the plasma levels of peptide YY. *Gastroenterology* 1993; 105: 733–39
25. Lin H C, Zhao X T, Wang L, Wong H. Fat-induced ileal brake in the dog depends on peptide YY. *Gastroenterology* 1996; 110: 1491–95
26. Raybould H E, Tabrizi Y, Meyer J H, Walsh J H. PYY immunoneutralization does not alter lipid-induced inhibition of gastric emptying in rats. *Regul Pept* 1999; 79: 125–130
27. Adrian T E, Savage A P, Bacarese-Hamilton A J, Wolfe K, Besterman H S, Bloom S R. Peptide YY abnormalities in gastrointestinal diseases. *Gastroenterology* 1986; 90: 379–384
28. Nightingale J M D, Kamm M A, van de Sijp J R M et al. Disturbed gastric emptying in the short bowel syndrome. Evidence for a 'colonic brake'. *Gut* 1993; 34: 1171–1176
29. Greeley G H, Jeng Y-J, Gomez G et al. Evidence for regulation of peptide YY release by the proximal gut. *Endocrinology* 1989; 124: 1438–1443
30. McFadden D W, Rudnicki M, Kuvshinov B, Fischer J E. Postprandial peptide YY release is mediated by cholecystokinin. *Surg Gynecol Obstet* 1992; 175: 145–150
31. Kuvshinov B, Rudnicki M, McFadden D W, Nussbaum M S, Fischer J E. Release of intraluminal and circulatory peptide YY after intravenous CCK-8 in conscious dogs. *Curr Surg* 1990; 94: 338–340
32. Greenberger N J, Rodgers J B, Isselbacher K J. Absorption of medium and long chain triglycerides: factors influencing their hydrolysis and transport. *J Clin Invest* 1966; 45: 217–227
33. Chow P B C, Shaffer E A, Parsons H G. Absorption of triglycerides in the absence of lipase. *Can J Physiol Pharmacol* 1990; 68: 519–523
34. Laver P, Von Der Ohe M, Holst J J, Jansen J B M J, Grandt D, Holtmann G, Goebell H. Altered postprandial motility in chronic pancreatitis: role of malabsorption. *Gastroenterology* 1997; 112: 1624–1634

Chapter 6

THE OSMOTIC LAXATIVE MAGNESIUM SULPHATE ACTIVATE THE ILEAL BRAKE

M.K. Vu, M.A.G. Nouwens, I. Biemond, C.B.H.W. Lamers, A.A.M. Masclee

Department of Gastroenterology-Hepatology, Leiden University Medical
Centre, the Netherlands

The osmotic laxative magnesium sulphate activates the ileal brake

M. K. VU, M. A. G. NOUWENS, I. BIEMOND, C. B. H. W. LAMERS & A. A. M. MASCLEE

Department of Gastroenterology–Hepatology, Leiden University Medical Centre, The Netherlands

Accepted for publication 23 December 1999

SUMMARY

Background: Alterations in gastrointestinal motility and hormone secretion, especially activation of the ileal brake, have been documented in malabsorption.

Aim: To investigate whether artificially-induced accelerated small intestinal transit activates the ileal brake mechanism.

Methods: Eight healthy volunteers (four female, four male; age 21 ± 3 years) participated in four experiments: (a) meal with either oral magnesium sulphate (MgSO_4) or placebo; and (b) fasting with either oral MgSO_4 or placebo. Antroduodenal motility was recorded by perfusion manometry. Duodenocaecal transit time was determined by the lactulose H_2 breath test. Gall-bladder volume was measured by ultrasound at regular intervals, and blood samples were drawn for determi-

nation of cholecystokinin and peptide YY (RIA). Twenty-four hour faecal weight and fat excretion were determined.

Results: MgSO_4 significantly accelerated duodenocaecal transit time and increased faecal fat and weight in all subjects. MgSO_4 significantly delayed the reoccurrence of phase III and affected antroduodenal motility during fasting but not after meal ingestion. Postprandial gall-bladder relaxation and postprandial peptide YY release were significantly increased during the MgSO_4 experiment compared to placebo.

Conclusions: The osmotic laxative MgSO_4 accelerates intestinal transit both in the fasting and fed state. MgSO_4 activates the ileal brake mechanism only in the fed state, with peptide YY release and inhibition of gall-bladder emptying.

INTRODUCTION

Both in humans and dogs, intra-ileal infusion of nutrients delays gastric emptying and small intestinal transit and inhibits exocrine pancreatic secretion.^{1–3} This phenomenon is called the 'ileal brake', a negative feedback response from the distal to the proximal gut. There is evidence suggesting that the ileal brake is hormonally mediated.⁴ Peptide YY is considered a hormonal representative of the ileal brake. Peptide YY is released from the ileo–colonic region in response to intraluminal unabsorbed nutrients.^{5, 6} Plasma peptide YY levels are increased in patients with malabsorptive

diseases and in patients with diarrhoea, compared to healthy subjects.^{7, 8} Although these findings could be explained by increased loads of unabsorbed nutrients to the distal gut, the increased levels of plasma peptide YY may also be related to, or result from, the underlying disease. The present study was performed to investigate whether artificially induced accelerated transit with reduced intestinal absorption of nutrients *per se* is able to stimulate peptide YY release and activate the ileal brake mechanism.

In healthy subjects, osmotic laxatives accelerate small intestinal transit and reduce the intestinal absorption of fat, protein and carbohydrates following ingestion of a solid meal.⁹ For this reason magnesium sulphate (MgSO_4), an osmotic laxative, was used in the present study. We hypothesized that accelerated small intestinal transit and subsequent reduced intestinal absorption

Correspondence to: Dr A. A. M. Masclee, Department of Gastroenterology–Hepatology, Leiden University Medical Centre, PO Box 9600, 2300 RC Leiden, The Netherlands.

E-mail: A.A.M.Masclee@LUMC.nl

induced by MgSO_4 would stimulate peptide YY release and activate the ileal brake mechanism in healthy subjects. In order to differentiate between the effects of reduced intraluminal nutrient absorption and those of MgSO_4 itself on gastrointestinal motility and secretion, experiments with MgSO_4 were performed both in the fed and fasted state. Antroduodenal and gall-bladder motility and proximal and distal gut hormone secretion were studied.

SUBJECTS AND METHODS

Subjects

Eight healthy volunteers (four men and four women, mean age 21 years, range 18–24 years) participated in this study. None of the subjects had a history of gastrointestinal disease or surgery and none was taking any medication. Informed consent was obtained from each subject and the protocol had been approved by the local ethical committee.

Experimental design

Each subject participated in four experiments performed in random order on separate days. Experiment 1: fasting and MgSO_4 ; 2: fasting and placebo; 3: meal and MgSO_4 ; 4: meal and placebo. The experiments started at 07:45 hours. After an overnight fast of at least 10 h subjects were intubated transnasally with the antroduodenal manometry catheter. Thereafter motility recording was started. An intravenous cannula was inserted into the antecubital vein of one arm for blood sampling. The spontaneous occurrence of a phase III in the duodenum marked the start point for all the experiments and was defined as $t = -30$ min. At $t = -15$ min, 15 g MgSO_4 dissolved in 50 mL water or placebo was ingested. In experiments 1 and 2, at time $t = 0$ min, the volunteers drank a 400-mL liquid meal (banana shake) containing 45 g fat, 35 g protein, 58 g carbohydrates and 780 kCal. In all experiments 6 g lactulose dissolved in 60 mL water was administered intraduodenally at time $t = 0$ min for measurements of small intestinal transit time. Antro-duodeno-jejunal motility was recorded for at least 6 h after MgSO_4 /placebo administration.

Stool collection

At the end of experiments 1 and 2 each subject received a standard evening meal consisting of potatoes, minced

meat, gravy, green beans, apple sauce and fruit cocktail (40 g protein, 42 g fat, 139 g carbohydrates, 1093 kcal). Stool was collected for 24 h (starting at 07:45 hours on the day of the experiment until 07:45 hours the next day) in a pre-weighed plastic bucket. Subjects were instructed to eat rice, cauliflower and chicken breast at dinner the evening before the day of the experiment. During the 24-h period of stool collection they did not consume other caloric items apart from the meal offered. Faecal weight and faecal fat were determined in gram per 24 h according to a previously described method.¹⁰

Antroduodenal manometry

Antroduodenal motility was recorded using a multilumen water perfused polyvinyl catheter (outer diameter 5 mm). The catheter incorporated eight side holes located at 0, 5, 10, 15, 20, 25, 30 and 35 cm from the distal tip. The manometry catheter was passed trans-nasally into the stomach and from there positioned into the duodenum-jejunum under fluoroscopic control. The tip of the catheter was located just distal to the ligament of Treitz so that one or two side hole openings were in the jejunum, three to four side hole openings were in the duodenum and at least two in the antrum. When the correct position had been verified the catheter was taped to the nose. At the end of each experiment the position of the catheter was checked again by fluoroscopy. Each lumen was connected to a pressure transducer and perfused with distilled water by a low compliance pneumo-hydraulic perfusion system (Arndorfer Medical Systems) at a rate of 0.5 mL/min. Outputs from pressure transducers were recorded by a polygraph (Synectics Medical, Stockholm, Sweden), displayed on a monitor and stored on a personal computer for automated and manual analysis.

Small intestinal transit

Small bowel transit time was determined by the lactulose hydrogen breath test after intraduodenal instillation of 6 g lactulose (Legendal, Inpharzam, Amersfoort, The Netherlands), dissolved in 60 mL water at time $t = 0$ min.¹¹ End expiratory breath samples were first collected under fasting conditions at time $t = -30$, and -15 min and every 10 min thereafter and were analysed immediately in a hydrogen breath test unit (Lactoscreen, Hoekloos, The Netherlands).

Duodenocaecal transit time was defined as the time between administration of lactulose and a sustained rise in breath H_2 concentration of at least 10 parts per million (p.p.m.) above basal levels. At our department the mean coefficient of variation for duodenocaecal transit using the lactulose hydrogen breath test with 6 g lactulose is $12 \pm 5\%$.

Measurements of gall-bladder volumes

Gall-bladder volumes were measured by real time ultrasonography (Toshiba, 3.75 MHz transducer) at $t = -30, -15, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 330$ and 360 min during experiments 1 and 2 and at $t = -30, -15, 0, 15, 30, 45, 60, 90, 120, 180, 240, 300$, and 360 min during experiments 3 and 4. Gall-bladder volumes were calculated by the sum of cylinders method using a computerized system.^{12, 13} In this method the longitudinal image of the gall-bladder is divided into series of equal height, with diameter perpendicular to the longitudinal axis of the gall-bladder image. The uncorrected volume is the sum of volumes of these separate cylinders. To correct for the displacement of the longitudinal image of the gall-bladder from the central axis, a correction factor is calculated from the longitudinal and transversal scans of the gall-bladder. Gall-bladder volume is calculated by multiplication of the uncorrected volume with the square of the correction factor; the mean of two measurements was used for analysis. The assumptions and the mathematical formula used to calculate gall-bladder volume have been described and validated previously.^{12, 13} Fasting gall-bladder volumes were expressed in millilitres. Gall-bladder emptying was calculated as a percentage of fasting gall-bladder volume.

Hormone assays

Blood samples for measurement of plasma pancreatic polypeptide, cholecystokinin and peptide YY were drawn at time $t = -0, -15, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 330$ and 360 min during experiments 1 and 2 and at $t = -30, -15, 0, 15, 30, 45, 60, 90, 120, 180, 240, 300$, and 360 min during experiments 3 and 4. The blood samples were collected in ice-chilled tubes containing EDTA. The samples were centrifuged at a rate of 3000 r.p.m. for 10 min at a temperature of $4^\circ C$. Plasma cholecystokinin was measured by a sensitive and specific radio-

immunoassay.¹⁴ This antibody binds to all cholecystokinin peptides including sulphated cholecystokinin octapeptide, but not gastrin. The detection limit of the assay is 0.1 pM plasma. Plasma peptide YY was measured by radioimmunoassay. Peptide YY antiserum was generated in rabbits by intracutaneous injections of synthetic human peptide YY (BACHEM AG, Bubendorf, Switzerland). Peptide YY was labelled with ¹²⁵Iodine using chloramine T. There is no cross-reactivity with pancreatic polypeptide or VIP; the detection limit is 10 pM plasma. Both peptide YY¹⁻³⁶ and peptide YY³⁻³⁶ bind to the antibody in dilutions up to 25 000.

Analysis of motility recording

Motility patterns from antroduodenal manometry were analysed both visually and by computer. The individual tracings were processed by special software (Polygram, Synectics Medical, Stockholm, Sweden) for adjusting baselines and extracting respiratory artefacts. However, the computer program does not recognize simultaneous pressure events as artefacts. Therefore, remaining artefacts due to increments in intra-abdominal pressure were identified visually and excluded from the analysis. Duodenal phases of the migrating motor complex (MMC) were defined as follows: phase I, no more than two contractions every 10 min for at least 5 min and preceded by phase III; phase II: irregular contractile activity at a frequency of more than two every 10 min and amplitude above 12 mmHg; phase III: regular contractile activity at a frequency of 10–12 contractions per min for at least 2 min. Phase III activity had to be propagated over at least two recording sites. Antral phase III activity was defined as rhythmic contractile activity at maximum frequency (three contractions/min) for at least 1 min in temporal relationship with duodenal phase III activity.¹⁵ Duration of the MMC cycle was taken as the interval between the end of phase III in the duodenum until the end of the next phase III cycle.

The postprandial period was defined as the time interval between the end of the meal and the occurrence of the first duodenal phase III propagated over at least two channels. Only pressure waves with an amplitude ≥ 10 mmHg and duration ≥ 1.5 s were considered as true contractions. The motility indices in the antrum and duodenum were calculated as the area under the contraction curves and expressed in mmHg.sec. In experiments 1 and 2, antral and duodenal motility

indices were calculated for the first three postprandial hourly intervals. For experiments 3 and 4, antral and duodenal motility indices were calculated during the last 30 min of phase II of the first MMC cycle and of the following MMC cycles.

Statistical analysis

Data are expressed as mean $\pm$ S.E.M. Differences in plasma cholecystokinin and peptide YY concentrations, gall-bladder volumes and postprandial antral and duodenal motility indices within and between groups were analysed for statistical significance using multiple analysis of variance (MANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student–Newman–Keuls analyses were performed to determine which values between or within subjects differ significantly. The remaining interdigestive, digestive, small intestinal transit, faecal weight, fat excretion and integrated values of cholecystokinin and peptide YY data were analysed by the Wilcoxon signed rank test or when appropriate by the two-tailed Student's *t*-test for paired results. Pearson's correlation was used to correlate the percentage of gall-bladder and plasma cholecystokinin level. The significant level was set at $P < 0.05$.

RESULTS

MgSO₄ in the fasting state

Duodenocaecal transit time. Three out of the eight studied subjects had watery diarrhoea within 3 h after the administration of MgSO₄. The duodenocaecal transit time was significantly ($P < 0.05$) shorter in the MgSO₄ (40 ± 6 min) compared to the placebo experiment (65 ± 8 min).

Antroduodenal motility. Twenty-nine and 24 complete MMC cycles were observed during the MgSO₄ and placebo experiment, respectively, in eight subjects during 50 h of recording after the administration of MgSO₄/placebo. The mean duration of the MMC cycles in the MgSO₄ experiment (158 ± 20 min) was significantly ($P < 0.05$) prolonged compared to placebo (104 ± 8 min) due to a significantly longer phase II (142 ± 18 min in the MgSO₄ and 83 ± 9 min in the placebo experiment). On further analysis, this prolongation of the MMC cycle length and of phase II was only

Table 1. Characteristics of MMC cycles (mean $\pm$ S.E.M., min) in eight healthy volunteers during experiments in the fasting state

	First MMC	Following MMC
Placebo		
MMC cycle length	99 ± 13	101 ± 9
Phase I	8 ± 2	15 ± 4
Phase II	86 ± 12	81 ± 15
Phase III	5 ± 1	5 ± 1
MgSO ₄		
Duration (min)	$211 \pm 37^*$	$137 \pm 32^\dagger$
Phase I (min)	10 ± 3	14 ± 3
Phase II (min)	$197 \pm 34^*$	$119 \pm 31^\dagger$
Phase III (min)	4 ± 1	4 ± 1

* $P < 0.05$ compared to placebo; $^\dagger P < 0.05$ compared to the first MMC cycle.

present in the first MMC cycle after the occurrence of the spontaneous phase III. No significant differences in the duration of the remaining MMC cycles and phase I, II, and III were found between the placebo and MgSO₄ experiment (Table 1). In addition, the duration of the first MMC cycle was significantly ($P < 0.05$) longer compared to the remaining MMC cycles during the MgSO₄ experiment (Table 1).

The antral motility index calculated during the last 30 min of phase II of the first MMC cycle was significantly lower in the MgSO₄ compared to the placebo experiment (Table 2). This lower antral motility indices in the MgSO₄ experiment was due to a decrease in number as well as amplitude of individual contractions (Table 2). The mean antral motility index of phase II of the remaining MMC cycles was not significantly different between the MgSO₄ and placebo experiments. Duodenal motility indices during the last 30 min of phase II were not significantly different between the MgSO₄ and the placebo experiments in either the first MMC cycle or the remaining MMC cycles (data not shown).

Gall-bladder emptying. Basal gall-bladder volumes were not significantly different between the placebo (16.9 ± 2.9 mL) compared to the MgSO₄ experiment (17.1 ± 2.5 mL). No significant changes in gall-bladder volume compared to basal values were observed after the administration of MgSO₄ or placebo.

Plasma cholecystokinin. Basal plasma cholecystokinin levels were not significantly different between the MgSO₄ and the placebo experiment (0.8 ± 0.2 pM vs.

Table 2. Antral motility characteristics during the last 30 min of phase II of the first MMC cycle and of the remaining MMC cycles in eight healthy subjects during experiments in the fasting state

	Antrum		Duodenum	
	Placebo	MgSO ₄	Placebo	MgSO ₄
First MMC				
Number of contractions	37 ± 10	17 ± 3*	109 ± 46	46 ± 12
Amplitude (mmHg)	72 ± 8	49 ± 4*	26 ± 1	27 ± 4
Motility index (mmHg.sec)	7750 ± 1342	2104 ± 806*	3857 ± 1097	2058 ± 604
Following MMC				
Number of contractions	21 ± 2	29 ± 6	46 ± 6	50 ± 11
Amplitude (mmHg)	98 ± 11	51 ± 6	29 ± 3	25 ± 3
Motility index (mmHg.sec)	5876 ± 1231	3407 ± 1146	2223 ± 511	1797 ± 535

* $P < 0.05$ compared to placebo.

Table 3. Postprandial antral and duodenal motility index (mean ± S.E.M., mmHg.sec) for the first three hourly intervals and for the total fed period after meal ingestion in eight healthy subjects

Motility indices	Placebo	MgSO ₄
Antrum 0–60 min	105 ± 37	149 ± 60
Antrum 60–120 min	303 ± 128	331 ± 85
Antrum 120–180 min	1012 ± 189	748 ± 299
Antrum, total fed period	1001 ± 138	1425 ± 201
Duodenum 0–60 min	2601 ± 737	2606 ± 1062
Duodenum 60–120 min	1687 ± 436	1752 ± 919
Duodenum 120–180 min	2130 ± 895	1614 ± 451
Duodenum, total fed period	2415 ± 601	2560 ± 847

* $P < 0.05$ compared to placebo.

0.7 ± 0.1 pM, respectively; Figure 1A). Plasma cholecystokinin levels at $t = 15$ and $t = 30$ min during the MgSO₄ experiment were slightly higher compared to basal values and compared to the placebo experiment but this difference did not reach significance (Figure 1A). The integrated incremental plasma cholecystokinin secretion was not significantly different between the MgSO₄ (29 ± 84 pM.360 min) compared to the placebo experiment (–13 ± 17 pM.360 min).

Plasma peptide YY. Basal plasma peptide YY levels were not significantly different between the MgSO₄ and the placebo experiment (19.2 ± 1.9 pM vs. 18.2 ± 2.0 pM, respectively; Figure 2A). No significant changes in plasma peptide YY levels compared to basal values were observed during either the MgSO₄ or the placebo experiment (Figure 2A). The integrated incremental

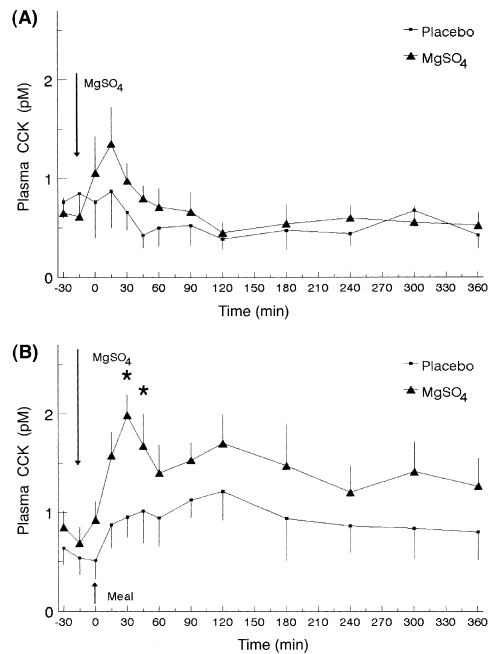


Figure 1. (A) Plasma cholecystokinin levels in the fasting state during the MgSO₄ (triangles) and the placebo experiment (squares). (B) Basal and postprandial plasma cholecystokinin levels during the MgSO₄ (triangles) and the placebo experiment (squares). * $P < 0.05$ compared to placebo.

plasma peptide YY secretion was also not significantly different between the MgSO₄ (56 ± 23 pM.360 min) and the placebo experiment (35 ± 30 pM.360 min).

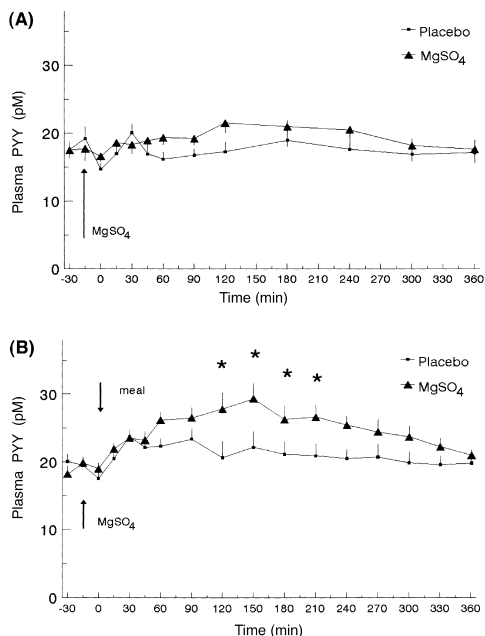


Figure 2. (A) Plasma peptide YY levels in the fasting state during the MgSO₄ (triangles) and the placebo experiment (squares). (B) Basal and postprandial plasma peptide YY levels during the MgSO₄ (triangles) and the placebo experiment (squares). * $P < 0.05$ compared to placebo.

MgSO₄ in the fed state

Duodenocaecal transit time and faecal parameters. MgSO₄ induced diarrhoea and significantly ($P < 0.05$) accelerated duodenocaecal transit time in all subjects compared to placebo. The mean duodenocaecal transit time was 31 ± 3 min after MgSO₄ administration compared to 54 ± 7 min with placebo. The 24 h faecal weight and fat excretion were significantly ($P < 0.05$) increased after the administration of MgSO₄ compared to placebo (395 ± 45 g per 24 h and 10.8 ± 1.4 g per 24 h vs. 128 ± 12 g per 24 h and 4.7 ± 0.6 g per 24 h, respectively).

Antroduodenal motility. The duration of the fed pattern was not significantly different between the placebo and MgSO₄ experiment (307 ± 31 min vs. 271 ± 46 min, respectively). In both experiments antral motor activity was significantly ($P < 0.01$) lower during the first two

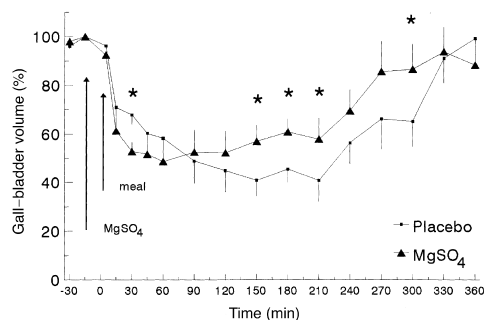


Figure 3. Percentage of postprandial gall-bladder emptying during the MgSO₄ (triangles) and the placebo experiment (squares). * $P < 0.05$ compared to placebo.

compared to the third postprandial hour (Table 1). Antral and duodenal motility indexes for the first three hourly intervals were not significantly different between the placebo and the MgSO₄ experiment (Table 1).

