Translational research in motor and sensory function of the upper GI tract

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Translationeel onderzoek naar de motorische en sensibele functie van het bovenste deel van het spijsverteringskanaal

(met een samenvatting in het Nederlands)

Proefschrift ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. W.H. Gispen, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 20 september 2007 des middags te 04.15 uur

door

Niels van Lelyveld

geboren op 20 juli 1976 te Groningen

Promotor Prof. dr. M. Samsom

Co-promotor Dr. J. ter Linde

Contents

Chapter 01

Introduction

Introduction

Functional dyspepsia

Functional dyspepsia (FD) is a common disorder seen in daily clinical practice, characterized by the presence of pain or discomfort in the upper abdomen in the absence of organic, systemic, or metabolic disease.[1;2] Functional dyspeptic patients complain about a variety of symptoms, which are frequently intermittent, and mostly related to food intake.[3] FD is associated with age, female gender, *H Pylori* infection, the use of NSAID's, heavy smoking, unemployment, and psychological disturbances.[4-6] Since reliable markers are not available (anatomic or biochemical abnormalities), the diagnosis is based upon symptoms, described in the Rome criteria.[1;2]

Functional dyspepsia and gastroduodenal motor and sensory abnormalities

Delayed gastric emptying is present in approximately 30% of FD patients.[7] The rate of gastric emptying is dependent on multiple factors, including storage of the ingested meal in the fundus of the stomach, grinding of the food particles in the antrum, pulsatile emptying of the stomach after relaxation of the pylorus, and duodenal motility.[8] The function of the proximal stomach is to temporarily store food and to regulate intragastric transport and antral filling. Adaptive proximal gastric relaxation is a vagally mediated reflex enabling a proximal gastric volume increase without a change in intragastric pressure and symptom induction.[9] Altered proximal gastric relaxation has been described in patients with FD, gastroesophageal reflux disease (GERD) (pre- and post-fundoplication), and diabetes mellitus.[10-14] Approximately 40% of FD patients have an impaired proximal gastric relaxation.[10]

Although visceral hypersensitivity is difficult to measure, and a gold standard is lacking, most agree that this is an important etiologic factor in the pathophysiology of FD. By inflating a bag in the proximal stomach (gastric barostat), the first perception or discomfort to gastric distention can act as a marker for visceral perception. FD patients reported upper abdominal sensations when the pressure in the balloon was raised above the minimal distending pressure, in contrast with healthy controls, who reported hardly any symptoms.[15;16] Hypersensitivity to fundic distension occurs in 34-48% of FD patients.[17;18] It has been demonstrated that FD patients are also hyper-

sensitive to antral distension.[19] The relative importance of proximal, as opposed to distal, gastric hypersensitivity in the induction of dyspeptic symptoms is not known.
In Addition stimulation of duodenal vagal afferents upon stimulation of small intestinal mechano- or specific chemoreceptors may be involved in gastric motor and sensory dysfunction and in the generation of upper abdominal symptoms in FD. Relaxation of the proximal stomach can be induced by distension of the duodenum and by infusion of nutrients into the duodenum.[20;21] It was shown that FD patients exhibit a less pronounced fundic relaxation in response to duodenal nutrients.[19] In addition, first perception of duodenal balloon distension occurs at significantly lower pressures in FD patients compared to healthy controls.[22]
Furthermore, it has been shown that intraduodenal infusion of acid induced a different response in patients compared to healthy controls. FD patients experienced nausea after the infusion and the duodenal motor activity was significantly altered.[23;24] Finally, an abnormal response to lipids in the duodenum has been suggested to play a role in the generation of dyspeptic symptoms.[21;25]

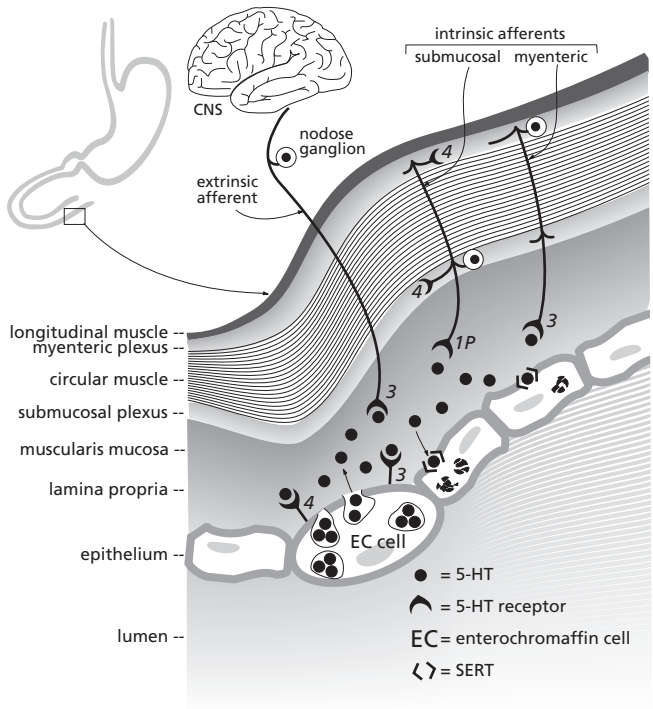
Aetiology of upper abdominal symptoms

It is well established that motoric and sensory abnormalities are highly prevalent in FD patients. However, while some investigators have reported statistically significant associations between dyspeptic symptoms and gastric motor and sensory abnormalities, these have in most cases been relatively weak, and there are at least as many studies that failed to do so. Impaired proximal gastric relaxation has been associated with early satiety and weight loss.[10;26] However, others were not able to confirm these findings.[18] Furthermore, a delayed gastric emptying has been associated with postprandial fullness and vomiting,[27] whereas others found no relationship between emptying rate and any upper abdominal symptoms.[28]
The key issue here is, whether gastric (dys)function is related to specific upper abdominal symptoms, and can be regarded as an etiologic factor of FD. This has been studied by various group, however no consensus has been reached so far. The clinical importance of this is evident, since gastric sensory or motor dysfunctions may serve as important therapeutic targets. To be considered an important therapeutic target there should be a clear causal relationship between (specific) symptoms and the pathophysiological

mechanism and correction should lead to symptom improvement.
The poor understanding of the relationships between dyspeptic symptoms and gastric motor and sensory abnormalities has hampered efforts to develop effective treatment modalities for functional dyspepsia.[29] Another major obstacle to the effective treatment of FD is that the molecular factors underlying the pathophysiological mechanisms and/or symptom generation are unknown. Identification of these molecular factors potentially provides a basis for the development of more targeted therapy and will ultimately improve the efficacy of treatment. Molecular factors may be related to a disordered function of receptors involved in the regulation of gastroduodenal motor and sensory function. An altered response may be due to specific receptor variants or abnormalities that are linked to the availability of the neurotransmitter or humoral factor at the receptor. In addition, an underlying second messenger abnormality may cause disordered receptor function. Genetic variants may also affect the response to the release of neurotransmitters or humoral factors. Considering its key role in regulating gastrointestinal motor and sensory function, we focused our attention on serotonin, in search of altered molecular factors or genetic variants in the pathogenesis of FD.

Serotonergic signalling

Within the bowel, serotonin (5-HT) is synthesized by the enterochromaffin (EC) cells in the mucosa and by myenteric neurons. EC cells are a subpopulation of the mucosal neuroendocrine cells, and they store serotonin in secretion granules at the base of the cell. Serotonin is excreted primarily into the lamina propria in response to intraluminal pressure or chemo specific properties of nutrients.[30] Tryptophan hydroxylase (TPH) catalyses the reaction of tryptophan to 5-hydroxy-L-tryptophan, which subsequently is converted to 5-HT. The expression level of TPH can be considered as a marker for serotonin synthesis, since TPH catalyses the rate-limiting step of the biosynthetic pathway. Two genes encoding TPH have been identified; TPH-1 and TPH-2.[31] TPH-1 is responsible for serotonin synthesis in non-neuronal cells such as neuroendocrine cells, whereas TPH-2 is expressed in neurons of the raphe nuclei and of the myenteric plexus.[32]
5-HT released from EC cells acts on 5-HT receptors situated on the mucosal projections of primary afferent neurons. These include extrinsic nerves, which transmit sensations of nausea and discomfort to the central nervous



Actions of serotonin released from enterochromaffin cells
Serotonin (5-HT) is synthesized by the enterochromaffin (EC) cells in the mucosa. Serotonin is excreted primarily into the lamina propria in response to intraluminal pressure or chemo specific properties of nutrients. 5-HT released from EC cells acts on 5-HT receptors situated on the mucosal projections of primary afferent neurons. These include extrinsic afferents, which transmit sensations of nausea and discomfort to the central nervous system (CNS) and the mucosal projections of intrinsic primary afferent neurons (IPANs). Submucosal IPANs initiate peristaltic and secretory reflexes, while myenteric IPANs initiate giant migrating contractions. 5-HT receptors are also situated on EC cells, modulating 5-HT release by autoregulatory mechanisms. 5-HT has to be removed rapidly from the neuroendocrine cell-sensory nerve junction to terminate responses and to prevent desensitisation of the receptors. A specific 5-HT transport protein (SERT), expressed by enterocytes, is responsible for this uptake.

system and the mucosal projections of intrinsic primary afferent neurons (IPANs).[33;34] Submucosal IPANs initiate peristaltic and secretory reflexes, while myenteric IPANs initiate giant migrating contractions.[35] 5-HT receptors are also situated on EC cells, modulating 5-HT release by autoregulatory mechanisms.[36-38] It has been shown that these mucosal non-neuronal

5-HT receptors modulate 5-HT induced fluid secretion.[39-41]
To date, seven 5-HT receptors have been identified, of which the 5-HT_3 and the 5-HT_4 receptor play an important role in gastrointestinal sensory and motor functions.[42;43] The 5-HT_4 receptor is a G protein-coupled receptor, linked to stimulation of adenylyl cyclase.[35] The 5-HT_3 receptor is a ligand-gated ion channel structured as a pentameric complex composed of 5 different subunits, termed A – E.[44-46]
5-HT has to be removed rapidly from the neuroendocrine cell-sensory nerve junction to terminate responses and to prevent desensitisation of the receptors. A specific 5-HT transport protein (SERT), expressed by enterocytes, is responsible for this uptake.[47]

Role of 5-HT in the pathophysiology of FD

Studies using agents intervening in serotonergic signalling indicate that serotonin is involved in upper GI motor functions, and upper abdominal symptom generation. Serotonin receptor agonists like the 5-HT_1P agonist sumatriptan, which reportedly enhanced relaxation of the gastric fundus,[48] and 5-HT_4 agonists, like cisapride, acting as a prokinetic drug enhancing gastric emptying rate,[49] are probably the best known examples. Furthermore, the 5-HT_3 receptor antagonist alosetron displayed a significant beneficial effect in relieving dyspeptic symptoms.[50] Selective serotonin reuptake inhibitors (SSRIs) were shown to increase meal induced relaxation of the gastric fundus.[51;52] Finally, tryptophan depletion is known to influence the rate of gastric emptying.[53] These findings point towards a possible role of serotonergic signalling in the pathogenesis of upper GI motoric and sensory abnormalities and the aetiology of dyspeptic symptoms. Serotonergic signalling abnormalities have been implicated in the pathophysiology of IBS, another functional GI disorder.[54-56] Altered expression of genes encoding components of serotonergic signalling at the mucosal level may result in abnormalities that are linked to synthesis (TPH-1) or the availability (SERT) of serotonin. An altered response may also be due to specific receptor variants. Seven 5-HT_4 receptor variants that differ in their C-termini due to alternative splicing have been identified.[57-59] Functional diversity, including level of constitutive activity, exists among the 5-HT_4 splice variants.[58;60;61] Thus, the distribution and expression levels of 5-HT_4 receptor splice variants affects 5-HT_4 mediated response. Furthermore, the subunit composition of the 5-HT_3 receptor influences the pharmacological and biophysical proper-

ties of the receptor, thereby determining excitability and receptor-mediated current.[62;63]

Genetic variants may also affect the response to the release of serotonin. A functional polymorphism has been identified in HTR3A, the gene coding for the 5-HT_3 receptor A subunit. An in vitro study has shown that this polymorphism is responsible for variations in HTR3A protein levels.[64] Changes in the subunit composition of the 5-HT_3 receptors lead to altered 5-HT receptor affinity and desensitization and hence in the response to 5-HT.[65]

A 44 bp insertion/deletion polymorphism is present in the 5' flanking promoter region of the SERT gene, creating a long (L) and short (S) allelic variant.[66] The presence of the short allele results in reduced SERT expression and 5-HT uptake capacity.[67] An association was found between the S/S genotype of the SERT promoter polymorphism and diarrhea predominant IBS.[55] Moreover, the genetic polymorphism in the SERT promoter region influences the response to serotonergic intervention in diarrhea predominant IBS.[68]

Putative second messenger candidates include the heterotrimeric G-proteins. A functional polymorphism is located in exon 10 (C825T) of the gene encoding the G protein β3 subunit (GNB3). The G protein affects the response to the release of serotonin and several other neurotransmitters modulating gastroduodenal sensory and motor function. The 825T allele is associated with alternative splicing of the gene, and an increased intracellular signal transduction.[69] In recent publications, an association with functional gastrointestinal disorders has been suggested.[70;71]

Aims and outline of this thesis

This thesis focuses on several topics: (1) upper abdominal symptoms and meal ingestion in patients with functional dyspepsia, all fulfilling the Rome II criteria for functional dyspepsia, (2) the relationship between gastrointestinal motility, and especially gastric emptying and proximal gastric relaxation, and upper abdominal symptoms, and (3) the role of serotonergic signalling in patients with functional dyspepsia.

We investigated the (patho)physiology of upper gastrointestinal motility and the generation of upper abdominal sensations in functional dyspeptic patients using several techniques. Three-dimensional ultrasonography (3D-US) is used to study meal-induced upper abdominal symptoms and total and partial gastric volume changes. 3D-US is a non-invasive tool for gastric

volume measurement, and allows us to differentiate between normal or impaired relaxation of the proximal and distal stomach. This technique has shown excellent in vitro and in vivo accuracy in volume estimation and a low inter observer variation.[12;13;72] The association between gastric function and chronic upper abdominal symptoms is assessed using the ^{13}C-octanoic breathtest for the measurement of gastric emptying.[73] Finally, the maximum drinking capacity of FD patients is determined by performing a nutrient drinktest. The nutrient drinktest has been suggested suitable for the measurement of meal-induced satiety, and as a non-invasive alternative for the detection of normal- or impaired accommodation of the stomach.[10;74] Many clinicians advice their FD patients to eat small size meals, in order to reduce upper abdominal symptoms. However, the rational of this advice is not fully understood. Therefore, we investigated the effect of the caloric content of a meal on total and partial gastric volumes and the generation of upper abdominal symptoms. In contrast with 5-HT$_4$ agonists, the effect of a 5-HT$_4$ antagonist on upper GI sensory-motoric function is unknown. In vitro studies have demonstrated an inhibitory effect of a 5-HT$_4$ receptor antagonist on SSRI induced contraction of the fundus, suggesting a beneficial effect on impaired accommodation of the proximal stomach, as seen in a subgroup of FD patients.[75] To investigate the effects of 5-HT$_4$ receptor antagonist we used R216073, a selective 5-HT$_4$ receptor antagonist and studied its mode of action on proximal gastric relaxation and maximum drinking capacity in patients with functional dyspepsia.

In our attempt to identify molecular factors underlying the gastroduodenal motor and sensory abnormalities and/or symptoms observed in patients with functional dyspepsia we focused on serotonergic signalling. The contribution of serotonergic signalling to normal gastroduodenal function is studied in several regions of the stomach and duodenum. Firstly, mRNA expression levels of genes encoding proteins responsible for 5-HT synthesis and inactivation, or affecting release are quantified by real-time PCR in mucosal biopsy specimens. In addition, by using immunohistochemistry, neuroendocrine cells and the subpopulation of 5-HT positive cells are counted in the three regions (fundus, antrum, and duodenum). Examination of these serotonergic signalling components in mucosal biopsy specimens from patients with idiopathic gastroparesis is performed to elucidate the role of abnormal serotonergic signalling in delayed gastric emptying and upper abdominal symptom generation. Serotonergic signalling may also be al-

tered as a consequence of genetic variants. Therefore, the genotype distribution of functional polymorphisms in the genes encoding the specific 5-HT transporter and one of the subunits of the 5-HT_3 receptor will be compared between healthy controls and tertiary referral FD patients. In addition, the association of a functional polymorphism in a gene involved in G-protein mediated signal transduction was tested.

In summary, the aim of the current thesis is to improve the understanding of the aetiology of dyspeptic symptoms. For that purpose, we have investigated:

(1) The relationship between dyspeptic symptoms and gastric motor and sensory function.
(2) Molecular factors potentially underlying the gastroduodenal motor and sensory abnormalities and/or the generation of upper abdominal symptoms.

References

1 Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR, Tytgat GN. Functional gastroduodenal disorders. Gut 1999; 45 Suppl 2:II37-II42.

2 Tack J, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR et al. Functional gastroduodenal disorders. Gastroenterology 2006; 130(5):1466-1479.

3 Feinle-Bisset C, Vozzo R, Horowitz M, Talley NJ. Diet, food intake, and disturbed physiology in the pathogenesis of symptoms in functional dyspepsia. Am J Gastroenterol 2004; 99(1):170-181.

4 Mahadeva S, Goh KL. Epidemiology of functional dyspepsia: a global perspective. World J Gastroenterol 2006; 12(17):2661-2666.

5 Wildner-Christensen M, Hansen JM, De Muckadell OB. Risk factors for dyspepsia in a general population: non-steroidal anti-inflammatory drugs, cigarette smoking and unemployment are more important than Helicobacter pylori infection. Scand J Gastroenterol 2006; 41(2):149-154.

6 Locke GR, III, Weaver AL, Melton LJ, III, Talley NJ. Psychosocial factors are linked to functional gastrointestinal disorders: a population based nested case-control study. Am J Gastroenterol 2004; 99(2):350-357.

7 Stanghellini V, Tosetti C, Paternic inverted question mA, Barbara G, Morselli-Labate AM, Monetti N et al. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. Gastroenterology 1996; 110(4):1036-1042.

8 Fraser RJ, Horowitz M, Maddox AF, Dent J. Postprandial antropyloroduodenal motility and gastric emptying in gastroparesis--effects of cisapride. Gut 1994; 35(2):172-178.

9 De Ponti F, Azpiroz F, Malagelada JR. Relaxatory responses of canine proximal stomach to esophageal and duodenal distension. Importance of vagal pathways. Dig Dis Sci 1989; 34(6):873-881.

10 Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998; 115(6):1346-1352.

11 Penagini R, Hebbard G, Horowitz M, Dent J, Bermingham H, Jones K et al. Motor function of the proximal stomach and visceral perception in gastro-oesophageal reflux disease. Gut 1998; 42(2):251-257.

12 Tefera S, Gilja OH, Olafsdottir E, Hausken T, Hatlebakk JG, Berstad A. Intragastric maldistribution of a liquid meal in patients with reflux oesophagitis assessed by three dimensional ultrasonography. Gut 2002; 50(2):153-158.

13 Scheffer RC, Gooszen HG, Wassenaar EB, Samsom M. Relationship Between Partial Gastric Volumes and Dyspeptic Symptoms in Fundoplication Patients: A 3D Ultrasonographic Study. Am J Gastroenterol 2004; 99(10):1902-1909.

14 Samsom M, Roelofs JM, Akkermans LM, Berge Henegouwen GP, Smout AJ. Proximal gastric motor activity in response to a liquid meal in type I diabetes mellitus with autonomic neuropathy. Dig Dis Sci 1998; 43(3):491-496.

15 Mearin F, Cucala M, Azpiroz F, Malagelada JR. The origin of symptoms on the brain-gut axis in functional dyspepsia. Gastroenterology 1991; 101(4):999-1006.

16 Salet GA, Samsom M, Roelofs JM, Berge Henegouwen GP, Smout AJ, Akkermans LM. Responses to gastric distension in functional dyspepsia. Gut 1998; 42(6):823-829.

17 Tack J, Caenepeel P, Fischler B, Piessevaux H, Janssens J. Symptoms associated with hypersensitivity to gastric distention in functional dyspepsia. Gastroenterology 2001; 121(3):526-535.

18 Boeckxstaens GE, Hirsch DP, Kuiken SD, Heisterkamp SH, Tytgat GN. The proximal stomach and postprandial symptoms in functional dyspeptics. Am J Gastroenterol 2002; 97(1):40-48.

19 Caldarella MP, Azpiroz F, Malagelada JR. Antro-fundic dysfunctions in functional dyspepsia. Gastroenterology 2003; 124(5):1220-1229.

20 Azpiroz F, Malagelada JR. Intestinal control of gastric tone. Am J Physiol 1985; 249(4 Pt 1):G501-G509.

21 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(3):G721-6.

22 Holtmann G, Goebell H, Talley J. Impaired small intestinal peristaltic reflexes and sensory thresholds are independent functional disturbances in patients with chronic unexplained dyspepsia. Am J Gastroenterol 1996; 91(3):485-91.

23 Samsom M, Verhagen MA, vanBerge Henegouwen GP, Smout AJ. Abnormal clearance of exogenous acid and increased acid sensitivity of the proximal duodenum in dyspeptic patients. Gastroenterology 1999; 116(3):515-520.

24 Schwartz MP, Samsom M, Smout AJ. Chemospecific alterations in duodenal perception and motor response in functional dyspepsia. Am J Gastroenterol 2001; 96(9):2596-2602.

25 Barbera R, Feinle C, Read NW. Abnormal sensitivity to duodenal lipid infusion in patients with functional dyspepsia. Eur J Gastroenterol Hepatol 1995; 7(11):1051-7.

26 Kim DY, Delgado-Aros S, Camilleri M, Samsom M, Murray JA, O'Connor MK et al. Noninvasive measurement of gastric accommodation in patients with idiopathic nonulcer dyspepsia. Am J Gastroenterol 2001; 96(11):3099-3105.

27 Sarnelli G, Caenepeel P, Geypens B, Janssens J, Tack J. Symptoms associated with impaired gastric emptying of solids and liquids in functional dyspepsia. Am J Gastroenterol 2003; 98(4):783-788.

28 Talley NJ, Verlinden M, Jones M. Can symptoms discriminate among those with delayed or normal gastric emptying in dysmotility-like dyspepsia? Am J Gastroenterol 2001; 96(5):1422-1428.

29 Cremonini F, Delgado-Aros S, Talley NJ. Functional dyspepsia: drugs for new (and old) therapeutic targets. Best Pract Res Clin Gastroenterol 2004; 18(4):717-733.

30 Bulbring E, Crema A. The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. J Physiol 1959; 146(1):18-28.

31 Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 2003; 299(5603):76.

32 Cote F, Thevenot E, Fligny C, Fromes Y, Darmon M, Ripoche MA et al. Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. Proc Natl Acad Sci U S A 2003; 100(23):13525-13530.

33 Pan H, Gershon MD. Activation of intrinsic afferent pathways in submucosal ganglia of the guinea pig small intestine. J Neurosci 2000; 20(9):3295-309.

34 Gershon MD. Nerves, reflexes, and the enteric nervous system: pathogenesis of the irritable bowel syndrome. J Clin Gastroenterol 2005; 39(5 Suppl):S184-93.