After the transition from a digestive into an interdigestive motility pattern, 10 complete MMC cycles in the placebo experiment and 13 complete MMC cycles in the MgSO₄ experiment were registered. The duration of MMC cycles was not significantly different between the placebo and MgSO₄ experiment (143 ± 12 min vs. 120 ± 17 min, respectively).

Gall-bladder emptying. Basal gall-bladder volumes were not significantly different between the placebo (17.8 ± 3.1 mL) and MgSO₄ experiment (17.1 ± 2.9 mL). In both the experiments gall-bladder volumes significantly ($P < 0.01$) decreased after meal ingestion and remained significantly decreased until $t = 300$ min in the placebo and until $t = 240$ min in the MgSO₄ experiment (Figure 3). Gall-bladder emptying at $t = 30$ min was significantly ($P < 0.05$) greater during the MgSO₄ compared to the placebo experiment. Gall-bladder emptying was significantly ($r = 0.55$; $P = 0.04$) correlated with plasma cholecystokinin levels from $t = 0$ to $t = 30$ min. In contrast, postprandial gall-bladder contraction between $t = 150$ and $t = 240$ min was significantly ($P < 0.05$) reduced in the MgSO₄ compared to the placebo experiment. In both experiments gall-bladder volumes had returned to basal levels at time $t = 360$ min.

Plasma cholecystokinin. Basal plasma cholecystokinin levels were not significantly different between the placebo (0.7 ± 0.1 pM) and MgSO_4 experiment (0.7 ± 0.2 pM; Figure 1B). Plasma cholecystokinin levels increased significantly over basal, starting from $t = 15$ min after meal ingestion and continuing until $t = 210$ min in the placebo and until $t = 240$ min in the MgSO_4 experiment. Plasma cholecystokinin levels during the first hour after meal ingestion were significantly higher in the MgSO_4 experiment compared to placebo (Figure 1B). The integrated incremental plasma cholecystokinin secretion during the total 6 h postprandial period was also significantly ($P < 0.01$) higher in the MgSO_4 (202 ± 69 pM) compared to the placebo experiment (95 ± 43 pM).

Plasma peptide YY. Basal plasma peptide YY levels were not significantly different between the placebo (19.5 ± 1.3 pM) and MgSO_4 experiment (19.9 ± 0.9 pM; Figure 2B). Plasma peptide YY levels increased significantly ($P < 0.05$) over basal starting from $t = 60$ min after meal ingestion until $t = 240$ min in the MgSO_4 experiment while no significant changes in plasma peptide YY levels were found in the placebo experiment. Plasma peptide YY concentrations from $t = 120$ min until $t = 240$ min were significantly ($P < 0.05$) higher in the MgSO_4 compared to the placebo experiment (Figure 2B). The integrated incremental plasma peptide YY secretion during the total 6 h postprandial period was significantly ($P < 0.01$) increased in the MgSO_4 (1667 ± 395 pM) compared to the placebo experiment (545 ± 125 pM).

DISCUSSION

This study shows that oral magnesium sulphate significantly accelerates small intestinal transit both in the fasting and fed state. During the interdigestive state, MgSO_4 significantly modulates antroduodenal motility without changes in intestinal hormone secretion. On the other hand, postprandial antroduodenal motility remains unaffected after the administration of MgSO_4 . When given in combination with a fatty meal, MgSO_4 induced diarrhoea in all healthy subjects with a significantly higher faecal weight and faecal fat excretion compared to placebo. Postprandial plasma levels of the distal gut hormone peptide YY were significantly increased in parallel with an increase in

gall-bladder volume (relaxation) after the administration of MgSO_4 .

It is apparent from the results that alterations in antroduodenal motility were present only in the early phase after the administration of MgSO_4 during the fasting state. MgSO_4 significantly increased the duration of the first MMC cycle by increasing the length of phase II and thus delaying the reoccurrence of phase III motor activity. In addition, the antroduodenal motility index of phase II of the first MMC cycle was significantly decreased. No significant differences in the remaining MMC cycles were found between the MgSO_4 and the placebo experiment. The exact mechanism(s) underlying these time-related changes is not obvious. MgSO_4 may affect antroduodenal motility through different mechanisms: increased intraluminal secretion; stimulation of cholecystokinin release; increased nitric oxide (NO) release; or a combination.^{1-4, 16-18} In the present study, plasma cholecystokinin levels during the first hour after MgSO_4 administration were slightly, although not significantly, increased compared to placebo. It has been shown that cholecystokinin interrupts the MMC cycle and induces a fed-like motor pattern.¹⁹ Thus, the prolonged phase II found in the present study could have resulted from cholecystokinin. The role of nitric oxide as a mediator of the laxative action of MgSO_4 has been recently recognized; MgSO_4 increases nitric oxide synthase activity.^{16, 18} Both in humans and animals, an increase of NO facilitates a postprandial-like motor pattern while NO synthase inhibitor induces a fasting-like motor pattern.²⁰ We did not measure NO but the congruency between motility changes found in the present study and reported changes induced by NO suggests that NO might be involved in delaying the reoccurrence of phase III and increasing the duration of the MMC cycle.

In contrast to the interdigestive state, when MgSO_4 was combined with a meal, it did not affect the postprandial antroduodenal motor pattern. The duration of the fed pattern and antroduodenal motility indices was similar in the placebo and the MgSO_4 experiment. The mechanisms responsible for the variable effects of MgSO_4 during the interdigestive and digestive states are unknown. However, our results are in agreement with those of a previous study reporting that oral administration of MgSO_4 modulates interdigestive motor pattern while digestive motility remains unaffected.²¹

The most striking effects induced by MgSO_4 after ingestion of a meal were observed with respect to intestinal gut hormone secretion. Postprandial release of the proximal gut hormone cholecystokinin was significantly increased after the administration of MgSO_4 compared to placebo. Because cholecystokinin release was only slightly increased by MgSO_4 during fasting, this finding suggests that the presence of intraluminal nutrients is the factor responsible for the significant increase in postprandial plasma cholecystokinin levels. It is conceivable that, due to an accelerated small intestinal transit induced by MgSO_4 , intraluminal nutrients are brought into contact with a larger area of the upper small bowel, permitting a greater number of cholecystokinin releasing cells to be activated, resulting in increased cholecystokinin release. For instance, postprandial plasma cholecystokinin concentrations are increased in patients with dumping syndrome who have accelerated small intestinal transit in addition to accelerated gastric emptying.²²

The same pattern was observed for the distal gut hormone peptide YY. After ingestion of the fatty meal, postprandial plasma levels of the distal gut hormone peptide YY were significantly higher after the administration of MgSO_4 compared to placebo. The fact that MgSO_4 itself did not stimulate peptide YY release during the fasting state indicates that changes in plasma levels of peptide YY after the administration of MgSO_4 in combination with a fatty meal are nutrient-related. Since peptide YY is released from the distal gut, the increased levels of plasma peptide YY suggests that nutrients were not completely absorbed but have reached the distal gut and stimulated peptide YY release.⁶ This is supported by the fact that all subjects had diarrhoea with increased faecal weight and that the faecal fat excretion was significantly increased after the administration of MgSO_4 .

Concerning gall-bladder motility, no significant changes in gall-bladder volume were observed after the administration of MgSO_4 during the interdigestive state. This finding is consistent with the insignificant changes in fasting plasma cholecystokinin levels. Our results, however, contrast with those found by Inoue *et al.* who documented that oral MgSO_4 induces gall-bladder contraction and increases cholecystokinin release.¹⁷ It is possible that MgSO_4 induced cholecystokinin release and subsequent gall-bladder contraction is a dose-dependent response. We have used 15 g MgSO_4 instead

of 25 g used by Inoue *et al.*; it has been reported in a previous study that only high dose intraduodenal MgSO_4 is able to increase bilirubin output.²³ Postprandial gall-bladder emptying in the MgSO_4 experiment, on the other hand, showed significant differences compared to placebo in two regards: an increase in gall-bladder emptying during the early phase and a decrease in gall-bladder emptying during the late phase. The latter was observed in parallel with an increase in plasma peptide YY levels. It could be suggested, based on this finding, that peptide YY is involved in stimulating gall-bladder relaxation in the late postprandial phase. This idea is in line with the hypothesis that unabsorbed nutrients in the distal small intestine stimulate the release of peptide YY which in turn exerts an inhibitory feedback on gall-bladder contraction. The observation that administration of peptide YY increases gall-bladder volume is evidence supporting this concept.²⁴ However, we cannot exclude the possibility that other distal gut hormones might also be involved.

In summary, oral MgSO_4 accelerates small intestinal transit, induces diarrhoea and increases faecal fat excretion in healthy subjects after ingestion of a meal. The increase in plasma peptide YY levels and gall-bladder relaxation in the late postprandial phase can be considered as evidence which indicates that MgSO_4 activates the ileal brake mechanism.

REFERENCES

- 1 Welch I, Cunningham KM, Read NW. Regulation of gastric emptying by ileal nutrients in humans. *Gastroenterology* 1988; 94: 401–4.
- 2 Lin HC, Zhao XT, Wang L. Intestinal transit is more potently inhibited by fat in the distal (ileal brake) than in the proximal (jejunal brake) gut. *Dig Dis Sci* 1997; 42: 19–25.
- 3 Layer P, Peschel S, Schlesinger T, Goebell H. Human pancreatic secretion and intestinal motility: effects of ileal nutrient perfusion. *Am J Physiol* 1990; 258: G196–201.
- 4 Lin HC, Zhao XT, Wang L, Wong H. Fat-induced ileal brake in the dog depends on peptide YY. *Gastroenterology* 1996; 110: 1491–5.
- 5 Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 1985; 89: 1070–7.
- 6 Spiller RC, *et al.* Further characteristics of the 'ileal brake' reflex in man. Effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY. *Gut* 1988; 29: 1042–51.

- 7 Adrian TE, Savage AP, Bacarese-Hamilton AJ, Wolfe K, Besterman HS, Bloom SR. Peptide YY abnormalities in gastrointestinal diseases. *Gastroenterology* 1986; 90: 379–84.
- 8 Adrian TE, Long RG, Fuessl HS, Bloom SR. Plasma peptide YY (PYY) in dumping syndrome. *Dig Dis Sci* 1985; 30: 1145–8.
- 9 Holgate AM, Read NW. Relationship between small bowel transit time and absorption of a solid meal. Influence of metoclopramide, magnesium sulfate, and lactulose. *Dig Dis Sci* 1983; 28: 812–9.
- 10 Hampton SM, Morgan LM, Lawrence N, *et al*. Postprandial hormone and metabolic responses in simulated shift work. *J Endocrinol* 1996; 151: 259–67.
- 11 Bond JH, Levitt MD. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H₂) measurements. *J Lab Clin Med* 1975; 85: 546–55.
- 12 Everson GT, Braverman DZ, Johnson ML, Kern F Jr. A critical evaluation of real-time ultrasonography for the study of gallbladder Volume and contraction. *Gastroenterology* 1980; 79: 40–6.
- 13 Hopman WPM, Brouwer WFM, Rosenbusch G, Jansen JBMJ, Lamers CBHW. A computerized method for rapid quantification of gallbladder volume from real-time sonograms. *Radiology* 1985; 154: 236–7.
- 14 Jansen JBMJ, Lamers CBHW. Radioimmunoassay of cholecystokinin in human tissue and plasma. *Clin Chim Acta* 1983; 131: 305–16.
- 15 Kellow JE, Borody TJ, Phillips SF, Tucker RL, Haddad AC. Human interdigestive motility: variations in patterns from esophagus to colon. *Gastroenterology* 1986; 91: 386–95.
- 16 Izzo AA, Gaginella TS, Capasso F. The osmotic and intrinsic mechanisms of the pharmacological laxative action of oral high doses of magnesium sulphate. Importance of the release of digestive polypeptides and nitric oxide. *Magnesium Res* 1996; 9: 133–8.
- 17 Inoue K, Wiener I, Fagan CJ, Watson LC, Thompson JC. Correlation between gallbladder size and release of cholecystokinin after oral magnesium sulfate in man. *Ann Surg* 1983; 197: 412–5.
- 18 Izzo AA, Gaginella TS, Mascolo N, Capasso F. Nitric oxide as a mediator of the laxative action of magnesium sulphate. *Br J Pharmacol* 1994; 113: 228–32.
- 19 Rodriguez-Membrilla A, Vergara P. Endogenous CCK disrupts the MMC pattern via capsaicin-sensitive vagal afferent fibers in the rat. *Am J Physiol* 1997; 272: G100–5.
- 20 Russo A, Fraser R, Adachi K, Horowitz M, Boeckxstaens G. Evidence that nitric oxide mechanisms regulate small intestinal motility in humans. *Gut* 1999; 44: 72–6. (GENERIC).
- 21 Stewart JJ, Gaginella TS, Olsen WA, Bass P. Inhibitory actions of laxatives on motility and water and electrolyte transport in the gastrointestinal tract. *J Pharmacol Exp Ther* 1975; 192: 458–67.
- 22 Vu MK, Vecht J, Lamers CBHW, Biemond I, Masclee AAM. Activation of 'ileal brake' hormones in dumping syndrome. *Gastroenterology* 1999; 116: A653(Abstract).
- 23 Malagelada JR, Holtermuller KH, McCall JT, Go VLW. Pancreatic, gallbladder, and intestinal responses to intraluminal magnesium salts in man. *Dig Dis Sci* 1978; 23: 481–5.
- 24 Conter RL, Roslyn JJ, Taylor IL. Effects of peptide YY on gallbladder motility. *Am J Physiol* 1987; 252: G736–41.

Chapter 7

ANTRODUODENAL MOTILITY IN CHRONIC PANCREATITIS: Are abnormalities related to exocrine insufficiency?

M.K. Vu¹, J. Vecht¹, E.H. Eddes², I. Biemond¹, C.B.H.W. Lamers¹, A.A.M. Masclee¹

Departments of Gastroenterology-Hepatology¹ and General Surgery²,
Leiden University Medical Center, the Netherlands

Antroduodenal motility in chronic pancreatitis: are abnormalities related to exocrine insufficiency?

M. K. VU,¹ J. VECHT,¹ E. H. EDDES,² I. BIEMOND,¹
C. B. H. W. LAMERS,¹ AND A. A. M. MASCLEE¹

Departments of ¹Gastroenterology-Hepatology and ²General Surgery,
Leiden University Medical Center, 2300 RC Leiden, The Netherlands

Vu, M. K., J. Vecht, E. H. Eddes, I. Biemond, C. B. H. W. Lamers, and A. A. M. Masclee. Antroduodenal motility in chronic pancreatitis: are abnormalities related to exocrine insufficiency? *Am. J. Physiol. Gastrointest. Liver Physiol.* 278: G458–G466, 2000.—In patients with chronic pancreatitis (CP) the relation among exocrine pancreatic secretion, gastrointestinal hormone release, and motility is disturbed. We studied digestive and interdigestive antroduodenal motility and postprandial gut hormone release in 26 patients with CP. Fifteen of these patients had pancreatic insufficiency (PI) established by urinary para-aminobenzoic acid test and fecal fat excretion. Antroduodenal motility was recorded after ingestion of a mixed liquid meal. The effect of pancreatic enzyme supplementation was studied in 8 of the 15 CP patients with PI. The duration of the postprandial antroduodenal motor pattern was significantly ($P < 0.01$) prolonged in CP patients (324 ± 20 min) compared with controls (215 ± 19 min). Antral motility indexes in the first hour after meal ingestion were significantly reduced in CP patients. The interdigestive migrating motor complex cycle length was significantly ($P < 0.01$) shorter in CP patients (90 ± 8 min) compared with controls (129 ± 8 min). These abnormalities were more pronounced in CP patients with exocrine PI. After supplementation of pancreatic enzymes, these alterations in motility reverted toward normal. Digestive and interdigestive antroduodenal motility are abnormal in patients with CP but significantly different from controls only in those with exocrine PI. These abnormalities in antroduodenal motility in CP are related to maldigestion.

pancreatic enzyme supplementation; cholecystokinin; peptide YY

IN THE FASTING STATE gastrointestinal motility is characterized by cyclic reoccurrence of a typical motor pattern, the migrating motor complex (MMC) (14, 38). Interdigestive exocrine pancreatic secretion cycles in close association with the various phases of the MMC in the duodenum (12, 27) but is dissociated from the MMC in chronic pancreatitis (CP) (29). After meal ingestion, gastrointestinal motility is converted to a feeding pattern and exocrine pancreatic secretion increases. The control of interdigestive and digestive motility and pancreatic secretion includes neural and hormonal components, several of which regulate both motility

and pancreatic secretion (35, 37). Recent studies indicate that in CP patients with impaired exocrine function alterations in gastrointestinal hormonal release and motility can be observed. Postprandial release of CCK and pancreatic polypeptide (PP) is reduced in patients with exocrine pancreatic insufficiency (PI) (10, 15), gallbladder contraction is impaired (24), and gastric emptying is accelerated (20). It has been suggested that the pancreas has a role in controlling antroduodenal motility (4, 17, 21–23). In patients with CP and PI interdigestive and digestive motility may be affected, as well as gastrointestinal transit (4, 17, 22).

However, results of studies on antroduodenal motility in patients with CP have been controversial. Both normal and increased interdigestive MMC cycle frequency have been observed (17, 22, 23). Duration of postprandial motility was reduced in one study (17), whereas in another study the postprandial antroduodenal motor pattern in patients with CP was not different from controls (23). These differences in results may be related to the presence of exocrine PI in CP patients.

Therefore, we have investigated digestive and interdigestive antroduodenal motility and release of the gastrointestinal hormones CCK, PP, and peptide YY (PYY) in a large group of CP patients. The patients were divided into groups with and without exocrine PI. To further elucidate the role of exocrine PI and subsequent maldigestion, we also studied the effect of exocrine pancreatic enzyme supplementation on the aforementioned parameters. Results were compared with those obtained in healthy control subjects.

METHODS

Subjects

Two groups of subjects were studied: 26 patients with CP (21 male, 5 female; mean age 47 ± 3 yr) and 15 healthy control subjects (9 male, 6 female; mean age 39 ± 5 yr). None of the patients with CP or control subjects had previously undergone abdominal surgery. The diagnosis of CP had been established in all patients by typical clinical history and characteristic abnormalities on ultrasonography, computed tomography, and endoscopic retrograde cholangiopancreatography. Exocrine pancreatic function was assessed by the indirect para-aminobenzoic acid (PABA) test and fecal fat excretion. Fifteen of twenty-six patients with CP had evidence of impaired exocrine pancreatic function, showing urinary PABA recovery of $<50\%$ and/or fecal fat excretion of >7 g/24 h. These patients were classified as having exocrine PI. Eleven patients with CP had no evidence of exocrine PI

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

(urinary PABA recovery >50% and fecal fat excretion <7 g/24 h). Six patients had had insulin-dependent diabetes mellitus for 5, 6, 7, 9, 10, and 23 yr, respectively. None of them had autonomic neuropathy as assessed by cardiovascular reflex tests described by Ewing and Clarke (6). Patient characteristics are listed in Table 1. Pancreatic enzyme supplementation and other medication possibly influencing antroduodenal motility were discontinued at least 4 days before the study. In eight patients with CP and exocrine PI, the study was repeated with pancreatic enzyme supplementation. Informed consent was obtained from each subject, and the study protocol was approved by the local ethical committee.

Antroduodenal Manometry

Antroduodenal motility was recorded using a multilumen water-perfused polyvinyl catheter (outer diameter 5 mm). The catheter incorporated eight side holes located at 3, 8, 13, 18, 23, 28, 33, and 38 cm from the distal tip. The manometry catheter was passed transnasally into the stomach and from there positioned into duodenum-jejunum under fluoroscopic control. The tip of the catheter was located just distal to the ligament of Treitz so that one or two side hole openings were in the jejunum, three to four side hole openings were in the duodenum, and at least two were in the antrum. When the correct position had been verified, the catheter was taped to the nose. At the end of each experiment, position of the catheter was checked again by fluoroscopy. Each lumen was connected to a pressure transducer and perfused with distilled water by a low-compliance pneumohydraulic perfusion system (Arndorfer Medical Systems) at a rate of 0.5 ml/min. Outputs from pressure transducers were recorded by a polygraph (Synectics Medical, Stockholm, Sweden), displayed on a monitor, and stored on a personal computer for automated and manual analysis.

Table 1. Clinical characteristics of patients with chronic pancreatitis and control subjects

	Chronic Pancreatitis			Controls
	All	With PI	Without PI	
n	26	15	11	15
Age	47 (22–67)	48 (22–67)	45 (31–66)	39 (21–50)
Gender, M/F	21/5	12/3	9/2	9/6
Etiology of CP				
Alcoholic, no. of subjects	16	9	7	
Unknown, no. of subjects	10	6	4	
Exocrine insufficiency				
Urinary PABA recovery <50%	15	15	0	
Fecal fat >7 g/24 h	15	15	0	
Exocrine pancreatic function				
Urinary PABA recovery, %	42 (3–89)	27 (3–44)	56 (54–89)	
Fecal fat, g/24 h	22 (2–95)	36 (8–95)	5 (2–7)	
Endocrine insufficiency				
Impaired glucose tolerance	10	6	4	
Insulin dependent	6	5	1	

Mean (range) parameter values are given; n, no. of subjects. CP, chronic pancreatitis; PI, pancreatic insufficiency; PABA, para-aminobenzoic acid.

Study Design

All subjects presented at our laboratory at 800 AM after an overnight fast. The manometry catheter was positioned as described in *Antroduodenal Motility*, and manometric recording was started. An intravenous cannula was inserted into the antecubital vein of one arm for blood sampling. At 0 min (around 900 AM) the study was started with oral ingestion of 400 ml of a commercially available polymeric liquid meal (Nutrison; Nutricia Zoetermeer) containing 16 g of casein, 48 g of carbohydrates (polysaccharides), and 12 g of saturated and unsaturated triglycerides (400 ml = 1,680 kJ; osmolality 260 mosmol/kg). Antroduodenal motility was recorded for at least 7 h after ingestion of the liquid meal.

To determine the effect of pancreatic enzyme supplementation on antroduodenal motility and gut hormone release, the study was repeated in 8 of 15 CP patients with exocrine PI. Each patient received the same liquid meal together with pancreatic enzymes [Pancrease, 30,000 Federal International Pharmaceutical (FIP) units lipase, 17,400 FIP units amylase, and 1,980 FIP units protease; Janssen-Cilag].

Hormone Assays

Blood samples for measurement of plasma CCK, PP, and PYY were drawn at –15 min, 0 min before meal ingestion, and thereafter at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min. Plasma CCK was measured by a sensitive and specific radioimmunoassay. This antibody binds to all CCK peptides, including sulfated CCK octapeptide, but not with gastrin. The detection limit of the assay is 0.3 pmol/l plasma. The intra-assay variation ranges from 4.6 to 11.5% and the interassay variation from 11.3 to 26.1% (9). Plasma PP concentrations were measured by a sensitive and specific radioimmunoassay as described previously (16). Plasma PYY was measured in our laboratory by a recently developed radioimmunoassay. PYY antiserum was generated in rabbits by intracutaneous injections of synthetic human PYY (Bachem Biochemica). PYY was labeled with ¹²⁵I with chloramine T. The assay is highly specific. There is no cross-reactivity with PP or VIP. The detection limit is 10 pmol/l. Both PYY(1–36) and PYY(3–36) bind to the antibody in dilutions up to 1:250,000.

Analysis of Manometric Data

Motility patterns from antroduodenal manometry were analyzed both visually and by computer. The individual tracings were processed by special software (Polygram, Synectics Medical, Stockholm, Sweden) for adjusting baselines and extracting respiratory artifacts. However, the computer program does not recognize simultaneous pressure events as artifacts. Therefore, remaining artifacts caused by increments in intra-abdominal pressure were identified visually and excluded from analysis. Duodenal phases of the MMC were defined as follows: *phase I*, no more than 2 contractions/10 min for at least 5 min and preceded by *phase III*; *phase II*, irregular contractile activity at a frequency of >2/10 min and amplitude >12 mmHg; *phase III*, regular contractile activity at a frequency of 10–12 contractions/min for at least 2 min. *Phase III* activity had to be propagated over at least 2 recording sites. Antral *phase III* activity was defined as rhythmic contractile activity at maximum frequency (3 contractions/min) for at least 1 min in temporal relationship with duodenal *phase III* activity (14). Duration of the MMC cycle was taken as the interval between the beginning of *phase III* in the duodenum and the beginning of the next *phase III* cycle. Antral or duodenal origin, duration, mean amplitude,

contraction frequency, propagation velocity, and area under the curve of *phase III* of the MMC were measured.