35 Gershon MD. Review article: serotonin receptors and transporters - roles in normal and abnormal gastrointestinal motility. Aliment Pharmacol Ther 2004; 20 Suppl 7:3-14.

36 Gebauer A, Merger M, Kilbinger H. Modulation by 5-HT3 and 5-HT4 receptors of the release of 5-hydroxytryptamine from the guinea-pig small intestine. Naunyn Schmiedebergs Arch Pharmacol 1993; 347(2):137-140.

37 Racke K, Reimann A, Schworer H, Kilbinger H. Regulation of 5-HT release from enterochromaffin cells. Behav Brain Res 1996; 73(1-2):83-87.

38 Schworer H, Ramadori G. Autoreceptors can modulate 5-hydroxytryptamine release from porcine and human small intestine in vitro. Naunyn Schmiedebergs Arch Pharmacol 1998; 357(5):548-552.

39 Borman RA, Burleigh DE. Human colonic mucosa possesses a mixed population of 5-HT receptors. Eur J Pharmacol 1996; 309(3):271-274.

40 Tuo BG, Sellers Z, Paulus P, Barrett KE, Isenberg JI. 5-HT induces duodenal mucosal bicarbonate secretion via cAMP- and Ca2+-dependent signaling pathways and 5-HT4 receptors in mice. Am J Physiol Gastrointest Liver Physiol 2004; 286(3): G444-G451.

41 Day J, King B, Haque SM, Kellum JM. A nonneuronal 5-hydroxytryptamine receptor 3 induces chloride secretion in the rat distal colonic mucosa. Am J Surg 2005; 190(5):736-738.

42 Foxx-Orenstein AE, Kuemmerle JF, Grider JR. Distinct 5-HT receptors mediate the peristaltic reflex induced by mucosal stimuli in human and guinea pig intestine. Gastroenterology 1996; 111(5):1281-1290.

43 Grider JR, Foxx-Orenstein AE, Jin JG. 5-Hydroxytryptamine4 receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. Gastroenterology 1998; 115(2):370-380.

44 Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG et al. The 5-HT3B subunit is a major determinant of serotonin-receptor function. Nature 1999; 397(6717):359-363.

45 Niesler B, Frank B, Kapeller J, Rappold GA. Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. Gene 2003; 310:101-111.

46 Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D. Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science 1991; 254(5030):432-437.

47 Wade PR, Chen J, Jaffe B, Kassem IS, Blakely RD, Gershon MD. Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract. J Neurosci 1996; 16(7):2352-2364.

48 Tack J, Coulie B, Wilmer A, Andrioli A, Janssens J. Influence of sumatriptan on gastric fundus tone and on the perception of gastric distension in man. Gut 2000; 46(4):468-473.

49 Veldhuyzen van Zanten SJ, Jones MJ, Verlinden M, Talley NJ. Efficacy of cisapride and domperidone in functional (nonulcer) dyspepsia: a meta-analysis. Am J Gastroenterol 2001; 96(3):689-696.

50 Talley NJ, Van Zanten SV, Saez LR, Dukes G, Perschy T, Heath M et al. A dose-ranging, placebo-controlled, randomized trial of alosetron in patients with functional dyspepsia. Aliment Pharmacol Ther 2001; 15(4):525-537.

51 Chial HJ, Camilleri M, Burton D, Thomforde G, Olden KW, Stephens D. Selective effects of serotonergic psychoactive agents on gastrointestinal functions in health. Am J Physiol Gastrointest Liver Physiol 2003; 284(1):G130-G137.

52 Tack J, Broekaert D, Coulie B, Fischler B, Janssens J. Influence of the selective serotonin re-uptake inhibitor, paroxetine, on gastric sensorimotor function in humans. Aliment Pharmacol Ther 2003; 17(4):603-608.

53 van Nieuwenhoven MA, Valks SD, Sobczak S, Riedel WJ, Brummer RJ. Acute tryptophan depletion slows gastric emptying in females. Br J Nutr 2004; 91(3):351-355.

54 Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H et al. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 2004; 126(7):1657-1664.

55 Yeo A, Boyd P, Lumsden S, Saunders T, Handley A, Stubbins M et al. Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. Gut 2004; 53(10):1452-1458.

56 Atkinson W, Lockhart S, Whorwell PJ, Keevil B, Houghton LA. Altered 5-hydroxytryptamine signaling in patients with constipation- and diarrhea-predominant irritable bowel syndrome. Gastroenterology 2006; 130(1):34-43.

57 Gerald C, Adham N, Kao HT, Olsen MA, Laz TM, Schechter LE et al. The 5-HT4 receptor: molecular cloning and pharmacological characterization of two splice variants. EMBO J 1995; 14(12):2806-2815.

58 Blondel O, Gastineau M, Dahmoune Y, Langlois M, Fischmeister R. Cloning, expression, and pharmacology of four human 5-hydroxytryptamine 4 receptor isoforms produced by alternative splicing in the carboxyl terminus. J Neurochem 1998; 70(6):2252-2261.

59 Bender E, Pindon A, van O, I, Zhang YB, Gommeren W, Verhasselt P et al. Structure of the human serotonin 5-HT4 receptor gene and cloning of a novel 5-HT4 splice variant. J Neurochem 2000; 74(2):478-489.

60 Mialet J, Berque-Bestel I, Sicsic S, Langlois M, Fischmeister R, Lezoualc'h F. Pharmacological characterization of the human 5-HT(4(d)) receptor splice variant stably expressed in Chinese hamster ovary cells. Br J Pharmacol 2000; 131(4):827-835.

61 Pindon A, van Hecke G, van Gompel P, Lesage AS, Leysen JE, Jurzak M. Differences in signal transduction of two 5-HT4 receptor splice variants: compound specificity and dual coupling with Galphas- and Galphai/o-proteins. Mol Pharmacol 2002; 61(1):85-96.

62 Stewart A, Davies PA, Kirkness EF, Safa P, Hales TG. Introduction of the 5-HT3B subunit alters the functional properties of 5-HT3 receptors native to neuroblastoma cells. Neuropharmacology 2003; 44(2):214-223.

63 Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC et al. The pharmacological and functional characteristics of the serotonin 5-HT(3A) receptor are specifically modified by a 5-HT(3B) receptor subunit. J Biol Chem 1999; 274(43):30799-30810.

64 Niesler B, Flohr T, Nothen MM, Fischer C, Rietschel M, Franzek E et al. Association between the 5' UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics 2001; 11(6):471-475.

65 Liu MT, Rayport S, Jiang Y, Murphy DL, Gershon MD. Expression and function of 5-HT3 receptors in the enteric neurons of mice lacking the serotonin transporter. Am J Physiol Gastrointest Liver Physiol 2002; 283(6):G1398-411.

66 Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996; 66(6):2621-2624.

67 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996; 274(5292):1527-1531.

68 Camilleri M, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE et al. Serotonin-transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. Gastroenterology 2002; 123(2):425-432.

69 Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R et al. Association of a human G-protein beta3 subunit variant with hypertension. Nat Genet 1998; 18(1):45-48.

70 Holtmann G, Siffert W, Haag S, Mueller N, Langkafel M, Senf W et al. G-protein beta 3 subunit 825 CC genotype is associated with unexplained (functional) dyspepsia. Gastroenterology 2004; 126(4):971-979.

71 Camilleri CE, Carlson PJ, Camilleri M, Castillo EJ, Locke GR, III, Geno DM et al. A study of candidate genotypes associated with dyspepsia in a U.S. community. Am J Gastroenterol 2006; 101(3):581-592.

72 Gilja OH, Detmer PR, Jong JM, Leotta DF, Li XN, Beach KW et al. Intragastric distribution and gastric emptying assessed by three-dimensional ultrasonography. Gastroenterology 1997; 113(1):38-49.

73 Minderhoud IM, Mundt MW, Roelofs JM, Samsom M. Gastric emptying of a solid meal starts during meal ingestion: combined study using 13C-octanoic acid breath test and Doppler ultrasonography. Absence of a lag phase in 13C-octanoic acid breath test. Digestion 2004; 70(1):55-60.

74 Tack J, Caenepeel P, Piessevaux H, Cuomo R, Janssens J. Assessment of meal induced gastric accommodation by a satiety drinking test in health and in severe functional dyspepsia. Gut 2003; 52(9):1271-1277.

75 James AN, Ryan JP, Parkman HP. Effects of the selective serotonin reuptake inhibitor, fluoxetine, on regional gastric contractility. Neurogastroenterol Motil 2005; 17(1):76-82.

Chapter 02

Partial gastric volumes and upper abdominal sensations in functional dyspeptic and GERD patients; a 3D ultrasonographic study

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Abstract

Background and aim

The aim of the study was to evaluate the change in proximal and distal gastric volumes after ingestion of a nutrient drink and its relationship to upper abdominal sensations using three-dimensional ultrasonography.

Methods

Fifty functional dyspeptic (FD) patients, 20 gastroesophageal reflux disease patients (GERD) patients and 35 healthy controls participated. Partial gastric volumes and sensations were assessed while fasting and after ingestion of a nutrient drink (500 ml, 300 kCal). Division of partial gastric volumes by total gastric volume calculated proximal and distal gastric volume ratios.

Results

The proximal gastric volume ratio was smaller in FD patients and larger in GERD patients compared to controls ($P < 0.001$ and $P = 0.008$ respectively). FD patients with impaired proximal relaxation (46%) had a larger increase in distal gastric volume ($P = 0.008$) and higher fullness sensations ($P = 0.027$) compared to FD patients with normal proximal relaxation. Fullness was related to distal gastric volume in both GERD patients and healthy controls ($r = 0.761$, $P < 0.001$ and $r = 0.674$, $P = 0.001$ respectively). In FD patients this relationship was not observed.

Conclusions

Impaired proximal gastric volume change after ingestion of a nutrient drink is associated with a larger distal gastric volume and increased fullness. In health and in GERD patients, the distal stomach is important in the regulation of fullness. However, in FD patients with normal or altered gastric volume distribution, this relationship is disturbed, implying that other causes are involved in the excessive generation of fullness.

Introduction

The function of the proximal stomach is to temporarily store food and to regulate intragastric transport and antral filling. Impaired proximal gastric relaxation has been described in patients with functional dyspepsia (FD), gastroesophageal reflux disease (GERD) (pre- and post-fundoplication) and diabetes mellitus.[1-5] Adaptive proximal gastric relaxation is a vagally mediated reflex enabling a proximal gastric volume increase without a change in intragastric pressure and symptom induction.[6]

Functional dyspepsia is a clinical entity commonly encountered by physicians. A decreased gastric emptying rate,[7] impaired proximal gastric relaxation[1;8] and hypersensitivity to gastric distension[9] have been described as pathophysiologic mechanisms in functional dyspepsia. Impaired proximal gastric relaxation has been associated with early satiety and weight loss.(1) However, a relationship between proximal gastric accommodation and other gastrointestinal sensations has not been reported.[10]

Despite many studies, the effect of impaired proximal gastric relaxation on symptom generation or on maximum tolerated volume is still controversial and the importance is matter of debate.[10-13] Many pharmaceutical agents, including sumatriptan (a 5-HT_1 receptor agonist)[14], cisapride (a 5-HT_4 agonists)[15] and glyceryl trinitrate[16], have been tested, in order to influence proximal gastric relaxation and upper GI symptoms. Although a fundus relaxing effect has been reported in several of these studies, most of these agents are not acting solely on the gastric fundus, but also influence gastric emptying.[17;18] Most importantly, the effect of these agents on upper abdominal symptoms remains to be elucidated.[10] Therefore, the question remains what the importance of impaired gastric accommodation is, and what is to be expected from therapeutic intervention in terms of relieve of upper abdominal symptoms.

Since impaired proximal relaxation is present in a large subset of patients with upper abdominal symptoms, we hypothesized a relationship between proximal or distal gastric relaxation and meal related symptoms. The aim of the study was to assess the change in proximal and distal gastric volumes in response to a nutrient drink using three-dimensional ultrasonography (3D-US), and to investigate the relationship between these volume changes and upper abdominal sensations in functional dyspeptic patients, GERD patients and healthy controls.

Materials and methods

Subjects

Three groups of subjects were studied: 50 FD patients (18 male; mean age 41 (19-64) years), 20 GERD patients (12 male; mean age 49 (29-69) and 35 healthy controls (16 male; mean age 31 (18-53) years.

All FD patients fulfilled the Rome II criteria, i.e. the presence of dyspeptic symptoms for at least 12 weeks in the last 12 months, in the absence of organic, systemic, or metabolic disease.[19] Before inclusion, each patient completed a symptom questionnaire. They were asked to score six different symptoms (pain or discomfort centered in the upper abdomen, early satiety, bloating in the upper abdomen, fullness, nausea and vomiting) from 0-5 (0=none, 1=very mild; awareness of symptoms but easily tolerated, 2=mild; tolerated without interference with usual activity, 3=moderate; enough to cause some interference with usual activity, 4=severe; enough to cause significant interference with usual activity, 5=very severe; incapacitating with inability to work or do usual activity). For inclusion, two of these symptoms had to be scored as moderate, severe or very severe and these symptoms needed to be present for at least 12 weeks, not necessary consecutive, in the preceding 12 months. Upper GI endoscopy was performed within 1 year prior to inclusion to rule out any upper gastrointestinal abnormalities.

The presence of GERD was established by symptom evaluation, upper GI endoscopy and 24-hour pH monitoring and defined by the presence of $\geq$ Los Angeles A oesophagitis on recent endoscopy (18 out of 20 patients) and/or abnormal 24-hour ambulatory pH monitoring using a cut-off value of 6.0% total time with pH $<$ 4 and a proven association between reflux and symptoms (symptom association probability index (SAP) $>$ 95%) (20 out of 20 patients).[20]

All FD and GERD patients were asked to discontinue any medication known to influence gastrointestinal motility (including PPI therapy) for at least 7 days prior to the study. None of the participants had a history of gastrointestinal surgery (other then appendectomy, inguinal hernia repair or haemorrhoidectomy). All patients were symptomatic at the time of the study. Healthy controls were recruited through advertisement and a medical history was obtained to rule out any history of gastrointestinal disease. The study was approved by the medical ethics committee of the University

Medical Center Utrecht and written informed consent was obtained from all participants.

Study Design

After an overnight fast of at least 10 hours, 3D-US was performed to assess total and partial gastric volumes. Subjects were comfortably seated in a wooden chair leaning slightly backward. The nutrient drink was ingested within three minutes. Ultrasonographic data were acquired while fasting and at 5, 15, 30, 45 and 60 minutes after ingestion of the nutrient drink. At all consecutive time points, upper abdominal sensations (hunger, nausea, fullness and upper abdominal pain) were scored using a visual analogue scale (VAS, 0-100 mm) varying from no sensations to unbearable sensations.[21;22] The sum of nausea, fullness and pain was calculated at every time point and referred to as the total score of upper abdominal sensations.

Nutrient drink

The nutrient drink (500 ml) consisted of a 200 ml lactose- and fibre-free milk drink, containing 12.0 gram proteins, 11.6 gram fat and 36.8 gram carbohydrate, 300 kcal (Nutridrink, Nutricia, Zoetermeer, The Netherlands) mixed with 300 ml water.

3D Ultrasonography Imaging System

The 3D imaging system consisted of an ultrasound scanner with a 3.5 MHz curved probe and a tracking system (Esaote-Pie Medical, Maastricht, The Netherlands). The tracking system consisted of a transmitter generating a spatially varying magnetic field and a small receiver, firmly attached to the ultrasound probe, containing three orthogonal coils to sense the magnetic field strength.[3]

The ultrasound probe with attached sensor was used to localize the left lateral and superior margins of the stomach and the pylorus. The depth of scanning was adjusted enabling an ultrasound scan of the stomach, superior mesenteric vein, aorta, left liver lobe and diaphragm on top of the gastric fundus. A standardized ultrasound scanning pattern was used, starting at the left lateral subcostal margin and then moving distally towards the pylorus having the probe in a vertical position. During the scan all participants suspended their breathing in inspiration. For each ultrasound scan approximately 300-400 2D ultrasound images were stored with a scan typ-

ically lasting 15-20 seconds. The 2D sagittal images were digitised and stored in the computer workstation.[4]

Total and Partial Gastric Volume

The gastric volume was measured using software with rendering and volume estimation capability (In Vivo ScanNT, Medcom GmbH, Darmstadt, Germany). The 2D sagittal frames were processed to construct 3D images, containing 60-70 sagittal planes. The sagittal planes were used to draw the region of interest. The inner layer of the stomach wall, corresponding to the interface between the outer profile of the gastric wall mucosa and the liquid nutrient, was outlined in an average of 10-20 planes. The computer using a triangulation technique generates gastric contours in the intermediate frames. Then a 3D reconstructed image of the stomach and volume was obtained.

In addition, partial gastric volumes were calculated. The proximal part was separated by a dividing plane 10 cm bellow the point where the fundic top reaches the diaphragm, perpendicular to the longitudinal axis of the stomach. A margin of 10 cm was chosen since this matches the diameter of a barostat balloon containing 500 ml of fluid. Similarly, a distal part was separated, defined as the gastric region between the antral area (the sagittal ultrasound plane in which the antrum, the left liver lobe, the superior mesenteric vein and the abdominal aorta are seen simultaneously) and the gastro duodenal junction. The antral area is a known anatomical landmark and therefore chosen as the margin of the distal gastric volume.[23]

3D-US Data Analysis

At every time point, we subtracted fasting total or partial gastric volume leaving the change in total or partial gastric volume. The gastric volume change was used for the comparison between groups.

In order to correct for differences in total gastric volume, partial gastric volumes were divided by total gastric volume: proximal/total gastric volume and distal/total gastric volume. The average proximal/total gastric volume of 5 and 15 minutes postprandially is referred to as the proximal gastric volume ratio. Impaired proximal relaxation was defined as a proximal gastric volume ratio smaller than the lower limit of the 95% confidence interval of healthy controls. In addition, we calculated a distal gastric volume ratio, by averaging the distal/total gastric volume of 5 and 15 minutes.

Statistical analysis

Data were summarized as mean ± SEM. Normality was tested using Kolmogorov-Smirnov test. Averaged 3D volumes and symptom scores were compared between FD and GERD patients and healthy controls using repeated measures of variance (ANOVA). Average fasting volumes and sensations were compared using One-way ANOVA. For comparison between separate groups, Dunnett's t-test was used post-hoc. The pearson correlation test was used to calculate correlation coefficients between upper GI sensations and partial gastric volumes. A $P < 0.05$ was considered significant. All statistical analysis was performed using commercially available software (SPSS 11.0 for Microsoft windows).

Results

Characteristics of the Patients

GERD patients were significantly older compared to FD patients and controls (both $P < 0.001$). FD patients were significantly older than controls ($P = 0.001$). Secondly, a significant difference in BMI between GERD patients (26.4 ± 0.8) and FD patients (22.9 ± 0.6) ($P < 0.001$), and between GERD patients and healthy controls (22.5 ± 0.5) ($P = 0.002$) was present. The BMI between FD patients and healthy controls was comparable. No significant differences in sex distribution were found between the three groups ($P > 0.05$). FD patients reported the following upper abdominal sensations as moderate or higher; upper abdominal pain (67%), early satiety (54%), fullness (77%), bloating (82%), and nausea (51%). Vomiting was present in 16% of patients.

Total Gastric Volume

Fasting total gastric volume was comparable between groups (all $P > 0.05$) (FD; 40 ± 3 ml, GERD; 34 ± 2 ml and controls; 34 ± 2 ml). However, after ingestion of the nutrient drink, total gastric volume was larger in GERD patients compared to controls ($P = 0.002$), and smaller in FD patients compared to controls ($P = 0.023$) (5 min postprandially: FD = 496 ± 5 ml, GERD = 515 ± 10 ml and controls = 497 ± 5 ml) (figure 1).

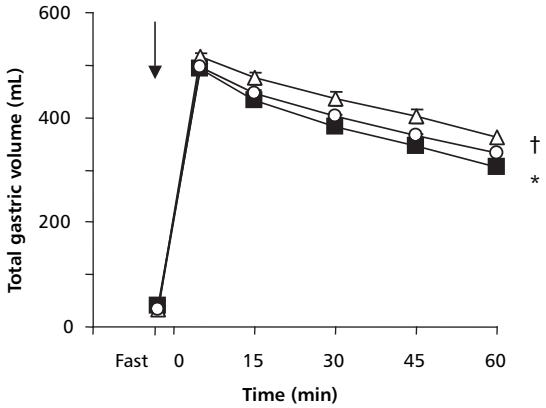

Figure 1
Total gastric volume measurements using 3D-US, before and after ingestion of a nutrient drink (arrow) in FD patients (■), GERD patients (Δ) and healthy controls (○); * P = 0.023, FD patients *vs* controls, † P = 0.002, GERD patients *vs* controls.

Partial Gastric Volumes

The proximal gastric volume change after ingestion of a nutrient drink was significantly smaller in FD patients compared to controls ($P < 0.001$). In contrast, GERD patients exhibited larger proximal gastric volume change than controls ($P < 0.001$) (figure 2). The distal gastric volume change after ingestion of a nutrient drink was significantly larger in both FD and GERD patients compared to controls ($P < 0.001$ and $P = 0.024$ respectively) (figure 2).

Partial Gastric Volume Ratios

The proximal gastric volume ratio was smaller in FD patients and larger in GERD patients compared to healthy controls ($P < 0.001$ and $P = 0.008$ respectively) (figure 3). Twenty-three FD patients (46%) had a proximal gastric volume ratio smaller than the 95% confidence interval of healthy controls (0.32 – 0.57). These FD patients had a larger increase in distal gastric volume compared to FD patients with normal proximal relaxation ($P = 0.008$). None of the GERD patients exhibited impaired proximal gastric relaxation.
The distal gastric volume ratio was larger in FD patients compared to healthy controls ($P < 0.001$), and comparable between GERD patients and healthy controls ($P > 0.05$).

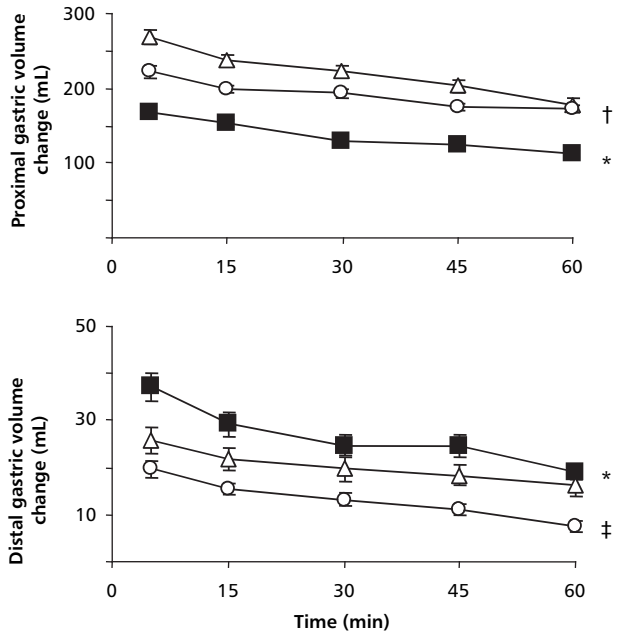

Figure 2
Proximal and distal gastric volume change after ingestion of a nutrient drink in FD patients (■), GERD patients (Δ) and healthy controls (○); Proximal gastric volume change: * P < 0.001, FD patients *vs* controls, † P < 0.001, GERD patients vs controls. Distal gastric volume change: * *P* < 0.001, FD patients *vs* controls, ‡ *P* = 0.024, GERD patients *vs* controls.