The postprandial period was defined as the time interval between the end of the meal and the occurrence of the first duodenal *phase III* propagated over at least two channels. Only pressure waves with an amplitude ≥ 10 mmHg and a duration ≥ 1.5 s were considered true contractions. The motility indexes (MI) of the postprandial period in antrum and duodenum were calculated as area under the contraction curves (expressed in mmHg·s·h⁻¹).

Data and Statistical Analysis

Integrated incremental CCK, PP, and PYY secretion in response to the meal were determined by calculating the area under the plasma concentration time curve after subtraction of the basal value at 0 min. Possible influences of diabetes mellitus on gastrointestinal motility and secretion were analyzed in two ways: 1) by comparing the results between patients with and without diabetes within the group of CP patients with PI and 2) by comparing the results between CP patients with and without PI after excluding the six patients with insulin-dependent diabetes mellitus. For all parameters, differences between and within groups were analyzed by repeated ANOVA. When this indicated a probability of <0.05 for the null hypothesis, Student-Newman-Keuls analysis was performed to determine which values between or within groups differed significantly. Statistical significance was defined as a P value <0.05 .

RESULTS

Antroduodenal Motility

Postprandial state. The duration of the fed motility pattern was significantly ($P < 0.01$) prolonged in CP patients (324 ± 20 min) compared with control subjects (215 ± 19 min). No significant difference in the duration of the fed motility patterns was found between patients with (345 ± 25 min) and without (294 ± 33 min) PI. The postprandial antral MI during the first hour after the meal was significantly ($P < 0.01$) reduced in the CP patient group compared with the control group (Table 2). Moreover, within the patient group, patients with PI had a significantly ($P < 0.01$) smaller MI compared with CP patients without PI. During the subsequent hourly intervals after the meal, the antral MI was not significantly different between CP patients and control subjects or between CP patients with and without PI (Table 2).

Table 3. Characteristics of interdigestive antroduodenal motility in CP patients and control subjects

	Chronic Pancreatitis			Controls (n = 15)
	All (n = 26)	PI+ (n = 15)	PI- (n = 11)	
MMC duration, min	90 ± 8*	72 ± 11*	104 ± 10	129 ± 8
Phase I, min	24 ± 3	20 ± 5	24 ± 5	22 ± 2
Phase II, min	62 ± 8*	47 ± 9*	77 ± 11	102 ± 9
Phase III, min	4 ± 0.4	5 ± 0.5	3 ± 0.6	5 ± 0.3

Values are means ± SE; n, no. of subjects. MMC, migrating motor complex. * $P < 0.05$ vs. controls.

The postprandial duodenal MI was not different between CP patients and control subjects during the first three subsequent hourly intervals after the meal or during the total fed period (Table 2). No differences were found in duodenal MI between CP patients with and without PI.

Interdigestive state. After transition from a digestive into an interdigestive motility pattern, 21 complete MMC cycles in the patient group and 22 complete MMC cycles in the control subjects were registered. The duration of complete MMC cycles was significantly ($P < 0.01$) reduced in CP patients compared with control subjects. The shorter duration of the MMC cycle in the patient group resulted from a significantly ($P < 0.05$) shorter *phase II* (Table 3). These differences were more pronounced in CP patients with PI compared with those without PI (Table 3). The amplitude of *phase III* in the CP patients (31 ± 3 mmHg) was significantly ($P < 0.01$) lower compared with the control group (39 ± 3 mmHg), but no significant difference was found between patients with and without exocrine PI (29 ± 4 mmHg vs. 32 ± 3 mmHg). Other *phase III* characteristics such as origin, duration, and propagation velocity were not significantly different between patients and controls (data not shown).

Pancreatic Enzyme Supplementation and Antroduodenal Motility

Digestive state. The duration of the fed motility pattern in the eight CP patients with PI was significantly ($P < 0.05$) shorter with enzyme supplementation

Table 2. Postprandial antral and duodenal motility index in 60-min periods and for total fed period after ingestion of a liquid meal in CP patients and control subjects

MI	Chronic Pancreatitis			Controls (n = 15)
	All (n = 26)	PI+ (n = 15)	PI- (n = 11)	
Antrum 0–60 min	825 ± 427*	305 ± 80*†	1,553 ± 1,004	2,473 ± 980
Antrum 60–120 min	1,299 ± 432	1,156 ± 488	1,507 ± 818	2,501 ± 688
Antrum 120–180 min	3,229 ± 1,229	3,327 ± 1,758	3,109 ± 1,795	2,760 ± 1,505
Antrum, total fed period	2,809 ± 702	2,357 ± 781	3,542 ± 1,366	3,468 ± 762
Duodenum 0–60 min	4,298 ± 864	4,001 ± 1,228	4,760 ± 1,180	4,325 ± 956
Duodenum 60–120 min	4,292 ± 613	4,121 ± 834	4,537 ± 943	4,096 ± 1,000
Duodenum 120–180 min	4,133 ± 658	4,145 ± 854	4,061 ± 1,103	4,013 ± 603
Duodenum, total fed period	4,377 ± 965	4,628 ± 1,454	3,968 ± 1,026	4,634 ± 752

Values (in mmHg·s) are means ± SE; n, no. of subjects. MI, motility index; PI+, with PI; PI-, without PI. * $P < 0.05$ vs. controls; † $P < 0.01$ vs. PI-.

Table 4. Postprandial antral and duodenal motility index in 60-min periods and for total fed period after ingestion of a liquid meal in PI patients with and without enzyme supplementation and control subjects

MI	No Enzymes (n = 8)	Enzymes (n = 8)	Controls (n = 15)
Antrum 0–60 min	271 ± 79	1,429 ± 318*	2,473 ± 980
Antrum 60–120 min	1,022 ± 241	1,296 ± 390	2,501 ± 688
Antrum 120–180 min	2,414 ± 643	2,217 ± 935	2,760 ± 1,505
Antrum, total fed period	2,592 ± 389	3,007 ± 1,107	3,468 ± 762
Duodenum 0–60 min	3,160 ± 961	3,584 ± 808	4,325 ± 956
Duodenum 60–120 min	3,738 ± 564	3,570 ± 542	4,096 ± 1,000
Duodenum 120–180 min	4,022 ± 528	4,000 ± 636	4,013 ± 603
Duodenum, total fed period	4,311 ± 542	3,881 ± 935	4,634 ± 752

Values (in mmHg·s) are means ± SE; n, no. of subjects. * $P < 0.05$ vs. no enzymes.

(254 ± 38 min) than without enzyme supplementation (356 ± 43 min). The duration of the fed pattern after enzyme supplementation was not significantly different from that in healthy controls (215 ± 19 min). Pancreatic enzyme supplementation markedly ($P < 0.05$) increased antral MI during the first postprandial hour compared with that without enzyme supplementation (Table 4). During the subsequent hourly intervals after the meal no significant differences were found in antral MI between PI patients with and without enzyme substitution. Furthermore, pancreatic enzyme substitution did not affect duodenal motility index (Table 4).

Interdigestive state. Pancreatic enzyme supplementation significantly ($P < 0.01$) increased the duration of the MMC cycle to values not different from controls (Table 5). This increase in MMC cycle length resulted from a prolonged duration of phase II. No changes were found in phase I or phase III characteristics when pancreatic enzymes were added.

Hormonal Responses

Plasma CCK. Basal plasma CCK levels in patients with CP (1.7 ± 0.2 pmol/l) were not significantly different from control subjects (1.9 ± 0.3 pmol/l; Fig. 1A). In both groups, plasma CCK levels increased significantly over basal levels starting from 15 min after meal ingestion and remained significantly increased until 120 min in the controls and 180 min in the patient group. Plasma CCK secretion during the first postprandial hour was significantly ($P < 0.05$) reduced

Table 5. Characteristics of interdigestive antroduodenal motility in PI patients with and without enzyme supplementation and control subjects

	No Enzymes (n = 8)	Enzymes (n = 8)	Controls (n = 15)
MMC duration, min	89 ± 10	119 ± 13*	129 ± 8
Phase I, min	18 ± 6	17 ± 4	22 ± 2
Phase II, min	66 ± 6	96 ± 11*	102 ± 9
Phase III, min	5 ± 1.0	6 ± 1.0	5 ± 0.3

Values are means ± SE; n, no. of subjects. * $P < 0.01$ vs. no enzymes.

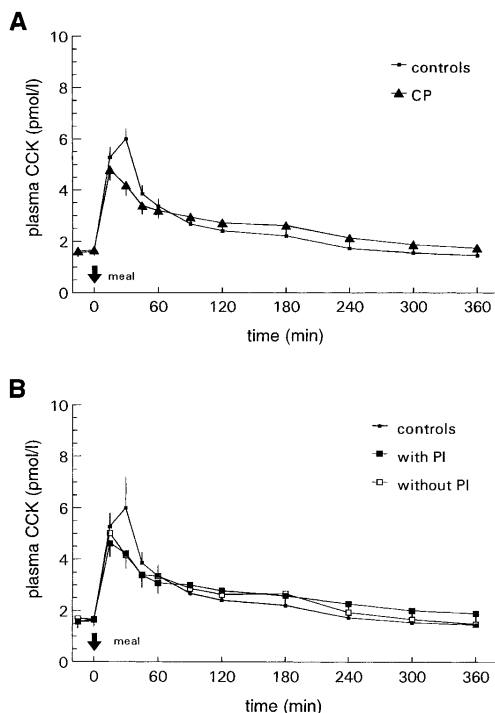


Fig. 1. A: fasting and postprandial plasma CCK levels (means ± SE) in patients with chronic pancreatitis (CP; $n = 26$) and controls ($n = 15$). B: fasting and postprandial plasma CCK levels in CP patients with exocrine pancreatic insufficiency (PI) ($n = 15$), CP patients without exocrine PI ($n = 11$), and controls ($n = 15$).

in the CP patients (118 ± 14 pmol·l⁻¹·60 min⁻¹) compared with the control group (168 ± 20 pmol·l⁻¹·60 min⁻¹). No difference in CCK secretion was found between patients with and without PI (Fig. 1B).

Plasma PP. Basal plasma PP levels in the CP patients (37 ± 7 pmol/l) were not significantly different from controls (41 ± 5 pmol/l; Fig. 2A). After meal ingestion plasma PP levels increased significantly ($P < 0.005$ – $P < 0.05$) in both groups and remained significantly elevated for 180 min. Postprandial PP secretion during the first hour was significantly ($P < 0.01$) reduced in CP patients ($2,220 \pm 397$ pmol·l⁻¹·60 min⁻¹) compared with controls ($3,198 \pm 709$ pmol·l⁻¹·60 min⁻¹). Compared with that in CP patients without PI, plasma PP secretion during the first postprandial hour was significantly ($P < 0.01$) reduced in CP patients with PI ($2,993 \pm 756$ vs. $1,640 \pm 345$ pmol·l⁻¹·60 min⁻¹; Fig. 2B).

Plasma PYY. Basal plasma PYY levels were not significantly different between CP patients (20 ± 2 pmol/l) compared with controls (18 ± 1 pmol/l). After meal ingestion plasma PYY levels increased significantly

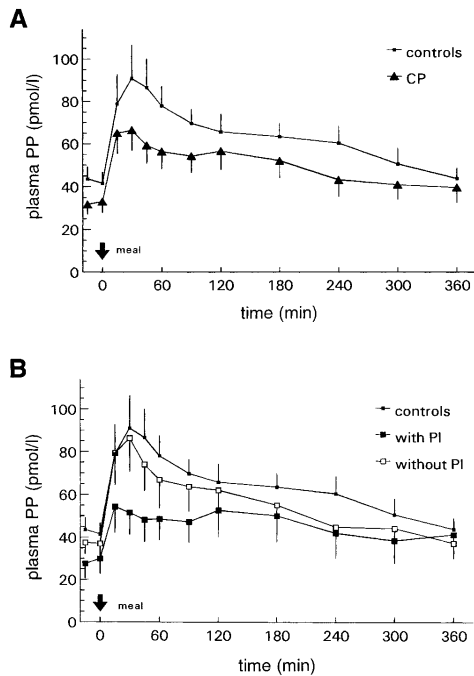


Fig. 2. A: fasting and postprandial plasma PP levels (means $\pm$ SE) in patients with CP ($n = 26$) and controls ($n = 15$). B: fasting and postprandial plasma PP levels in CP patients with exocrine PI ($n = 15$), CP patients without exocrine PI ($n = 11$), and controls ($n = 15$).

cantly ($P < 0.01$) over basal levels starting from 15 min until 240 min in controls and until 300 min in the CP patients (Fig. 3A). Plasma PYY levels in CP patients were significantly ($P < 0.05$) increased over controls from 30 to 180 min after meal ingestion. Integrated postprandial PYY secretion in CP patients was also significantly ($P < 0.05$) higher compared with controls ($2,840 \pm 470$ vs. $1,380 \pm 340$ $\text{pmol} \cdot \text{l}^{-1} \cdot 360 \text{ min}^{-1}$). When analyzed separately according to exocrine function, only in the patients with PI were plasma PYY levels significantly increased over those in controls. This was true for basal plasma PYY levels and those from 15 to 240 min after meal ingestion (Fig. 3B). Plasma PYY levels were higher in CP patients with exocrine PI compared with patients without PI, although this difference was not statistically significant.

Pancreatic Enzyme Supplementation and Hormone Responses

Plasma CCK. Pancreatic enzyme supplementation significantly ($P < 0.05$) increased postprandial plasma CCK levels in CP patients over those without enzyme supplementation (Fig. 4). After enzyme supplementation integrated plasma CCK secretion during the first postprandial hour (148 ± 16 $\text{pmol} \cdot \text{l}^{-1} \cdot 60 \text{ min}^{-1}$) was

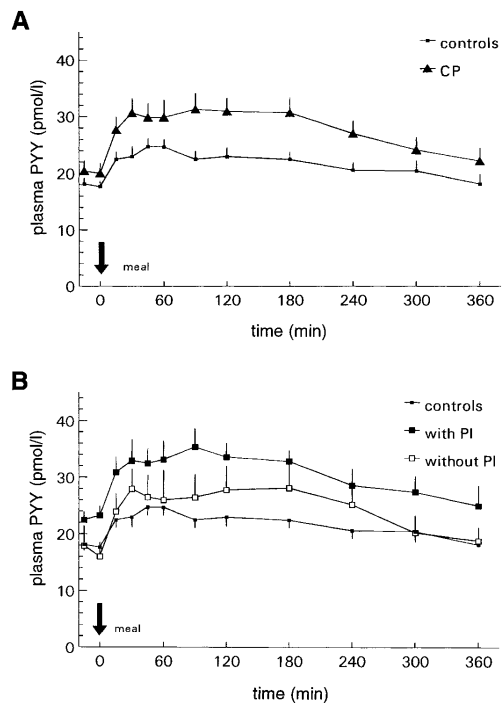


Fig. 3. A: fasting and postprandial plasma peptide YY (PYY) levels (means $\pm$ SE) in patients with CP ($n = 26$) and controls ($n = 15$). B: fasting and postprandial plasma PYY levels in CP patients with exocrine PI ($n = 15$), CP patients without exocrine PI ($n = 11$), and controls ($n = 15$).

not significantly different from controls (168 ± 20 $\text{pmol} \cdot \text{l}^{-1} \cdot 60 \text{ min}^{-1}$) but was significantly ($P < 0.05$) higher compared with those without enzyme supplementation (77 ± 17 $\text{pmol} \cdot \text{l}^{-1} \cdot 60 \text{ min}^{-1}$).

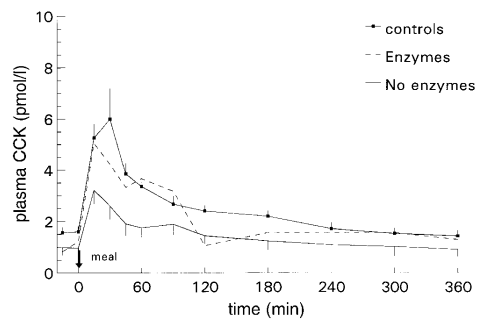


Fig. 4. Fasting and postprandial plasma CCK levels (means $\pm$ SE) in 8 PI patients with and without enzyme supplementation and controls ($n = 15$).

Plasma PP. Supplementation of pancreatic enzymes did not alter postprandial PP release. The integrated plasma PP secretions during the first postprandial hour were $1,205 \pm 515$ and $1,242 \pm 335$ pmol $\cdot$ l $^{-1}$ $\cdot$ 60 min $^{-1}$, respectively, with and without addition of pancreatic enzymes.

Plasma PYY. Postprandial plasma PYY decreased significantly ($P < 0.05$) to levels comparable with controls after supplementation of pancreatic enzymes (Fig. 5). Integrated postprandial PYY release after enzyme supplementation (839 ± 127 pmol $\cdot$ l $^{-1}$ $\cdot$ 360 min $^{-1}$) was significantly ($P < 0.05$) lower compared with that without enzyme supplementation ($2,683 \pm 315$ pmol $\cdot$ l $^{-1}$ $\cdot$ 360 min $^{-1}$) and was not significantly different from healthy controls ($1,380 \pm 340$ pmol $\cdot$ l $^{-1}$ $\cdot$ 360 min $^{-1}$).

Role of Endocrine Insufficiency

Of the 15 CP patients with exocrine PI, 5 patients had insulin-dependent diabetes mellitus. Within this group, the mean duration of the fed pattern was not significantly different between CP patients with exocrine PI either with (361 ± 37 min) or without (345 ± 32 min) diabetes compared with controls (215 ± 19 min). Antral hypomotility during the first hour of the fed period was present ($P \leq 0.05$) in both groups compared with controls ($2,473 \pm 980$ mmHg $\cdot$ s $\cdot$ h $^{-1}$), but no significant difference was found between CP patients with (368 ± 165 mmHg $\cdot$ s $\cdot$ h $^{-1}$) and without (257 ± 92 mmHg $\cdot$ s $\cdot$ h $^{-1}$) diabetes mellitus. In addition, no significant differences in the duration of MMC cycle phases I, II, and III were found between patients with exocrine PI with and without diabetes (data not shown). When the results were analyzed between CP patients with and without exocrine PI after excluding six CP patients with diabetes mellitus from the study, differences in gastrointestinal motility and secretion still existed and remained significant between CP patients with and without exocrine PI (Table 6).

DISCUSSION

Our results demonstrate that antroduodenal motility is altered in patients with CP. The duration of the

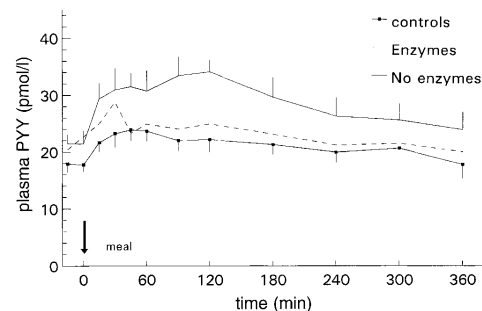


Fig. 5. Fasting and postprandial plasma PYY levels (means $\pm$ SE) in 8 PI patients with and without enzyme supplementation and controls ($n = 15$).

Table 6. Parameters that remain different between CP patients with and without PI after excluding 6 patients with insulin-dependent diabetes mellitus

	Chronic Pancreatitis		Controls ($n = 15$)
	PI+ ($n = 10$)	PI- ($n = 10$)	
Duration fed pattern, min	$331 \pm 35^*$	$326 \pm 34^*$	215 ± 19
MI antrum 0-60 min, mmHg $\cdot$ s	$295 \pm 105^{*\dagger}$	$1,671 \pm 1,115$	$2,473 \pm 980$
MMC duration, min	$77 \pm 16^*$	106 ± 12	129 ± 8
Plasma PP, pmol $\cdot$ l $^{-1}$ $\cdot$ 60 min $^{-1}$	$1,788 \pm 222^{*\dagger}$	$2,476 \pm 422^*$	$3,918 \pm 709$
Plasma CCK, pmol $\cdot$ l $^{-1}$ $\cdot$ 60 min $^{-1}$	$120 \pm 17^*$	133 ± 19	168 ± 20
Plasma PYY, pmol $\cdot$ l $^{-1}$ $\cdot$ 360 min $^{-1}$	$2,915 \pm 154^*$	$2,218 \pm 479$	$1,380 \pm 340$

Values are means $\pm$ SE; n , no. of subjects. PP, pancreatic polypeptide; PYY, peptide YY. $^*P < 0.05$ vs. controls; $^{\dagger}P < 0.05$ vs. PI-.

postprandial motor pattern was significantly prolonged and the interdigestive motility pattern was characterized by shorter duration of the MMC cycle because of a reduction in duration of phase II. These abnormalities were more pronounced in CP patients with PI. After addition of pancreatic enzymes, these alterations in antroduodenal motility reverted toward normal.

Recently, several studies were published about antroduodenal motility in patients with chronic pancreatitis. Malfertheiner et al. (22, 23) and Pieramico et al. (29) did not observe any changes in interdigestive motility in patients with CP vs. controls, whereas Layer et al. (17) found that the duration of the interdigestive motor cycle was significantly reduced. We were able to confirm the results of Layer et al. by finding a shorter duration of the MMC cycle with shorter phase II in CP patients. Differences in results between the studies of Layer et al. (17) and ours compared with those of Malfertheiner (22, 23) and Pieramico (29) may be related to several factors. First, the degree of exocrine PI in CP patients may have an important role. Whereas Malfertheiner et al. and Pieramico et al. studied 15 CP patients without steatorrhea, Layer et al. investigated patients with severely impaired exocrine function. In the present study, the duration of the MMC cycle was shorter compared with controls only in patients with and not in those without exocrine PI. Several explanations could be considered. This finding could be caused by autonomic neuropathy secondary to diabetes mellitus. However, none of the six patients with insulin-dependent diabetes mellitus had evidence of autonomic neuropathy and similar motility results were obtained between patients with and without diabetes. Therefore, the possibility that autonomic neuropathy affects antroduodenal motility seems unlikely. Furthermore, Samson and Smout (33) showed that MMC cycle length is actually prolonged, not shortened, in diabetic patients with autonomic neuropathy. Second, the presence of endocrine PI and subsequent hyperglycemia may be a confounding factor because it has been demonstrated that hyperglycemia shortens MMC cycle length by

shortening the duration of *phase II* (7). During the experiments plasma glucose levels were kept in the euglycemic range of 4–8 mmol/l. It is more likely that exocrine PI and subsequent malabsorption activated “ileal brake” mechanisms that facilitate the occurrence of *phase III* and thereby shorten MMC cycle length. Normalization of the MMC cycle length after pancreatic enzyme supplementation further supports this idea.

The occurrence of antroduodenal *phase III* activity is associated with peaks in plasma motilin concentrations but is also dependent on vagal cholinergic neural input (11). Pieramico et al. (29) found a dissociation between interdigestive antroduodenal motility and cyclic exocrine pancreatic enzyme output and PP secretion. It is not known whether cyclic fluctuations in plasma motilin concentrations occur at higher frequency in patients with PI. Output of pancreaticobiliary juice into the duodenum releases plasma motilin (28). Although pancreatic enzyme output to the duodenum is reduced in PI, this does not affect plasma motilin concentrations (22). In dogs after total pancreatectomy, plasma motilin concentrations remain unchanged and continue to cycle in association with the duodenal MMC (21). However, it should be noted that in dogs total pancreatectomy has little effect on cycling and characteristics of the MMC. Apart from motilin, plasma PP concentrations cycle synchronously with interdigestive antroduodenal motor activity (27). Interdigestive PP release is already impaired in early stages of CP (30). PP is known to inhibit pancreatic secretion and gallbladder motility but does not appear to have a role in the regulation of interdigestive motility (21).

The duration of the fed pattern was significantly prolonged in patients with CP. When analyzed separately, the difference compared with controls was significant only in CP patients with and not in those without exocrine PI. This finding is not in line with a recent study by Layer et al. (17), who reported that the duration of the fed pattern was significantly shorter in CP patients compared with controls. This discrepancy could be explained by differences in the degree of exocrine PI and subsequent malabsorption. In cases of severe exocrine PI the amount of unabsorbed nutrients in the distal gut will be higher compared with mild exocrine PI, resulting in different effects on gastrointestinal motility and secretion. Layer et al. (19) showed that intraileal infusion with nutrients at a rate of 4 kcal/min converted the fed motility to the interdigestive motility pattern. On the other hand, Keller et al. (13) suggested a positive correlation between the duration of the fed pattern and relative increase in ileal nutrient concentration after ingestion of a semiliquid meal. Apart from the duration, differences in postprandial motor pattern were also observed. In the CP patients with PI the antral motility index in the first postprandial hour was significantly reduced. As a consequence of antral hypomotility gastric emptying of nutrients may be delayed. Recently, Layer et al. (17) found that in the late postprandial phase gastric emptying was accelerated but in the early postprandial phase

gastric emptying in patients with PI was delayed compared with controls. Our results of an initial postprandial antral hypomotility are in line with the observed delay in gastric emptying in the early postprandial phase (17).