Upper GI Sensations

Preprandially, FD patients and GERD patients have a higher total score of upper abdominal sensations than controls ($P < 0.001$ and $P = 0.013$ respectively). After ingestion of the nutrient drink, FD patients showed higher fullness, nausea and pain sensation scores when compared to controls (P = 0.041, $P < 0.001$ and $P < 0.001$ respectively). In addition, FD patients had significantly higher fullness and nausea scores compared to GERD patients ($P = 0.01$ and $P = 0.025$ respectively). The pain scores in GERD and FD patients were not significantly different. Consequently, FD patients have a higher total score of upper abdominal sensations compared to controls and to GERD patients ($P < 0.001$ and $P = 0.007$ respectively). Postprandial fullness, nausea and pain sensations were comparable between GERD patients and controls (Fig 4). In a fasting state, fullness scores in FD patients with impaired relaxation of the proximal stomach and FD patients

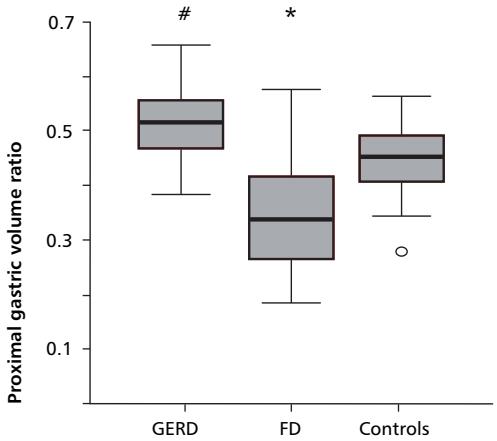

Figure 3
Proximal gastric volume ratio in FD patients, GERD patients and healthy controls; * $P < 0.001$, FD patients *vs* controls, # $P = 0.008$, GERD patients *vs* controls.

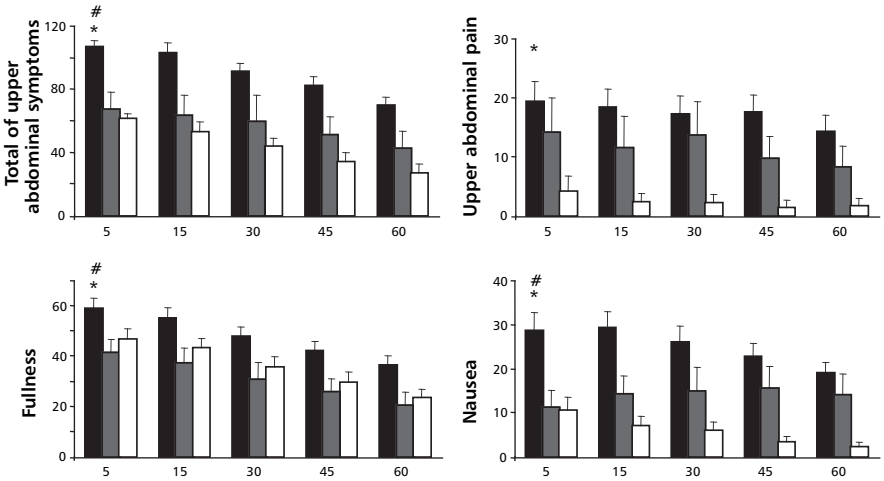

Figure 4
Effect of ingestion of a nutrient drink on upper abdominal sensations in FD patients (■), GERD patients (▩) and healthy controls (❑). After ingestion of a nutrient drink, FD patients showed a higher total score of upper abdominal sensations compared to healthy controls and GERD patients (* $P < 0.001$ and # $P = 0.007$ respectively). Fullness, pain and nausea sensation scores were higher in FD patients compared to controls (* $P = 0.041$, $P < 0.001$ and $P < 0.001$ respectively). In addition, fullness and nausea scores were higher in FD patients compared to GERD patients (# $P = 0.01$ and $P = 0.025$ respectively). Pain scores were comparable between GERD and FD patients.

with normal proximal relaxation were comparable (P = ns). After the nutrient drink, FD patients with impaired relaxation of the proximal stomach had significantly higher fullness sensations compared to FD patients with normal proximal relaxation ($P = 0.027$) (figure 5). No differences for upper abdominal pain, nausea, or hunger were found between the two groups.

Relationship between Partial Gastric Volumes and Sensations

In healthy controls, a significant relationship between the increase in distal gastric volume and the increase in fullness was observed (5 min postprandially $r = 0.761$, $P < 0.001$; 15 min $r = 0.783$, $P < 0.001$). For GERD patients a similar relationship was found (5 min $r = 0.674$, $P = 0.001$; 15 min $r < 0.759$, $P < 0.001$). In contrast, in FD patients this relationship was not seen at any of the time points (5 min $r = 0.215$, $P = 0.133$; 15 min $r = 0.181$, $P = 0.208$) (figure 6). Neither was this relationship present in FD patients with a normal change in proximal gastric volume (5 min $r = 0.02$, $P = 0.72$). Healthy controls also show a positive relationship between the increase in the total score of upper abdominal sensations and the increase in distal gastric volume (5 min $r = 0.413$, $P = 0.014$; 15 min $r = 0.510$, $P = 0.002$). Again this relationship was not seen in FD patients. We did not find a relationship between distal gastric volume and hunger, nausea or pain in any of the subject groups. No relationship between an increase in proximal gastric volume and upper abdominal sensations was found in any subject group.

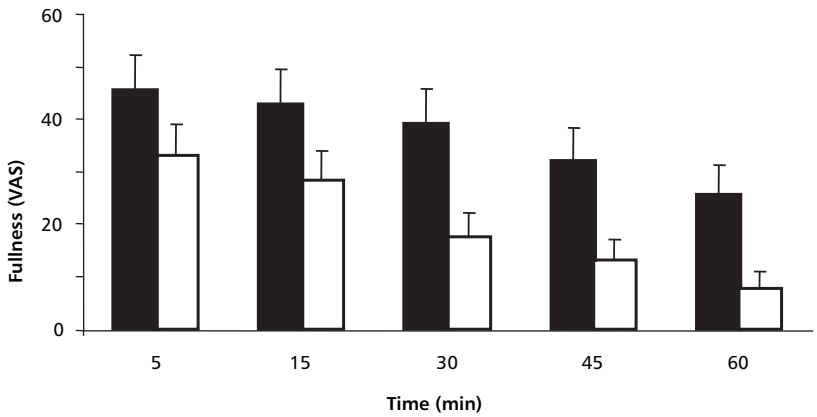

Figure 5

FD patients with impaired proximal gastric relaxation (n=23) (■) have a higher fullness sensation score compared to FD patients with normal proximal gastric relaxation (n=27) (❑) ($P = 0.027$).

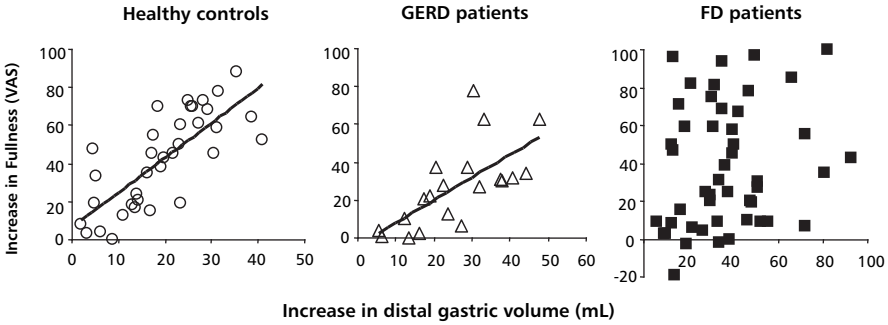

Figure 6
Relationship between the increase in distal gastric volume and the increase in fullness, 5 minutes postprandially. Healthy controls; (r = 0.761, $P < 0.001$), GERD patients; (r = 0.674, $P = 0.001$) and FD patients; (r = 0.215, $P > 0.05$).

Discussion

FD patients and GERD patients show remarkable differences in partial gastric volumes compared to healthy controls. After ingestion of the nutrient drink, FD patients exhibit a smaller proximal gastric volume, whereas GERD patients have a larger proximal gastric volume compared to controls. These findings are confirmatory with other studies using either barostat, SPECT imaging, or 3D-US.[1;3;24-27] FD patients with impaired proximal relaxation show a larger distal gastric volume and score higher fullness sensations compared to FD patients with normal proximal gastric relaxation. A strong positive relationship between the increase in distal gastric volume and fullness was found in healthy controls and in GERD patients, whereas no such relationship was found between proximal gastric volume and fullness. Interestingly, in FD patients, the relationship between distal gastric volume and fullness was lacking.

Although the volume of the ingested nutrient drink was similar in all participants, we did find differences in total gastric volume between the three groups. The differences in total gastric volume were most pronounced between 5 and 60 minutes postprandially. The total gastric volume was larger in GERD patients, and smaller in FD patients compared to healthy controls. Since we have not determined the rate of gastric emptying, we can only speculate that the change in total gastric volume is explained by differences in gastric emptying rate.[28]

In the present study, forty six percent of the FD patients have been identified as having impaired relaxation of the proximal stomach. This number is in concordance with studies using either barostat technique or SPECT imaging.[1;8;27] We choose to define impaired relaxation of the proximal stomach as the average proximal/total gastric volume of 5 and 15 minutes after ingestion of the nutrient drink smaller than 0.32 (lower limit 95% CI healthy volunteers). From literature is known that the maximal accommodation response occurs between 10-20 minutes postprandially.[1;29;30]

An increase in distal gastric volume or antral area after ingestion of a nutrient drink was observed in all three groups.[31] However, we observed a larger increase in distal gastric volume ratio after ingestion of the nutrient drink in FD patients compared to healthy controls and GERD patients. In addition, FD patients show a higher increase of upper abdominal sensations, including fullness, after a meal. FD patients with impaired relaxation of the proximal stomach have a larger distal gastric volume compared to FD patients with normal proximal relaxation.[24;32] Under physiological conditions, antral filling and nutrients in the proximal gut leads to an increase in relaxation of the gastric fundus contributing to the accommodation response. In a subgroup of FD patients however, antral distension fails to induce this response, which is likely to be due to disturbed antro-fundic and/or entero-gastric reflexes.[33]

In healthy controls and GERD patients, a strong positive correlation between fullness and distal gastric volume was found, whereas no such relation was found between the proximal gastric volume and fullness. This finding indicates that the distal stomach is important in the regulation of the sensation fullness during physiological conditions, and is supported by studies reporting a positive relationship between fullness and the increase in antral area or scintigraphic content of the distal stomach in healthy controls.[34;35]

However, a dramatic change occurs in patients with functional dyspepsia, in whom no relationship could be observed between antral, or fundal volume changes and fullness sensation. One could speculate that this is caused by impaired relaxation of the proximal stomach. FD patients with impaired relaxation have an enlarged antrum after meal ingestion, which may underlie the change in sensitivity in this region. At best this is partially true, since the relationship between the change in distal gastric volume and fullness is not restored when leaving out the FD patients with impaired relaxation of

the proximal stomach from the analysis. This indicates that other mechanisms must be involved. In addition, we observed no relationship between any of the other upper abdominal sensations and changes in partial gastric volumes. In our opinion, these observations somewhat downplay the role of impaired proximal gastric relaxation (and an increased gastric antrum) in the symptom generation in functional dyspepsia, or at least indicate the very complex interaction between gastric motor and sensory function. Other mechanisms, such as an increased visceral sensitivity, most likely play an important role in symptom generation.[30]

We found significant differences in age and BMI between the three groups. When categorizing all participants according to age (younger then 30 years, between 30 and 50 years or older than 50 years) or according to their BMI (BMI < 20, BMI 20 – 25, and BMI > 25), no effects of age or BMI were found on any of the end points of the study (total gastric volume, partial gastric volumes and upper abdominal sensations). This is in line with a recent publication, in which no effect of age, and merely any effect of BMI on gastric volumes was found.[36] We therefore believe it is unlikely that the differences of age or BMI between the groups had any effect on the outcome of this study.

The technique used in the current study, 3D-US, can be used to measure total and partial gastric volumes in a non-invasive way.[37] 3D-US has shown excellent in vitro and in vivo accuracy in volume estimation and a low inter observer variation.[3;4;37] A head to head comparison between barostat and 3D-US has shown a large overlap in the evaluation of proximal gastric function.[38]

In summary, 3D-US is a non-invasive tool for gastric volume measurement and allows us to differentiate between normal or impaired relaxation of the proximal and distal stomach. GERD patients have an increased proximal gastric volume, whereas forty six percent of FD patients have an impaired proximal relaxation. FD patients with impaired proximal relaxation exhibit a larger distal gastric volume and an increased fullness sensation. In health and GERD patients, an increase in fullness sensation is strongly related to an increase in distal gastric volume. This relationship was not observed in FD patients with normal or altered volume distribution, implying that other causes than altered meal distribution are involved in the excessive generation of fullness, such as increased visceral sensitivity.

References

1 Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998; 115(6):1346-1352.

2 Penagini R, Hebbard G, Horowitz M, Dent J, Bermingham H, Jones K et al. Motor function of the proximal stomach and visceral perception in gastro-oesophageal reflux disease. Gut 1998; 42(2):251-257.

3 Tefera S, Gilja OH, Olafsdottir E, Hausken T, Hatlebakk JG, Berstad A. Intragastric maldistribution of a liquid meal in patients with reflux oesophagitis assessed by three dimensional ultrasonography. Gut 2002; 50(2):153-158.

4 Scheffer RC, Gooszen HG, Wassenaar EB, Samsom M. Relationship Between Partial Gastric Volumes and Dyspeptic Symptoms in Fundoplication Patients: A 3D Ultrasonographic Study. Am J Gastroenterol 2004; 99(10):1902-1909.

5 Samsom M, Roelofs JM, Akkermans LM, Berge Henegouwen GP, Smout AJ. Proximal gastric motor activity in response to a liquid meal in type I diabetes mellitus with autonomic neuropathy. Dig Dis Sci 1998; 43(3):491-496.

6 De Ponti F, Azpiroz F, Malagelada JR. Relaxatory responses of canine proximal stomach to esophageal and duodenal distension. Importance of vagal pathways. Dig Dis Sci 1989; 34(6):873-881.

7 Stanghellini V, Tosetti C, Paternic inverted question mA, Barbara G, Morselli-Labate AM, Monetti N et al. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. Gastroenterology 1996; 110(4):1036-1042.

8 Kim DY, Delgado-Aros S, Camilleri M, Samsom M, Murray JA, O'Connor MK et al. Noninvasive measurement of gastric accommodation in patients with idiopathic nonulcer dyspepsia. Am J Gastroenterol 2001; 96(11):3099-3105.

9 Tack J, Caenepeel P, Fischler B, Piessevaux H, Janssens J. Symptoms associated with hypersensitivity to gastric distention in functional dyspepsia. Gastroenterology 2001; 121(3):526-535.

10 Boeckxstaens GE, Hirsch DP, Kuiken SD, Heisterkamp SH, Tytgat GN. The proximal stomach and postprandial symptoms in functional dyspeptics. Am J Gastroenterol 2002; 97(1):40-48.

11 Boeckxstaens GE, Hirsch DP, van den Elzen BD, Heisterkamp SH, Tytgat GN. Impaired drinking capacity in patients with functional dyspepsia: relationship with proximal stomach function. Gastroenterology 2001; 121(5):1054-1063.

12 Tack J, Caenepeel P, Piessevaux H, Cuomo R, Janssens J. Assessment of meal induced gastric accommodation by a satiety drinking test in health and in severe functional dyspepsia. Gut 2003; 52(9):1271-1277.

13 Delgado-Aros S, Camilleri M, Cremonini F, Ferber I, Stephens D, Burton DD. Contributions of gastric volumes and gastric emptying to meal size and postmeal symptoms in functional dyspepsia. Gastroenterology 2004; 127(6):1685-1694.

14 Tack J, Coulie B, Wilmer A, Andrioli A, Janssens J. Influence of sumatriptan on gastric fundus tone and on the perception of gastric distension in man. Gut 2000; 46(4):468-473.

15 Tack J, Broeckaert D, Coulie B, Janssens J. The influence of cisapride on gastric tone and the perception of gastric distension. Aliment Pharmacol Ther 1998; 12(8):761-766.

16 Gilja OH, Hausken T, Bang CJ, Berstad A. Effect of glyceryl trinitrate on gastric accommodation and symptoms in functional dyspepsia. Dig Dis Sci 1997; 42(10):2124-2131.

17 Coulie B, Tack J, Maes B, Geypens B, De Roo M, Janssens J. Sumatriptan, a selective 5-HT1 receptor agonist, induces a lag phase for gastric emptying of liquids in humans. Am J Physiol 1997; 272(4 Pt 1):G902-G908.

18 Konturek JW, Thor P, Domschke W. Effects of nitric oxide on antral motility and gastric emptying in humans. Eur J Gastroenterol Hepatol 1995; 7(2):97-102.

19 Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR, Tytgat GN. Functional gastroduodenal disorders. Gut 1999; 45 Suppl 2:II37-II42.

20 Weusten BL, Roelofs JM, Akkermans LM, Berge-Henegouwen GP, Smout AJ. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. Gastroenterology 1994; 107(6):1741-1745.

21 Ohnhaus EE, Adler R. Methodological problems in the measurement of pain: a comparison between the verbal rating scale and the visual analogue scale. Pain 1975; 1(4):379-384.

22 McCormack HM, Horne DJ, Sheather S. Clinical applications of visual analogue scales: a critical review. Psychol Med 1988; 18(4):1007-1019.

23 Bolondi L, Bortolotti M, Santi V, Calletti T, Gaiani S, Labo G. Measurement of gastric emptying time by real-time ultrasonography. Gastroenterology 1985; 89(4):752-759.

24 Gilja OH, Hausken T, Wilhelmsen I, Berstad A. Impaired accommodation of proximal stomach to a meal in functional dyspepsia. Dig Dis Sci 1996; 41(4):689-696.

25 Zerbib F, des Varannes SB, Ropert A, Lamouliatte H, Quinton A, Galmiche JP. Proximal gastric tone in gastro-oesophageal reflux disease. Eur J Gastroenterol Hepatol 1999; 11(5):511-515.

26 Tefera S, Gilja OH, Hatlebakk JG, Berstad A. Gastric accommodation studied by ultrasonography in patients with reflux esophagitis. Dig Dis Sci 2001; 46(3):618-625.

27 Bredenoord AJ, Chial HJ, Camilleri M, Mullan BP, Murray JA. Gastric accommodation and emptying in evaluation of patients with upper gastrointestinal symptoms. Clin Gastroenterol Hepatol 2003; 1(4):264-272.

28 Moragas G, Azpiroz F, Pavia J, Malagelada JR. Relations among intragastric pressure, postcibal perception, and gastric emptying. Am J Physiol 1993; 264(6 Pt 1): G1112-G1117.

29 Kuiken SD, Samsom M, Camilleri M, Mullan BP, Burton DD, Kost LJ et al. Development of a test to measure gastric accommodation in humans. Am J Physiol 1999; 277(6 Pt 1):G1217-G1221.

30 Salet GA, Samsom M, Roelofs JM, Berge Henegouwen GP, Smout AJ, Akkermans LM. Responses to gastric distension in functional dyspepsia. Gut 1998; 42(6):823-829.

31 Sturm K, Parker B, Wishart J, Feinle-Bisset C, Jones KL, Chapman I et al. Energy intake and appetite are related to antral area in healthy young and older subjects. Am J Clin Nutr 2004; 80(3):656-667.

32 Troncon LE, Bennett RJ, Ahluwalia NK, Thompson DG. Abnormal intragastric distribution of food during gastric emptying in functional dyspepsia patients. Gut 1994; 35(3):327-332.

33 Caldarella MP, Azpiroz F, Malagelada JR. Antro-fundic dysfunctions in functional dyspepsia. Gastroenterology 2003; 124(5):1220-1229.

34 Jones KL, Doran SM, Hveem K, Bartholomeusz FD, Morley JE, Sun WM et al. Relation between postprandial satiation and antral area in normal subjects. Am J Clin Nutr 1997; 66(1):127-132.

35 Santangelo A, Peracchi M, Conte D, Fraquelli M, Porrini M. Physical state of meal affects gastric emptying, cholecystokinin release and satiety. Br J Nutr 1998; 80(6):521-527.

36 Delgado-Aros S, Cremonini F, Castillo JE, Chial HJ, Burton DD, Ferber I et al. Independent influences of body mass and gastric volumes on satiation in humans. Gastroenterology 2004; 126(2):432-440.

37 Gilja OH, Detmer PR, Jong JM, Leotta DF, Li XN, Beach KW et al. Intragastric distribution and gastric emptying assessed by three-dimensional ultrasonography. Gastroenterology 1997; 113(1):38-49.

38 Mundt M, Samsom M. Fundal Dysaccommodation in Functional Dyspepsia: Head-to-head comparison between the Barostat and 3D Ultrasonographic Technique. Gut 2006; in press.

Chapter 03

Lack of relationship between chronic upper abdominal symptoms and gastric function in functional dyspepsia

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Abstract

Background and aim

To determine the relationship between gastric function and upper abdominal sensations.

Methods

Sixty FD patients (43 female) were studied. All patients underwent three gastric function tests: ^{13}C octanoic gastric emptying test, three-dimensional ultrasonography (proximal and distal gastric volume), and the nutrient drinktest. Upper abdominal sensations experienced in daily life were scored using questionnaires.

Results

Impaired proximal gastric relaxation (23%) and a delayed gastric emptying (33%) are highly prevalent in FD patients, however only a small overlap exists between the two pathophysiologic disorders (5%). No relationship was found between chronic upper abdominal symptoms and gastric function (proximal gastric relaxation, gastric emptying rate, or drinking capacity) (all $P > 0.01$). Proximal gastric relaxation or gastric emptying rate had no effect on maximum drinking capacity ($P > 0.01$).

Conclusions

The lack of relationship between chronic upper abdominal sensations and gastric function questions the role of these pathophysiologic mechanisms in the generation of symptoms.

Introduction

Functional dyspepsia (FD) is a common disorder seen in daily clinical practice, characterized by the presence of pain or discomfort in the upper abdomen in the absence of organic, systemic, or metabolic disease.[1] Functional dyspeptic patients complain about a variety of symptoms, which are frequently intermittent, and mostly related to food intake.[2] Fore that reason, a subdivision of patients has been proposed, in order to clarify the heterogeneity of this disorder and to direct treatment options.[3] Attempts have been made to subdivide patients according to their symptoms, however a large overlap of symptoms exists and many patients do not fit into one of the subgroups.[1]

Currently, many efforts are being made to subdivide patients according to gastric (dys)function, and to find new ways of treating these proposed pathophysiologic disorders.[4] Three pathophysiologic mechanisms have been described as possible etiologic factors; (1) a delayed gastric emptying, (2) impaired proximal gastric accommodation, and (3) visceral hypersensitivity. Delayed gastric emptying is present in approximately 30% of FD patients, and may be one of the underlying mechanisms for symptoms.[5] Impaired proximal gastric relaxation may be an important etiologic factor in the pathophysiology of functional dyspepsia, considering the high prevalence of approximately 40% in FD patients, and a possible association with specific upper abdominal symptoms.[6;7] Finally, an increased visceral sensitivity is highly frequent in FD patients, and even though difficult to measure, an important etiologic factor in the pathophysiology of FD.[8] However, when targeting specific pathophysiologic mechanisms by the use of pharmacologic agents, the effect on symptoms is questionable.[6;9;10] In other words, the relationship between specific upper abdominal sensations and the above described mechanisms remains to be matter of debate.