The differences in antroduodenal interdigestive and digestive motility pattern that we found between patients with and without exocrine PI suggest that the observed abnormalities are related to exocrine PI and subsequent maldigestion but not to CP per se. The observed changes in the duration of the fed pattern, MMC cycle, and *phase II* toward normal with the addition of pancreatic enzymes support this concept. Recently, Bassotti et al. (5) reported abnormalities in interdigestive antroduodenal pattern in adult patients with untreated celiac sprue similar to those we have observed in CP patients with PI. Combining the results of these studies, it is tempting to relate the abnormalities in antroduodenal motility to intraluminal conditions rather than to the disease per se.

Ingestion of the liquid meal induced a rapid increase in plasma CCK levels both in patients and controls. CCK may be involved in the conversion of a fasted into a fed antroduodenal motor pattern (25, 35, 37). Postprandial CCK release is impaired in CP patients with PI (10, 24). In the present study, integrated plasma CCK secretion was significantly reduced in CP patients in the first postprandial hour. Maldigestion of triglycerides and proteins as a result of exocrine PI could be responsible for this finding. Hildebrand et al. (8) showed that an adequate digestion of triglycerides by pancreatic lipase is necessary for release of CCK in response to food, particularly during the immediate postprandial phase. In support of this concept and in line with earlier studies (10, 24) we have found that pancreatic enzyme supplementation increased postprandial plasma CCK levels toward control values. Postprandial CCK levels remained elevated over basal for a longer period in CP patients than in controls, possibly contributing to prolonged duration of the fed motor pattern. Our findings contrast with those of others who found a significantly shorter duration of the fed motility pattern in CP (17). These differences are not easily explained and may have been influenced by patient characteristics, degree of exocrine PI, meal composition (higher fat and caloric content in our study), CCK secretion, or activation of the ileal brake.

In contrast to the proximal gut hormone CCK, basal and postprandial plasma levels of the distal gut hormone PYY were significantly increased in CP patients with exocrine PI. PYY is found in highest concentrations in the mucosa of the distal gut (2) and is considered one of the mediators of the so-called ileal brake (18, 31). In the present study, PYY was chosen as a marker of the ileal brake because there is substantial evidence suggesting that plasma PYY levels correlate with ileal fat-induced delayed gastric emptying (31), prolonged small intestinal transit, and inhibition of small intestinal motility (32, 36). In humans, infusion of PYY delays gastric emptying and small intestinal transit in a dose-dependent manner (34). Elevated

plasma PYY levels have been found in diseases associated with malabsorption such as celiac sprue, cystic • brosis, and dumping syndrome (1, 3, 26). These • ndings support the idea that alterations in plasma PYY secretion in PI patients result from malabsorption. The presence of undigested and unabsorbed nutrients in the distal gut activates the ileal brake with concomitant PYY release. This results in feedback regulation of proximal gut motor function such as prolongation of the fed pattern to optimize nutrient uptake and absorption. Normalization of postprandial plasma PYY secretion and duration of the fed pattern after pancreatic enzyme supplementation should be considered as evidence supporting this concept.

It is concluded that in patients with CP and exocrine PI, but not in those with normal exocrine function, 1) duration of postprandial antroduodenal motility is significantly prolonged and early postprandial antral motility is significantly reduced; 2) interdigestive MMC cycle length is significantly reduced because of shortening of phase II; 3) endogenous secretion of CCK and PP is decreased, whereas PYY secretion is increased; and 4) alterations in antroduodenal motility and hormone responses in CP patients are related to intraluminal maldigestion and malabsorption and revert toward normal with enzyme supplementation.

Address for reprint requests and other correspondence: A. A. M. Masclee, Dept. of Gastroenterology-Hepatology, Leiden Univ. Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands (E-mail: a.a.m.masclee@lumc.nl).

Received 7 April 1999; accepted in • nal form 5 November 1999.

REFERENCES

- Adrian, T. E., A. P. Savage, A. J. Bacarese-Hamilton, K. Wolfe, H. S. Besterman, and S. R. Bloom. Peptide YY abnormalities in gastrointestinal diseases. *Gastroenterology* 90: 379–384, 1986.
- Adrian, T. E., G. L. Ferri, A. J. Bacarese-Hamilton, H. S. Fuessl, J. M. Polak, and S. R. Bloom. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 89: 1070–1077, 1985.
- Adrian, T. E., R. G. Long, H. S. Fuessl, and S. R. Bloom. Plasma peptide YY (PYY) in dumping syndrome. *Dig. Dis. Sci.* 30: 1145–1148, 1985.
- Armbrecht, U., J. Svanvik, and R. Stockbr•gger. Enzyme substitution in chronic pancreatitis: effects on clinical and functional parameters and on the hydrogen (H₂) breath test. *Scand. J. Gastroenterol.* 21: 55–59, 1986.
- Bassotti, G., G. Castellucci, C. Betti, C. Fusaro, M. L. Cavalletti, A. Bertotto, F. Spinozzi, A. Morelli, and M. A. Pelli. Abnormal gastrointestinal motility in patients with celiac sprue. *Dig. Dis. Sci.* 39: 1947–1954, 1994.
- Ewing, D. J., and B. F. Clarke. Autonomic neuropathy: its diagnosis and prognosis. *Clin. Endocrinol. Metab.* 15: 855–888, 1986.
- Gielkens, H. A. J., M. Verkijk, M. Fr•lich, C. B. H. W. Lamers, and A. A. M. Masclee. Is the effect of acute hyperglycemia on interdigestive antroduodenal motility and small bowel transit time mediated by insulin? *Eur. J. Clin. Invest.* 27: 703–710, 1997.
- Hildebrand, P., C. Petrig, B. Burckhardt, S. Ketterer, H. Lengsfeld, A. Fleury, P. Hadvary, and C. Beglinger. Hydrolysis of dietary fat by pancreatic lipase stimulates cholecystokinin release. *Gastroenterology* 114: 123–129, 1998.
- Jansen, J. B. M. J., and C. B. H. W. Lamers. Radioimmunoassay of cholecystokinin in human tissue and plasma. *Clin. Chim. Acta* 131: 305–316, 1983.
- Jansen, J. B. M. J., M. C. W. Jebbink, H. J. A. Mulders, and C. B. H. W. Lamers. Effect of pancreatic enzyme supplementation on postprandial plasma cholecystokinin secretion in patients with pancreatic insufficiency. *Regul. Pept.* 25: 333–342, 1989.
- Janssens, J., J. Hellemaans, T. E. Adrian, S. R. Bloom, T. L. Peeters, N. Christo•des, and G. R. Vantrappen. Pancreatic polypeptide is not involved in the regulation of the migrating motor complex in man. *Regul. Pept.* 3: 41–49, 1982.
- Keane, F. B., E. P. DiMagno, R. R. Dozois, and V. L. W. Go. Relationship among canine interdigestive exocrine pancreatic and biliary •ow, duodenal motor activity, plasma pancreatic polypeptide and motilin. *Gastroenterology* 78: 310–316, 1980.
- Keller, J., M. Runzi, H. Goebell, and P. Layer. Duodenal and ileal nutrient deliveries regulate human intestinal motor and pancreatic responses to a meal. *Am. J. Physiol. Gastrointest. Liver Physiol.* 272: G632–G637, 1997.
- Kellow, J. E., J. F. Borody, S. F. Phillips, R. L. Tucker, and A. C. Haddad. Human interdigestive motility: variations in patterns from esophagus to colon. *Gastroenterology* 91: 386–395, 1986.
- Lamers, C. B. H. W., C. M. Diemel, and J. B. M. J. Jansen. Comparative study of plasma pancreatic polypeptide responses to food, secretin and bombesin in normal subjects and in patients with chronic pancreatitis. *Dig. Dis. Sci.* 29: 102–108, 1984.
- Lamers, C. B. H. W., J. M. Diemel, E. van Leer, R. van Leusen, and J. Peetoom. Mechanisms of elevated serum pancreatic polypeptide concentrations in chronic renal failure. *J. Clin. Endocrinol. Metab.* 55: 922–926, 1982.
- Layer, P., M. R. Van der Ohe, J. J. Holst, J. B. M. J. Janssens, D. Grandt, G. Holtmann, and H. Goebell. Altered postprandial motility in chronic pancreatitis: role of malabsorption. *Gastroenterology* 112: 1624–1634, 1997.
- Layer, P., T. Schlesinger, G. Gr•ger, and H. Goebell. Modulation of human periodic interdigestive gastrointestinal motor function and pancreatic function by the ileum. *Pancreas* 8: 426–432, 1993.
- Layer, P., S. Peschel, T. Schlesinger, and H. Goebell. Human pancreatic secretion and intestinal motility: effects of ileal nutrient perfusion. *Am. J. Physiol. Gastrointest. Liver Physiol.* 258: G196–G201, 1990.
- Long, W. B., and J. B. Weiss. Rapid gastric emptying of fatty meals in pancreatic insufficiency. *Gastroenterology* 67: 920–925, 1974.
- Malfertheiner, P., M. G. Sarr, and E. P. DiMagno. Role of the pancreas in the control of interdigestive gastrointestinal motility. *Gastroenterology* 96: 200–205, 1989.
- Malfertheiner, P., O. Pieramico, D. K. Nelson, H. Friess, M. B•chler, R. Lorch, and H. Ditschuneit. Does pancreatic disease influence gastrointestinal motility? (Abstract). *J. Gastrointest. Motil.* 2: 151, 1990.
- Malfertheiner, P., O. Pieramico, M. B•chler, D. K. Nelson, and H. Ditschuneit. Gastrointestinal motility in chronic pancreatitis. In: *Chronic Pancreatitis*, edited by H. G. Beger, M. B•chler, H. Ditschuneit, and P. Malfertheiner. Berlin: Springer, 1990, p. 232–234.
- Masclee, A. A. M., J. B. M. J. Jansen, F. H. M. Corstens, and C. B. H. W. Lamers. Reversible gallbladder dysfunction in severe pancreatic insufficiency. *Gut* 30: 866–872, 1989.
- Masclee, A. A. M., M. Beeren, L. C. Rovati, and C. B. H. W. Lamers. Effect of cholecystokinin on small intestinal motility and transit time in humans (Abstract). *Gastroenterology* 106: A537, 1994.
- Murphy, M. S., A. L. Brunetto, A. D. J. Pearson, M. A. Ghatei, R. Nelson, R. J. Eastham, S. R. Bloom, and A. Aynsley Green. Gut hormones and gastrointestinal motility in children with cystic • brosis. *Dig. Dis. Sci.* 37: 187–192, 1992.
- Owyang, C., S. R. Achem-Karam, and A. I. Vinik. Pancreatic polypeptide and intestinal migrating motor complex in humans: effect of pancreatobiliary secretion. *Gastroenterology* 84: 10–17, 1983.
- Peeters, T. L., G. Vantrappen, and J. Janssens. Fasting plasma motilin levels are related to the interdigestive motor complex. *Gastroenterology* 79: 716–719, 1980.

29. **Pieramico, O., J. E. Dominguez-Muñoz, D. K. Nelson, W. Böck, M. Büchler, and P. Malfertheiner.** Interdigestive cyclic in chronic pancreatitis: altered coordination among pancreatic secretion, motility and hormones. *Gastroenterology* 109: 224–230, 1995.
30. **Pieramico, O., K. Nelson, B. Glasbrenner, and P. Malfertheiner.** Impaired interdigestive pancreatic polypeptide release: early hormonal disorder in chronic pancreatitis? *Dig. Dis. Sci.* 39: 69–74, 1994.
31. **Pironi, L., V. Stanghellini, M. Miglioli, R. Corinaldesi, R. De Giorgio, E. Ruggeri, G. Tosetti, G. Poggioli, A. M. M. Labate, N. Monetti, G. Gozetti, L. Barbara, and V. L. W. Go.** Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma level of peptide YY. *Gastroenterology* 105: 733–739, 1993.
32. **Read, N. W., A. MacFarlane, and R. Kinsman.** Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon in man. *Gastroenterology* 86: 274–280, 1984.
33. **Samsom, M., and A. J. Smout.** Abnormal gastric and small intestinal motor function in diabetes mellitus. *Dig. Dis.* 15: 263–274, 1997.
34. **Savage, A. P., T. E. Adrian, G. Carolan, V. K. Chatterjee, and S. R. Bloom.** Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut* 28: 166–170, 1987.
35. **Schmidt, W. E., W. Creutzfeldt, A. Schleser, A. R. Choudhury, R. Nustede, M. Höcker, R. Nitsche, H. Sostamann, L. C. Rovati, and U. R. Fölsch.** Role of CCK in regulation of pancreaticobiliary functions and GI motility in humans: effects of loxiglumide. *Am. J. Physiol. Gastrointest. Liver Physiol.* 260: G197–G206, 1991.
36. **Spiller, R. C., I. F. Trotman, T. E. Adrian, S. R. Bloom, J. J. Misiewicz, and D. B. A. Silk.** Further characterisation of the ileal brake reflex in man: effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY. *Gut* 29: 1042–1051, 1988.
37. **Thor, P., J. Laskiewicz, P. Konturek, and S. J. Konturek.** Cholecystokinin in the regulation of intestinal motility and pancreatic secretion in dogs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 255: G498–G504, 1988.
38. **Vantrappen, G., J. Janssens, J. Hellemans, and Y. Goos.** The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine. *J. Clin. Invest.* 59: 1158–1166, 1977.



Chapter 8

GASTROINTESTINAL MOTILITY AND PEPTIDE SECRETION IN SYSTEMIC SCLEROSIS

M.K. Vu¹, J. M. van Laar², J.J. Haans¹, I. Biemond¹, A.A.M. Masclee¹

Departments of Gastroenterology-Hepatology¹ and Rheumatology², Leiden
University Medical Center, the Netherlands

ABSTRACT

Gastrointestinal (GI) symptoms are common in systemic sclerosis (SSc). It is not known whether GI involvement differs between limited SSc and diffuse SSc. The aim of the study was to evaluate GI motility and gut hormone secretion in SSc patients of both subtypes. Motility of the oesophageal, antrum and proximal small intestine and secretion of cholecystokinin (CCK), motilin and peptide YY (PYY) were studied in 23 patients with SSc (limited form N=11, diffuse form N=12). Fifteen healthy subjects served as controls. *Results:* All SSc patients had prolonged duration of the postprandial motor pattern 322 ± 22 min vs 215 ± 19 min in controls ($p < 0.01$). Postprandial antral and duodenal motility indices (MI) were significantly ($p < 0.05$) reduced in the SSc patient group compared to controls (1203 ± 225 vs 3468 ± 762 mmHg*sec per hour and 2195 ± 305 vs 4634 ± 752 mmHg*sec per hour respectively). Duodenal MI was significantly reduced in the limited subtype compared to the diffuse subtype (1406 ± 280 vs 2591 ± 480 mmHg*sec per hour). Interdigestive motility represented by migrating motor cycles was also reduced in SSc patients. Esophageal motility was affected in all SSc patients. In SSc patients, postprandial plasma CCK was significantly ($p < 0.05$) reduced while motilin and PYY secretion was significantly ($p < 0.05$) increased compared to controls. *Conclusions:* Whereas oesophageal and gastric motility is affected to a similar extent in the limited and diffuse SSc, alterations in intestinal motility are more pronounced in patients with limited SSc. Qualitatively, intestinal motility in the limited type is characterised by myopathic changes and in the diffuse type neuropathic changes.

INTRODUCTION

Systemic sclerosis (SSc) is a multisystem disorder characterized by excessive deposition of collagen and other matrix elements in skin and frequently also in tissue of other organs. Two main subsets of scleroderma have been identified: the limited and diffuse cutaneous form of SSc. Limited cutaneous SSc (lSSc) is characterised by skin involvement limited to the face, hand, forearms and feet and includes the CREST variant (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, teleangiectasia). Diffuse cutaneous SSc (dSSc) is characterised by diffuse skin involvement including the trunk.

Gastrointestinal (GI) tract involvement is common in SSc and frequently gives rise to intestinal symptoms. Esophageal motor abnormalities leading to dysphagia and reflux esophagitis are observed in up to 90% of patients with systemic sclerosis (1-3). Involvement of the stomach and small intestine is less common but abdominal symptoms are reported by 25%-50% of SSc patients (3-6). Not only gastrointestinal motor function, secretory function including gut peptide release may also be affected in SSc (7,8). It has been assumed that GI tract involvement occurs late in the course of the disease of patients with limited SSc while in the diffuse cutaneous form visceral involvement occurs at an early stage. However, up to now studies have not dealt with a comparison of subsets of SSc patients with respect to GI motility.

The aim of the present study was to investigate antroduodenojejunal motility and proximal and distal gut peptide release in a group of patients with SSc and compare data from patients with limited and diffuse cutaneous form of scleroderma and healthy controls.

SUBJECTS

Two groups of subjects were studied: 23 patients with SSc (eight males, 15 females; mean age 51 ± 4 yr) and 15 healthy control subjects (nine males, six females; mean age 39 ± 5 yr). Twelve out of 23 patients had diffuse cutaneous SSc and eleven patients suffered from the limited type of systemic sclerosis. None of the patients or controls had taken any kind of proton pump inhibitors, motility modulating drugs or antacids up to three days prior to the investigation. Patient characteristics are listed in Table 1. Disease severity score was calculated as described previously (9)

METHODS

Oesophageal manometry

Oesophageal body motility and lower esophageal sphincter pressure (LESP) were recorded by perfusion manometry with a small polyvinyl multi-lumen composite side-hole catheter using a low-compliance capillary tube perfusion pump (Arndorfer Medical Specialist, Greendale, WI). A stepwise pull-through technique was used to record end- expiratory LESP. Intraluminal esophageal pressures were recorded at 5, 10, 15 and 20 cm above the upper margin of the lower esophageal sphincter (LES).

Antroduodenojejunal manometry

Antroduodenojejunal (ADJ) motility was recorded using a multilumen water perfused polyvinyl catheter (outer diameter 5 mm). The catheter incorporated eight side holes located at 3, 8, 13, 18, 23, 28, 38 and 43 cm from the distal tip. The manometry catheter was passed transnasally into the stomach and from

there positioned into duodenum-jejunum under fluoroscopic control. The tip of the catheter was located 5-10 cm distal to the ligament of Treitz so that one or two side hole openings were in the jejunum, three to four side hole openings were in the duodenum and at least two in the antrum. When the correct position had been verified the catheter was taped to the nose. At the end of each experiment position of the catheter was checked again by fluoroscopy. Each lumen was connected to a pressure transducer and perfused with distilled water by a low compliance pneumohydraulic perfusion system (Arndorfer Medical Systems) at a rate of 0.5 ml/min. Outputs from pressure transducers were recorded by a polygraph (Synectics Medical, Skovlunde, Denmark), displayed on a monitor, stored on a personal computer for automated and manual analysis.

Study design

All subjects presented at our laboratory at 08.00 AM after an overnight fast. The manometry catheter was positioned as described above and manometric recording was started. An intravenous cannula was inserted into the antecubital vein of one arm for blood sampling. At time 0 min (around 09.00 AM) the study was started with oral ingestion of 400 ml of a commercially available polymeric liquid meal (Nutrison; Nutricia Zoetermeer, The Netherlands) containing 16 g long chain triglyceride (LCT) fat, 48 g lactose-free carbohydrates and 16 g protein per 400 ml (400 ml = 1680 kJ; osmolality 260 mOsm). Antro-duodeno-jejunal motility was recorded for at least 6 hr after ingestion of the liquid meal.

Hormone assays

Blood samples for measurement of plasma cholecystokinin (CCK) and peptide YY (PYY) were drawn at time -15, and 0 min before meal ingestion and at

regular intervals thereafter at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min. Plasma CCK was measured by a sensitive and specific radioimmunoassay (10). The detection limit of the assay is 0.1 pM plasma. Plasma PYY was measured by radioimmunoassay. PYY antiserum was generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM AG, Bubendorf, Switzerland). PYY was labelled with ¹²⁵Iodine using chloramine T. There is no cross-reactivity with pancreatic polypeptide (PP) or vasoactive intestinal peptide (VIP). The detection limit is 10 pM plasma. Both PYY (1-36) and PYY (3-36) bind to the antibody in dilutions up to 25000 (11). Plasma motilin was measured by a specific radioimmunoassay using ¹²⁵I-labelled motilin and rabbit antiserum to highly purified porcine motilin (both: Euro-diagnostica AB, Malmo, Sweden). The antiserum was directed towards the middle portion of the motilin molecule and did not show any-reactivity with gastrin, human synthetic secretin (Sigma, St. Louis, MO, USA), CCK, vasoactive intestinal peptide, gastric inhibitory polypeptide, neuropeptide Y or PYY. ¹²⁵I-motilin binds in a reverse proportion to the concentration of motilin in standards and samples. The sensitivity of the radioimmunoassay is 10 pM of plasma (12).

Analysis of manometric data

Motility patterns from antroduodenojejunal manometry were analyzed both visually and by computer. The individual tracings were processed by special software (Polygram^R, Syntectics Medical, Skovlunde, Denmark) for adjusting baselines and extracting respiratory artifacts. Artifacts due to increments in intra-abdominal pressure were identified visually and excluded from analysis. Duodenal phases of the motor migrating complex (MMC) were defined as

follows: phase I, no more than 1 contractions per 5 min and preceded by phase III; phase II: irregular contractile activity at a frequency of more than 1 per 5 min and amplitude above 12 mmHg; phase III: regular contractile activity at a frequency of 10-12 contractions per min for at least 2 min. Phase III activity had to be propagated over at least 2 recording sites. Antral phase III activity was defined as rhythmic contractile activity at maximum frequency (3 contractions/min) for at least 1 min in temporal relationship with duodenal phase III activity (13). Duration of the MMC cycle was taken as the interval between the beginning of phase III in the duodenum until the beginning of the next phase III cycle. Duration, mean amplitude, contraction frequency, propagation velocity and areas under the curve (AUC) of phases III of the MMC's were measured. The postprandial period was defined as the time interval between the end of the meal and the occurrence of the first duodenal phase III propagated over at least two channels. Only pressure waves with an amplitude ≥ 10 mmHg and duration ≥ 1.5 s were considered as true contractions. The motility indices (MI) of the postprandial period in antrum and duodenum were calculated as area under the contraction curves and expressed in mmHg.sec per hour.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Parameters of digestive and interdigestive antroduodenojejunal motility and plasma CCK, PYY and motilin secretion between and within groups were analyzed by repeated analysis of variance. When this indicated a probability of less than 0.05 for the null hypothesis Student-Newman-Keuls analyses were performed to determine which values between or within groups differed significantly. Coefficient of linear correlation (Spearman) was used to calculate the correlation between the

disease duration and motility parameters. Statistical significance was defined as a P value <0.05.

RESULTS

Baseline characteristics of the SSc patients

There were no significant differences in age, disease duration and disease severity score between patients with lSSc and dSSc (Table 1). Upper gastrointestinal symptoms such as heartburn and dysphagia were reported by 83% and 25% respectively in dSSc patients vs 90% and 36% respectively in lSSc patients. Intestinal symptom such as diarrhea was present in 33% of patients with dSSc en in 54% of patients with lSSc (Table 1).

Table 1. *Characteristics of the SSc patients*

	Diffuse SSc (N=12)	Limited SSc (N=11)
Age, years (mean±SEM)	50±4	52±5
Male/Female ratio	4/8	4/7
Disease duration, years (mean±SEM)	6.2±1	6.0±1
Severity score (mean±SEM)	6.8±0.6	6.6±0.8
Heartburn	10/12	10/11
Dysphagia	3/12	4/11
Diarrhea	4/12	6/11

Antroduodenojejunal motility

Postprandial state

The duration of the postprandial motility pattern was significantly ($p<0.01$) prolonged in the SSc patient group (322 ± 22 min) compared to control subjects (215 ± 19 min). No significant differences were observed in the duration of the postprandial pattern between patients with limited versus diffuse type scleroderma (328 ± 28 min and 316 ± 36 min respectively). Postprandial antral and duodenal motility indices (MI) were significantly ($p<0.05$) reduced in the SSc patient group compared to controls (Table 2). No significant difference in postprandial antral MI was found between patients with diffuse and limited disease. Postprandial duodenal MI was, on the other hand, significantly reduced in patients with limited disease compared to patients with diffuse scleroderma (Table 2). This difference resulted from a significant reduction in the number of contractions in patients with limited disease (Table 2).

Neither disease duration nor disease severity score was correlated with motility parameters such as the duration of the fed pattern ($r=0.3$, $p=0.5$ and $r=-0.2$; $p=0.4$ respectively), antral MI ($r=-0.3$; $p=0.4$ and $r=0.05$, $p=0.8$ respectively) and duodenal MI ($r=-0.3$, $p=0.4$ and $r=0.2$, $p=0.3$ respectively).