We conducted a study to assess the relationship between gastric function and upper abdominal sensations. Gastric emptying rate, proximal gastric relaxation, and maximum drinking capacity were assessed in FD patients. The symptoms experienced in daily life (chronic upper abdominal symptoms) were assessed and related to the primary outcome parameters of the gastric function tests. We hypothesised that chronic upper abdominal symptoms and specific pathophysiologic mechanisms have no correlation in functional dyspepsia.

Materials and Methods

Patients

All patients visiting the outpatient clinics at our hospital, fulfilling the Rome II criteria for functional dyspepsia,[1] were subjected to three non-invasive functional tests of the stomach. A total of sixty functional dyspeptic patients were prospectively evaluated; 43 female (median age 40 years; range 18 - 65) and 17 male (median age 37 years; range 21 - 64).

The inclusion criteria were (a) the presence of dyspeptic symptoms, assessed using the questionnaire described below; (b) no evidence of macroscopic inflammation of the esophageal mucosa or focal lesions of the esophagogastroduodenal mucosa at upper gastrointestinal endoscopy (performed within1 year prior to inclusion); (c) no abnormalities seen during upper abdominal ultrasonography (performed within 1 year prior to inclusion); (d) absence of serious concomitant illness; and (e) the absence of major gastrointestinal surgery (excluding appendectomy).

The protocol was approved by the ethics committee of the University Medical Center Utrecht. All patients gave written informed consent for inclusion in the trial.

Chronic dyspeptic symptoms questionnaire

Each patient completed a reproducible dyspepsia questionnaire.[5;6;11] Patients were asked to score six different symptoms (pain or discomfort centered in the upper abdomen, early satiety, bloating in the upper abdomen, fullness, nausea, and vomiting) from 0-5 (0=none, 1=very mild; awareness of symptoms but easily tolerated, 2=mild; tolerated without interference with usual activity, 3=moderate; enough to cause some interference with usual activity, 4=severe; enough to cause significant interference with usual activity, 5=very severe; incapacitating with inability to work or do usual activity). For inclusion, two of these symptoms had to be scored as moderate, severe or very severe and these symptoms needed to be present for at least 12 weeks, not necessary consecutive, in the preceding 12 months.

Study protocol

All patients underwent three functional tests of the stomach on three separate days; the ^{13}C-octanoic breathtest, three-dimensional ultrasonography of the stomach, and a nutrient drinktest. The order of the three study days

was arbitrary. Each of the study days started at 08:00 h after an overnight fast of at least 10 hours. Patients were asked to discontinue any medication known to influence gastrointestinal motility or sensitivity for at least 7 days prior to the study, including PPI therapy.

^{13}C-octanoic breathtest

The rate of gastric emptying was assessed using the ^{13}C-octanoic breathtest. The test meal consisted of two fried eggs, one slice of bread, 5 g margarine and 150 ml water (total caloric value of 294 kcal and a nutrient composition of 16 g protein, 16 g carbohydrate, 18 g fat).[12] The egg yolk of one egg was labelled with 100 mg ^{13}C-sodium-octanoic acid (598 μmol; Campro Scientific, Veenendaal, The Netherlands), dissolved in 1 ml distilled water. Breath samples were taken at baseline, before the meal and from start of ingestion of the meal every 2 min the first 30 min, every 5 min for the next 30 min and every 15 min thereafter up to 4 h.

Three-dimensional ultrasonography

Total -, proximal -, and distal gastric volumes were assessed before and after ingestion of a nutrient drink using 3D-US.[13-16] Ultrasonographic data was acquired in a sitting position, while fasting and at 5, 15, 30, 45, and 60 minutes after ingestion of a nutrient drink. The nutrient drink (500 ml) consisted of 200 ml lactose- and fiber-free milk drink, containing 12.0 gram proteins, 11.6 gram fat and 36.8 gram carbohydrate (300 kcal) (Nutridrink, Nutricia, Zoetermeer, The Netherlands) mixed with 300 ml of water, and was ingested within 3 minutes.

The 3D imaging system consisted of an ultrasound scanner with a 3.5 MHz curved probe and a tracking system (Esaote-Pie Medical, Maastricht, The Netherlands). The tracking system consisted of a transmitter generating a spatially varying magnetic field and a small receiver, firmly attached to the ultrasound probe, containing three orthogonal coils to sense the magnetic field strength.[17] A standardized ultrasound-scanning pattern was used, starting at the left lateral subcostal margin and then moving distally towards the pylorus having the probe in a vertical position.[14] The 2D sagittal planes were used to draw the region of interest, corresponding to the inner layer of the stomach wall (the interface between the outer profile of the gastric wall mucosa and the liquid nutrition). A 3D reconstructed image of the stomach and the gastric volume was obtained using software with ren-

dering and volume estimation capability (In Vivo ScanNT, Medcom GmbH, Darmstadt, Germany).
The proximal gastric volume was defined as the gastric volume between the diaphragm and a dividing plane 10 cm below the point where the fundic top reaches the diaphragm. Similarly, a distal part was separated, defined as the gastric region between the antral area and the gastro duodenal junction.[18] At every time point, we subtracted fasting total or partial gastric volume leaving the change in total or partial gastric volume. Proximal and distal gastric volume ratios were calculated by dividing proximal or distal gastric volume by total gastric volume. Recently, we defined impaired proximal relaxation as the average of the proximal gastric volume ratios of 5 and 15 minutes smaller than the lower limit of the 95% confidence interval of healthy controls (0.32 – 0.57).[7] All measurements were made by a single investigator (N.v.L.) who was blinded for the results of the gastric emptying test and the drinktest.

Nutrient drinktest

The nutrient drinktest was used to measure the drinking capacity and the symptoms evoked by a nutrient drink.[19;20] Patients were asked to ingest a nutrient drink (Nutridrink; 1.5 Kcal/mL) at a constant rate of 15 mL/min. At 5-minute intervals, they scored satiety using a graphic rating scale that combines verbal descriptors on a scale graded 0-5 (0 = no satiety, 5 = maximum satiety). The test ends when the subject reaches maximum satiety. Upper abdominal sensations were scored in a fasting state and 30 minutes after reaching maximum satiety using a VAS scale.
In healthy controls, maximum satiety occurs after ingestion of 1005 ml (1508 kcal). The lower limit of normal w as 653 ml (979 kcal).[19] In the same study, FD patients ingested 361 ml (542 kcal) before reaching maximum satiety.

Statistical analysis

The main focus of our analysis was; (a) to analyse a possible relationship between the rate of gastric emptying, total or partial gastric volumes after meal ingestion, and drinking capacity, and (b) to compare the outcome of the ^{13}C-octanoic breathtest, the 3D-US test, and the drinktest with the symptoms of patients experienced during daily life (chronic symptoms).
The primary end points of the gastric function tests are dichotomous and

continuous. The relationship between the outcomes of the three gastric function tests was studied using a Pearson's correlation between continuous variables (half-emptying time, retentions after 120 minutes, fasting gastric volume, proximal gastric volume ratio, distal gastric volume ratio, and maximum drinking capacity). Secondly, patients were subdivided in two groups, according to postprandial proximal gastric relaxation (normal or impaired), and according to the rate of gastric emptying (normal or delayed), in order to compare multiple variables between the sub-groups using the Students' T-test.

Chronic upper abdominal symptoms (ordinal variables) were compared between FD patients with normal- or delayed gastric emptying or a normal or impaired proximal gastric relaxation (dichotomous variable), using the Chi-Square test. Secondly, the relationship between chronic upper abdominal symptoms and half-emptying time, retention after 120 minutes, fasting gastric volume, proximal gastric volume ratio, distal gastric volume ratio, and maximum tolerated volume (continuous variable) was analysed using one-way analysis of variance (Anova).

We have analysed the effect of age, height, weight, BMI, and sex on the outcome of the gastric function tests and the chronic symptoms using a regression analysis. All variables were tested in single models and using multivariable analysis.

Due to the high number of comparisons made, we considered a *P* value of < 0.01 as statistically significant. All statistical analysis was performed using commercially available software (SPSS 11.0 for Microsoft windows). For the Chi-square test and the Students' T-test, on the basis of a Cohen's effect size of 0.8, a power of 0.80 was obtained with 60 subjects (considering $\alpha = 0.01$).[21] With the same number of subjects, there was good power (0.80) to detect an R value of 0.40 with an $\alpha = 0.01$.

Results

Combined assessment of the ^{13}C-octanoic breathtest, 3D-US, and the nutrient drinktest was performed in 60 FD patients. The frequency of upper abdominal sensations scored as moderate or higher was; upper abdominal pain (80%), early satiety (59%), bloating (77%), fullness (71%), and nausea (51%). Vomiting was present in 13% of patients (table 1).

Gastric emptying and proximal gastric relaxation

Figure 1 shows the frequency of a delayed gastric emptying rate, defined as a half emptying time ≥ 120 minutes and/or a retention after 120 minutes ≥ 40%, and impaired proximal gastric relaxation, defined as an average proximal gastric volume ratio of 5 and 15 minutes postprandially ≤ 0.32 (95% CI healthy controls) in the patient group.[7] A delayed gastric emptying with a normal proximal gastric relaxation was found in 33% of patients. Impaired proximal gastric relaxation with a normal gastric emptying rate was observed in 23% of patients. In 38% of all patients, none both pathophysiologic

Table 1
Frequency of severity grading for each of six dyspeptic symptoms in 60 dyspeptic patients (chronic symptoms).

	0 (None)	1-2 (Very mild – mild)	3 (Moderate)	4-5 (Severe – very severe)
Upper abdominal pain	1 (2)	11 (18)	18 (30)	30 (50)
Early satiety	7 (12)	18 (30)	19 (32)	16 (27)
Bloating	4 (7)	10 (17)	27 (45)	19 (32)
Fullness	2 (3)	15 (25)	26 (43)	17 (28)
Nausea	10 (17)	19 (32)	14 (23)	17 (28)
Vomiting	46 (77)	6 (10)	2 (3)	6 (10)

Numbers in parentheses represent row percentages.

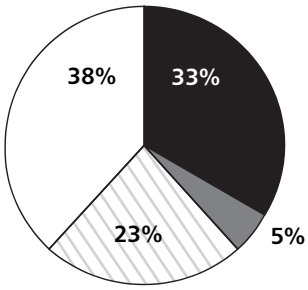

Figure 1
Gastric emptying and proximal gastric relaxation in 60 FD patients. 33% of patients have a delayed gastric emptying and normal proximal gastric relaxation (black), 23% of patients have impaired proximal gastric relaxation and a normal gastric emptying rate (striped), 38% of patients have none of the pathophysiologic disorders (white), and 5% of patients have both disorders (grey).

disorders were found. Finally, only a small overlap exists between the two pathophysiologic disorders (5% of patients). These numbers are in concordance with literature.[5;6]

Nutrient drinktest

The average amount of nutridrink ingested before reaching maximum satiety was 399.8 (344 – 447) ml (600 kcal). Table 2 summarizes the results of the nutrient drinktest. No effect of age or BMI on MTV and upper abdominal sensations was observed (all $P > 0.01$). Male patients had a maximum tolerated volume (MTV) of 500 (381 – 618), and female patients of 359 (301 – 417) ($P = 0.017$).

Relationship between gastric emptying, total and partial gastric volume, and drinking capacity

Table 3 displays some of the patient characteristics in FD patients with normal or impaired proximal gastric relaxation and with normal or delayed gastric emptying. Age or BMI did not influence proximal gastric relaxation or the rate of gastric emptying. Patients with a normal gastric emptying rate had a MTV of 439 ml (359 – 519) whereas patients with a delayed gastric emptying had a MTV of 334 ml (279 – 389) ($P = 0.032$). MTV in patients with normal or impaired proximal relaxation was very similar (404 and 384 ml respectively). In patients with normal proximal gastric relaxation, an av-

Table 2
The effect of age, BMI, and sex on maximum tolerated volume (MTV) and the change in upper abdominal sensations after the nutrient drinktest.

	Age		BMI		Sex	
	β_0	β_1	β_0	β_1	Female	Male
MTV	329 (160–499)	1.7 (-2.2–5.6)	363 (3–725)	1.6 (-14–18)	359 (301–417)	500 (381–618)
Delta symptoms						
Pain	11 (0–35)	0.02 (-0.5–0.6)	24 (0–74)	-0.5 (-3–2)	10 (0–20)	14 (0–28)
Fullness	49 (23 – 76)	-0.1 (-0.7 – 0.5)	49 (0 – 100)	-0.1 (-2 – 2)	48 (37 – 59)	42 (29 – 55)
Nausea	46 (20–72)	-0.5 (-1–0.1)	28 (0–85)	-0.2 (-3–2)	21 (11–31)	31 (12–50)
Hunger	-28 (-50–0)	0.09 (-0.4–0.6)	-7 (-52–40)	-0.8 (-3–1.2)	-24 (-33– -15)	-25 (-37– -14)

β_0: intercept of the model. β_1: slope of the corresponding variable. Numbers in parenthesis represent the 95% confidence interval of β_i. MTV and delta symptoms in female and male patients are presented as mean (95% confidence interval for mean). No effect of age, BMI, or sex on MTV or the change in upper abdominal sensations after the nutrient drinktest was observed (all $P > 0.01$).

erage fasting gastric volume of 50 ml (41 – 59) was determined, opposed to 34 ml (24 – 44) in patients with impaired proximal relaxation ($P = 0.029$). The fasting gastric volume in patients with normal or delayed gastric emptying was 44 and 47 ml respectively.

Twenty out of 43 female patients had a delayed gastric emptying (47%) and 18% of all male patients had a delayed gastric emptying ($P = 0.038$). The prevalence of impaired proximal gastric relaxation in male and female patients was 21% and 35% respectively (not shown in the table).

A positive correlation was observed between the proximal gastric volume

Table 3
Characteristics of FD patients, subdivided according to the extent of proximal gastric relaxation or the rate of gastric emptying (n = 60).

	Proximal gastric relaxation		Gastric emptying	
	Normal (n = 43)	Impaired (n = 17)	Normal (n = 37)	Delayed (n = 23)
Age	41 (36 – 45)	42 (35 – 49)	40.9 (36 – 45)	41 (35 – 48)
BMI	22 (21 – 23)	23 (21 – 25)	22 (21 – 23)	22 (21 – 23)
MTV (ml)	404 (346 – 461)	384 (237 – 530)	439 (359 – 519)	334 (279 – 389)
Fasting gastric volume (ml)	50 (41 – 59)	34 (24 – 44)	44 (33 – 55)	47 (37 – 56)

Data are presented as mean (95% confidence interval for mean). No effect of age and BMI on proximal gastric relaxation or gastric emptying was observed.

ratio (3D-US) and half emptying time (r = 0.32, $P = 0.015$) and the retention after 120 minutes (r = 0.30, $P = 0.024$) (13C breathtest). No correlation was found between the distal gastric volume ratio and the main outcome parameters of the gastric emptying test.

Relationship between chronic symptoms and gastric function

No effect of age, sex, or BMI on any of the chronic upper abdominal sensations was observed, except that patients with a higher BMI or a higher weight scored lower on symptoms of early satiety (both $P = 0.007$). Figure 2 shows the chronic upper abdominal symptoms in FD patients with normal or delayed gastric emptying and normal or impaired proximal gastric relaxation. No differences were observed in the percentage of patients who scored any of the upper abdominal symptoms as moderate or higher between these groups (all $P > 0.01$). We did not find any correlation between half emptying time, retention after 120 minutes, proximal gastric volume

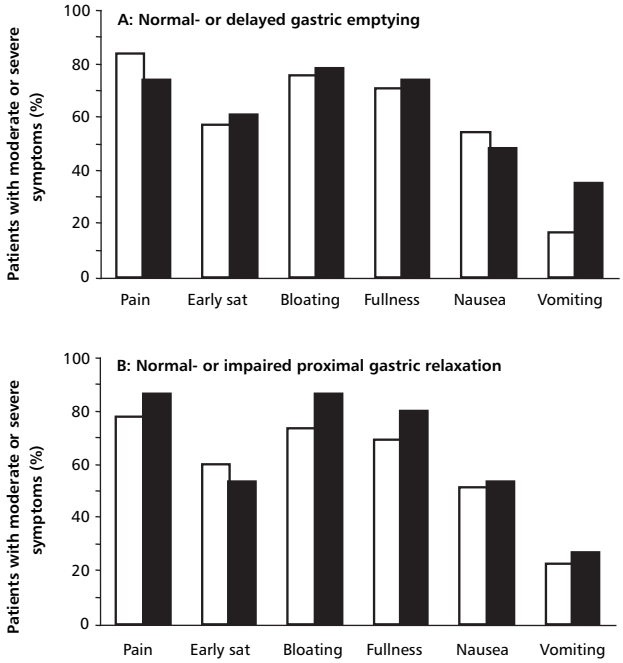

Figure 2

Chronic upper abdominal symptoms scored from 0 (non existent) to 5 (very severe; incapacitating with inability to work or do usual activity). The figure shows the number of the patients who scored three (moderate) or higher on the questionnaire (expressed as a percentage of the total) in subgroups with; (figure 1A) normal- (❑) or delayed (■) gastric emptying, and (figure 1B) normal- (❑) or impaired (■) proximal relaxation.

ratio, distal gastric volume ratio, fasting gastric volume, and any of the chronic upper abdominal symptoms (all $P > 0.01$).

Figure two depicts all FD patients categorized according to their chronic complaints; mild (1 – 2), moderate (3), and severe (4 – 5). No difference in maximum drinking capacity was observed between the three groups (all $P > 0.01$) (figure 3). Interestingly, patients who reported early satiety as moderate or higher, have a comparable drinking capacity with patients who do not experience this symptom in daily life.

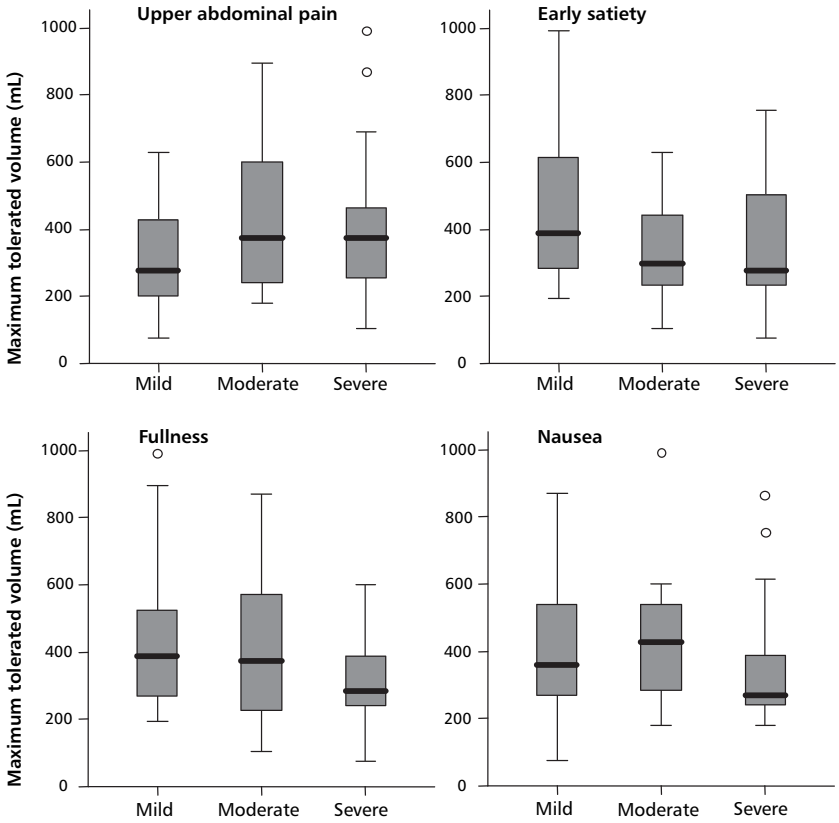

Figure 3
Nutrient drinktest: maximum tolerated volume in FD patients, categorized according to the chronic symptoms. No difference in drinking capacity was observed between patients experiencing mild (1-2), moderate (3), or severe (4-5) pain, early satiety, fullness, or nausea in daily life (all $P > 0.01$).

Discussion

The most important findings of this study were: (1) no relationship was found between chronic upper abdominal symptoms and gastric function (proximal gastric relaxation, gastric emptying rate, or drinking capacity), (2) a third of all FD patients have a normal gastric emptying rate and a normal proximal gastric relaxation, and only a small overlap exists between the two pathophysiologic disorders (7%), and (3) the absence of any relationship between maximum drinking capacity and proximal gastric relaxation or gastric emptying rate.

The observation that approximately 40% of FD patients had a delayed gastric emptying, and approximately 30% of FD patients had impaired proximal gastric relaxation, is confirmatory of previous studies.[5;6] The relative small overlap between the two pathophysiologic mechanisms may suggest that one abnormality excludes the other. However, no significant positive correlation between the proximal gastric volume ratio and half emptying time or retention after 120 minutes was found. The rate of gastric emptying is most likely dependent on many factors, including fundal, antral, pyloric, and duodenal motility.[22] For that reason, gastric emptying and postprandial gastric relaxation should be considered as two separate mechanisms.[23]

The nutrient drinktest has been suggested as a tool to measure meal-induced satiety, and as a non-invasive alternative for the detection of normal- or impaired accommodation of the stomach.[6;19] Furthermore, a positive relationship between the rate of gastric emptying and the amount of Kcal ingested during the nutrient drinktest has been described, thereby suggesting that the maximum tolerated volume is not only influenced by gastric accommodation.[24] However, many studies have shown conflicting results, displaying no relationship between drinking capacity and barostat or SPECT findings,[25;26] and a negative relationship between gastric emptying rate and maximum tolerated volume.[27]

Since the results from different studies do not correspond, it remains a mystery what it is we are testing with the nutrient drinktest. The suggestion that the nutrient drinktest can be used to discriminate between FD patients with normal or impaired visceral sensitivity, is disputable.[9] In the current study, we did not observe any relationship between MTV and chronic upper abdominal symptoms or between MTV and proximal gastric relaxation or gastric emptying rate. Most studies do agree that the drinktest differenti-

ates between FD patients and healthy controls, as we have found in the present study. Notably, the average amount of nutridrink ingested until maximum satiety, was very similar to what others have found (approximately 360 ml),[19] which is below the 95% confidence interval of healthy controls. No effect of age or BMI on MTV was observed in the current study, however we did observe a modest effect of gender, although this did not reach statistical significance ($P = 0.017$).[20]

The nutrient drinktest is also being used in pharmacological trials, and a resemblance between symptoms evoked by the meal challenge and symptoms experienced in daily life has been observed.[27] Recently, we have performed a double blind, placebo controlled, crossover trial, in which the activity of a new drug was tested, using the outcome of the nutrient drink test as one of the end points in the study.[28] FD patients who participated in the pharmacological trial drank significantly more compared to the FD patients in the current study; 569 ± 90 ml and 360 ± 30 ml respectively, ($P < 0.001$). A strong placebo effect, and cognitive influences like motivation, should therefore be considered as confounding factors. No differences in age, sex, BMI, chronic symptoms, or upper abdominal sensations experienced during the drinktest were observed between the patients who participated in the pharmacological trial and those who did not.

In summary, the maximum drinking capacity of FD patients, seen at a tertiary referral practice, is not influenced by gastric emptying rate or proximal gastric relaxation. The question is raised what usefulness this test has, in terms of diagnosis or treatment options in FD patients and as a tool to analyze gastric function or upper abdominal sensations. In our opinion, many subjective factors, like motivation, probably play an important disturbing factor in the outcome of the test.

In conclusion, in spite of a high prevalence of impaired proximal accommodation and delayed gastric emptying in FD patients, the lack of correlation between chronic upper abdominal sensations and gastric function questions the role of these pathophysiologic mechanisms in the generation of symptoms. Consequently, gastric function does not serve as a clear marker for the symptoms experienced by FD patients in daily life, and limited effect on symptoms may be expected when targeting these specific mechanisms. Finally, despite many efforts, no (measurable) motoric disorder can be appointed as a possible pathophysiologic mechanism underlying the presence of upper abdominal symptoms. Most likely, other factors like visceral perception play a vital role in functional dyspepsia.

References

1 Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR, Tytgat GN. Functional gastroduodenal disorders. Gut 1999; 45 Suppl 2:II37-II42.

2 Feinle-Bisset C, Vozzo R, Horowitz M, Talley NJ. Diet, food intake, and disturbed physiology in the pathogenesis of symptoms in functional dyspepsia. Am J Gastroenterol 2004; 99(1):170-181.

3 Cremonini F, Delgado-Aros S, Talley NJ. Functional dyspepsia: drugs for new (and old) therapeutic targets. Best Pract Res Clin Gastroenterol 2004; 18(4):717-733.

4 Tack J, Lee KJ. Pathophysiology and treatment of functional dyspepsia. J Clin Gastroenterol 2005; 39(4 Suppl 3):S211-S216.

5 Stanghellini V, Tosetti C, Paternic inverted question mA, Barbara G, Morselli-Labate AM, Monetti N et al. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. Gastroenterology 1996; 110(4):1036-1042.

6 Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998; 115(6):1346-1352.

7 van Lelyveld N, Scheffer R, Mundt M, Samsom M. Partial gastric volumes and upper abdominal sensations in functional dyspeptic and GERD patients: a 3D ultrasonographic study. Am J Gastroenterol 2006; 101(8):1845-52.

8 Salet GA, Samsom M, Roelofs JM, Berge Henegouwen GP, Smout AJ, Akkermans LM. Responses to gastric distension in functional dyspepsia. Gut 1998; 42(6):823-829.

9 Boeckxstaens GE, Hirsch DP, Kuiken SD, Heisterkamp SH, Tytgat GN. The proximal stomach and postprandial symptoms in functional dyspeptics. Am J Gastroenterol 2002; 97(1):40-48.

10 Verhagen MA, Samsom M, Maes B, Geypens BJ, Ghoos YF, Smout AJ. Effects of a new motilide, ABT-229, on gastric emptying and postprandial antroduodenal motility in healthy volunteers. Aliment Pharmacol Ther 1997; 11(6):1077-86.

11 Tucci A, Corinaldesi R, Stanghellini V, Tosetti C, Di Febo G, Paparo GF et al. Helicobacter pylori infection and gastric function in patients with chronic idiopathic dyspepsia. Gastroenterology 1992; 103(3):768-774.

12 Samsom M, Vermeijden JR, Smout AJ, Van Doorn E, Roelofs J, Van Dam PS et al. Prevalence of delayed gastric emptying in diabetic patients and relationship to dyspeptic symptoms: a prospective study in unselected diabetic patients. Diabetes Care 2003; 26(11):3116-3122.

13 Gilja OH, Detmer PR, Jong JM, Leotta DF, Li XN, Beach KW et al. Intragastric distribution and gastric emptying assessed by three-dimensional ultrasonography. Gastroenterology 1997; 113(1):38-49.

14 Scheffer RC, Gooszen HG, Wassenaar EB, Samsom M. Relationship Between Partial Gastric Volumes and Dyspeptic Symptoms in Fundoplication Patients: A 3D Ultrasonographic Study. Am J Gastroenterol 2004; 99(10):1902-1909.

15 Scheffer RC, Gooszen HG, Hebbard GS, Samsom M. The role of transsphincteric pressure and proximal gastric volume in acid reflux before and after fundoplication. Gastroenterology 2005; 129(6):1900-1909.

16 Mundt MW, Samsom M. Fundal dysaccommodation in functional dyspepsia: head-to-head comparison between the barostat and three-dimensional ultrasonographic technique. Gut 2006; 55(12):1725-30.

17 Tefera S, Gilja OH, Olafsdottir E, Hausken T, Hatlebakk JG, Berstad A. Intragastric maldistribution of a liquid meal in patients with reflux oesophagitis assessed by three dimensional ultrasonography. Gut 2002; 50(2):153-158.

18 Bolondi L, Bortolotti M, Santi V, Calletti T, Gaiani S, Labo G. Measurement of gastric emptying time by real-time ultrasonography. Gastroenterology 1985; 89(4):752-759.

19 Tack J, Caenepeel P, Piessevaux H, Cuomo R, Janssens J. Assessment of meal induced gastric accommodation by a satiety drinking test in health and in severe functional dyspepsia. Gut 2003; 52(9):1271-1277.

20 Chial HJ, Camilleri C, Delgado-Aros S, Burton D, Thomforde G, Ferber I et al. A nutrient drink test to assess maximum tolerated volume and postprandial symptoms: effects of gender, body mass index and age in health. Neurogastroenterol Motil 2002; 14(3):249-253.

21 Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, New Jersey: Erlbaum, 1988.

22 Fraser RJ, Horowitz M, Maddox AF, Dent J. Postprandial antropyloroduodenal motility and gastric emptying in gastroparesis--effects of cisapride. Gut 1994; 35(2):172-178.

23 Troncon LE, Bennett RJ, Ahluwalia NK, Thompson DG. Abnormal intragastric distribution of food during gastric emptying in functional dyspepsia patients. Gut 1994; 35(3):327-332.

24 Cuomo R, Sarnelli G, Grasso R, Bruzzese D, Pumpo R, Salomone M et al. Functional dyspepsia symptoms, gastric emptying and satiety provocative test: analysis of relationships. Scand J Gastroenterol 2001; 36(10):1030-1036.

25 Boeckxstaens GE, Hirsch DP, van den Elzen BD, Heisterkamp SH, Tytgat GN. Impaired drinking capacity in patients with functional dyspepsia: relationship with proximal stomach function. Gastroenterology 2001; 121(5):1054-1063.

26 Gonenne J, Castillo EJ, Camilleri M, Burton D, Thomforde GM, Baxter KL et al. Does the nutrient drink test accurately predict postprandial gastric volume in health and community dyspepsia? Neurogastroenterol Motil 2005; 17(1):44-50.

27 Delgado-Aros S, Camilleri M, Cremonini F, Ferber I, Stephens D, Burton DD. Contributions of gastric volumes and gastric emptying to meal size and postmeal symptoms in functional dyspepsia. Gastroenterology 2004; 127(6):1685-1694.

28 van Lelyveld N, Ter Linde J, Baron A, Mundt M, Wajs E, Samsom M. The 5-HT4 antagonist R216073 does not affect gastric motor and sensory function in patients with functional dyspepsia. Aliment Pharmacol Ther 2006; 24(4):669-77.

Chapter 04

The effect of the energy content of a meal on partial gastric volumes and upper abdominal sensations in patients with functional dyspepsia

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Submitted

Abstract

Background and aim

In order to reduce postprandial symptoms, many functional dyspeptic (FD) patients reduce the energy content of a meal. The aim of this study was to evaluate the influence of the energy content of a nutrient drink on upper abdominal sensations and total and partial gastric volumes.

Methods

Fourteen FD patients participated. Three-dimensional ultrasonography of the stomach was performed twice, after a high-energy (500 ml, 300kcal) and a low-energy (500ml, 20kcal) nutrient drink. Total, proximal, and distal gastric volumes were assessed while fasting and at 5, 15, 30, 45, and 60 minutes postprandially. At all consecutive time points, upper abdominal sensations were scored using a visual analogue scale (VAS).

Results

Total, proximal, and distal gastric volumes were significantly larger after the high-energy nutrient drink. After the high-energy nutrient drink, satiation scores were higher compared to the low-energy nutrient drink ($P = 0{\cdot}030$). However, no differences in fullness, nausea, and upper abdominal pain were observed (all $P > 0.05$).

Conclusion

The energy content of a liquid nutrient drink influences gastric motility, but this effect seems independent from the effect on visceral perception. In this study, the energy content of a nutrient drink could not be regarded as a specific trigger for symptom generation in FD.

Introduction

Functional dyspepsia (FD) is a functional disorder, characterized by pain or discomfort localized in the upper abdomen in the absence of organic disease.[1] Symptoms often include upper abdominal pain, early satiety, postprandial fullness, nausea, and vomiting. The aetiology of FD is unclear, but delayed gastric emptying,[2] impaired gastric accommodation,[3] and visceral hypersensitivity[4;5] have been suggested as possible mechanisms for postprandial symptoms.

In health, the energy intake is influenced by a number of different factors, including gastric distension, and exposure of small-intestinal receptors to nutrients and hormones.[6-8] Distension of the proximal stomach by the inflation of a barostat bag increases the perception of fullness.[9] However, there is accumulating evidence that the distal stomach plays a key role in the generation of fullness. Jones et al. showed that in health, the antral area is positively correlated with postprandial fullness sensation.[10] In addition, fullness was found to be related to distal gastric volume rather than proximal gastric volume, studied by three dimensional ultrasonography.[11]

In FD patients the relationship between distal gastric volume and postprandial sensations is less clear. A larger distal gastric volume in FD patients has been reported in previous studies.[12-14] Furthermore, most FD patients have a higher postprandial sensation of fullness. The presence of profound changes in gastric volume and upper abdominal symptoms suggest a relationship. Previously, the relationship between the increase in distal gastric volume and fullness was studied in GERD patients, FD patients, and healthy controls. After ingestion of a liquid nutrient drink (500ml, 300kcal) a significant relationship between distal gastric volume and fullness was found in GERD patients and in healthy controls. In FD patients however, this relationship was lacking.[15] Apparently, the physiological regulation of fullness sensation, in part regulated by the distal stomach, is disturbed in FD patients.

In order to reduce postprandial symptoms, many FD patients reduce the energy content of a single meal; however the rational of this is not fully understood. Since the energy content of a meal is a large contributor to the generation of postprandial symptoms in FD patients, we hypothesized an equivalent effect on total and partial gastric volumes. In the current study, we aimed to evaluate the effect of the energy content of a liquid nutrient drink on upper abdominal sensations and total and partial gastric volumes, by simultaneous measurement.

Materials and methods

Patients

Fourteen patients (10 women, 4 men, mean age of 46 years; range 20-62) were included in the study. Functional dyspeptic patients, entering the gastrointestinal motility outpatient clinic, were asked to participate in the study. Functional dyspepsia was defined according to the Rome II criteria; upper abdominal symptoms (pain or discomfort) for at least 3 months in the last 12 months, which did not need to be consecutive. Upper GI endoscopy was performed within 1 year prior to inclusion, to rule out any upper gastrointestinal abnormalities. Additional investigations, including abdominal ultrasonography and laboratory testing, all showed negative results. Patients were asked to discontinue the use of any medication known to influence gastrointestinal motility for at least 3 days prior to the study days. None of the patients had concomitant illness or a history of abdominal surgery.

All patients completed a questionnaire concerning upper abdominal symptoms experienced in daily life; upper abdominal pain, fullness, bloating, early satiety, nausea, and vomiting. The severity of symptoms was scored from 0-5 (0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe). For inclusion, two symptoms had to be scored as 3 or higher.[16] In addition, the relationship between ingestion of food and the generation of symptoms was assessed by clinical interview. All patients experienced postprandial upper abdominal sensations.

The study was approved by the medical ethics committee of the University Medical Center Utrecht and written informed consent was obtained from all participants.

Study design

Patients were asked to visit our outpatient clinic on two separate occasions. The maximum interval between two measurements was 3 weeks. After a ten hour fasting period, patients randomly received either the high-energy, or the low-energy drink. Patients who received the high-energy drink at the first visit, received a low-energy drink at the second visit and vice versa. The nutrient drink was presented in three covered mugs with a straw and both patients and investigators were blinded for the content of the liquid nutrient drink.

Three-dimensional ultrasound (3D-US) of the stomach was performed while fasting, and at 5, 15, 30, 45, and 60 minutes after ingestion of the nutrient drink. At all consecutive time points patients were asked to score satiation, hunger, upper abdominal pain, fullness, and nausea using a visual analogue scale (VAS) varying from no symptoms to unbearable symptoms (0 – 100 mm).

Nutrient drink

The high-energy drink (500ml) consisted of 200 ml nutrient drink (300kcal) (Nutridrink, Nutricia, Zoetermeer, the Netherlands), mixed with 300 ml water. The low-energy drink had a energy load of 20 kcal, similar to other studies.[17] The drink (500ml) consisted of 13 ml nutrient drink mixed with water to a volume of 500ml.

3D ultrasonography imaging system

The 3D imaging system consisted of an ultrasound scanner with a 3.5 MHz curved probe and a tracking system (Esaote-Pie Medical, Maastricht, The Netherlands). The tracking system consisted of a transmitter generating a spatially varying magnetic field and a small receiver, firmly attached to the ultrasound probe, containing three orthogonal coils to sense the magnetic field strength. The ultrasound probe with attached sensor was used to localize the left lateral and superior margins of the stomach and the pylorus. The depth of scanning was adjusted enabling an ultrasound scan of the stomach, superior mesenteric vein, aorta, left liver lobe and diaphragm on top of the gastric fundus. A standardized ultrasound-scanning pattern was used, starting at the left lateral subcostal margin and then moving distally towards the pylorus having the probe in a vertical position. During the scan, all patients suspended their breathing in inspiration.[18;19]

Partial gastric volumes

The two-dimensional (2D) frames were processed to construct 3D images. The 2D sagittal planes were used to draw the region of interest, corresponding to the inner layer of the stomach wall (the interface between the outer profile of the gastric wall mucosa and the liquid nutrition). A 3D reconstructed image of the stomach and the gastric volume was obtained using software with rendering and volume estimation capability (In Vivo ScanNT, Medcom GmbH, Darmstadt, Germany).

In addition, partial gastric volumes were calculated. Proximal gastric volume was defined as the gastric volume between the diaphragm and a dividing plane 10cm below the point where the fundic top reaches the diaphragm. Similarly, distal gastric volume was defined as the gastric region between the antral area (the sagittal ultrasound plane in which the antrum, the left liver lobe, the superior mesenteric vein and the gastroduodenal junction) and the gastroduodenal junction. The proximal to distal gastric volume ratios were calculated at all time points.
Analysis of total and partial gastric volumes was performed by a single investigator (N.B.) who was blinded for the energy content of the liquid nutrient drink.

Statistical analysis

Data were shown as mean ± standard error of the mean (SEM). Normality was tested using Kolmogorov-Smirnov-test. Gastric volumes and upper abdominal sensations were compared between the high-energy and the low-energy drink using the paired sample t-test. At consecutive time points averaged 3D volumes and averaged symptom scores were compared between the high-energy and the low-energy drink using repeated measures analysis of variance (ANOVA). Pearson's correlation test was used to calculate correlation coefficients between upper gastrointestinal sensations and partial gastric volumes. $P < 0.05$ was considered significant. All statistical analysis was performed using commercially available software (SPSS 11.0 for Microsoft windows).

Results

The following symptoms were reported as moderate, severe or very severe; upper abdominal pain (93%), early satiety (71%), bloating (86%), fullness (64%), nausea (71%), and vomiting (21%) (table 1). All patients reported to have increased upper abdominal sensations within 30 minutes after meal ingestion in daily life.

Total gastric volumes

The 3D-US images of two patients were not suitable for analysis and were therefore left out of the analysis. Fasting total gastric volume was similar

Table 1
Frequency of severity grading of each of six upper abdominal symptoms in 14 patients with FD.

	0-2 (none-Mild)	3-5 (Moderate-very severe)
Upper abdominal pain	1 (7%)	13 (93%)
Early satiety	4 (29%)	10 (71%)
Bloating	2 (14%)	12 (86%)
Fullness	5 (36%)	9 (64%)
Nausea	4 (29%)	10 (71%)
Vomiting	11 (79%)	3 (21%)

Numbers in parentheses represent row percentages.

before ingestion of the high-energy (45 ml ± 6) and the low-energy drink (47 ml ± 6), $P > 0.05$. Total gastric volume was significantly larger at all consecutive time points after ingestion of the high-energy drink compared to the low-energy drink, $P < 0.001$. Five minutes after ingestion, total gastric volume was 505 ml ± 12 after the high-energy drink compared to 425 ml ± 27 after the low-energy drink, $P = 0.017$ (figure 1).

Partial gastric volumes

At 45 and 60 minutes after ingestion of the low-energy drink, proximal gastric volume was very low, and could not be reliably quantified. Until 30 minutes postprandially, proximal gastric volume was larger after the high-energy nutrient drink compared to the low-energy nutrient drink ($P = 0.029$). Distal gastric volume was larger at all consecutive time points after the high-energy nutrient drink ($P = 0.039$) (figure 1).

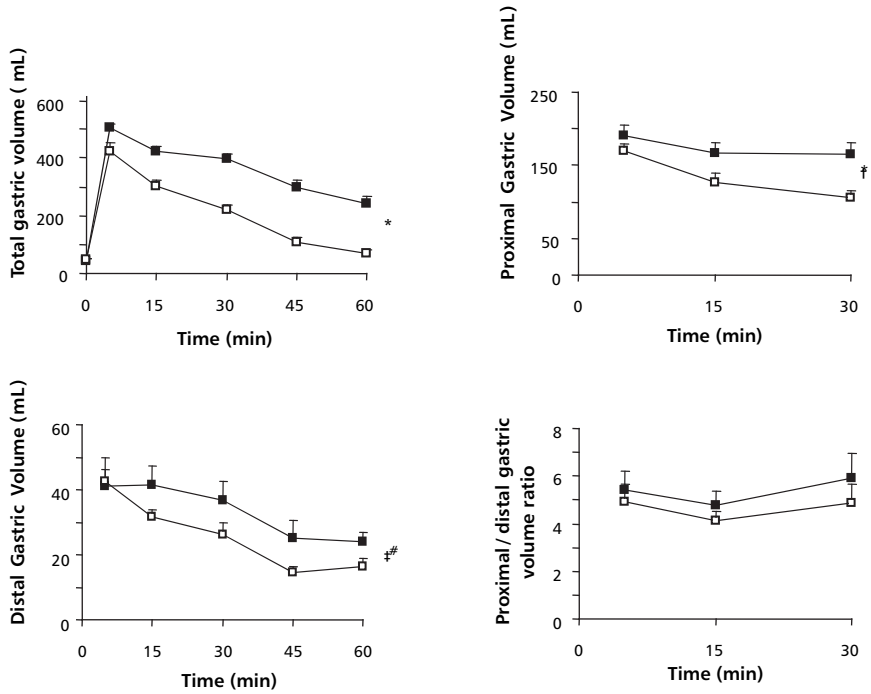

Figure 1

Total gastric volume was larger after ingestion of a high-energy (■) nutrient drink compared to a low-energy (❑) nutrient drink. * $P < 0.001$. Similarly, proximal and distal gastric volumes were larger after ingestion of a high-energy nutrient drink compared to a low-energy nutrient drink. †$P = 0.029$; ‡$P = 0.039$. There is no significant difference in proximal to distal gastric volume ratio between the high-energy and low-energy drink.

Proximal to distal gastric volume ratio

Proximal to distal gastric volume ratios were calculated at 5, 15, and 30 minutes postprandially. Proximal to distal gastric volume ratios were comparable between the low-energy and the high-energy drink ($P = 0.268$) (Figure 1).

Upper abdominal sensations

Five minutes after ingestion of the high-energy nutrient drink, a significant increase in satiation ($P < 0.01$), fullness ($P < 0.01$), nausea ($P = 0.02$), and upper abdominal pain ($P < 0{\cdot}01$) was observed. Five minutes after ingestion of the low-energy nutrient drink, scores for satiation ($P < 0.01$), fullness ($P < 0.01$) and nausea ($P = 0.02$) increased significantly, except for upper abdominal pain ($P = 0.38$).

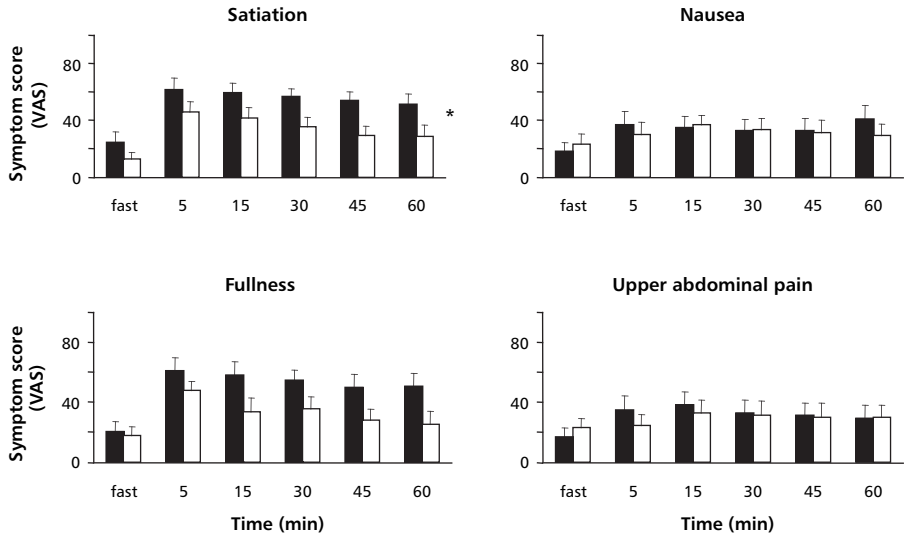

Figure 2
Symptom scores for satiation were higher after a high-energy (■) nutrient drink compared to a low-energy (❑) nutrient drink. *P = 0.03. For fullness, nausea and upper abdominal pain there was no significant difference.

At all time points postprandially, higher satiation scores were shown after the high-energy nutrient drink compared to the low-energy nutrient drink (P = 0.030) whereas fullness (P = 0.076), nausea (P = 0.866), and upper abdominal pain (P = 0.688) were not significantly different (figure 2).