With respect to a qualitative motility analysis we observed that the characteristics of the postprandial antroduodenojejunal motility patterns were different between the patient group with diffuse and limited disease. Postprandial antroduodenojejunal motility in 7 out of 12 patients (58%) with diffuse type scleroderma was characterised by the occurrence of non-propagated clustered contractions and duodenal discrete clustered contractions (Figure 1; upper panel). Patients with limited type scleroderma exhibited a motility pattern characterised by periods of motor quiescence in the duodenum and jejunum (Figure 1; lower panel). This motility pattern was seen in six out of eleven patients (54%) with limited scleroderma.

Table 2. Antral and duodenal postprandial motility characteristics in systemic sclerosis patients and healthy controls. * $p < 0.05$ compared to controls; # $p < 0.05$ compared to diffuse disease. MI: Motility Index

	Controls (N=15)	SSc patients (N=23)	Diffuse SSc (N=12)	Limited SSc (N=11)
Antrum				
Contractions (number/hour)	32±6	19±2*	17±3*	20±5*
Amplitude (mmHg)	50±5	35±4*	34±5*	36±7*
MI (mmHg*sec) per hour	3468±762	1203±225*	948±177*	1482±257*
Duodenum				
Contractions (number/hour)	122±14	56±9*	71±9*	41±7*#
Amplitude (mmHg)	29±1	22±1*	21±1*	23±1*
MI (mmHg*sec) per hour	4634±752	2195±305*	2591±580*	1406±280*#

Interdigestive state

After transition from a digestive into an interdigestive motility pattern, 11 complete MMC cycles in the patient group and 22 complete MMC cycles in the control subjects were registered. Of the 11 MMC cycles in the SSc patient group 8 MMC cycles were observed in five patients with the diffuse type SSc. The other three MMC cycles were found in two patients with the limited type SSc. The duration of the MMC cycles was not significantly different between the SSc patient group and controls (Table 3). In addition, no significant

differences were found in the duration of phase I, II and III between the patient and control group. However, the amplitude of phase III was significantly reduced ($p<0.05$) in the SSc patient group (25 ± 5 mmHg) compared to controls (39 ± 3 mmHg).

Esophageal manometry

Mean LESp was 8.4 ± 1.4 mmHg (normal range 15-25 mmHg). There was no significant difference in LESp between patients with diffuse and limited disease (8.5 ± 2.1 mmHg and versus 8.3 ± 1.8 mmHg respectively). Peristaltic wave amplitude of the SSC patients was 33 ± 4.8 mmHg (normal 50-60mmHg) in the upper portion, 6.7 ± 2.8 mmHg (normal 40-50 mmHg) in the mid portion and 6.4 ± 3.4 mmHg (normal 50-60 mmHg) in the distal portion of the esophagus. No significant differences were found between patients with limited and diffuse scleroderma.

Table 3. Characteristics of the migrating motor complex (MMC) cycles (mean $\pm$ SEM) found in patients with systemic sclerosis and in 15 healthy controls

	SSc patients	Diffuse SSc	Limited SSc	Controls
Number of cycles	11	8	3	22
MMC cycle duration (min)	132 $\pm$ 36	122 $\pm$ 25	140 $\pm$ 39	129 $\pm$ 9
Phase I (min)	14 $\pm$ 2	16 $\pm$ 3	13 $\pm$ 2	21 $\pm$ 3
Phase II (min)	112 $\pm$ 35	98 $\pm$ 26	123 $\pm$ 29	101 $\pm$ 9
Phase III (min)	6 $\pm$ 0.9	6 $\pm$ 0.8	5 $\pm$ 0.6	5 $\pm$ 0.5

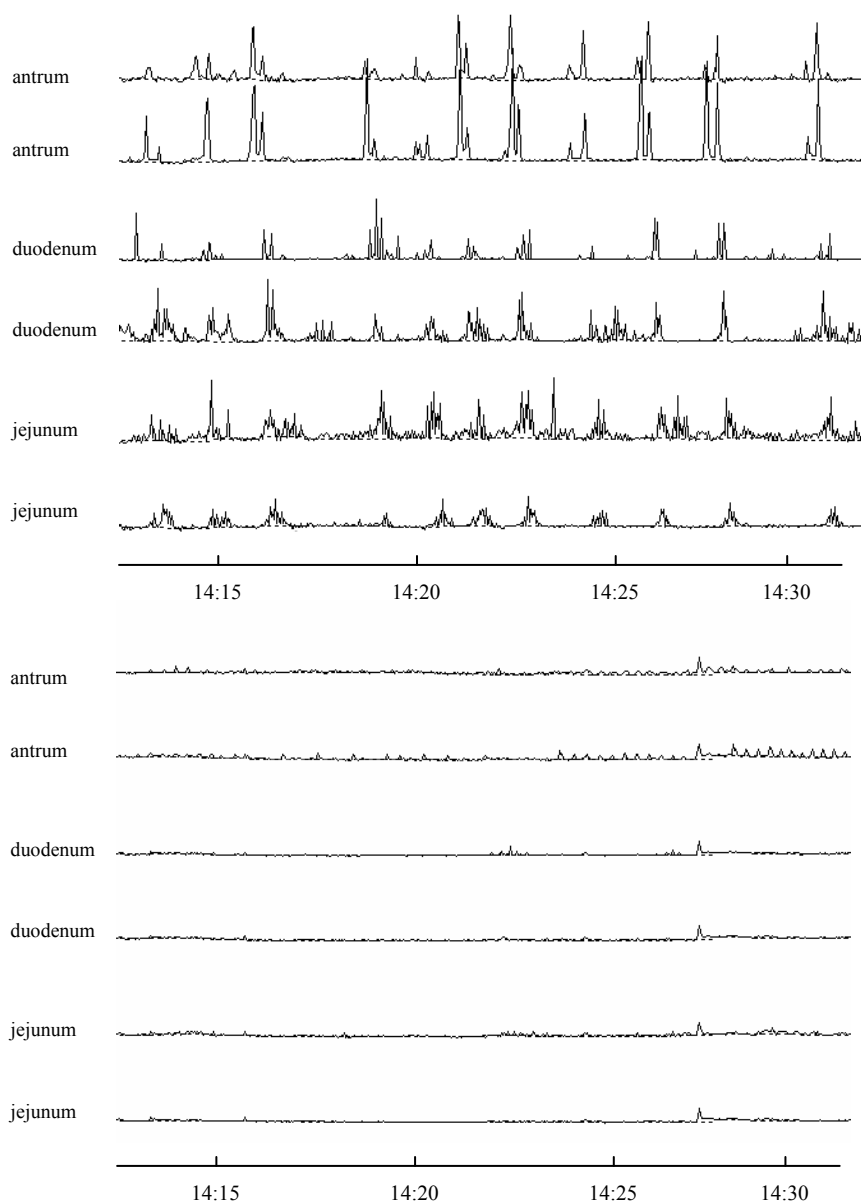


Figure 1. Postprandial antroduodenojejunal motility in patients with diffuse type scleroderma (upper panel) and in patients with limited type scleroderma (lower panel)

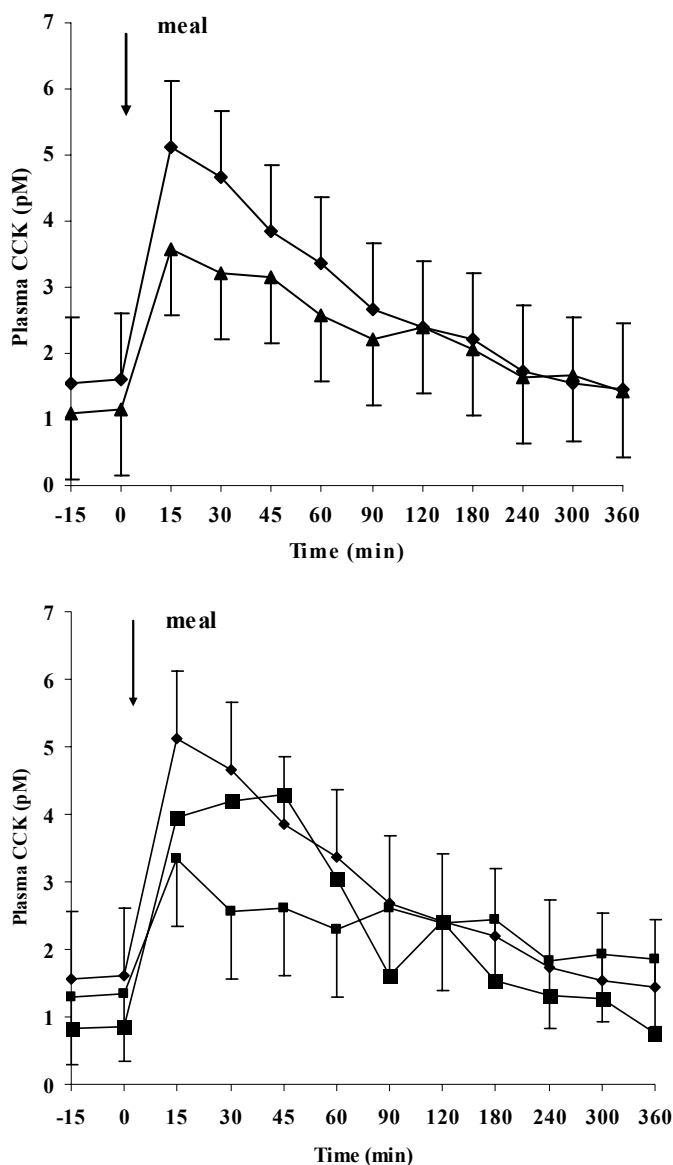


Figure 2. Fasting and postprandial plasma CCK levels (mean $\pm$ SEM) in patients with systemic sclerosis (n=23; triangles) and controls (n=15; small squares) (upper panel) and in SSc patients with the diffuse type scleroderma (n=12; big squares), patients with the limited scleroderma (n=11, crosses) and controls (n=15; small squares) (lower panel).

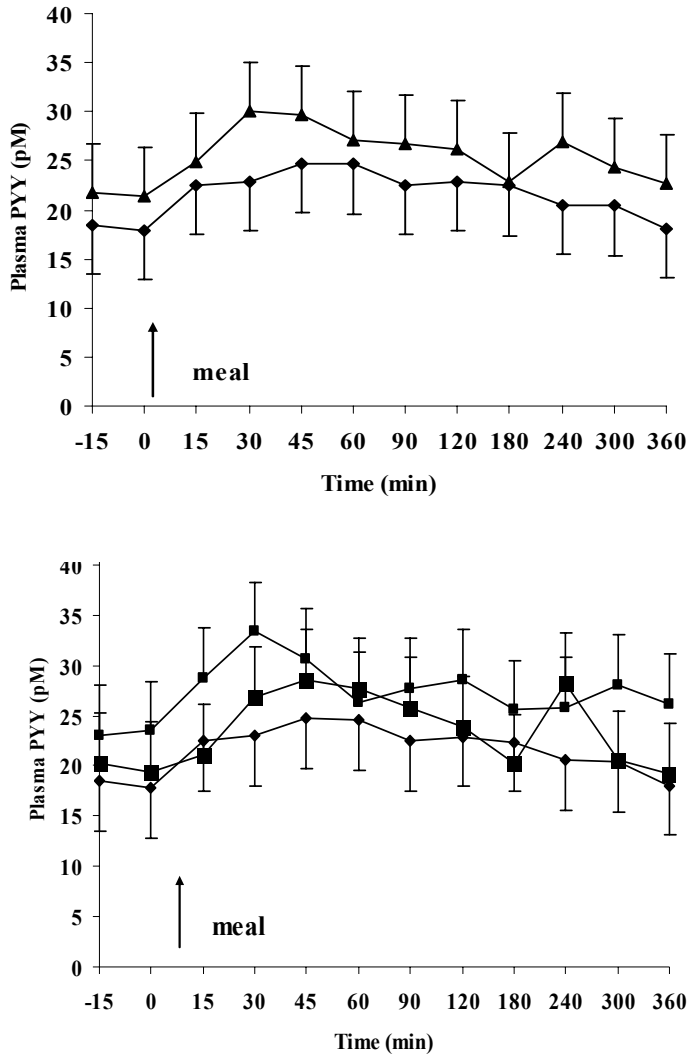


Figure 3. Fasting and postprandial plasma PYY levels (mean $\pm$ SEM) in patients with systemic sclerosis (n=23; triangles) and controls (n=15; small squares) (upper panel) and in SSc patients with the diffuse type scleroderma (n=12; big squares), patients with the limited scleroderma (n=11, crosses) and controls (n=15; small squares) (lower panel).

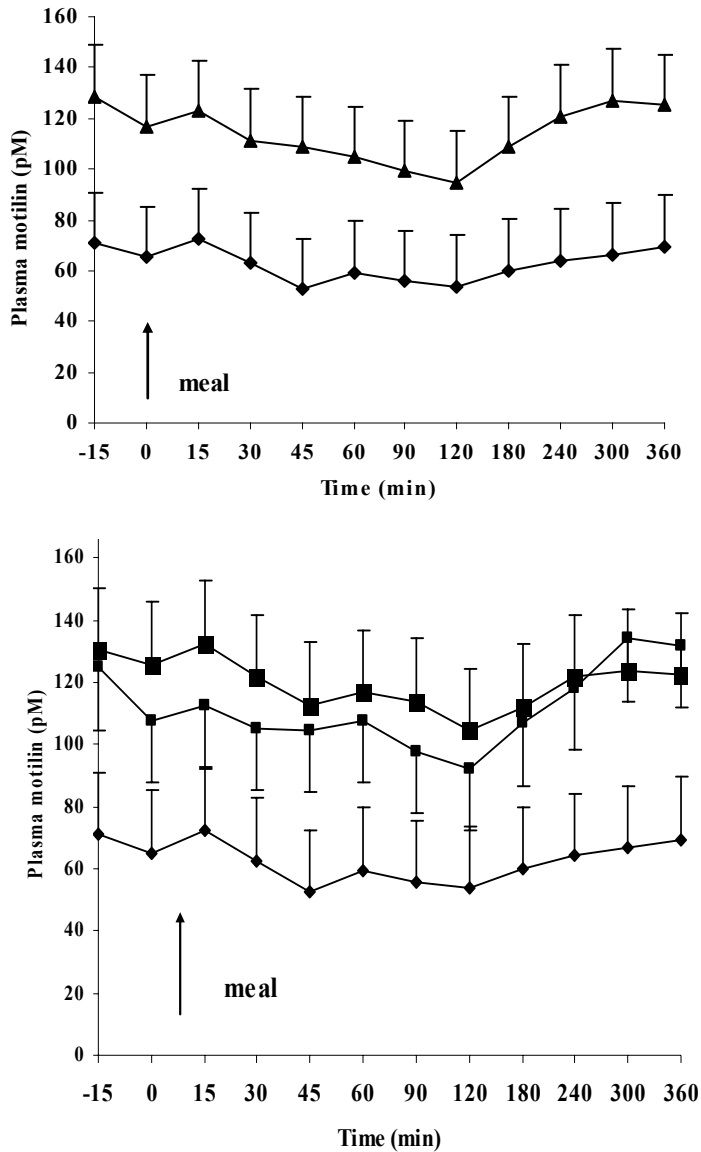


Figure 4. Fasting and postprandial plasma motilin levels (mean $\pm$ SEM) in patients with systemic sclerosis (n=23; triangles) and controls (n=15; small squares) (upper panel) and in SSc patients with the diffuse type scleroderma (n=12; big squares), patients with the limited scleroderma (n=11, crosses) and controls (n=15; small squares) (lower panel).

Plasma CCK

Basal plasma CCK levels in patients with SSc (1.3 ± 0.6 pM) were not significantly different from control subjects (1.5 ± 0.2 pM). In both groups plasma CCK levels increased significantly ($p < 0.05$) over basal starting from 15 min after meal ingestion and remained significantly increased until time 120 min (Figure 3; upper panel). Postprandial plasma CCK levels at $t=15$ min and $t=30$ min were significantly ($p < 0.05$) reduced in the patient group compared to controls (Figure 2; upper panel). These differences resulted from significant ($p < 0.05$) reductions in plasma CCK levels of the patients with limited disease and not of those with diffuse type disease (Figure 2; lower panel).

Plasma PYY

Basal plasma PYY levels were not significantly different between SS patients (21 ± 1 pM) compared to controls (18 ± 1 pM). After meal ingestion plasma PYY levels increased significantly ($p < 0.01$) over basal starting from 15 min until 120 min in the controls and until 180 min in the SSc patients (Figure 3, upper panel). No significant difference was found in postprandial plasma PYY secretion between SSc patients and controls. When comparing the subsets of SSc patients, in those with limited disease, basal plasma PYY levels were higher compared to controls (although not statistically significant) and postprandial plasma PYY levels at $t=15$, 30 and 45 min were significantly ($p < 0.05$) higher compared to controls (Figure 3; lower panel).

Plasma motilin

Basal and postprandial motilin levels were significantly ($p < 0.05$) higher in the scleroderma patients compared to control subjects (Figure 4; upper panel).

There was no significant difference in basal and postprandial plasma motilin levels between patients with limited and diffuse type scleroderma (Figure 4; lower panel).

DISCUSSION

The results of the present study show that small intestinal motility is more severely affected in patients with limited compared to those with diffuse type scleroderma, irrespective of disease duration and disease severity score. Esophageal and gastric motor function are, on the other hand, equally impaired among patients with diffuse and limited SSc.

Although GI motility disorders in patients with SSc have been well documented, it is not clear whether the frequency and type GI dysmotility differ between patients with diffuse and limited type scleroderma. Esophageal motility was impaired in all but one patient with SSc and the degree of impairment was equal among patients with diffuse and limited disease. Antral motility was impaired in both groups of patients to an equal extent. With respect to duodenojejunal motility, disturbance was, however, more pronounced in patients with limited type scleroderma.

It has been suggested that gastrointestinal changes in SSc occur in various stages. Neural dysfunction appears to be the earliest gastrointestinal change induced by SSc. The second stage is muscle atrophy and the final stage is characterized by muscle fibrosis (3,14). In a study of eight patients with diffuse type SSc, Greydanus *et al* have demonstrated two distinct gastrointestinal motility patterns (15). One was characterized by non-propagated, uncoordinated clustered contractions, suggestive of the neuropathic stage. These motility abnormalities resemble those observed in diseases associated with neuropathy

such as diabetes mellitus (16). The other motility pattern was characterized by a reduced number of contractions with low amplitude, suggestive of the myopathic lesions of the small intestine (15). Another study by Sjölund *et al* showed that small intestinal motility patterns in eight out of ten patients with diffuse and limited SSc were characterized by both neuropathic and myopathic patterns (17). In a most recent study Marie *et al* have shown a rapid deterioration of small bowel motor function with neurogenic abnormalities preceding myopathic motility pattern in eight SSc patients (two with diffuse and six with limited SSc) at 5-year follow-up (18). However, it is not clear from these aforementioned studies whether small intestinal motility differs between patients with limited and diffuse SSc. In the present study patients with limited SSc exhibited a myopathic pattern with postprandial hypomotility whereas the majority of patients with diffuse SSc had a neuropathic pattern characterised by the presence of non-propagated, uncoordinated clustered contractions. According to the concept that the neuropathic stage occurs early in the course of SSc and precedes the myopathic stage in SSc, these findings imply that scleroderma is in a more advanced stage in patients with limited compared to diffuse disease. Against this concept is the observation that neither disease severity score nor disease duration differed significantly between the two groups of SSc patients. Therefore one could also argue that the differences in GI motility between patients with diffuse and limited type scleroderma represent the natural course of two variants of the disease and are not related to the duration or the stage of scleroderma. There are arguments in favor of the concept that the diffuse and limited forms of scleroderma are different diseases: 1) the two classical SSc-selective autoantibodies clearly identify the SSc subsets. The limited SSc is associated with anticentromere antibodies and the

diffuse SSc with antitopoisomerase antibodies; 2) the transition from one form of SSc to the other is seldom seen (19).

Not only gastrointestinal motility but also gastrointestinal peptide secretion was abnormal in patients with SSc. Plasma levels of the proximal gut hormone CCK were significantly decreased in SSc. When analysed separately, only the patients with limited disease showed a significant reduction in plasma CCK levels compared to controls. This reduction in plasma CCK levels can be due to several factors such as: 1) reduced number of CCK producing cells in the upper small intestine as a result of fibrosis and atrophy or 2) inadequate intraluminal nutrient stimulation. Previous studies have shown that fatty acids rather than intact triglycerides stimulate CCK release (20). One may argue that the hydrolysis of triglycerides to fatty acids is reduced or delayed in these patients due to changes in intraluminal contents resulting from delayed gastric emptying, reduced intraluminal exocrine pancreatic enzyme and gallbladder bile acid concentrations. The observation that 73% of the patients with limited disease had intestinal involvement with diarrhea supports this concept.

In contrast with plasma CCK, plasma levels of the distal gut peptide PYY during the first 45 min of the postprandial period were significantly higher in the patients with limited scleroderma compared to controls. PYY represents the so-called “ileo-colonic brake”, a negative feedback control mechanism from the distal to the proximal gut (21-23). Increased plasma PYY levels are found in diseases associated with malabsorption such as exocrine pancreatic insufficiency, coeliac sprue and dumping syndrome (11,24,25). The observation that a majority of the patients with limited disease had gastrointestinal symptoms including steatorrhoea is in line with findings in other gastrointestinal disorders with malabsorption. We therefore believe that changes in CCK and

PYY secretion are secondary to changes in GI function induced by SSc and do not primarily relate to SSc.

Basal and postprandial plasma levels of motilin were significantly higher in SSc patients compared to healthy controls. Motilin is released from the proximal bowel and cyclically peaks in close association with phase III of the MMC cycle (26-28). Based on the action of motilin in the interdigestive state we hypothesize that increased plasma motilin levels in SSc patients result from a prolonged postprandial motility period and the delayed occurrence or complete absence of phase III in these patients. Our results are in line with those of Akesson *et al* who found that plasma motilin levels were higher in SSc patients compared to controls (8). There was no difference between patients with diffuse and limited type scleroderma.

It is concluded that alterations in gastrointestinal motility and gut hormone secretion are frequent in patients with systemic sclerosis. Whereas esophageal and gastric motility are affected to the same extent in the limited and diffuse type SSc, alterations in duodenojejunal motility and gut hormone secretion are more pronounced among patients with the limited type SSc. Qualitatively, intestinal motility in the limited type scleroderma is characterized by myopathic changes and the diffuse type by neuropathic changes.

REFERENCES

1. Abu-Shakra M, Guillemin F, Lee P. Gastrointestinal manifestations of systemic sclerosis. *Semin Arthritis Rheum* 1994;24(1):29-39.
2. Zamost BJ, Hirschberg J, Ippoliti AF, Furst DE, Clements PJ, Weinstein WM. Esophagitis in scleroderma. Prevalence and risk factors. *J Lab Clin Med* 1987;92(2):421-8.

3. Rose S, Young MA, Reynolds JC. Gastrointestinal manifestations of scleroderma. *Gastroenterol Clin North Am* 1998;27:563-94.
4. Madsen JL, Hendel L. Gastrointestinal transit times of radiolabeled meal in progressive systemic sclerosis. *Dig Dis Sci* 1992;37(9):1404-8.
5. Trezza M, Krogh K, Egekvist H, Bjerring P, Laurberg S. Bowel problems in patients with systemic sclerosis. *Scand J Gastroenterol* 1999;34:409-13.
6. Weston S, Thumshirn M, Wiste J, Camilleri M. Clinical and upper gastrointestinal motility features in systemic sclerosis and related disorders. *Am J Gastroenterol* 1998;93(7):1085-9.
7. Akesson A, Ekman R, Prytz H, Sundler F. Tissue concentrations of gastrointestinal regulatory peptides in the duodenal mucosa in systemic sclerosis. *Clin Exp Rheumatol* 1998;16(2):141-8.
8. Akesson A, Ekman R. Gastrointestinal regulatory peptides in systemic sclerosis. *Arthritis Rheum* 1993;36(5):698-703.
9. Medsger TA, Silman A, Steen V, Black C, Akesson A, Bacon P, Harris CA, et al. A disease severity scale for systemic sclerosis: development and testing. *J Rheumatol* 1999; 26:2159-67.
10. Jansen JBMJ, Lamers CBHW. Radioimmunoassay of cholecystokinin in human tissue and plasma. *Clin Chim Acta* 1983;131:305-16.
11. Vu MK, Vecht J, Eddes EH, Biemond I, Lamers CB, Masclee AA. Antroduodenal motility in chronic pancreatitis: are abnormalities related to exocrine insufficiency? *Am J Physiol Gastrointest Liver Physiol*. 2000;278(3):G458-66.
12. Kamerling IM, Van Haarst AD, Burggraaf J, Schoemaker HC, Biemond I, Jones R, Cohen AF, Masclee AA. Dose-related effects of motilin on proximal gastrointestinal motility. *Aliment Pharmacol Ther*

2002;16(1):129-35.

13. Kellow JE, Borody TJ, Phillips SF, Tucker RL, Haddad AC. Human interdigestive motility: variations in patterns from esophagus to colon. *J Lab Clin Med* 1986;91:386-95.
14. Sjogren RW. Gastrointestinal motility disorders in scleroderma. *Arthritis Rheum* 1994;37(9):1265-82.
15. Greydanus MP, Camilleri M. Abnormal postcibal antral and small bowel motility due to neuropathy or myopathy in systemic sclerosis. *J Lab Clin Med* 1989;96(1):110-5.
16. Camilleri M, Malagelada JR. Abnormal intestinal motility in diabetics with the gastroparesis syndrome. *Eur J Clin Invest* 1984;14:420-7.
17. Sjolund K, Bartosik I, Lindberg G, Scheja A, Wildt M, Akesson A. Small intestinal manometry in patients with systemic sclerosis. *Eur J Gastroenterol Hepatol* 2005; 17:1205-12.
18. Marie I, Ducrotté P, Denis P, Hellot M.F, Levesque H. Outcome of small-bowel motor impairment in systemic sclerosis: a prospective manometric 5-yr follow-up. *Rheumatology* 2007;46:150-53.
19. Wollheim FA. Classification of systemic sclerosis. Visions and reality. *Rheumatology (Oxford)* 2005;44(10):1212-6.
20. Hildebrand P, Petrig C, Burckhardt B, Ketterer S, Lengsfeld H, Fleury A, Hadvary P, Beglinger C. Hydrolysis of dietary fat by pancreatic lipase stimulates cholecystokinin release. *J Lab Clin Med* 1998;117:123-9.
21. Welch.I, Cunningham.K.M, Read.N.W. Regulation of gastric emptying by ileal nutrients in humans. *J Lab Clin Med* 1988;94:401-4.
22. Lin HC, Zhao XT, Wang L. Intestinal transit is more potently inhibited by fat in the distal (ileal brake) than in the proximal (jejunal brake) gut. *Dig*

- Dis Sci 1997;42(1):19-25.
23. Layer P, Peschel S, Schlesinger T, Goebell H. Human pancreatic secretion and intestinal motility: effects of ileal nutrient perfusion. *Am J Physiol* 1990;258:G196-201.
 24. Adrian TE, Long RG, Fuessl HS, Bloom SR. Plasma peptide YY (PYY) in dumping syndrome. *Dig Dis Sci* 1985;30:1145-8.
 25. Adrian TE, Savage AP, Bacarese-Hamilton AJ, Wolfe K, Besterman HS, Bloom SR. Peptide YY abnormalities in gastrointestinal diseases. *J Lab Clin Med* 1986;90:379-84.
 26. Peeters TL, Vantrappen G, Janssens J. Fasting plasma motilin levels are related to the interdigestive motility complex. *J Lab Clin Med* 1980;79:716-9.
 27. Nilsson BI, Svenberg T, Tollstrom T, Hellstrom PM, Samuelson K, Schnell PO. Relationship between interdigestive gallbladder emptying, plasma motilin and migrating motor complex in man. *Acta Physiol Scand* 1990;139(1):55-61.
 28. Stolk MF, van Erpecum KJ, Smout AJ, Akkermans LM, Jansen JB, Lamers, CB, Peeters TL, vanBerge-Henegouwen GP. Motor cycles with phase III in antrum are associated with high motilin levels and prolonged gallbladder emptying. *Am J Physiol* 1993;264:G596-G600.

Chapter 9

GALLBLADDER MOTILITY IN CROHN'S DISEASE: Influence of disease localisation and bowel resection

M. K. Vu¹, H. A. J. Gielkens¹, R. A. van Hogezaand¹, J. A. van Oostayen², C. B. H. W. Lamers¹, A. A. M. Masclee¹

Departments of Gastroenterology-Hepatology¹ and Radiology², Leiden University Medical Center, Leiden, the Netherlands

Gallbladder Motility in Crohn Disease: Influence of Disease Localization and Bowel Resection

M. K. Vu, H. A. J. Gielkens, R. A. van Hogezaand, J. A. van Oostayen, C. B. H. W. Lamers
& A. A. M. Masclee

Dept. of Gastroenterology–Hepatology and Radiology, Leiden University Medical Center, Leiden, The Netherlands

Vu MK, Gielkens HAJ, van Hogezaand RA, van Oostayen JA, Lamers CBHW, Masclee AAM. Gallbladder motility in Crohn disease: influence of disease localization and bowel resection. *Scand J Gastroenterol* 2000;35:1157–1162.

Background: Patients with Crohn disease (CD) have an increased risk of developing gallstones. Among other factors, gallbladder motility may have a role in the pathogenesis of gallstone formation. We have evaluated whether gallbladder motor function is affected in Crohn disease with special emphasis on the influence of disease localization and previous bowel resection. **Methods:** Thirty-seven patients (20 females and 17 males, age 36 ± 2 years) with inactive Crohn disease (CDAI < 150) were studied: 15 patients after ileocecal resection and 22 non-operated patients; 12 had small bowel disease and 10 had large bowel disease. Nineteen healthy subjects (10 female; 9 male, age 30 ± 2 years) served as controls. Gallbladder volumes were measured in the fasting state and at regular intervals for 2 h after ingestion of a solid meal (780 kcal). Blood samples were drawn at regular intervals for determination of cholecystokinin (CCK) and peptide YY (PYY). **Results:** Fasting gallbladder volumes were significantly ($P < 0.05$) reduced in patients with large bowel disease (20.8 ± 2.1 ml) or after ileocecal resection (18.3 ± 2.4 ml) compared to patients with small bowel disease (28.0 ± 2.1 ml) and controls (27.2 ± 1.8 ml). Fasting plasma CCK levels were significantly ($P < 0.05$) higher in patients with large bowel disease or after ileocecal resection compared to patients with small bowel disease and controls. Postprandial gallbladder emptying and endogenous plasma CCK and PYY secretion in patients with Crohn disease were not different from controls. **Conclusions:** Fasting gallbladder volume is decreased and fasting plasma CCK levels are increased in patients with Crohn disease of the large bowel and patients after ileocecal resection. Postprandial gallbladder motility, CCK and PYY release were not affected in patients with Crohn disease.

Key words: Gallbladder motility; Crohn disease; cholecystokinin; peptide YY

A. A. M. Masclee, M.D., Ph.D., Dept. of Gastroenterology–Hepatology, Leiden University Medical Center, P.O. Box 9600, 2300 RC, Leiden, The Netherlands (fax: +31-71-5248115)

Patients with Crohn disease have an increased risk of developing gallstones (1–5). Several studies have reported a gallstone prevalence of about 30% in patients with Crohn disease compared to 8% among age-matched healthy subjects (1–3). This reported prevalence is, however, not equally distributed among the group of patients with Crohn disease. Patients with Crohn disease confined to the ileum or after ileal resection have a higher prevalence of gallstones of around 34% while the prevalence is only 5% in patients with the disease confined to the colon (1, 2).

Pathophysiological factors underlying cholesterol gallstone disease in general are: excess biliary cholesterol secretion resulting in cholesterol supersaturation; increase in nucleation-promoting factors; and alterations in gallbladder motility (6, 7). In patients with Crohn disease, disturbances in the enterohepatic circulation of bile salts owing to disease or

resection of the ileum resulting in bile salt malabsorption and increased biliary cholesterol have been proposed as the most important mechanism (8, 9). The role of gallbladder motility in the pathogenesis of gallstone formation in Crohn disease is less clear because the published data are conflicting (10–13). In three studies, impaired postprandial gallbladder contraction in patients with Crohn disease was reported while no evidence for abnormal gallbladder emptying was found in other studies (10–13). The aim of the present study was, therefore, to evaluate fasting and meal-stimulated gallbladder motility in a large group of Crohn disease patients with different disease localization and with or without ileocecal resection. As the gallbladder motor response to a meal is mainly controlled by various gut hormones released during the intestinal phase, basal and meal-stimulated plasma concentrations of proximal and distal gut hormones were also measured.

Subjects and Methods

Subjects

Thirty-seven patients with inactive Crohn disease (Crohn disease activity index (CDAI) median 63; range, 15–149) were included. Patients with concomitant diseases such as diabetes mellitus and hypertension were excluded from the study. None of the patients had clinical symptoms or ultrasonographical evidence of gallstones. Fifteen of the 37 patients had previously undergone ileocecal resection (median 6 years; range, 2–10 years). The mean length of the resected ileocecal segment was 42 ± 10 cm. Twelve of the remaining 22 patients had small bowel disease and 10 had colonic disease. At the time of the experiment 26 patients were on medication among which 6 patients were on prednisone, 9 on 5-ASA derivatives and 11 on both prednisone and 5-ASA derivatives. Nine of the female patients used oral contraceptives. All medications were continued except on the morning of the experiment. Further clinical characteristics of the three patient groups are presented in Table I.

Nineteen healthy subjects without any history of gastrointestinal disease or abdominal surgery served as controls (Table I). The use of oral contraceptives was reported in 7 of the 9 female healthy subjects. Studies were performed irrespective of the time of the menstrual cycle. The study protocol had been approved by the ethics committee of the Leiden University Medical Center.

Study protocol

All subjects were studied at 0830 h after an overnight fast. After two measurements of basal gallbladder volume an intravenous cannula was inserted into the antecubital vein of one arm for blood sampling. A solid meal consisting of 50 g fat, 42 g protein and 38 g carbohydrates (780 kcal) was ingested at time $t = 0$ min. Gallbladder volumes were measured and blood samples for determination of gut hormones cholecystokinin (CCK), pancreatic polypeptide (PP) and peptide YY (PYY) were drawn at $t = -15, 0, 10, 20, 30, 45, 60, 75, 90, 105$ and 120 min after meal ingestion.

Gallbladder volume

Gallbladder volumes measured by real-time ultrasonography (Toshiba, 3.5 MHz transducer) were calculated by the

sum of cylinders method using a computerized system (14, 15). In this method the longitudinal image of the gallbladder is divided into series of equal height, with diameter perpendicular to the longitudinal axis of the gallbladder image. The uncorrected volume is the sum of volumes of these separate cylinders. To correct for the displacement of the longitudinal image of the gallbladder from the central axis, a correction factor is calculated from the longitudinal and transversal scans of the gallbladder. Gallbladder volume is calculated by multiplication of the uncorrected volume with the square of the correction factor. The mean of two measurements was used for analysis. The assumptions and the mathematical formula used to calculate gallbladder volume have been described and validated previously (14, 15).

Hormone assays

Blood samples for measurement of plasma pancreatic polypeptide (PP), cholecystokinin (CCK) and peptide YY (PYY) were drawn at time $t = -15, 0, 15, 30, 45, 60, 90, 120, 150$ and 180 min during each experiment. The blood samples were collected in EDTA containing ice-chilled tubes. The samples were centrifuged at a rate of 3000 rpm for 10 min at a temperature of 4 °C. Plasma CCK was measured by a sensitive and specific radioimmunoassay (16). This antibody binds to all CCK peptides including sulphated CCK octapeptide, but not gastrin. The detection limit of the assay is 0.3 pM plasma. Plasma PYY was measured by radioimmunoassay. PYY antiserum was generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM A.G., Switzerland). PYY was labelled with ^{125}I using chloramine T. There is no cross-reactivity with PP or VIP. The detection limit is 10 pM plasma. Both PYY (1–3) and PYY (3–36) bind to the antibody in dilutions up to 25,000. Plasma PP was determined by radioimmunoassay as described previously (17).

Data and statistical analysis

Data are expressed as mean $\pm$ standard error of the mean. Postprandial gallbladder emptying was calculated as the percentage of the basal volume. Integrated incremental values for plasma hormone secretion were calculated as the area under the plasma concentration curve after subtraction of the basal value at $t = 0$. Multiple analysis of variance (MANO-

Table I. Characteristics of the three patient groups and of the healthy controls. Disease duration and CDAI are expressed as medians and ranges; age and body mass index are expressed as mean $\pm$ standard error of the mean

	Controls (N = 19)	Ileal CD (N = 12)	Colonic CD (N = 10)	Ileocecal resection (N = 15)
Age (years)	30 $\pm$ 2	34 $\pm$ 4	40 $\pm$ 4	37 $\pm$ 4
Sex (F/M)	9/10	5/7	7/3	6/9
Body mass index (kg/m ²)	23 $\pm$ 1	22 $\pm$ 3	24 $\pm$ 3	23 $\pm$ 2
Disease duration (years)	—	6 (2–23)	7 (3–22)	6 (2–25)
CDAI	—	76 (9–149)	90 (18–134)	80 (12–139)

Table II. Fasting and residual gallbladder volumes and maximal percentage of postprandial gallbladder emptying in the three patient groups and healthy controls. Data are expressed as mean $\pm$ standard error of the mean

	Controls (N = 19)	Ileal CD (N = 12)	Colonic CD (N = 10)	Ileocecal resection (N = 15)
Fasting volume (ml)	27.2 $\pm$ 1.8	28.0 $\pm$ 3.3	20.8 $\pm$ 2.1*	18.3 $\pm$ 2.4*
Residual volume (ml)	8.0 $\pm$ 0.9	9.6 $\pm$ 2.8	7.0 $\pm$ 1.3	6.0 $\pm$ 1.0
Maximal emptying (%)	70 $\pm$ 4	66 $\pm$ 8	67 $\pm$ 4	68 $\pm$ 3

* $P < 0.05$ compared to patients with ileal Crohn disease (CD) and controls.

VA) was used to compare gallbladder volume, plasma PP and CCK levels between and within groups and to analyse the influence of age, gender and the use of medication on gallbladder volume. Coefficient of linear correlation (Spearman) was used to calculate the correlations between fasting gallbladder volumes and the length of the resected segment and CDAI. The level of significance was set at $P < 0.05$.

Results

Fasting gallbladder volume

Mean basal gallbladder volume was significantly ($P < 0.05$) smaller in patients with large bowel disease and after ileocecal resection compared to patients with small bowel disease and to controls (Table II). Individual data of fasting gallbladder volumes are shown in Fig. 1. No correlation was found between fasting gallbladder volume and CDAI ($r = 0.16$; $P = 0.2$) or length of the resected segment ($r = 0.048$; $P = 0.7$).

Postprandial gallbladder emptying

After meal ingestion, gallbladder volume decreased significantly ($P < 0.01$) in all Crohn disease patients and healthy

controls (Fig. 2). The degree of postprandial gallbladder emptying was not significantly different between the patient groups and controls. Residual gallbladder volumes and maximal postprandial gallbladder emptying for all groups are given in Table II.

Plasma PP

Basal plasma PP levels were not significantly different between the four groups (Table III). Plasma PP levels significantly increased ($P < 0.001$) in response to the meal in all patients and control subjects. Postprandial integrated plasma PP concentration was not significantly different between the groups (Table III).

Plasma CCK

Basal plasma CCK levels were significantly ($P < 0.05$) higher in patients with large bowel Crohn disease and after ileocecal resection compared to patients with small bowel disease and controls (Table III). Plasma CCK levels significantly ($P < 0.01$) increased in response to the meal in all four groups (Fig. 3). Postprandial-integrated plasma CCK concentrations were not significantly different between patients and controls (Table III).

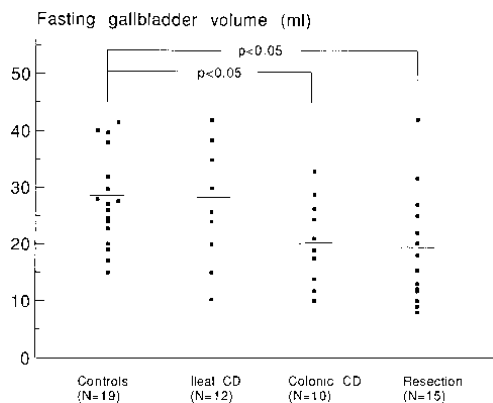


Fig. 1. Individual data of fasting gallbladder volume (ml; mean $\pm$ standard error of the mean) in patients with Crohn disease of the ileum and colon, patients after ileocecal resection and healthy controls.

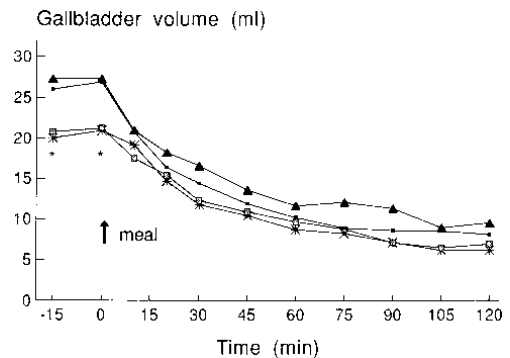


Fig. 2. Gallbladder volume (ml; mean $\pm$ standard error of the mean) during fasting and after meal ingestion in patients with Crohn disease of the ileum (closed triangles), of the colon (big squares), in patients after ileocecal resection (crosses) and healthy controls (small squares). * $P < 0.05$ compared to patients with ileal Crohn disease and controls.

Table III. Basal and postprandial integrated plasma concentrations of PP, CCK and PYY in patients with small and large bowel Crohn disease, patients after ileocecal resection and healthy controls

	Controls (N = 19)	Ileal CD (N = 12)	Colonic CD (N = 10)	Ileocecal resection (N = 15)
Fasting PP (pM)	49 ± 3	52 ± 6	41 ± 9	43 ± 8
Fasting CCK (pM)	0.7 ± 0.2	0.8 ± 0.1	1.6 ± 0.3*	1.5 ± 0.2*
Fasting PYY (pM)	19 ± 1	21 ± 3	15 ± 1	21 ± 1
AUC PP (pM*120 min)	8908 ± 1597	7732 ± 1190	7697 ± 1203	7929 ± 717
AUC CCK (pM*120 min)	137 ± 15	145 ± 46	217 ± 33	204 ± 40
AUC PYY (pM*120 min)	406 ± 120	515 ± 200	514 ± 133	400 ± 107

* $P < 0.05$ compared to patients with ileal Crohn disease and to controls.

Plasma PYY

Basal plasma PYY levels were not significantly different between Crohn disease patients and control subjects (Table III). A significant ($P < 0.05$) rise in plasma levels of PYY was found in the last 30 min after meal ingestion ($t = 90, 105$ and 120 min) in all the patient groups and in the healthy controls. Postprandial-integrated plasma PYY concentrations were not significantly different between the four groups (Table III).

Discussion

The results of the present study show that fasting gallbladder volume is significantly smaller in patients with Crohn disease of the large bowel and in patients after ileocecal resection compared to patients with small bowel disease and to controls. On the other hand, gallbladder emptying in response to a meal is not significantly different between the patients and the controls. We have included in this study only patients with inactive Crohn disease and without evidence of cholesterol gallstones. Patients who had developed cholesterol gallstones were excluded since it has been documented that cholesterol gallstones affect gallbladder motility (18).

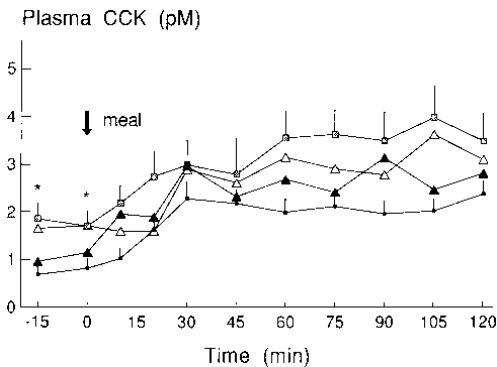


Fig. 3. Basal and postprandial plasma CCK levels (pM; mean $\pm$ standard error of the mean) in patients with Crohn disease of the ileum (closed triangles), of the colon (big squares), in patients after ileocecal resection (open triangles) and healthy controls (small squares). * $P < 0.05$ compared to patients with ileal Crohn disease and controls.

Fasting gallbladder tone and volume are controlled by both hormonal and neural pathways. Alterations in one of these factors may affect gallbladder motility. Indeed, plasma CCK levels were significantly higher in patients with large bowel disease or after ileocecal resection compared to patients with small bowel disease and to controls. CCK is released from the upper small bowel and is the most important hormonal mediator of gallbladder motility (19). CCK is not only involved in postprandial gallbladder motility but also plays an important role in regulating basal gallbladder volume. In humans, administration of the CCK antagonist loxiglumide significantly increases gallbladder volume (20). The elevated fasting plasma CCK levels in patients with large bowel disease and after ileocecal resection correlate with small fasting gallbladder volume found in these groups. This finding is in agreement with a previous study by Salemans et al. (21) who found that basal plasma CCK is increased and fasting gallbladder volume is decreased in patients after colectomy with ileal pouch anal anastomosis, suggesting a role for the colon in controlling plasma CCK release from the proximal gut.

Gastrointestinal peptides are released also from the distal small bowel and large bowel. Peptide YY (PYY) is such a distal gut hormone. It is a mediator of the so-called ileal and colonic brake—a negative feedback from the distal to the proximal gastrointestinal tract (22, 23). The highest concentrations of PYY-producing cells are found in the ileum, colon and rectum (23). It has been shown in dogs that PYY infusion induces gallbladder relaxation after CCK-stimulated gallbladder contraction (24). In the present study fasting plasma PYY levels were not significantly different between patients with large bowel disease or after ileocecal resection compared to patients with small bowel disease and to controls, suggesting that PYY does not account for the differences in fasting gallbladder volume found between these patient groups.

The vagus nerve is an important cholinergic neural mediator of fasting gallbladder motor activity (25). In both humans and dogs, gallbladder volume is increased after truncal vagotomy or during cholinergic blockage with atropine while it is decreased during administration of the vagal cholinergic stimulus bethanechol (26, 27). Lindgren et al. have shown that autonomic nerve dysfunction is frequently

present in patients with Crohn disease especially vagus dysfunction (28). Based on the results of these studies theoretically, the gallbladder volume should be increased in patients with Crohn disease compared to controls. However, the finding of a smaller fasting gallbladder volume in patients with large bowel disease and after ileocecal resection does not support the hypothesis of autonomic dysfunction as a factor accounting for the smaller fasting gallbladder volume.

In contrast to fasting gallbladder volume, no significant difference was found in postprandial gallbladder emptying between patients with colonic disease or after ileocecal resection compared to patients with small bowel disease and controls. Although several studies on postprandial gallbladder emptying in patients with Crohn disease have been performed, no consistent results have been obtained (10–13). Murray et al. documented that gallbladder emptying in response to a fatty meal is impaired in patients with Crohn disease. The most pronounced impairment has been observed in patients with both large and small bowel disease or after a previous resection (10). Consistent with the results of Murray et al., Damiao et al. have shown that gallbladder emptying in response to a solid meal is significantly reduced in patients with Crohn disease, an abnormality which is not influenced by gastric emptying (13). Unfortunately, differences in gallbladder emptying related to the localization of the disease or after resection were not analysed in the latter study. On the other hand, Maurer et al. found no evidence for abnormal postprandial gallbladder emptying in a group of 17 Crohn disease patients with small and/or large bowel disease or after previous resection (12). The discrepancy in results between these studies could be related to differences in the composition of the test meals and differences in patient population. It has previously been shown that the pattern and magnitude of postprandial gallbladder emptying are determined by the type, composition and caloric value of the meal (29). Differences in patient population due to the heterogeneous nature of Crohn disease with respect to disease duration and localization, bowel resection and the use of medication are probably the most important factors contributing to the different results found between the studies. In the present study, we have measured gallbladder volume in patients with inactive Crohn disease and data were analysed according to disease localization and bowel resection.

Since no differences were found in postprandial gallbladder emptying and residual volume, questions must be raised concerning the role of gallbladder motility in the formation of gallstones in patients with Crohn disease. Residual volume and fractional emptying have been documented to be the most important factors promoting stasis and gallstone formation (30). It is not known whether smaller fasting gallbladder volumes are relevant for gallstone formation. Instead, there is evidence suggesting that patients with cholesterol gallstones are characterized by enlarged fasting gallbladder volumes (31). Given the higher prevalence of gallstones in patient with Crohn ileitis or after ileocecal resection, it is obvious that

other pathogenetic factor(s) for gallstone formation must be involved. Excess of biliary cholesterol in relation to phospholipids and bile acids due to bile acid malabsorption in patients with Crohn disease in the ileum or after ileocecal resection has been reported (8, 9). However, recent studies have documented that cholesterol saturation of bile is not increased in patients with Crohn disease after ileocecal resection (32, 33). Not only the size but also the composition of the bile acid pool is relevant for cholesterol gallstone formation. The secondary bile acid deoxycholic acid stimulates cholesterol secretion to a larger extent than primary bile acids (34). The biliary concentration of deoxycholic acid is decreased in patients with Crohn colitis who have a low incidence of gallstones (35, 36). Data on the concentration of deoxycholic acid in patients with Crohn disease of the ileum or after ileocecal resection are less consistent (32, 36, 37). Lapidus & Einarsson have found that in addition to the decreased concentration of deoxycholic acid in duodenal bile, the amount of ursodeoxycholic acid is increased in patients after ileocecal resection (32). It is also possible that pigment rather than cholesterol gallstones are present in patients with ileal dysfunction or after ileal resection. It has been shown in animal models that after ileal resection especially pigment gallstones are formed (38). More recently, Brink et al. have shown that gallbladder bile of patients with ileal Crohn disease is saturated with unconjugated bilirubin (39).