Relationship between partial gastric volumes and upper abdominal sensations

After the high-energy drink, no significant correlation was found between distal gastric volume and fullness (5 min r = 0.467, P = 0.147; 15 min r = 0.028, P = 0.932). In addition, fullness did not correlate with distal gastric volume after the low-energy drink (5 min r = -0.440, P = 0.152; 15 min r = -0.220, P = 0.542) nor with any of the other sensations.

Discussion

The most important finding of this study is that despite large differences in total and partial gastric volumes after ingestion of a high energy drink compared to a low energy drink, no change in upper abdominal sensations fullness, nausea, and upper abdominal pain was observed. Symptom scores for satiation, which is a physiological upper abdominal sensation, were different between the high-energy and the low-energy drink. However, nausea, fullness, and upper abdominal pain were comparable. Thus, there is a discrepancy between the change in gastric volume and the change in symptom scores.

The intragastric distribution, as evaluated by the proximal to distal gastric volume ratios, was not different for the two drinks. Intragastric distribution is thus independent from the energy content of a nutrient drink. This is in concordance with results from a study where intragastric distribution was compared between functional dyspepsia patients and healthy controls.[20] In this study, patients and controls were given a solid meal (413 kcal) and a liquid meal, consisting of 170 ml of water. Proximal to distal gastric volume ratios of the solid phase initially showed a more proximal distribution, but comparison of distribution ratios at different time points was not significant.

Both after the low-energy and the high-energy drink, we did not find a relationship between distal gastric volume and fullness. We showed that this relationship could not be restored when a low-energy drink is given, suggesting that the disturbance between the perception of abdominal sensations and distal gastric volume in FD patients persists, irrespective of the energy content of a meal. It has been suggested that altered meal distribution is partially responsible for the excessive generation of fullness, however other causes such as visceral hypersensitivity most likely play a major role.[20]

The selection of patients was based purely on the Rome II criteria for functional dyspepsia. We did not select patients based on proximal gastric relaxation or gastric emptying rate. We believe it is unlikely that the presence of impaired proximal relaxation influences the outcome of our study since the relationship between proximal stomach function and postprandial fullness, nausea and upper abdominal pain is unclear, and remains to be matter of debate.[3;21] As well, no convincing evidence was found for the

relationship between gastric emptying and upper abdominal symptoms.[14]
To control the intake of the nutrient drink, we chose for the physiological way of administration rather than using an intraduodenal catheter. Patients were blinded for the energy content of the nutrient drink, but could still taste the difference in density between the two nutrient drinks. Although the blinding is arguably less accurate, we chose to stay close to daily practise.
The proximal gastric volume at 45 and 60 minutes after ingestion of the low-energy drink were too small to be measured accurately. The relationship between proximal gastric volume and postprandial symptoms was only assessed until 30 minutes postprandially. Nevertheless, all patients reported to have postprandial symptoms in daily life within 30 minutes after meal ingestion. Therefore, an indication of a relationship would most likely be present within 30 minutes.
The absence of any influence of the energy content of a nutrient drink on upper abdominal sensations is probably the most surprising finding of this study. In daily practise, FD patients are advised to decrease the energy content of a meal and the proportion of a meal, as this may reduce postprandial symptoms. In this study, we could not substantiate this with evidence. A possible explanation for the lack of relation between the energy content and upper abdominal sensations is the sudden volume load during the test. This may trigger the generation of dyspeptic symptoms, and thereby reducing the influence of the energy content.
In summary, the energy content of a liquid nutrient drink influences gastric motility, but this effect seems independent from the effect on visceral perception. In this study, the energy content of a nutrient drink could not be regarded as a specific trigger for symptom generation in functional dyspeptics.

References

1 Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR, Tytgat GN. Functional gastroduodenal disorders. Gut 1999; 45 Suppl 2:II37-II42.

2 Quartero AO, de Wit NJ, Lodder AC, Numans ME, Smout AJ, Hoes AW. Disturbed solid-phase gastric emptying in functional dyspepsia: a meta-analysis. Dig Dis Sci 1998; 43(9):2028-2033.

3 Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998; 115(6):1346-1352.

4 Salet GA, Samsom M, Roelofs JM, Berge Henegouwen GP, Smout AJ, Akkermans LM. Responses to gastric distension in functional dyspepsia. Gut 1998; 42(6):823-829.

5 Tack J, Caenepeel P, Fischler B, Piessevaux H, Janssens J. Symptoms associated with hypersensitivity to gastric distention in functional dyspepsia. Gastroenterology 2001; 121(3):526-535.

6 Kissileff HR, Pi-Sunyer FX, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in man. Am J Clin Nutr 1981; 34(2):154-160.

7 MacIntosh CG, Horowitz M, Verhagen MA, Smout AJ, Wishart J, Morris H et al. Effect of small intestinal nutrient infusion on appetite, gastrointestinal hormone release, and gastric myoelectrical activity in young and older men. Am J Gastroenterol 2001; 96(4):997-1007.

8 Read N, French S, Cunningham K. The role of the gut in regulating food intake in man. Nutr Rev 1994; 52(1):1-10.

9 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(3 Pt 1): G721-G726.

10 Jones KL, Doran SM, Hveem K, Bartholomeusz FD, Morley JE, Sun WM et al. Relation between postprandial satiation and antral area in normal subjects. Am J Clin Nutr 1997; 66(1):127-132.

11 Mundt MW, Hausken T, Smout AJ, Samsom M. Relationships between gastric accommodation and gastrointestinal sensations in healthy volunteers. A study using the barostat technique and two- and three-dimensional ultrasonography. Dig Dis Sci 2005; 50(9):1654-1660.

12 Hausken T, Berstad A. Wide gastric antrum in patients with non-ulcer dyspepsia. Effect of cisapride. Scand J Gastroenterol 1992; 27(5):427-432.

13 Troncon LE, Bennett RJ, Ahluwalia NK, Thompson DG. Abnormal intragastric distribution of food during gastric emptying in functional dyspepsia patients. Gut 1994; 35(3):327-332.

14 Pallotta N, Pezzotti P, Corazziari E. Relationship between antral distension and postprandial symptoms in functional dyspepsia. World J Gastroenterol 2006; 12(43):6982-6991.

15 van Lelyveld N, Scheffer R, Mundt M, Samsom M. Partial gastric volumes and upper abdominal sensations in functional dyspeptic and GERD patients: a 3D ultrasonographic study. Am J Gastroenterol 2006; 101(8):1845-1852.

16 Stanghellini V, Tosetti C, Paternico A, Barbara G, Morselli-Labate AM, Monetti N et al. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. Gastroenterology 1996; 110(4):1036-1042.

17 Hjelland IE, Ofstad AP, Narvestad JK, Berstad A, Hausken T. Drink tests in functional dyspepsia: which drink is best? Scand J Gastroenterol 2004; 39(10):933-937.

18 Tefera S, Gilja OH, Olafsdottir E, Hausken T, Hatlebakk JG, Berstad A. Intragastric maldistribution of a liquid meal in patients with reflux oesophagitis assessed by three dimensional ultrasonography. Gut 2002; 50(2):153-158.

19 Scheffer RC, Gooszen HG, Wassenaar EB, Samsom M. Relationship between partial gastric volumes and dyspeptic symptoms in fundoplication patients: a 3D ultrasonographic study. Am J Gastroenterol 2004; 99(10):1902-1909.

20 Piessevaux H, Tack J, Walrand S, Pauwels S, Geubel A. Intragastric distribution of a standardized meal in health and functional dyspepsia: correlation with specific symptoms. Neurogastroenterol Motil 2003; 15(5):447-455.

21 Boeckxstaens GE, Hirsch DP, Kuiken SD, Heisterkamp SH, Tytgat GN. The proximal stomach and postprandial symptoms in functional dyspeptics. Am J Gastroenterol 2002; 97(1):40-48.

Chapter 05

The 5-HT_4 antagonist R216073 does not affect gastric motor and sensory function in patients with functional dyspepsia

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Abstract

Background

Serotonin (5-HT) and the 5-HT_4 receptor play an important role in gastrointestinal motor and sensory functions. While 5-HT_4 agonists are known for their prokinetics properties, the effect of 5-HT_4 antagonists on upper gastrointestinal functions is largely unknown.

Aim

To assess the effect of a 5-HT_4 receptor antagonist (R216073) on gastric relaxation and visceral sensitivity in patients with functional dyspepsia (FD). Secondly, the influence of a functional polymorphism in the gene encoding the serotonin transport protein (SERT) on drug response was determined.

Methods

A double blind, randomised, placebo controlled, 2-period crossover study was performed in 20 FD patients. The effect of a single dose of 2000 mg R216073 on gastric relaxation and sensitivity was tested using three-dimensional ultrasonography and a nutrient drinktest.

Results

R216073 did not affect partial gastric volumes or upper abdominal sensations scored during 3D-US ($P > 0.05$). The maximum tolerated volume or upper abdominal sensations induced by the drinktest were not affected by R216073 ($P > 0.05$). The SERT promoter polymorphism was not associated with any of the end-points of the study.

Conclusion

A single dose of R216073 had no effect on fundic relaxation, drinking capacity, or upper abdominal symptoms in patients with functional dyspepsia.

Introduction

Several pathophysiologic mechanisms associated with functional dyspepsia have been described including: delayed gastric emptying, impaired gastric accommodation, and visceral hypersensitivity.[1-3] Serotonin (5-HT) plays a key role in regulating gastrointestinal sensory motor function.[4] Mucosal enterochromaffin cells (EC cells) release 5-HT in response to intraluminal stimulation after which 5-HT acts on 5-HT receptors, situated on afferent nerve endings in the lamina propria.[5] Subsequently, the afferent nerve endings generate a peristaltic or sensory reflex.[6-8]

There are 7 classes of 5-HT receptors.[9] Of these, the 5-HT$_4$ receptor plays an important role in the gastrointestinal sensory and motor functions.[6,7] In vitro studies suggest that 5-HT$_4$ receptor agonists potentiate fast excitatory postsynaptic potentials and also trigger the peristaltic reflex.[6,10,11]

Serotonergic agents are widely used to modulate gastrointestinal sensory motor function in patients with functional gastrointestinal disorders.[12] These include serotonin reuptake inhibitors (SSRI), which were shown to increase meal induced relaxation of the gastric fundus[13,14], the 5-HT$_1$P agonist sumatriptan, which reportedly enhanced relaxation of the gastric fundus[15], and 5-HT$_4$ agonists, like cisapride, acting as a prokinetic.[16]

In contrast with human studies, fluoxetine (SSRI) induced a concentration dependent contractile response in guinea pig muscle strips, which was greatest in the fundus.[17] The contractile response was reduced by GR113808, a 5-HT$_4$ antagonist.[17] R216073, the 5-HT$_4$ antagonist used in the current study, also prevented the inhibitory effects of an SSRI on canine fundus relaxation.[18] These studies suggest that a 5-HT$_4$ antagonist may affect gastric fundic relaxation. While 5-HT$_4$ receptor antagonists have very minor effects on gastrointestinal transit in healthy subjects,[19] the effect of a 5-HT$_4$ antagonist on gastric fundic relaxation is unknown.

5-HT has to be removed rapidly from the EC cell afferent nerve junction to terminate responses and to prevent desensitisation of the receptors. The 5-HT transport protein (SERT) is responsible for this uptake.[20] A 44-bp promoter polymorphism in the SERT gene displays two allelic forms, a long and a short variant.[21,22] The short variant is associated with reduced transcription of the gene and a lower 5-HT reuptake activity leading to increased availability of 5-HT for receptor stimulation. These variations in the gene encoding for SERT have been shown to affect treatment outcome after

alosetron (a 5-HT_3 receptor antagonist) dosage in IBS patients.[23]
The present study was undertaken to test the effect of R216073, a 5-HT_4 receptor antagonist, on proximal gastric relaxation and visceral sensitivity in patients with functional dyspepsia. Furthermore, the influence of the genotype of the SERT promoter on response to R216073 was evaluated. Three-dimensional ultrasonography (3D-US) was used for measuring fasting and postprandial gastric volumes after active or placebo dosage.[24,25] A nutrient drinktest was used for measuring maximum tolerated volume and meal induced sensations.[26]

Materials and Methods

Study subjects

Twenty patients (13 female) with a mean age of 42 (23-64) years and a mean weight of 70 (49-86) kg participated in the study. All patients fulfilled the Rome II criteria i.e. the presence of dyspeptic symptoms for at least 12 weeks in the last 12 months, in the absence of organic, systemic, or metabolic disease.[27]
Before inclusion, each patient completed a symptom questionnaire. They were asked to score six different symptoms (pain or discomfort centered in the upper abdomen, early satiety, bloating in the upper abdomen, fullness, nausea and vomiting) from 0-5 (0=none, 1=very mild; awareness of symptoms but easily tolerated, 2=mild; tolerated without interference with usual activity, 3=moderate; enough to cause some interference with usual activity, 4=severe; enough to cause significant interference with usual activity, 5=very severe; incapacitating with inability to work or do usual activity). For inclusion, two of these symptoms had to be scored as moderate, severe or very severe and these symptoms needed to be present for at least 12 weeks, not necessary consecutive, in the preceding 12 months.
Upper GI endoscopy was performed within 1 year prior to inclusion, to rule out any upper gastrointestinal abnormalities. A clinical interview, physical examination and laboratory testing were performed during the screening visit and two weeks after the study was completed. None of the patients had a history of gastrointestinal surgery. Female patients with childbearing potential without adequate use of contraception were excluded. Delayed gastric emptying, assessed within 6 months prior to inclusion using the ^{13}C-

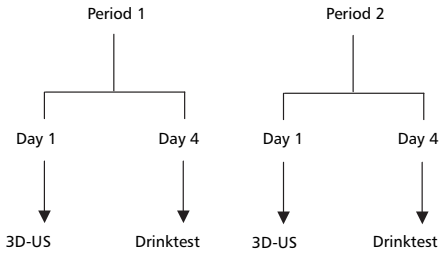

Figure 1
A randomised, double blind, placebo-controlled, 2-period crossover study in 20 functional dyspeptic patients. Subjects received the same treatment in one period, either R216073 or placebo.

octanoic breathtest, was considered an exclusion criterion.[28,29]
Patients were asked to discontinue any medication known to influence gastrointestinal motility or sensitivity for at least 7 days prior to the study. The protocol was approved by the ethics committee of the University Medical Centre Utrecht. All patients gave written informed consent for participation in the trial.

Study design

Drug efficacy was tested in a randomised, double blind, placebo-controlled, 2-period crossover study (figure 1). Each period of the crossover design consisted of 2 single dose administrations on two different study days. Randomisation was done at the level of the study periods, meaning that all patients received the same treatment in one period.
Previously, the pharmacokinetics of R216073 have been studied in 49 healthy subjects after a single dose of 100 – 2000 mg.[30] Plasma concentrations increased in proportion with the dose. The plasma concentration-time profiles displayed a biphasic decline, with a rapid decrease over the first 12 hours and a slower decline from 12 hours onwards. The median terminal half-life was about 16 hours. Peak plasma concentrations and systemic exposure increased fairly proportional throughout the investigated dose range.
On each treatment day a single dose of either 2000 mg of R216073 or placebo was administered. The mean peak plasma concentration reached in the current study (1008 ng/ml), was in the same range as the mean peak

plasma measured in the canine study (678 ng/ml), in which an effect on fundic accommodation was detected. The study medication was taken orally with 100 mL of water. Before- and 4 hours after dosage, ECG testing was performed to detect any abnormalities. Treatment periods were separated by an interval of at least 2 weeks and at the most 4 weeks. Within each period, treatment days were separated by an interval of at least 3 days and at the most 7 days. Each study day started at 8.00 am after an overnight fast of at least 10 hours.

Treatment day 1: three-dimensional ultrasonography

One hour after dosage, 3D-US was performed to assess total and partial gastric volumes. Subjects were comfortably seated in a wooden chair leaning slightly backward. A liquid nutrient test meal (500 ml) consisted of 200 ml lactose- and fiber-free milk drink, containing 12.0 gram proteins, 11.6 gram fat and 36.8 gram carbohydrate (300 kcal) (Nutridrink, Nutricia, Zoetermeer, The Netherlands) mixed with 300 ml of water. The liquid nutrient was ingested within 3 minutes. Ultrasonographic data were acquired while fasting and at 5, 15, 30, 45 and 60 minutes after meal ingestion. At all consecutive time points, upper abdominal sensations (upper abdominal pain, nausea, fullness and hunger) were scored using a visual analogue scale (VAS, 0-100 mm) varying from no sensation to unbearable sensations. The sum of nausea, fullness and pain was calculated at every time point and referred to as the total of upper abdominal sensations.

Treatment day 2: nutrient drinktest

The nutrient drinktest was performed one hour after dosage.[26,31] Subjects were asked to ingest a liquid nutrient (1.5 Kcal/mL, Nutridrink, Nutricia, Zoetermeer, The Netherlands) at a constant rate of 15 mL/min. At 5-minute intervals, they scored satiety using a graphic rating scale that combines verbal descriptors on a scale graded 0-5 (0 = no satiety, 5 = maximum satiety). The test ends when the subject reaches maximum satiety[26]. Upper abdominal sensations (upper abdominal pain, nausea, fullness and hunger) were scored using a VAS before the start and 30 minutes after reaching maximal satiety.

On each study day, peripheral blood samples, urine samples, ECG and vital signs were collected to evaluate drug safety before- and 2.5 hours after

dosage. Extra peripheral blood samples were taken for pharmacokinetic analysis of the drug before dosage and at 1, 1.5 and 2 hours after dosage. In addition, 10 ml peripheral blood was withdrawn for genetic analysis.

3D Ultrasonography Imaging System

The 3D imaging system consisted of an ultrasound scanner with a 3.5 MHz curved probe and a tracking system (Esaote-Pie Medical, Maastricht, The Netherlands). The tracking system consisted of a transmitter generating a spatially varying magnetic field and a small receiver, firmly attached to the ultrasound probe, containing three orthogonal coils to sense the magnetic field strength.[32]

The ultrasound probe with attached sensor was used to localize the left lateral and superior margins of the stomach and the pylorus. The depth of scanning was adjusted enabling an ultrasound scan of the stomach, superior mesenteric vein, aorta, left liver lobe and diaphragm on top of the gastric fundus. A standardized ultrasound scanning pattern was used, starting at the left lateral subcostal margin and then moving distally towards the pylorus having the probe in a vertical position. During the scan all participants suspended their breathing in inspiration. For each ultrasound scan approximately 300-400 2D ultrasound images were stored with a scan typically lasting 15-20 seconds. The 2D sagittal images were digitised and stored in the computer workstation.[33]

Total and Partial Gastric Volumes

The gastric volume was measured using software with rendering and volume estimation capability (In Vivo ScanNT, Medcom GmbH, Darmstadt, Germany). The 2D sagittal frames were processed to construct 3D images, containing 60-70 sagittal planes. The sagittal planes were used to draw the region of interest. The inner layer of the stomach wall, corresponding to the interface between the outer profile of the gastric wall mucosa and the liquid nutrition, was outlined in an average of 10-20 planes. The computer generates gastric contours in the intermediate frames using a triangulation technique. Then a reconstructed 3D image of the stomach and the gastric volume was obtained.

In addition, partial gastric volumes were calculated. The proximal part was separated by a dividing plane 10 cm below the point where the fundic top reaches the diaphragm, perpendicular to the longitudinal axis of the stomach. The 10 cm margin matches the diameter of a barostat balloon contain-

ing 500 ml of fluid. Similarly, a distal part was separated, defined as the gastric region between the antral area (the sagittal ultrasound plane in which the antrum, the left liver lobe, the superior mesenteric vein and the abdominal aorta are seen simultaneously) and the gastro duodenal junction. The antral area is a known anatomical landmark and therefore chosen as the margin of the distal gastric volume.[34]
At every time point, we subtracted fasting total or partial gastric volume leaving the change in total or partial gastric volume. The change in gastric volume was used for comparison between the two treatment periods. A proximal gastric volume ratio was calculated by dividing proximal gastric volume by total gastric volume. Recently, we defined impaired proximal relaxation as the average of the proximal gastric volume ratios of 5 and 15 minutes smaller than the lower limit of the 95% confidence interval of healthy controls (0.32 – 0.57).[35] Proximal relaxation was assessed after placebo dosage.

SERT promoter polymorphism

Genomic DNA was isolated from whole blood from all participants using the QIAamp DNA blood minikit (Qiagen Inc., Valencia, California, USA). PCR across the SERT promoter insertion/deletion polymorphism was performed using the primers and reaction conditions described by Camilleri et al.[23] However, we performed PCR amplification in a total volume of 25 μL, containing 100 ng genomic DNA. The size of the amplified fragments was determined by electrophoresis on a 2.5% low range ultra agarose gel (Biorad Inc, USA) stained with ethidium bromide; 572 bp and 528 bp products were typed as long (L) and short (S) alleles respectively.

Statistical analysis

The focus of the analysis was to determine differences in response between R216073 and placebo dosage on the primary end points of the study; proximal gastric volume, distal gastric volume, maximum tolerated volume and upper abdominal sensations scored during the 3D-US or nutrient drinktest. Possible differences in pharmacological response in relation to the genotype of the SERT promoter polymorphism were analysed by comparing gastric volume data, upper abdominal sensations and maximum tolerated volume for the three genotypes.
Data was summarized as mean ± SEM. Normality was tested using the Kolmogorov-Smirnov test. At consecutive time points, average 3D volume data

and upper abdominal sensation scores were compared between periods (R216073 or placebo dosage) using repeated measures analysis of variance (ANOVA). Fasting gastric volumes and sensations were compared using independent samples T-test. A $p < 0.05$ was considered significant. All statistical analysis was performed using commercially available software (SPSS 11.0 for Microsoft windows).

Results

On both study days, high plasma concentrations of R216073 were achieved at the time of the start of the assessments, at one hour post dose. The mean peak plasma concentrations of 1008 ± 781 ng/mL in the first period and 937 ± 464 ng/mL in the second period were comparable with previous findings following a 2000 mg dosage in healthy volunteers (1008 ± 573 ng/mL).[30] There was a substantial inter-subject variability in the peak plasma concentration. However, no correlation between the pharmacodynamic effect and plasma concentrations of the drug was observed. FD patients reported the following upper abdominal sensations as moderate or higher; upper abdominal pain (77%), early satiety (72%), bloating (83%), fullness (78%), and nausea (67%). Vomiting was present in 22% of patients (table 1). R216073

Table 1
Frequency of severity grading for each of six dyspeptic symptoms in 18 patients with functional dyspepsia.

	0 (Absent)	1-2 (Mild)	3 (Moderate)	4-5 (Severe)
UA Pain	1 (6)	3 (17)	8 (44)	6 (33)
Early sat	2 (11)	3 (17)	8 (44)	5 (28)
Bloating	0 (0)	3 (17)	11 (61)	4 (22)
Fullness	1 (6)	3 (17)	11 (61)	3 (17)
Nausea	3 (17)	3 (17)	5 (28)	7 (39)
Vomiting	14 (78)	0 (0)	0 (0)	4 (22)

Numbers in parentheses represent row percentages.