In conclusion, patients with inactive Crohn disease of the large bowel and patients after ileocecal resection have smaller fasting gallbladder volumes and increased basal plasma CCK levels. Gallbladder motility and subsequent hormone release in response to a meal are, on the other hand, not affected in patients with inactive Crohn disease irrespective of disease localization and previous bowel resection. Gallbladder (dys)motility does not seem to contribute to gallstone formation in patients with Crohn disease.

References

1. Cohen S, Kaplan M, Gottlieb L, Patterson J. Liver disease and gallstones in regional enteritis. *Gastroenterology* 1971;60:237–45.
2. Baker AL, Kaplan M, Norton AP, Patterson J. Gallstones in inflammatory bowel disease. *Am J Dig Dis* 1974;19:109–12.
3. Whorwell JP, Hawkins R, Dewbury K, Wright R. Ultrasound survey of gallstones and other hepatobiliary disorders in patients with Crohn's disease. *Dig Dis Sci* 1984;29:930–3.
4. Lorusso D, Silvana L, Mossa A, Misciagna G, Guerra V. Cholelithiasis in inflammatory bowel disease. *Dis Colon Rect* 1990;33:791–4.
5. Lapidus A, Bangstad M, Åström M, Muhrbeck O. The prevalence of gallstone disease in a defined cohort of patients with Crohn's disease. *Am J Gastroenterol* 1999;94:1261–6.
6. Paumgartner G, Sauerbruch T. Gallstones: pathogenesis. *Lancet* 1991;338:1117–21.
7. Levy PF, Smith BF, LaMont JT. Human gallbladder mucine accelerates nucleation of cholesterol in artificial bile. *Gastroenterology* 1984;87:270–5.
8. Dowling RH, Bell GD, White J. Lithogenic bile in patients with ileal dysfunction. *Gut* 1972;13:415–20.
9. Marks JW, Conley DR, Capretta TL, Bonorris GG, Chunng A,

- Coyne MJ, et al. Gallstone prevalence and biliary lipid composition in inflammatory bowel disease. *Dig Dis Sci* 1977; 22:1097-100.
10. Murray FE, McNicholas M, Stack W, O'Donoghue DP. Impaired fatty-meal-stimulated gallbladder contractility in patients with Crohn's disease. *Clin Sci* 1992;83:689-93.
11. Anness V, Vantrappen G. Gallstones in Crohn's disease: other hypothesis. *Gut* 1994;35:1676.
12. Maurer P, Haag K, Roth M, Kuder C, Schölmerich J. No evidence for abnormal gallbladder emptying in Crohn's disease. *Hepato-gastroenterology* 1996;43:807-12.
13. Damiao AOMC, Sipahi AM, Vezozzo DP, Goncalves PL, Fukui P, Laudanna AA. Gallbladder hypokinesia in Crohn's disease. *Digestion* 1997;58:458-63.
14. Everson GT, Braverman DZ, Johnson ML, Kern F Jr. A critical evaluation of real-time ultrasonography for the study of gallbladder volume and contraction. *Gastroenterology* 1980;79:40-6.
15. Hopman WPM, Brouwer WFM, Rosenbusch G, Jansen JBMJ, Lamers CBHW. A computerized method for rapid quantification of gallbladder volume from real-time sonograms. *Radiology* 1985;154:236-7.
16. Jansen JBMJ, Lamers CBHW. Radioimmunoassay of cholecystokinin in human tissue and plasma. *Clin Chim Acta* 1983;131: 305-16.
17. Lamers CBHW, Diemel CM, Van Leer E, Van Leusen R, Peetoom JJ. Mechanism of elevated serum pancreatic polypeptide concentrations in chronic renal failure. *J Clin Endocrinol Metab* 1982;55:922-6.
18. Masclee AA, Jansen JB, Driessen WM, Geuskens LM, Lamers CB. Plasma cholecystokinin and gallbladder responses to intraduodenal fat in gallstone patients. *Dig Dis Sci* 1989; 34(3):353-9.
19. Hopman WPM, Kerstens PJSM, Jansen JBMJ, Rosenbusch G, Lamers CBHW. Effect of graded physiologic doses of cholecystokinin on gallbladder contraction measured by ultrasonography. Determination of threshold, dose-response relationships and comparison with intraduodenal bilirubin output. *Gastroenterology* 1985;89:1242-7.
20. Jebbink MCW, Masclee AAM, van der Kleij FG, Schipper J, Rovati LC, Jansen JB, et al. Effect of loxiglumide and atropine on erythromycin-induced reduction in gallbladder volume in human subjects. *Hepatology* 1992;16(4):937-42.
21. Salemans JM, Thirister PW, Hopman WP, Kuijpers HC, Rosenbusch G, Nagangast FM, et al. Plasma cholecystokinin levels and gallbladder volumes after proctocolectomy with ileal pouch-anal anastomosis. *Surgery* 1995;117:705-11.
22. Pironi L, Stanghellini V, Miglioli M, et al. Fat-induced ileal resection in humans: a dose-dependent phenomenon correlated to the plasma levels of peptide YY. *Gastroenterology* 1993;105: 733-9.
23. Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 1985;89: 1070-7.
24. Conter RL, Roslyn JJ, Taylor IL. Effects of peptide YY on gallbladder motility. *Am J Physiol* 1987;252:G736-G741.
25. Fisher RS, Rock E, Malmud LS. Cholinergic effects on gallbladder emptying in humans. *Gastroenterology* 1985;89: 716-22.
26. Hanse WE, Maurer H, Haberland H. The effect of sham-feeding on gallbladder volume and circulation of bile acids. *Hepato-gastroenterology* 1982;29:108-10.
27. Fisher RS, Rock E, Malmud LS. Gallbladder emptying in response to sham feeding in humans. *Gastroenterology* 1986;90: 1854-7.
28. Lindgren S, Lilja B, Rosen I, Sundkvist G. Disturbed autonomic nerve function in patients with Crohn's disease. *Scand J Gastroenterol* 1991;26:361-6.
29. Froehlich F, Gonvers JJ, Fried M. Role of nutrient fat and cholecystokinin in regulation of gallbladder emptying in man. *Dig Dis Sci* 1995;40:529-33.
30. Everson GT. Gallbladder function in gallstone disease. *Gastroenterol Clin N Am* 1991;20:85-110.
31. Festi D, Frabboni R, Bazzoli F, Sangermano A, Ronchi M, Rossi L, et al. Gallbladder motility in cholesterol gallstone disease. *Gastroenterology* 1990;99:1779-85.
32. Lapidus A, Einarsson K. Effects of ileal resection on biliary lipids and bile acid composition in patients with Crohn's disease. *Gut* 1991;32:1488-91.
33. Lapidus A, Einarsson C. Bile composition in patients with ileal resection due to Crohn's disease. *Inflamm Bowel Dis* 1998;4: 89-94.
34. Carulli NP, Loria P, Bertolotti M, Ponz de Leon M, Menozzi D, Medici G, et al. Effects of acute changes of bile acid pool composition of biliary lipid secretion. *J Clin Invest* 1984;74: 614-24.
35. Rutgeerts P, Ghooys Y, Vantrappen G. Bile acid studies in patients with Crohn's colitis. *Gut* 1979;20:1072-7.
36. Rutgeerts P, Ghooys Y, Vantrappen G. Kinetics of primary bile acids in patients with non-operated Crohn's disease. *Eur J Clin Invest* 1982;12:135-43.
37. Kruis W, Kalek HD, Stellaard F, Paumgartner G. Altered faecal bile acid pattern in patients with inflammatory bowel disease. *Digestion* 1986;35:189-98.
38. Pitt HA, Lewenski MA, Muller EL, Porter-Frink V, Den Besten L. Ileal resection-induced gallstones: altered bilirubin or cholesterol metabolism. *Surgery* 1984;96:154-62.
39. Brink MA, Slors JRM, Keulemans YC, Mok KS, de Waart DR, Carey MC, et al. Enterohepatic cycling of bilirubin: a putative mechanism for pigment gallstone formation in patients with ileal Crohn's disease. *Gastroenterology* 1999; 116:1420-7.

Chapter 10

SUMMARY

SAMENVATTING

SUMMARY

Since its initial description in 1984, several aspects of the action of the ileal brake have been investigated in animal models but information on the ileal brake in humans is still scarce and the obtained results are not always consistent. The studies presented in this thesis were performed to gain more insight into the physiology and the pathophysiology of the ileal brake in humans.

Ileal brake under physiological conditions

In **chapter II**, we studied and compared the effects of the fat induced ileal and jejunal brake on digestive and interdigestive motor patterns and postprandial gallbladder motility. Intrajejunal and intraileal administration of fat both prolonged the duration of the fed pattern induced by an oral liquid meal and reduced duodenal and jejunal motility index (MI). The reduction in MI was more pronounced during intraileal compared to intrajejunal fat perfusion. The observation that the duration of the fed pattern correlated significantly only with postprandial plasma PYY but not with plasma CCK release may suggest a role of PYY in the transition from the digestive into the interdigestive state. This is in contrast with CCK which has a role in the transition from fasting into fed motility patterns. Concerning the interdigestive motor pattern, intraileal fat shortened the MMC cycle length while this was prolonged in the intrajejunal fat experiment. Although the underlying mechanisms are not obvious, these findings are in support of previous results and are in line with the observation that MMC cycle length is shortened in malabsorptive diseases with increasing ileal fat delivery. Up to now, little is known about the effects of ileal and jejunal

brakes on gallbladder motility. Our results showed that ileal fat significantly decreased postprandial gallbladder emptying while this was enhanced during intrajejunal fat infusion. The observation that, postprandial gallbladder emptying significantly correlated with postprandial plasma CCK but not with plasma PYY release, suggests an insignificant role of PYY in the regulation of postprandial gallbladder motility. These findings are in agreement with previous results demonstrating that PYY did not inhibit gallbladder contraction stimulated by exogenous or endogenous CCK. Altogether, results of the study in chapter II demonstrate that intestinal feedback control mechanisms evoked by the fat induced ileal brake on proximal small intestine, postprandial gallbladder motility and hormone release differ qualitatively and quantitatively from those evoked by the fat induced jejunal brake.

In line with the concept of a braking mechanism from the distal gut, we hypothesized that the more distally the nutrients are delivered into the intestine, the less activation of the exocrine pancreatic secretion will be found. This concept is of particular importance in the treatment of acute pancreatitis since the ideal therapy for these patients should consist of enteral nutrients thereby maintaining gut barrier function without affecting exocrine pancreatic secretion. The study in **chapter III** was, therefore, designed to compare the effect of a commercially available nutrient solution on exocrine pancreatic secretion in healthy volunteers, gallbladder contraction and subsequent release of proximal and distal gut hormones, when administered either in the proximal or in the distal jejunum. We used Nutrison standard as the test meal which was given continuously at the same rate as clinically used in patients on enteral nutrition. Our results demonstrated that continuous administration of a mixed liquid meal in the distal jejunum did not stimulate pancreatic enzyme secretion in contrast to

nutrients in the proximal jejunum. Gallbladder contraction, on the other hand, was induced by distal jejunal nutrients, though to a lesser extent compared to when nutrients were given in the proximal jejunum. The decrease in gallbladder contraction was in parallel with the decrease in intraduodenal bilirubin output during distal enteral nutrient delivery. Because no differences were found in plasma CCK, neurotensin and PYY release during proximal and distal jejunal nutrient infusion, it seems unlikely that these changes were regulated by a hormonal mediated pathway but probably due to reduced activation of the enteropancreatic reflexes. From a clinical point of view, results of this study have provided useful information concerning the nutritional support route in the acute phase of pancreatitis. Distal jejunal delivery of nutrients does not activate exocrine pancreatic secretion while gallbladder motility is preserved. We do not know whether these findings can be extrapolated to patients with acute pancreatitis. This concept needs to be further explored in patients with acute pancreatitis

Although recently much attention has been focused on PYY as a potential anorexogenic substance, an association between ileal brake activation and satiety has not been clearly defined. Since PYY is a candidate hormonal mediator of the ileal brake, it is conceivable that activation of the ileal brake with subsequent endogenous PYY release also induces satiety and inhibits food intake. Therefore **in chapter IV** we compared the effects of ileal brake activation with ileal fat (endogenous PYY release) versus exogenous PYY₃₋₃₆ infusion on satiety and on proximal gastric motor function using two protocols. In the first protocol, the effect of ileal fat and subsequent endogenous PYY release was studied and in the second protocol, we investigated the dose-response relationship of exogenous PYY. The results

clearly demonstrated that, in healthy volunteers, ileal fat induced satiety in the fasting state and increased satiety in the fed state. Furthermore, ileal fat significantly increased fasting proximal gastric volume, enhanced postprandial proximal gastric relaxation and stimulated PYY release. In contrast, exogenous PYY₃₋₃₆ infusion, at both low and high doses, did not affect satiety nor proximal gastric motor and sensory function. These findings, therefore, do not support a role for PYY as a physiological mediator of ileal fat induced gastric relaxation and accommodation. Our data concerning exogenous PYY and satiety were not in agreement with previous results showing that PYY markedly reduced food intake. The most likely explanation for this discrepancy may be related to the doses of PYY given. Indeed, a recent study showed that exogenous PYY₃₋₃₆ only significantly reduced feelings of hunger and decreased food intake at high, pharmacological doses. The plasma levels of PYY reached with exogenous PYY in our study were in the physiological postprandial range. Thus, based on the results of our study, we suggest that the doses of PYY required to produce a significant effect on satiety and food intake are in the supraphysiological or pharmacological range.

In **chapter V** we used the existence of the ileal brake with subsequent PYY release to test the hypothesis that medium chain triglycerides (MCT) are less rapidly absorbed, in contrast to the general belief that the absorption of MCT is more rapid and complete compared to long chain triglycerides (LCT). This hypothesis derived from the observations that patients receiving MCT frequently complain of nausea, borborygmi, cramps, abdominal pain and diarrhoea. The results confirmed that MCT significantly accelerated small intestinal transit compared to saline and LCT. Furthermore, intraduodenally

administered MCT induced a significant increase in the plasma levels of the distal gut hormone PYY but not of the proximal gut hormone CCK. The elevated plasma levels of PYY after intraduodenal administration of MCT even at the low dose suggest that MCT were not completely absorbed but reached the distal gut and stimulated PYY release. These findings have raised questions about the concept of rapid hydrolysis and absorption of MCT. Furthermore, the accelerated small intestinal transit induced by MCT may even have detrimental effects on absorption since small intestinal transit is often already accelerated in disorders associated with malabsorption.

Altered activation of the ileal brake with enhanced endogenous PYY release has been reported in malabsorptive diseases. This could be due to increased loads of unabsorbed nutrients in the distal gut. However, increased levels of plasma PYY may also be related to, or result from the underlying disease. The study in **chapter VI** was performed to investigate whether artificially induced accelerated transit with reduced intestinal absorption of nutrients per se is able to stimulate PYY release and activate the ileal brake mechanism. The osmotic laxative magnesium sulphate (MgSO_4) was used to induce the artificial malabsorptive state in healthy subjects. Antroduodenal and gallbladder motility and proximal and distal gut hormone secretion were measured. In order to differentiate between the effects of reduced intraluminal nutrient absorption and those of MgSO_4 itself on gastrointestinal motility and secretion, experiments with MgSO_4 were performed both in the fed and fasted state. The results showed that oral magnesium sulphate significantly accelerated small intestinal transit both in the fasting and fed state. During the interdigestive state, MgSO_4 significantly modulates antroduodenal motility without changes in intestinal hormone secretion. On the other hand, postprandial antroduodenal motility

remains unaffected after the administration of MgSO_4 . When given in combination with a fatty meal, MgSO_4 induced diarrhoea in all healthy subjects with a significantly higher faecal weight and faecal fat excretion compared to placebo. Postprandial plasma levels of the distal gut hormone PYY were significantly increased in parallel with an increase in gallbladder volume (relaxation) after the administration of MgSO_4 . The increase in plasma PYY levels and gallbladder relaxation in the late postprandial phase can be considered as evidence which indicates that MgSO_4 induced malabsorption activates the ileal brake mechanism in healthy subjects.

Ileal brake under pathophysiological conditions

Chronic pancreatitis with exocrine pancreatic insufficiency is one of many causes leading to maldigestion and malabsorption. Gastrointestinal motility and hormonal changes have been reported in association with chronic pancreatitis but results of published studies are not in line. We hypothesized that these differences in results may be related to the presence of exocrine insufficiency in chronic pancreatitis patients. Therefore we investigated in **chapter VII** digestive and interdigestive antroduodenal motility and release of gastrointestinal hormones CCK, PP and peptide YY (PYY) in a large group of CP patients. The patients were divided into groups with and without exocrine pancreatic insufficiency. In order to further elucidate the role of exocrine insufficiency and subsequent maldigestion, we also studied the effect of exocrine pancreatic enzyme supplementation on the aforementioned parameters. Results were compared with those obtained in healthy controls. Our results demonstrated that in patients with chronic pancreatitis 1) the duration of postprandial antroduodenal motility is significantly prolonged and early

postprandial antral motility was significantly reduced; 2) the interdigestive MMC cycle length is significantly reduced due to shortening of phase II and 3) endogenous secretion of CCK and PP is decreased while PYY secretion was increased. These abnormalities were found in patients with exocrine pancreatic insufficiency but not in those with normal exocrine pancreatic function. Differences in antroduodenal interdigestive and digestive motility pattern and enhanced PYY release that we found between patients with and without exocrine insufficiency suggest that the observed abnormalities are related to exocrine insufficiency and subsequent maldigestion but not to chronic pancreatitis per se. Normalization of antroduodenal motility and postprandial plasma PYY secretion after pancreatic enzyme supplementation should be considered as evidence supporting this concept.

Malabsorption and abdominal symptoms are also frequently found in systemic sclerosis (SSc), a multisystem disorder, with gastrointestinal (GI) tract involvement. There are two forms of SSc, the limited and the diffuse cutaneous form. It has been assumed that GI tract involvement occurs late in the course of the disease of patients with limited SSc whereas in the diffuse cutaneous form there is early visceral involvement. However, there are no data available comparing GI motility disturbances between these two subsets of SSc patients. In **Chapter VIII** we investigated antroduodenal motility and proximal and distal gut hormone release in patients with SSc with limited and diffuse cutaneous forms. Data were related to esophageal manometry findings and gastrointestinal symptoms. We found that esophageal motor function abnormalities (low LESP and peristaltic wave amplitudes) were equally distributed among patients with diffuse and limited type disease. Alterations in antroduodenal motility, on the other hand, were more pronounced among

patients with the limited type SSc. In addition, our results also demonstrated that antroduodenojunal motility differed qualitatively between patients with limited and diffuse cutaneous forms of SSc. Intestinal motility in the limited type scleroderma is characterized by myopathic changes (reduced number of contractions with low amplitude) and the diffuse type by neuropathic changes (non-propagated, uncoordinated contractions). Not only gastrointestinal motility but also gastrointestinal hormone secretion was affected in patients with SSc. Plasma levels of CCK were significantly decreased in patients with limited disease. This might be explained by the reduced number of CCK producing cells in the upper small intestine as a result of fibrosis and atrophy or an inadequate intraluminal nutrient stimulation due to pancreaticobiliary insufficiency which is frequently found in patients with SSc. The observations that plasma levels of PYY were only increased in patients with limited disease and that a majority of these patients had gastrointestinal symptoms including steatorrhoea suggest that the enhanced PYY release is secondary, resulting from changes in GI function induced by SSc and not primarily related to SSc.

Apart from changes associated with maldigestion and malabsorption, the feedback function of the ileal brake could also be altered due to mucosal defects or after resection of distal small intestine, as in patients with Crohn's disease. The fact that the prevalence of gallstones is higher in Crohn's patients compared to age-matched healthy subjects gives rise to questions concerning possible mechanisms underlying the gallstone formation in these patients. Because the role of gallbladder motility in the pathogenesis of gallstone formation in Crohn's disease is not clearly ascertained the study in **chapter IX** was performed to evaluate fasting and meal-stimulated gallbladder motility in Crohn's disease. In order to answer the question whether changes in

gallbladder motility are explained by altered ileal brake function due to disease localisation or bowel resection we investigated gallbladder motility in a large group of Crohn's disease patients with different disease localisation (ileal or colonic) and with or without ileocecal resection. As the gallbladder motor response to a meal is mainly controlled by various gut hormones released during the intestinal phase, basal and meal-stimulated plasma concentrations of proximal and distal gut hormones are also measured. The results showed that patients with inactive Crohn's disease of the large bowel and patients after ileocecal resection have smaller fasting gallbladder volumes and increased basal plasma CCK levels. Although in line with previous results demonstrating that basal plasma CCK was increased and fasting gallbladder volume was decreased in patients after colectomy with ileal pouch anal anastomosis, the functional significance of these findings remains unclear. Furthermore, the observation that fasting plasma PYY levels were not significantly different between the patient groups compared to controls suggests that PYY did not account for the differences in fasting gallbladder volume found between these patient groups. In addition, gallbladder motility and subsequent hormone release in response to a meal were not affected in patients with inactive Crohn's disease irrespective of disease localization and previous bowel resection. Thus, we conclude that gallbladder (dys)motility does not seem to be a factor contributing to gallstone formation in patients with Crohn's disease.

CONCLUSIONS AND CONSIDERATIONS

1. Feedback control mechanisms from the distal to the proximal gut differ with respect to the site of stimulation. Effects of the fat induced ileal brake on

proximal small intestine, postprandial gallbladder motility and hormone release are qualitatively and quantitatively different from those evoked by the fat induced jejunal brake.

2. Nutrients in the distal jejunum do not activate exocrine pancreatic secretion while gallbladder motility is preserved. From a clinical point of view, this provides useful information concerning the nutritional support route in the acute phase of pancreatitis. Future study in patients with acute pancreatitis is needed to elaborate on this concept.
3. Activation of the ileal brake reduces gallbladder emptying, or in other words, stimulates gallbladder relaxation, especially in the late postprandial phase. It remains, however, debatable whether this action is hormonally mediated through PYY release.
4. Gallbladder (dys)motility does not seem to contribute to gallstone formation in patients with Crohn's disease. Disease localisation and ileocecal resection do not appear to significantly affect fasting nor meal stimulated plasma PYY release.
5. Ileal brake induced satiety and proximal gastric relaxation are not mediated through PYY release. In order to produce a significant effect on satiety and food intake the dose of exogenous PYY infusion must probably be in the supraphysiological range.
6. Medium chain triglycerides accelerate intestinal transit and stimulate PYY release. The latter implies that MCT are not completely absorbed but reached the distal gut. This observation, thus, casts doubt on the concept of rapid hydrolysis and absorption of MCT.
7. Activation of the ileal brake with subsequent PYY release in malabsorptive diseases is most likely secondary, resulting from malabsorption rather than

primarily due to the underlying disease. The findings that laxative induced malabsorption activates the ileal brake mechanism in healthy subjects and that pancreatic enzyme supplementation normalises antroduodenal motility and postprandial plasma PYY secretion in chronic pancreatitic patients with exocrine pancreatic secretion are clear evidence supporting this concept.

SAMENVATTING

Sinds de eerste omschrijving van de “ileal brake” in 1984 zijn verscheidene aspecten ervan in diermodellen onderzocht. Over de “ileal brake” bij de mens is echter weinig bekend en de huidige gegevens spreken elkaar vaak tegen. De experimenten die in dit proefschrift staan beschreven werden verricht om meer inzicht te krijgen in de fysiologie en de pathofysiologie van de “ileal brake” bij de mens.

De “ileal brake” onder fysiologische omstandigheden

In **hoofdstuk II** wordt een vergelijkend onderzoek beschreven naar het effect van de door vet geïnduceerde “ileal brake” en “jejunal brake” op de motoriek van de maag, de dunne darm en de galblaas in de nuchtere toestand en na de maaltijd. Intra-jejunale en intra-ileale toediening van vet verlengt de duur van het gevoed patroon en vermindert de duodenale en jejunale motiliteitsindex (MI). De afname in MI is meer uitgesproken tijdens intra-ileale dan tijdens intra-jejunale vettoediening. De bevinding dat de duur van het gevoed patroon alleen met postprandiale plasma PYY maar niet met postprandiale plasma CCK spiegels correleert, doet een rol van PYY in de overgang van gevoed naar nuchter motoriek patroon vermoeden. Dit staat tegenover de rol die CCK speelt bij de overgang van nuchter naar gevoed patroon. De zogenaamde “migrating motor complex” cycli (MMC), het interdigestieve motoriek patroon, zijn korter tijdens intra-ileale vet infusie terwijl intra-jejunale vet infusie de MMC cyclus verlengt. Hoewel de onderliggende mechanismen niet geheel duidelijk zijn, komen deze bevindingen overeen met de resultaten van eerdere studies. De MMC

cycluslengte is bijvoorbeeld korter bij patiënten met malabsorptie met toegenomen hoeveelheid onverteerd voedsel in de distale dunne darm. Tot op heden is er weinig bekend over de effecten van de aanwezigheid van vet in het ileum op de motoriek van de galblaas. De resultaten van deze studie laten zien dat intra-ileale vet infusie een significante afname van de postprandiale galblaascontractiliteit geeft terwijl intra-jejunale vet infusie tot een toename van de galblaascontractie leidt. Onze bevinding dat de galblaasmotiliteit alleen met plasma CCK spiegels maar niet met plasma PYY spiegels correleert, suggereert dat PYY geen belangrijke rol speelt bij de regulatie van de postprandiale galblaasmotoriek. Ook eerdere studies hebben aangetoond dat PYY geen inhiberend effect heeft op de CCK geïnduceerde galblaascontractie. Samengevat laten de resultaten beschreven in hoofdstuk II zien dat in het geval van de door vet geïnduceerde “ileal brake” het feedback mechanisme op de proximale dunne darm motoriek, de postprandiale galblaasmotoriek en de hormoon afgifte zowel kwalitatief als kwantitatief anders verloopt dan bij de door vet geïnduceerde “jejunal brake”.

Uitgaande van het concept dat het distale darmkanaal een remmende werking heeft op het proximale deel veronderstelden we stimulatie van de exocriene pancreassecretie afneemt als nutriënten distaler in de dunne darm worden toegediend. Dit is vooral van belang bij de behandeling van acute pancreatitis aangezien de beste behandeling voor deze patiënten bestaat uit het behouden van de integriteit van het darmslijmvlies en tegelijkertijd minimale stimulatie van de exocriene enzymsecretie. De studie beschreven in **hoofdstuk III** werd uitgevoerd om het effect te vergelijken van nutriënten in het proximale versus het distale jejunum op de exocriene pancreassecretie, galblaas contractie en hormoonsecretie in gezonde vrijwilligers. Als testmaaltijd gebruikten we

Nutrison standaard sondevoeding welke continu en in dezelfde hoeveelheid gegeven werd bij patiënten met acute pancreatitis. De resultaten tonen aan dat continu toediening van sondevoeding in het distale jejunum de exocriene pancreassecretie niet stimuleert, terwijl toediening van sondevoeding in het proximale jejunum dit wel doet. Toediening van nutriënten in het distale jejunum induceert een grotere mate van galblaascontractie dan toediening in het proximale jejunum. De afname van galblaascontractie gaat gepaard met een daling van de intraduodenale bilirubine uitscheiding tijdens distale enterale voeding. Omdat er geen verschillen in plasma CCK, neurotensin en PYY concentraties gevonden worden, is het onwaarschijnlijk dat de beschreven veranderingen hormonaal gemedieerd worden maar eerder het gevolg zijn van verminderde activatie van enteropancreatische reflexen. Deze bevindingen kunnen een belangrijke bijdrage leveren aan de vraag hoe patiënten met acute pancreatitis gevoed moeten worden. Toediening van voeding in het distale jejunum leidt niet tot exocriene pancreas enzymensecretie terwijl de galblaasmotoriek gehandhaafd blijft. Dit concept kan echter niet zomaar naar de klinische praktijk worden vertaald en dient dan ook verder in patiënten met acute pancreatitis te worden onderzocht.

Hoewel de laatste jaren veel aandacht is besteed aan het hormoon PYY als potentiële eetlust remmer, is een duidelijke associatie tussen de “ileal brake” en verzadiging niet beschreven. Aangezien PYY de “ileal brake” mogelijk medieert, is het denkbaar dat activatie van de “ileal brake” met endogene PYY secretie ook tot verzadiging leidt en voedselinname remt. Om dit te onderzoeken wordt in **hoofdstuk IV** het effect van “ileal brake” activatie door middel van intra-ileale vet infusie (endogene PYY secretie) vergeleken met exogene infusie van PYY-36 op verzadiging en op proximale maag

motoriek. Ten eerste werd het effect van intra-ileale vet infusie en de daarop volgende endogene PYY secretie bestudeerd. Vervolgens onderzochten we het dosis-respons effect van exogeen PYY. De resultaten laten duidelijk zien dat in nuchtere gezonde vrijwilligers intra-ileale vet infusie verzadiging en het verzadigingsgevoel in de gevoede toestand versterkt. Voorts leidt intra-ileale vet infusie tot een significante toename van het nuchtere proximale maagvolume en de proximale maagrelaxatie na de maaltijd en wordt de endogene PYY secretie gestimuleerd. Toediening van lage en hoge doseringen exogeen PYY heeft echter geen invloed op verzadiging, noch op motoriek en sensibiteit van de proximale maag. Deze resultaten zijn in tegenstelling tot exogene PYY welke bij zowel lage als hoge dosering, geen invloed heeft op verzadiging noch proximale maag motoriek en secretie functies. Deze bevindingen pleiten daarom tegen een rol voor PYY als fysiologische mediator van de door intra-ileale vet infusie geïnduceerde relaxatie en accommodatie van de maag. Onze bevindingen wat betreft exogene PYY infusie zijn niet in overeenstemming met eerdere studies waarin wordt beschreven dat PYY duidelijk de voedselinname remt. De meest waarschijnlijke verklaring voor deze discrepantie is een verschil in de dosering van exogene PYY. Een recente studie toonde aan dat alleen een hoge, farmacologische dosering van PYY een significante toename van verzadiging en een significante afname van voedselinname geeft. In onze studie zijn de plasma spiegels van PYY na exogene toediening in de fysiologische postprandiale range. Waarschijnlijk zijn suprafysiologische doseringen van PYY vereist om een effect op verzadiging en voedselinname uit te oefenen.

In **hoofdstuk V** gebruiken we het concept van de “ileal brake” en endogene PYY secretie om de hypothese te testen dat middellange-keten triglyceriden (MCT) minder snel worden geabsorbeerd, hoewel in het algemeen vanuit wordt gegaan, dat de absorptie van MCT vollediger en sneller is dan van lange-keten triglyceriden (LCT). Deze hypothese komt voort uit de observatie dat patiënten die MCT gebruiken vaak klagen over misselijkheid, borborygmi, krampen, buikpijn en diarree. De resultaten bevestigen dat MCT een significante afname van de dunne darm passagetijd geeft in vergelijking met LCT en fysiologisch zout. Intraduodenale toediening van MCT leidt tot een significante stijging in de plasma concentratie van het distale darm hormoon PYY maar niet van het proximale darm hormoon CCK. De stijging van plasma PYY, ook bij een lage dosering MCT in het duodenum, suggereert dat MCT niet volledig geabsorbeerd wordt maar gedeeltelijk de distale dunne darm bereikt en aldaar de secretie van PYY stimuleert. Deze bevindingen roepen vragen op omtrent de veronderstelling dat MCT volledig en snel geabsorbeerd wordt. De versnelde dunne darm passage, geïnduceerd door MCT, kan zelfs de absorptie aanzienlijk verslechteren aangezien de dunne darm passage reeds versneld is bij patiënten met malabsorptie.

Veranderde activatie van de “ileal brake” en toegenomen endogene PYY secretie zijn reeds beschreven bij patiënten met malabsorptie. Dit kan veroorzaakt worden door een toegenomen hoeveelheid onverteerd voedsel in de distale dunne darm. Toegenomen PYY secretie kan echter ook gerelateerd zijn aan of juist het gevolg zijn van de onderliggende ziekte. De studie beschreven in **hoofdstuk VI** onderzoekt of een kunstmatig geïnduceerde versnelde dunne darm passagetijd en de bijbehorende afgenomen intestinale absorptie in staat is om PYY secretie te stimuleren en de “ileal brake” te

activeren. Het osmotisch laxans magnesiumsulfaat (MgSO_4) werd gebruikt om een malabsorptieve toestand bij gezonde vrijwilligers te creëren. Antroduodenale motiliteit, galblaasmotoriek en proximale en distale hormoonsecretie door de dunne darm werden gemeten. De experimenten met MgSO_4 werden uitgevoerd zowel in gevoede als in nuchtere toestand om onderscheid te kunnen maken tussen het effect van verminderde intraluminale absorptie en het effect van MgSO_4 op gastrointestinale motiliteit en secretie. De resultaten laten zien dat orale toediening van MgSO_4 een significante versnelling geeft van de dunne darm passagetijd zowel in gevoede als in nuchtere toestand. Antroduodenale motiliteit wordt beduidend beïnvloed door magnesiumsulfaat in de nuchtere toestand terwijl de hormoonsecretie onveranderd blijft. Postprandiale antroduodenale motiliteit daarentegen verandert niet na toediening van magnesiumsulfaat. In combinatie met een vetrijke maaltijd veroorzaakt MgSO_4 in alle gezonde vrijwilligers diarree met een beduidend hoger fecaal gewicht en toegenomen fecale vet excretie in vergelijking met placebo. Postprandiale plasmaspiegels van het distale darmhormoon PYY zijn significant hoger na toediening van MgSO_4 . Hogere plasma PYY spiegels gaan gepaard met een stijging van galblaas volumina. Deze bevindingen wijzen erop dat de door MgSO_4 veroorzaakte malabsorptie de “ileal brake” in gezonde vrijwilligers activeert.

De “ileal brake” onder pathofysiologische omstandigheden

Chronische pancreatitis (CP) met exocriene insufficiëntie is één van vele oorzaken die tot maldigestie en malabsorptie kunnen leiden. Veranderingen in gastrointestinale motiliteit en hormoonsecretie zijn beschreven bij patiënten met chronische pancreatitis maar de gegevens zijn niet eenduidig.

Mogelijkerwijs is de aanwezigheid van exocriene insufficiëntie bij patiënten met CP de oorzaak van de verschillen in eerdere resultaten. Daarom onderzochten we in **hoofdstuk VII** de digestieve en interdigestieve antroduodenale motiliteit en secretie van de gastrointestinale hormonen CCK, PP en PYY in een grote groep CP patiënten. Patiënten werden verdeeld in een groep met en een groep zonder exocriene insufficiëntie. Om de rol van exocriene pancreasinsufficiëntie en maldigestie te onderzoeken bestudeerden wij ook het effect van exocriene pancreasenzym-suppletie op de bovengenoemde parameters. De resultaten werden vergeleken met die verkregen in gezonde controles. We vonden dat bij patiënten met chronische pancreatitis 1) de duur van het gevoed patroon langer is en de postprandiale antrale motiliteit verminderd is in de vroeg postprandiale fase; 2) de interdigestieve MMC cycluslengte beduidend korter is door een verkorting van fase II en 3) de secretie van CCK en PP afgenomen is terwijl de PYY secretie toegenomen is. Deze bevindingen zijn gevonden bij patiënten met exocriene pancreasinsufficiëntie maar niet bij die met normale exocriene pancreasfunctie. De gevonden verschillen in interdigestieve en digestieve antroduodenale motiliteit en PYY secretie tussen patiënten met en zonder exocriene pancreasinsufficiëntie suggereren dat deze vooral samenhangen met exocriene insufficiëntie en bijbehorende maldigestie, en niet zozeer met chronische pancreatitis zelf. De normalisering van de antroduodenale motiliteit en de postprandiale PYY secretie na pancreasenzym-suppletie ondersteunt deze hypothese. Malabsorptie en buikklachten komen vaak voor bij systemische sclerose (SSc), een systeemziekte waarbij de tractus digestivus betrokken is. SSc komt voor in de gelimiteerde en de diffuus-cutane vorm. Hoewel gesuggereerd is dat de tractus digestivus pas laat in de

ziekte betrokken raakt bij patiënten met gelimiteerd SSc maar vroeg bij diffuus SSc, zijn er geen vergelijkende beschikbaar. In **hoofdstuk VIII** onderzochten we de antroduodenojejunale motiliteit en de proximale en distale darmhormoonsecretie in patiënten met gelimiteerd en diffuus SSc. De resultaten werden gecorreleerd met bevindingen bij slokdarmmanometrie en gastrointestinale symptomen. Wij vonden dat afwijkingen in de slokdarmmotoriek (lage druk in de onderste slokdarmsfincter en peristaltische amplitude) niet verschilden tussen patiënten met diffuus en gelimiteerd SSc. Veranderingen in antroduodenojejunale motiliteit zijn echter meer uitgesproken bij patiënten met gelimiteerd SSc. Bovendien verschilt de antroduodenojejunale motiliteit kwalitatief tussen patiënten met gelimiteerd en diffuus SSc. Intestinale motiliteit in gelimiteerd SSc wordt gekenmerkt door myopathische veranderingen (verminderd aantal contracties met lage amplitude) en die van het diffuse type wordt gekenmerkt door neuropathische veranderingen (niet-voortgeleide, ongecoördineerde contracties). Niet alleen gastrointestinale motiliteit maar ook gastrointestinale hormoonsecretie is veranderd in patiënten met SSc. Plasma CCK spiegels zijn aanzienlijk lager in patiënten met gelimiteerd SSc. Dit zou verklaard kunnen worden door een afgenomen aantal CCK-producerende cellen in de proximale dunne darm als gevolg van bindweefsel-formatie en atrofie, of een onvoldoende intraluminale vertering van voedsel door pancreasinsufficiëntie die vaak bij patiënten met SSc wordt gevonden. De observatie dat plasma PYY spiegels alleen verhoogd zijn in patiënten met gelimiteerd SSc en dat de meerderheid van deze patiënten gastrointestinale symptomen heeft suggereert dat toegenomen PYY secretie het gevolg is van een SSc-gerelateerde verandering in gastrointestinaal functioneren maar niet primair gerelateerd is aan de ziekte

zelf.

De terugkoppelingsfunctie van de “ileal brake” wordt niet alleen beïnvloed door maldigestie en malabsorptie, maar ook door mucosale afwijkingen en/of darmresecties, zoals bij patiënten met de ziekte van Crohn. Het feit dat de prevalentie van galstenen hoger is in Crohn patiënten in vergelijking met een gezonde groep van gelijke leeftijd roept vragen op omtrent de mogelijke mechanismen die aan de galsteenvorming in deze patiënten ten grondslag liggen. Omdat de rol van galblaasmotiliteit bij het ontstaan van galsteen bij de ziekte van Crohn nog niet duidelijk is wordt in **hoofdstuk IX** een onderzoek naar nuchtere en maaltijd-gestimuleerde galblaasmotiliteit in patiënten met de ziekte van Crohn uitgevoerd. Om de vraag te beantwoorden of veranderingen in de galblaasmotiliteit toe te schrijven zijn aan de veranderingen in de “ileal brake” die het gevolg zijn van ziekte localisatie of darmresectie, onderzochten wij de galblaasmotiliteit in een groep patiënten met de ziekte van Crohn met verschillende ziekte localisaties (ileum of dikke darm) en met of zonder ileocecaal-resectie. Aangezien postprandiale galblaasmotiliteit door diverse darmhormonen gereguleerd wordt, werden de nuchtere en postprandiale plasmaconcentraties van proximale en distale darmhormonen gemeten. Deze laten zien dat patiënten met een inactieve Crohn van de dikke darm en patiënten na ileocecaal-resectie kleinere nuchtere galblaasvolumina en hogere nuchtere plasma CCK spiegels hebben. Hoewel deze bevindingen in overeenstemming zijn met eerdere studies, waarin gevonden werd dat nuchtere plasma CCK spiegels verhoogd en nuchtere galblaasvolumina verlaagd zijn bij patiënten na een colectomie met een ileo-anale anastomose met aanleggen van een pouch, blijft de functionele betekenis ervan onduidelijk. Voorts suggereren de gelijke nuchtere plasma PYY

spiegels in patiënten en controles dat PYY niet verantwoordelijk is voor het gemeten verschil in nuchtere galblaasvolumina. Postprandiale galblaasmotiliteit en hormoonsecretie zijn bovendien niet veranderd bij patiënten met inactieve Crohn, ongeacht localisatie van de ziekte en/of voorafgaande darmresectie. De conclusie van deze studie luidt dan ook dat galblaas (dis)motiliteit waarschijnlijk geen rol speelt bij de vorming van galsteen bij patiënten met de ziekte van Crohn.

CONCLUSIES

1. Het terugkoppelingsmechanisme van het distale naar het proximale deel van de tractus digestivus hangt af van het punt waar stimulatie plaatsvindt. De effecten van vet infusie in het ileum op de proximale dunne darm, postprandiale galblaasmotoriek en hormoonsecretie zijn kwalitatief en kwantitatief verschillend van die geïnduceerd door intrajeunale vet infusie.
2. De aanwezigheid van nutriënten in het distale jejunum leidt niet tot activatie van exocriene pancreasenzymsecretie terwijl galblaasmotoriek gehandhaafd blijft. Voor de kliniek levert dit gegeven een belangrijke bijdrage aan de vraag hoe patiënten met acute pancreatitis het beste gevoed kunnen worden.
3. Activatie van de “ileal brake” remt de galblaascontractie en bevordert de galblaasrelaxatie vooral in de vroege postprandiale fase. Het blijft echter onduidelijk of dit effect hormonaal gemedieerd wordt door PYY secretie.
4. Galblaas (dis)motiliteit lijkt niet bij te dragen aan galsteenvorming bij patiënten met de ziekte van Crohn. De localisatie van de ziekte en voorgaande ileocecaal-resectie beïnvloeden de nuchtere en postprandiale PYY secretie niet.

5. De door de “ileal brake” veroorzaakte verzadiging en proximale maagrelaxatie worden niet gemedieerd door PYY secretie. Om een significant effect op verzadiging en voedselinname te bereiken moet de exogene PYY infusie zich waarschijnlijk in de suprafysiologische range bevinden.
6. Middellange-keten triglyceriden (MCT) induceren een versnelde dunne darm passage en stimuleren PYY secretie. Dit laatste impliceert dat MCT niet volledig geabsorbeerd worden en de distale darm kunnen bereiken. Deze bevinding pleit tegen het concept van een snelle hydrolyse en absorptie van MCT.
7. Activatie van de “ileal brake” met PYY secretie in aandoeningen die gepaard gaan met malabsorptie is zeer waarschijnlijk het gevolg van de malabsorptie zelf en niet primair als gevolg van de onderliggende ziekte. Dit concept wordt ondersteund door de bevindingen dat 1) de “ileal brake” bij gezonde vrijwilligers kan worden geactiveerd door malabsorptie ten gevolge van laxantia-gebruik en 2) suppletie van exocriene pancreasenzymen bij patiënten met exocriene pancreasinsufficiëntie de antroduodenale motiliteit en postprandial plasma PYY secretie normaliseert.

ACKNOWLEDGMENTS

First and foremost, I thank the many healthy volunteers and patients for their great contributions. Without them it would have been impossible to write this thesis.

Sincere thanks to Eveline Muller and to the late Jan Paul Gilliams, whom I remember with gratitude, for their endless effort in processing the vast numbers of blood samples.

Planning the experiments was sometimes even more complicated than the experiments themselves. For all their help in this, I thank Carlien de Jong, Cindy Magdalena and Jolet Kerkvliet.

Thanks to my college researchers, Hugo Gielkens, Marco Verkijk, Jan Willem Straathof, Banafsche Mearadji, André van Peterson and especially Corine Penning, for their peer support.

I have enjoyed a great deal working with the many students. I appreciate their enthusiasm and diligence.

And last but not least, I thank Patrick van de Veek for making the “samenvatting” legible.

PUBLICATIONS

- Masclee AA, **Vu MK**. Gallbladder motility in inflammatory bowel diseases. *Dig Liver Dis*. 2003; 35 Suppl 3:S35-8. Review
- Lindeboom MY, **Vu MK**, Ringers J, Masclee AA. Function of the proximal stomach after partial versus complete laparoscopic fundoplication. *Am J Gastroenterol*. 2003; 98(2):284-90
- Symersky T, **Vu MK**, Biemond I, Masclee AA. The effect of equicaloric medium-chain and long-chain triglycerides on pancreas enzyme secretion. *Clin Physiol Funct Imaging*. 2002 Sep; 22(5):307-11.
- Penning C, **Vu MK**, Delemarre JB, Masclee AA. Proximal gastric motor and sensory function in slow transit constipation. *Scand J Gastroenterol*. 2001 Dec;36(12):1267-73.
- Mearadji B, Penning C, **Vu MK**, van der Schaar PJ, van Petersen AS, Kamerling IM, Masclee AA. Influence of gender on proximal gastric motor and sensory function. *Am J Gastroenterol*. 2001 Jul;96(7):2066-73.
- **Vu MK**, Berkhoudt J, Van Oostayen JA, Lamers CB, Masclee AA. Effect of triglycerides with different fatty acid chain length on superior mesenteric artery blood flow. *Acta Physiol Scand*. 2001 Jan;171(1):37-41.
- **Vu MK**, Van Oostayen JA, Biemond I, Masclee AA. Effect of somatostatin on postprandial gallbladder relaxation. *Clin Physiol*. 2001 Jan;21(1):25-31.
- **Vu MK**, Gielkens HA, van Hogezaand RA, van Oostayen JA, Lamers CB, Masclee AA. Gallbladder motility in Crohn disease: influence of

disease localization and bowel resection. *Scand J Gastroenterol.* 2000 Nov;35(11):1157-62.

- van Petersen AS, **Vu MK**, Lam WF, Lamers CB, Ringers J, Masclee AA. Effects of hyperglycaemia and hyperinsulinaemia on proximal gastric motor and sensory function in humans. *Clin Sci (Lond).* 2000 Jul;99(1):37-46.
- **Vu MK**, Nouwens MA, Biemond I, Lamers CB, Masclee AA. The osmotic laxative magnesium sulphate activates the ileal brake. *Aliment Pharmacol Ther.* 2000 May;14(5):587-95.
- **Vu MK**, Ringers J, Arndt JW, Lamers CB, Masclee AA.
Prospective study of the effect of laparoscopic hemifundoplication on motor and sensory function of the proximal stomach. *Br J Surg* 2000 Mar;87(3):338-43.
- **Vu MK**, Vecht J, Eddes EH, Biemond I, Lamers CB, Masclee AA. Antroduodenal motility in chronic pancreatitis: are abnormalities related to exocrin insufficiency? *Am J Physiol Gastrointest Liver Physiol.* 2000 Mar;278(3):G458-66.
- **Vu MK**, Verkijk M, Muller ES, Biemond I, Lamers CB, Masclee AA. Medium chain triglycerides activate distal but not proximal gut hormones. *Clin Nutr.* 1999 Dec;18(6):359-63.
- **Vu MK**, van der Veek PP, Frolich M, Souverein JH, Biemond I, Lamers CB, Masclee AA. Does jejunal feeding activate exocrine pancreatic secretion? *Eur J Clin Invest.* 1999 Dec;29(12):1053-9.
- **Vu MK**, Straathof JW, vd Schaar PJ, Arndt JW, Ringers J, Lamers CB, Masclee AA. Motor and sensory function of the proximal

stomach in reflux disease and after laparoscopic Nissen fundoplication. *Am J Gastroenterol.* 1999 Jun;94(6):1481-9.

CURRICULUM VITAE

Name: My Kieu Vu

Born: Hanoi, Vietnam, December 1970

1988	Graduated high school, Hanoi
1989	Colloquium Doctum, Leiden University
1993-1994	Research student, Cardiovascular Research Unit, Edinburgh University, Scotland
1994	Master of Science, Medical Faculty, Leiden University
1994-1996	Rotating internship, Leiden University Medical Center
1996-1997	Research student, Department of Gastroenterology-Hepatology, Leiden University Medical Center
1997	M.D.
1997-1999	Research fellow, Department of Gastroenterology-Hepatology, Leiden University Medical Center
2000-2004	Resident, Internal Medicine, Groene Hart Hospital, Gouda and Leiden University Medical Center
2004-2007	Resident, Gastroenterology-Hepatology, Leiden University Medical Center. Thesis "Physiology and pathophysiology of the ileal brake in humans"
From Jan. 2008	Gastroenterologist, Rijnland Hospital, Leiderdorp