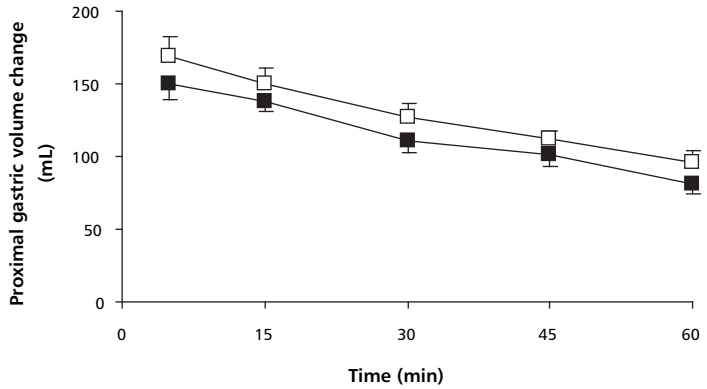

Figure 2
Proximal gastric volume change after a liquid nutrient, measured by 3D-US. No differences were observed between R216073 (■) or placebo dosage (❑) ($P > 0.05$).

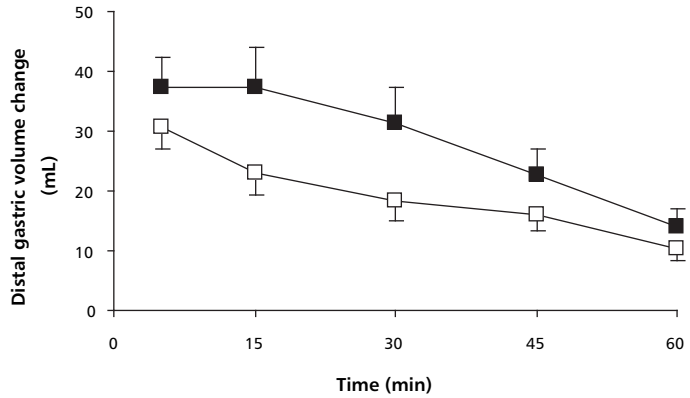

Figure 3
Distal gastric volume change after a liquid nutrient, measured by 3D-US. No differences were observed between R216073 (■) or placebo dosage (❑) ($P > 0.05$).

was well tolerated by all subjects. One female subject was diagnosed to suffer from diabetes during the study and was excluded. A total of 19 subjects completed the study. Due to technical problems, the 3D-US data of one male subject was unsuitable for analysis. Therefore, combined assessment of 3D-US and the nutrient drinktest was completed in a total of 18 subjects.

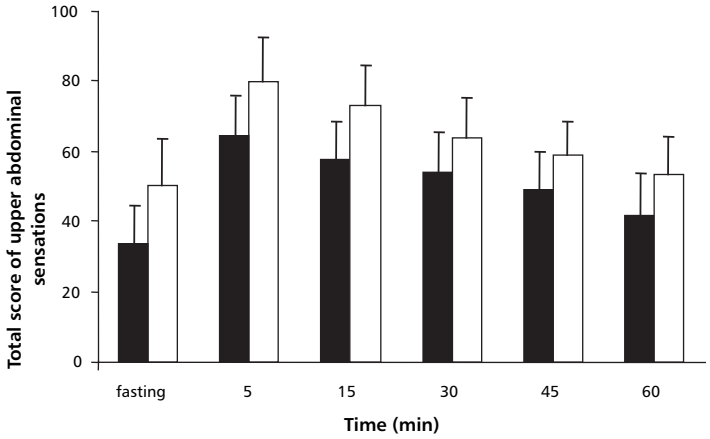

Figure 4
Effect of a liquid nutrient on upper abdominal sensations. R216073 (black bars) had no effect on preprandial or postprandial upper abdominal sensations compared to placebo ($P > 0.05$).

Three-dimensional ultrasonography

R216073 did not affect fasting total gastric volume (R216073; 46.2 ± 4.8 and placebo; 36.2 ± 3.9, $P > 0.05$). Directly after ingestion of the liquid nutrient, the change in total gastric volume was comparable between R216073 and placebo (5 minutes: R216073; 430.0 ± 10.7 ml, placebo; 441.5 ± 8.8 ml, $P > 0.05$).

The change in proximal gastric volume after meal ingestion was comparable after R216073 or placebo dosage ($P > 0.05$) (figure 2). Likewise, administration of R216073 did not significantly affect the change in distal gastric volume after meal ingestion ($P = 0.073$) (figure 3).

A total of 6 out of 18 patients had a proximal gastric volume ratio smaller then 0.32 after placebo dosage (33%). These patients were classified as having impaired proximal gastric relaxation. No differences in pharmacological response were observed between patients with normal or impaired proximal relaxation. R216073 did not affect upper GI sensations scored during 3D-US. Upper abdominal pain, fullness, nausea and hunger sensation scores were comparable at all time points ($P > 0.05$). Consequently, no change in the sum of upper abdominal sensations was observed (figure 4). In addition, no change in hunger score was seen after R216073 or placebo dosage.

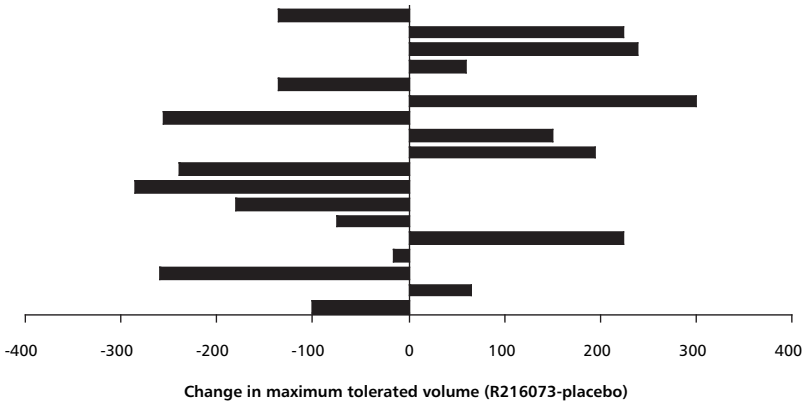

Figure 5
Nutrient drinktest; each bar represents the difference in maximum drinking capacity between the two periods, after either R216073 or placebo dosage. Eight patients drank less after R216073 dosage and 10 patients drank more. Overall, R216073 had no effect on maximum drinking capacity ($P > 0.05$).

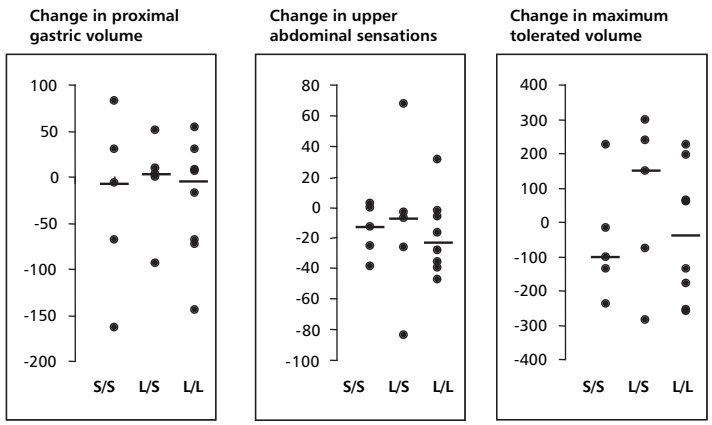

Figure 6
The effect of R216073 on proximal gastric volume, upper abdominal sensations and maximum tolerated volume, plotted in three groups of patients with a different genotype of the SERT promoter; (1) S/S homozygous and (2) L/L homozygous and (3) L/S heterozygous ($P > 0.05$). The horizontal bars indicate the mean.

Nutrient drinktest

R216073 did not affect maximum tolerated volume (R216073; 561.7 ± 52.4 ml, placebo; 573.9 ± 56.7 ml, $P > 0.05$). Ten patients drank less while eight patients drank more after R216073 dosage (figure 5).

The sensations of upper abdominal pain, fullness and nausea before and after the drinktest were comparable after R216073 or placebo dosage (all $P > 0.05$). The increase in the total of upper abdominal sensations was also comparable between R216073 or placebo administration ($P > 0.05$).

SERT promoter polymorphism

Five patients were heterozygous (28%), five patients had the S/S genotype (28%), and eight patients carried the L/L genotype (44%). We observed no differences when comparing 3D-US data, upper abdominal sensations or maximum tolerated volume between the three genotypes ($P > 0.05$) (figure 6).

Discussion

This is the first study to assess the effect of a 5-HT$_4$ receptor antagonist on gastric relaxation and visceral sensitivity in patients with functional dyspepsia. A single dose of 2000 mg of R216073 had no effect on proximal or distal gastric volume change after a liquid nutrient nor did it effect upper abdominal sensations scored during 3D-US in FD patients. In addition, R216073 did not influence maximum tolerated volume or upper abdominal sensations induced by the intake of the liquid nutrient during the drinktest. Finally, the SERT promoter polymorphism was not associated with any of the end-points of the study.

R216073 is a selective, competitive and reversible 5-HT$_4$ antagonist, as demonstrated in various in vitro and in vivo animal studies.[18,36] The exposure level of R216073 was 10 times higher then the exposure needed to have 90% occupancy of the receptor.[18] In a canine model, R216073 has been shown to be able to restore abnormal fundus relaxation induced by a serotonin reuptake inhibitor (SSRI), with a comparable peak plasma concentration reached in the current study.[18] This led to the hypothesis that an increased stimulation of 5-HT$_4$ receptors induces fundic contraction whereas antagonising the 5-HT$_4$ receptor would result in enhanced relaxation of the gastric fundus.

In the present study, impaired proximal gastric relaxation was found in 6 out of 18 patients. The highest effect of R216073 was to be expected in this group of patients in terms of restoring fundus relaxation. We choose to study various end points, including the effect on drinking capacity and upper abdominal sensations. Considering the lack of agreement between various publications describing the relationship between impaired proximal relaxation, upper abdominal sensations and maximum tolerated volume, normal proximal relaxation was not an exclusion criterion.[26,37-39]

We have considered several potential factors that might explain the lack of efficacy of R216073 on gastric relaxation and visceral sensitivity in FD patients. Firstly, the canine study does not necessarily serve as a model for dyspeptic patients. In the canine study, the level of SERT was diminished leading to higher availability of serotonin. However, in our studied population, we do not know if the activity of SERT or the availability of serotonin is altered.

Secondly, there are at least 21 different variants of the 5-HT receptor. Several of which are likely to be involved in the regulation of gastric accommodation or visceral sensitivity. By administrating sumatriptan, a 5-HT_1 receptor agonist, the gastric fundus relaxes immediately.[15] However, sumatriptan failed to relieve postprandial symptoms in FD patients with impaired proximal accommodation.[40] A 5-HT_3 receptor agonist has been shown to delay gastric emptying in combination with a relaxation of the gastric fundus.[41] An improvement of global symptoms, pain and discomfort was observed after administration of alosetron, a 5-HT_3 receptor antagonist, in non-constipated female IBS patients, suggesting an important role of the 5-HT_3 receptor in visceral sensitivity.[42] In FD patients, a modest relief of symptoms was found after alosetron dosage compared to placebo.[43] 5-HT_4 receptor agonists, like cisapride or tegaserod, are known to accelerate gastric emptying and gastrointestinal transit, however also enhanced both the perception of gastric distension and the gastric accommodation to a meal.[44,45]

Another possible explanation for the absence of effect of R216073 is the design of the trial in terms of the duration of dosing. Although high plasma concentrations were reached and high receptor occupancy is expected, a single dose might not be sufficient for regulating gastric motor and sensory properties. Finally, the techniques being used might not be sensitive enough for detecting a drug effect.

3D-US is a non-invasive technique, which can be used for the assessment of total and partial gastric volumes.[35] 3D-US has shown excellent in vitro and in vivo accuracy in volume estimation and a low inter observer variation.[25,32,33] We have recently shown that 3D-US can be used for the identification of impaired proximal relaxation and the assessment of distal gastric function.[35] A large overlap in the detection of impaired proximal relaxation was shown in a head to head comparison between the barostat technique and 3D-US.[46] All together, 3D-US has proven to be a reliable and sensitive technique for the evaluation of proximal and distal gastric volumes.

The nutrient drinktest was developed as a non-invasive alternative of the barostat technique for the evaluation of gastric motor function and sensitivity.[26,31] The drinktest does not differentiate between hypersensitivity and impaired proximal relaxation. Moreover, a relationship between the increase in total or partial gastric volume and maximum tolerated volume is lacking[37-39]. However, it has recently been shown that symptoms scored after a nutrient drinktest mimic those reported by patients as chronic symptoms.[38] Secondly, the nutrient drinktest has been established as a valid tool for measuring meal induced satiation[47]. Therefore, we believe that the nutrient drinktest is a suitable instrument for the comparison before and after treatment, as has been done in the current study.

The S and L variants differentially modulate transcriptional activity of the SERT promoter and thereby determine 5-HT uptake capacity. The S variant is associated with lower expression and activity of SERT compared to the L variant.21,22 Therefore, we choose to categorize patients in three groups for the comparison of various end points: (1) S/S homozygous (2) L/S heterozygous and (3) L/L homozygous. We found the different genotypes to be present in 28%, 28%, and 44% respectively in the studied population. The absence of any differences in the primary end points between the three groups indicates that the lack of effect of R216073 cannot be attributed to the genotype of the SERT promoter polymorphism.

In conclusion, R216073 has no effect on proximal or distal gastric relaxation, upper abdominal sensations or maximum tolerated volume. Although high plasma concentrations of R216073 were reached, the current study suggests that R216073 has no therapeutic potential in FD patients. Further studies, in which 3D-US can be used to assess gastric relaxation, are required to facilitate the development of new therapeutic agents on specific motoric or sensory gastric functions.

References

1) 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.

2 Kim DY, Delgado-Aros S, Camilleri M, Samsom M, Murray JA, O'Connor MK et al. Noninvasive measurement of gastric accommodation in patients with idiopathic nonulcer dyspepsia. Am J Gastroenterol 2001;96:3099-3105.

3 Salet GA, Samsom M, Roelofs JM, Berge Henegouwen GP, Smout AJ, Akkermans LM. Responses to gastric distension in functional dyspepsia. Gut 1998;42:823-829.

4 Gershon MD. Review article: serotonin receptors and transporters - roles in normal and abnormal gastrointestinal motility. Aliment Pharmacol Ther 2004;20 Suppl 7:3-14.

5 Bulbring E, Crema A. The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. J Physiol 1959;146:18-28.

6 Grider JR, Foxx-Orenstein AE, Jin JG. 5-Hydroxytryptamine4 receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. Gastroenterology 1998;115:370-380.

7 Foxx-Orenstein AE, Kuemmerle JF, Grider JR. Distinct 5-HT receptors mediate the peristaltic reflex induced by mucosal stimuli in human and guinea pig intestine. Gastroenterology 1996;111:1281-1290.

8 Grider JR, Kuemmerle JF, Jin JG. 5-HT released by mucosal stimuli initiates peristalsis by activating 5-HT4/5-HT1p receptors on sensory CGRP neurons. Am J Physiol 1996;270:G778-G782.

9 Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999;38:1083-1152.

10 Pan H, Galligan JJ. 5-HT1A and 5-HT4 receptors mediate inhibition and facilitation of fast synaptic transmission in enteric neurons. Am J Physiol 1994;266:G230-G238.

11 Sanger GJ, Banner SE, Smith MI, Wardle KA. SB-207266: 5-HT4 receptor antagonism in human isolated gut and prevention of 5-HT-evoked sensitization of peristalsis and increased defaecation in animal models. Neurogastroenterol Motil 1998;10:271-279.

12 Cremonini F, Delgado-Aros S, Talley NJ. Functional dyspepsia: drugs for new (and old) therapeutic targets. Best Pract Res Clin Gastroenterol 2004;18:717-733.

13 Chial HJ, Camilleri M, Burton D, Thomforde G, Olden KW, Stephens D. Selective effects of serotonergic psychoactive agents on gastrointestinal functions in health. Am J Physiol Gastrointest Liver Physiol 2003;284:G130-G137.

14 Tack J, Broekaert D, Coulie B, Fischler B, Janssens J. Influence of the selective serotonin re-uptake inhibitor, paroxetine, on gastric sensorimotor function in humans. Aliment Pharmacol Ther 2003;17:603-608.

15 Tack J, Coulie B, Wilmer A, Andrioli A, Janssens J. Influence of sumatriptan on gastric fundus tone and on the perception of gastric distension in man. Gut 2000;46:468-473.

16 Veldhuyzen van Zanten SJ, Jones MJ, Verlinden M, Talley NJ. Efficacy of cisapride and domperidone in functional (nonulcer) dyspepsia: a meta-analysis. Am J Gastroenterol 2001;96:689-696.

17 James AN, Ryan JP, Parkman HP. Effects of the selective serotonin reuptake inhibitor, fluoxetine, on regional gastric contractility. Neurogastroenterol Motil 2005;17:76-82.

18 Bosmans JP, Meulemans A. Compound monograph R184798, version 2.0. Janssen Research Foundation 2000.

19 Bharucha AE, Camilleri M, Haydock S, Ferber I, Burton D, Cooper S et al. Effects of a serotonin 5-HT(4) receptor antagonist SB-207266 on gastrointestinal motor and sensory function in humans. Gut 2000;47:667-674.

20 Wade PR, Chen J, Jaffe B, Kassem IS, Blakely RD, Gershon MD. Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract. J Neurosci 1996;16:2352-2364.

21 Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621-2624.

22 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527-1531.

23 Camilleri M, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE et al. Serotonin-transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. Gastroenterology 2002;123:425-432.

24 Gilja OH, Hausken T, Wilhelmsen I, Berstad A. Impaired accommodation of proximal stomach to a meal in functional dyspepsia. Dig Dis Sci 1996;41:689-696.

25 Gilja OH, Detmer PR, Jong JM, Leotta DF, Li XN, Beach KW et al. Intragastric distribution and gastric emptying assessed by three-dimensional ultrasonography. Gastroenterology 1997;113:38-49.

26 Tack J, Caenepeel P, Piessevaux H, Cuomo R, Janssens J. Assessment of meal induced gastric accommodation by a satiety drinking test in health and in severe functional dyspepsia. Gut 2003;52:1271-1277.

27 Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR, Tytgat GN. Functional gastroduodenal disorders. Gut 1999;45 Suppl 2:II37-II42.

28) Minderhoud IM, Mundt MW, Roelofs JM, Samsom M. Gastric emptying of a solid meal starts during meal ingestion: combined study using 13C-octanoic acid breath test and Doppler ultrasonography. Absence of a lag phase in 13C-octanoic acid breath test. Digestion 2004;70:55-60.

29 Samsom M, Vermeijden JR, Smout AJ, Van Doorn E, Roelofs J, Van Dam PS et al. Prevalence of delayed gastric emptying in diabetic patients and relationship to dyspeptic symptoms: a prospective study in unselected diabetic patients. Diabetes Care 2003;26:3116-3122.

30 Johnson & Johnson Pharmaceutical Research & Development. Key Biometric Results for 216073-CDE-002. On file at J&J PR&D 2003.

31 Chial HJ, Camilleri C, Delgado-Aros S, Burton D, Thomforde G, Ferber I et al. A nutrient drink test to assess maximum tolerated volume and postprandial symptoms: effects of gender, body mass index and age in health. Neurogastroenterol Motil 2002;14:249-253.

32 Tefera S, Gilja OH, Olafsdottir E, Hausken T, Hatlebakk JG, Berstad A. Intragastric maldistribution of a liquid meal in patients with reflux oesophagitis assessed by three dimensional ultrasonography. Gut 2002;50:153-158.

33 Scheffer RC, Gooszen HG, Wassenaar EB, Samsom M. Relationship Between Partial Gastric Volumes and Dyspeptic Symptoms in Fundoplication Patients: A 3D Ultrasonographic Study. Am J Gastroenterol 2004;99:1902-1909.

34 Bolondi L, Bortolotti M, Santi V, Calletti T, Gaiani S, Labo G. Measurement of gastric emptying time by real-time ultrasonography. Gastroenterology 1985;89:752-759.

35 van Lelyveld N, Scheffer RCH, Mundt M, Samsom M. Impaired Proximal Gastric Volume Change in Patients with Functional Dyspepsia is Related to Fullness; A 3D-Ultrasonography Study (abstr). Gastroenterology 2005;10:A543.

36 Megens AAHP, Canters LLJ, Vermote PCM. Stereoselective antipropulsive effect of the 5-HT4 antagonist R184798 in rats: a comparison with other 5-HT4 antagonists. Janssen Research Foundation, Non-Clinical Research Report 2001;EDMS-BEBE-2824824.

37 Boeckxstaens GE, Hirsch DP, van den Elzen BD, Heisterkamp SH, Tytgat GN. Impaired drinking capacity in patients with functional dyspepsia: relationship with proximal stomach function. Gastroenterology 2001;121:1054-1063.

38 Delgado-Aros S, Camilleri M, Cremonini F, Ferber I, Stephens D, Burton DD. Contributions of gastric volumes and gastric emptying to meal size and postmeal symptoms in functional dyspepsia. Gastroenterology 2004;127:1685-1694.

39 Gonenne J, Castillo EJ, Camilleri M, Burton D, Thomforde GM, Baxter KL et al. Does the nutrient drink test accurately predict postprandial gastric volume in health and community dyspepsia? Neurogastroenterol Motil 2005;17:44-50.

40 Boeckxstaens GE, Hirsch DP, Kuiken SD, Heisterkamp SH, Tytgat GN. The proximal stomach and postprandial symptoms in functional dyspeptics. Am J Gastroenterol 2002;97:40-48.

41 Coleman NS, Marciani L, Blackshaw E, Wright J, Parker M, Yano T et al. Effect of a novel 5-HT3 receptor agonist MKC-733 on upper gastrointestinal motility in humans. Aliment Pharmacol Ther 2003;18:1039-1048.

42 Cremonini F, Delgado-Aros S, Camilleri M. Efficacy of alosetron in irritable bowel syndrome: a meta-analysis of randomized controlled trials. Neurogastroenterol Motil 2003;15:79-86.

43 Talley NJ, Van Zanten SV, Saez LR, Dukes G, Perschy T, Heath M et al. A dose-ranging, placebo-controlled, randomized trial of alosetron in patients with functional dyspepsia. Aliment Pharmacol Ther 2001;15:525-537.

44 Tack J, Broeckaert D, Coulie B, Janssens J. The influence of cisapride on gastric tone and the perception of gastric distension. Aliment Pharmacol Ther 1998;12:761-766.

45 Degen L, Matzinger D, Merz M, Appel-Dingemanse S, Osborne S, Luchinger S et al. Tegaserod, a 5-HT4 receptor partial agonist, accelerates gastric emptying and gastrointestinal transit in healthy male subjects. Aliment Pharmacol Ther 2001;15:1745-1751.

46 Mundt M, Samsom M. Fundal Dysaccommodation in Functional Dyspepsia: Head-to-head comparison between the Barostat and 3D Ultrasonographic Technique (abstr). Neurogastroenterol Motil 2004;16:A41.

47 Cuomo R, Sarnelli G, Grasso R, Bruzzese D, Pumpo R, Salomone M et al. Functional dyspepsia symptoms, gastric emptying and satiety provocative test: analysis of relationships. Scand J Gastroenterol 2001;36:1030-1036.

Chapter 06

Regional differences in expression of TPH-1, SERT, 5-HT_3, and 5-HT_4 receptors in the human stomach and duodenum

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Abstract

Background and aim

To increase the understanding of the role of serotonergic signalling in normal gastroduodenal function at a molecular level.

Methods

Mucosal biopsy specimens were collected from the fundus, antrum, and duodenum of 11 healthy subjects. Serotonin (5-HT) positive cells were counted and the mRNA levels of tryptophan hydroxylase (TPH), serotonin transporter (SERT), 5-HT_4 receptor, and 5-HT_3 receptor subunits were quantified by real time RT-PCR.

Results

The number of serotonin positive cells was larger in the duodenum compared to the stomach ($P < 0.001$). SERT expression was 19-fold higher in the duodenum compared to the antrum and 457-fold higher compared to the fundus ($P < 0.001$). TPH-1 expression was lower in the duodenum compared to the antrum and fundus (regional differences –2.3 and –3.6 respectively). The 5-HT_4 receptor and the 5-HT_{3C} and 5-HT_{3E} receptor subunits were more abundantly expressed in duodenum compared to the stomach ($P < 0.001$).

Conclusions

The larger number of 5-HT positive cells, the higher expression of 5-HT_3 and 5-HT_4 receptors, and in particularly the higher uptake capacity of serotonin in the duodenum, point out to a more prominent role of serotonergic signalling at the mucosal level in the duodenum compared to the stomach.

Introduction

Studies using agents intervening in serotonergic signalling, such as serotonin (5-HT) receptor agonists and antagonists, selective serotonin reuptake inhibitors (SSRIs), and tryptophan depletion, demonstrate that serotonergic signalling is involved in the process of regulating the orderly and controlled delivery of nutrients to the small intestine.[1-6] Serotonin is most likely involved in both motoric functions and in visceral sensitivity of the gastroduodenal region.[7]

Subpopulations of the mucosal neuroendocrine cells (enterochromaffin cells) synthesise and store 5-HT in secretion granules at the base of the cell. 5-HT is excreted primarily into the lamina propria in response to intraluminal pressure or chemo specific properties of nutrients.[8] Tryptophan hydroxylase (TPH) catalyses the reaction of tryptophan to 5-hydroxy-L-tryptophan, which subsequently is converted to 5-HT. The expression level of TPH can be considered as a marker for serotonin synthesis, since TPH catalyses the rate-limiting step of the biosynthetic pathway. Two genes encoding TPH have been identified; TPH-1 and TPH-2.[9] TPH-1 is responsible for serotonin synthesis in non-neuronal cells such as neuroendocrine cells, whereas TPH-2 is expressed in neurons of the raphe nuclei and of the myenteric plexus.[10]

To date, seven 5-HT receptors have been identified, of which the 5-HT_3 and the 5-HT_4 receptor play an important role in gastrointestinal sensory and motor functions.[11;12] The 5-HT_4 receptor is a G protein-coupled receptor, linked to stimulation of adenylyl cyclase.[7] The 5-HT_3 receptor is a ligand-gated ion channel structured as a pentameric complex composed of 5 different subunits, termed A – E.[13-15] The subunit composition influences the pharmacological and biophysical properties of the receptor, thereby determining excitability and receptor-mediated current.[16;17] Thus, knowledge of the expression patterns for 5-HT_3 subunit genes is fundamental for understanding the possible structural composition and functional characteristics of 5-HT_3 receptors on different cells or in the various regions of the gastrointestinal tract.

5-HT has to be removed rapidly from the neuroendocrine cell-sensory nerve junction to terminate responses and to prevent desensitisation of the receptors. The 5-HT transport protein (SERT), expressed by enterocytes, is responsible for this uptake.[18]

The key elements of 5-HT signalling have recently been studied in human

rectal mucosal biopsies showing interesting molecular changes in patients with ulcerative colitis and irritable bowel syndrome.[19] We anticipated that determining the number of neuroendocrine cells, and specifically 5-HT producing cells, and the mRNA levels of genes encoding components of the serotonergic system in the human stomach would increase the understanding of the role of serotonergic signalling in normal gastroduodenal function at a molecular level. For this purpose, mRNA expression levels of TPH-1, SERT, the 5-HT_4 receptor, and 5-HT_3 receptor subunits were analysed in the fundus, antrum, and duodenum.

Methods

Participants

Eleven healthy subjects, 9 female (mean age 46.3 years, range 35 – 61), were included. Healthy subjects were recruited through advertisement. A series of questions about the medical history served to check the health status of subjects. None of the participants had a history of gastrointestinal disease or abdominal surgery. During upper endoscopy, no signs of abnormality or inflammation were seen. None of the participants used medication known to influence serotonergic signaling.

Study protocol

During upper GI endoscopy, mucosal biopsy specimens of the duodenum, antrum, and fundus were obtained. Biopsies were either immediately snap frozen in liquid nitrogen and subsequently stored at -80°C for mRNA expression analysis or fixed in 4% formaline for histopathology and immunohistochemistry. The study was approved by the medical ethics committee of the University Medical Centre Utrecht and written informed consent was obtained from all participants.

mRNA expression analysis

Total RNA isolation from biopsies was performed using the RNeasy micro kit (Qiagen, Hilden, Germany). Spectrophotometric quantification of total RNA was performed and A260/A280 ratios were within normal range. Subsequently, the integrity of total RNA was checked by denaturing agarose gel electrophoresis. First strand cDNA was synthesized from 1 μg of total RNA

Table 1
Oligonucleotides and thermal cycling conditions for mRNA expression analysis.

Gene	Forward primer Reverse primer	PCR product	Amplification		
PBGD	Hs00609297	64 bp	15 sec 95°C	1 min 60°C	
SERT	5'-tggttctatggcatcactcagttc-3' 5'-gttgtggcgggctcatcag-3'	148 bp	15 sec 95°C	30 sec 60° C	30 sec 72°C
TPH-1	5'-tgcaaaggagaagatgagagaatttac-3' 5'-ctggttatgctcttggtgtctttc-3'	114 bp	15 sec 95°C	30 sec 60° C	30 sec 72°C
HTR4	5'-caaggctggaataacattggcata-3' 5'-gttgaccatgaagacacagtacg-3'	93 bp	15 sec 95°C	30 sec 58° C	30 sec 72°C
HTR3C	5'-acacttctgctgggctacaac-3' 5'-tgaccaccatcagggacagg-3'	115 bp	10 sec 95°C	30 sec 60°C	
HTR3E	5'-aacgctcctgctgggctac-3' 5'-agggcgaagtagacaccgatg-3'	93 bp	10 sec 95°C	30 sec 60°C	

using the iScript cDNA synthesis kit (BioRad, Hercules, CA, USA) in a volume of 20 μl. Expression analysis was performed by quantitative real time RT-PCR, using the iCycler iQ system (BioRad, Hercules, CA, USA), and mRNA levels were monitored using SYBR green based detection.

Prior to real-time PCR analysis, cDNA samples were diluted 1:10 (duodenum) or 1:2.5 (antrum and fundus) with RNAse free water. The PCR reactions were set up in a volume of 25 μl, containing 5 μl of the diluted cDNA and 12.5 μl of 2x iQ SYBR Green Supermix (BioRad, Hercules, CA, USA). Specific primers and reaction conditions for amplification are listed in table 1. The primers were mRNA/cDNA specific (designed on intron/exon boundaries or flanking an intron) to prevent signal formation from contaminating genomic DNA. All protocols consisted of a 3 min 95°C initial denaturation

and enzyme-activating step. The amplification was followed by a melting curve analysis; performed by increasing the temperature by 0.5°C increments from 55°C to 95°C and measuring fluorescence at each temperature for a period of 10 sec. All cDNA samples were analysed in duplicate.
In every run, a relative standard curve was included. The standard curve allows comparison of the expression levels across runs and takes differences in PCR efficiency for the mRNAs analysed into account. cDNA synthesised from total RNA extracted from full thickness jejunum resection material was used to generate the relative standard curve. Because of the low expression level of HTR3C and HTR3E in this material, we used purified PCR product to generate the relative standard curve. Expression levels in the various biopsy specimens were quantified by calculating initial target concentrations using the obtained threshold cycle values and the relative standard curve.
Expression levels of all genes were normalized against the endogenous reference gene porphobilinogen deaminase (PBGD). Quantification of PBGD was carried out using 5 μl of diluted cDNA, 12.5 μl 2x iQ Supermix (BioRad, Hercules, CA, USA), and 1.25 μl 20x Assays-on-demand gene expression assay mix Hs00609297 (Applied Biosystems, Foster City, CA, USA). $MgCl_2$ was added to obtain a final concentration of 4mM in a total volume of 25 μl. Thermal cycling conditions consisted of a 3 min 95°C initial denaturation step, followed by 40 cycles of amplification (table 1).

Histopathology and immunohistochemistry

The number of chromogranin-immunoreactive (neuroendocrine) cells and 5-HT immunoreactive cells per gastric pit or crypt/villous complex were evaluated in 12 biopsy specimens obtained from four healthy subjects. After routine fixation and dehydration procedures the biopsy specimens were cut in serial sections of 5 mu, stained with haematoxylin-eosin (HE) and immunohistochemistry staining with monoclonal antibodies for chromogranin (DAKO A430, rabbit-anti-human, 1/500) and serotonin (Eurodiagnostica, rabbit-anti-human, PSE 1/200). By microscopic evaluation of duodenum, antrum, and fundus mucous membrane the total amount of chromogranin- and serotonin positive cells were counted in a blinded way.

Statistics

The mRNA expression data was analyzed using univariate analysis of vari-

ance (one-way Anova) and a post-hoc test (Bonferroni correction). In order to obtain normally distributed data for evaluation of differences between the three regions, the normalized mRNA levels were transformed by taking the natural logarithm. The relative differences between the regions are expressed as a fold change (95% confidence interval). The correlation between different genes was analyzed by performing a Spearman's correlation test, giving a Spearman's correlation coefficient (rho). A $P < 0.05$ was considered significant. All statistical analysis was performed using commercially available software (SPSS 11.0 for Microsoft windows).

Results

No abnormalities were seen during upper endoscopy in any of the subjects. Biopsy specimens were obtained without any complications. For all SYBR Green assays, the amplification yielded a single product which size was equivalent to that predicted from the relevant sequence. PCR efficiency was comparable between runs.

The biopsies examined in the current study consist of the mucosal layer (epithelium and lamina propria) as shown by HE staining. Most of the biopsies just reached the mucosal muscular layer, however none of the biopsies contained submucosal tissue. Therefore the biopsy specimens do not contain neuronal mRNA.

Neuroendocrine cell count

The number of chromogranin- and serotonin positive cells was larger in the duodenum compared to the stomach (all $P < 0.001$), however comparable between the antrum and the fundus (figure 1).

Regional differences in mRNA expression

The level of expression of PBGD, the reference gene used for normalization of the mRNA expression, was comparable between the duodenum, the antrum, and the fundus (all $P > 0.05$).

TPH-1: In duodenum, the expression of TPH-1 was lower compared to antrum and fundus ($P = 0.017$ and $P < 0.001$ respectively). The relative differences of TPH-1 expression were -2.3 (-1.1 - -4.6) (duodenum versus antrum) and -3.6 (-1.8 - -7.2) (duodenum versus fundus). No differences were observed between antrum and fundus (figure 2).

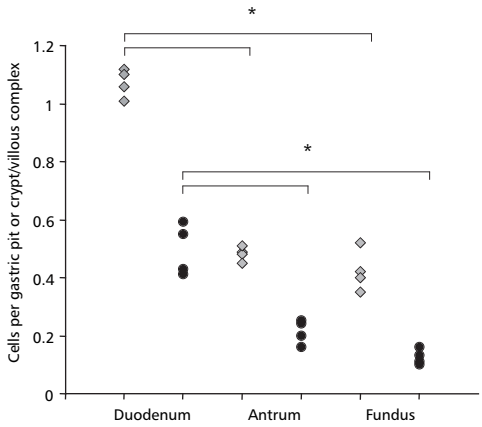

Figure 1
The number of chromogranin-immunoreactive (neuroendocrine) cells (grey) and 5-HT immunoreactive cells (black) per gastric pit or crypt/villous complex evaluated in 12 biopsy specimens. The number of neuroendocrine cells and serotonin positive cells was larger in the duodenum compared to the stomach (* $P < 0.001$), however comparable between the antrum and the fundus.

SERT: The expression of SERT was found to be highest in the duodenum ($P < 0.001$ compared to antrum and fundus) and lowest in the fundus ($P < 0.001$ compared to antrum) (figure 2). The relative differences in SERT expression were 19 (9.2 – 40) (duodenum versus antrum), 24 (12– 49) (antrum versus fundus), and 457 (220 – 947) (duodenum versus fundus).

5-HT$_3$ receptor subunits: Since we found very low expression of the 5-HT$_{3A}$ and 5-HT$_{3B}$ in the stomach and the duodenum at the mucosal level, and 5-HT$_{3D}$ was reported not to be expressed in the stomach and small intestine,(14) we have focused on the 5-HT$_{3C}$ and 5-HT$_{3E}$ receptor subunits.
The expression of 5-HT$_{3C}$ was found to be higher in the duodenum compared to the antrum and fundus, with relative differences of 14 (7.7 – 23) and 6.9 (4.0 – 12) respectively) (all $P < 0.001$). The expression in the fundus was higher compared to the antrum, with a relative difference of 2.0 (1.1 – 3.4) ($P = 0.024$).
The 5-HT$_{3E}$ receptor subunit was expressed at a higher level in duodenum compared to both regions in the stomach (all $P < 0.001$). The relative differences of 5-HT$_{3E}$ expression were 18 (10 – 31) (duodenum versus antrum)

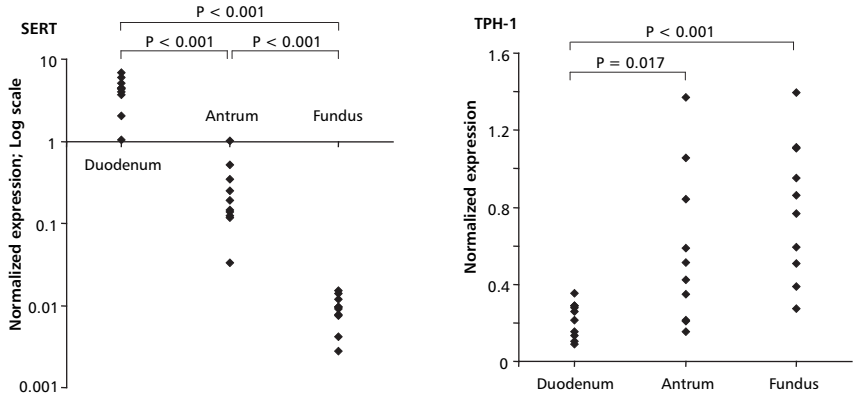

Figure 2
Normalized expression of TPH-1 and SERT in the duodenum, antrum, and fundus. Although TPH-1 expression is significantly higher in antrum and fundus compared to the duodenum, the relative differences are much smaller compared to those of SERT in the three regions. SERT expression in the fundus was low but could be reliably quantified.

and 14 (8.2 – 24) (duodenum versus fundus). We found no differences in expression between the antrum and the fundus (table 2).

5-HT$_4$ receptor: The expression of 5-HT$_4$ in the duodenum was higher compared to the antrum and the fundus with relative differences of 87 (46 – 165) and 84 (45 – 157) respectively (all $P < 0.001$). No differences were observed between antrum and fundus (table 2).

Correlations in mRNA expression

No correlation was found between the mRNA expression of SERT and TPH-1. Neither did we find any correlation between SERT or TPH-1 and any of the 5-HT receptors. However, a strong correlation was observed between the 5-HT$_{3C}$ and 5-HT$_{3E}$ subunits in duodenum (rho = 0.918, $P < 0.001$), antrum (rho = 0.806, $P = 0.003$), and fundus (rho = 0.923, $P < 0.001$) (figure 3).

Table 2
The mRNA expression of the 5-HT$_4$ receptor and the 5-HT$_3$ receptor subunits, normalized against PBGD.

	Duodenum	Antrum	Fundus
HTR3C	0.36 (0.25 – 0.75) *	0.032 (0.020 – 0.050) **	0.056 (0.043 – 0.092)
HTR3E	0.11 (0.09 – 0.26) †	0.010 (0.005 – 0.013)	0.010 (0.008 – 0.015)
HTR4	1.71 (1.53 – 2.01) ‡	0.028 (0.010 – 0.043)	0.020 (0.016 – 0.029)

All values represent median (25th –75th percentiles). HTR3C, * duodenum vs antrum/fundus: $P < 0.001$, ** antrum vs fundus $P = 0.024$; HTR3E, † duodenum vs antrum/fundus: $P < 0.001$; HTR4, ‡ duodenum vs antrum/fundus: $P < 0.001$. The mRNA expression cannot be compared between the different genes, since different standard curves were used.

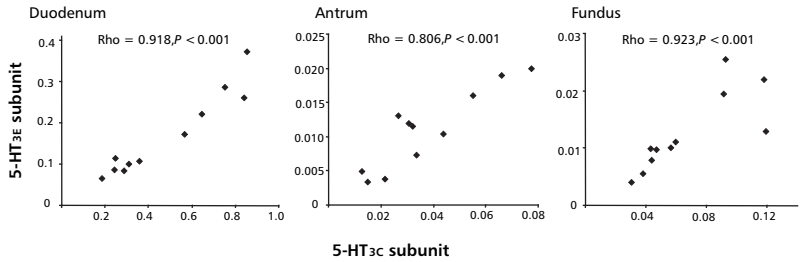

Figure 3
Correlation between the mRNA expression of the 5-HT$_{3C}$ and the 5-HT$_{3E}$ subunits in duodenum, antrum, and fundus.

Discussion

This study shows large regional differences in mRNA expression levels of the various proteins involved in serotonergic signalling. SERT expression level was found to be highest in the duodenum, lower in the antrum, and lowest in the fundus, with relative differences of approximately 20 between duodenum and antrum, and antrum and fundus, up to 450 between duodenum and fundus. The 5-HT_3 receptor subunits have a higher expression in duodenum compared to both regions in the stomach, with relative differences ranging from seven up to eighteen. In addition, the expression of the 5-HT_4 receptor was higher in the duodenum compared to both regions of the stomach, with relative differences of approximately 85. In spite of a larger number of 5-HT positive cells in the duodenum, TPH-1 expression in the duodenum was lower compared to the antrum and fundus, and comparable between the antrum and fundus. However, relative differences for TPH-1 (approximately three between the duodenum and the stomach) were low compared to the relative differences of SERT expression. Clearly, the expression of SERT does not correlate with the expression of TPH-1.

Serotonin is involved in the regulation of both motoric and sensory functions of the upper gastrointestinal tract. Gastric emptying follows a pulsatile pattern, requiring coordination of the antral wall and pyloric-duodenal resistive forces.[20] The proximal stomach has the capacity to relax in anticipation of food without a rise of intragastric pressure, the so-called accommodation reflex, which is initiated by a feedback mechanism from the duodenum after the arrival of nutrients.[21] In addition to the markedly different function of the regions, the actions of the 5-HT receptors in fundus and antrum differ upon stimulation.[22] Interestingly, we found many similarities in expression profiles of the genes involved in serotonergic signalling in the antrum and fundus, with the exception of SERT. Recently it was shown that THP-1 expression level, and 5-HT content in the neuroendocrine cells, are not very good markers for 5-HT release.[19] We postulate that the high expression of SERT, and not so much TPH-1, is indicative of increased activity of serotonergic signalling (5-HT activity and/or 5-HT release). The functional role of SERT, which is to control the extracellular level of 5-HT and to prevent accumulation of 5-HT, fits such a mechanism. Analysis of the distribution of SERT using immunohistochemistry might shed some light on possible regional differences in activity. Conceivably, the storage of 5-HT by

neuroendocrine cells allows an enhanced 5-HT release without concomitantly increasing 5-HT synthesis. Immunolocalization and in situ hybridization studies in rat and guinea pig showed that SERT is primarily expressed by enterocytes.[18;23] However, the recent transcriptome analysis of *Mastomys* 5-HT positive neuroendocrine cells established the presence of SERT.[24] This suggests that additionally 5-HT recycling may explain a lacking correlation between 5-HT synthesis and release.

The biopsy specimens used in the current study were taken from the mucosa, implying that our data give insight into 5-HT synthesis by neuroendocrine cells, 5-HT uptake capacity by SERT expressed by epithelial cells, and the 5-HT receptors expressed by neuroendocrine cells. Therefore, 5-HT synthesis and uptake capacity by serotonergic neurons, which are situated within the myenteric plexus, are not assessed in the current study. It should be emphasized that 5-HT is situated mainly (>90%) in neuroendocrine cells.(7) Expression of 5-HT receptors present on afferent nerve endings in the lamina propria could not be studied either, since mRNA for these receptors is situated in the neuronal cell bodies and is therefore not acquired in a mucosal biopsy. Progress may be made in this area by using receptor specific antibodies.

Most studies using agents intervening in serotonergic signalling are performed in whole bodies or tissues, and the effect on gastrointestinal function is largely attributed to 5-HT receptors situated on afferent nerve endings, submucosal and myenteric neurons. However, 5-HT receptors are also situated on neuroendocrine mucosal cells, modulating 5-HT release by autoregulatory mechanisms.[25-27] In addition, it has been shown that mucosal non-neuronal 5-HT receptors modulate 5-HT induced fluid secretion.[28-30] To fully elucidate the mechanisms by which 5-HT regulates gastrointestinal function, the response mediated by neuronal and non-neuronal 5-HT receptors should be studied separately by using muscle-stripped mucosa, mucosa free preparations, or by applying neuronal blockade. Determination of functional characteristics of neuronal and non-neuronal 5-HT receptors would provide further insight into this issue.

This study has shown a higher expression of both 5-HT_3 and 5-HT_4 receptors in the duodenum compared to the stomach. This finding is in line with the larger number of 5-HT positive cells in the duodenum. Our results indicate that the 5-HT_3 receptors on epithelial cells have a distinct structural compo-

sition. In contrast with brain tissue and full thickness material from the gastrointestinal tract,[14] we found very low levels of mRNA encoding the 5-HT_{3A} and 5-HT_{3B} receptor subunits. Consistent with a distinct subunit composition, electrophysiological evidence showed that 5-HT_3 receptors on epithelial cells are functionally different from those on neurons.[27] The remarkable correlation between the mRNA expression of the 5-HT_{3C} and 5-HT_{3E} subunits raises the possibility that the prevalent form of 5-HT_3 receptor synthesized by cells within the gastroduodenal mucosa is a heteromeric 5-$HT_{3C/E}$ receptor. However, the fact that HTR3C and HTR3E reside in a region of less than 100 kb on chromosome 3q27,[14] and consequently tend easily to be co-expressed, may simply underlie this finding. Cellular analysis of the distribution pattern of 5-HT_{3C} and 5-HT_{3E} subunits using subunit-specific riboprobes or antibodies for in situ hybridisation may reveal the prevalence of 5-HT_{3C} or 5-HT_{3E} homomeric versus heteromeric receptors.

In summary, TPH-1 (5-HT synthesis) and SERT (5-HT uptake) expression do not correlate. Serotonin synthesis varies only marginally between the regions, whereas the uptake capacity of serotonin differs tremendously between the fundus, antrum, and duodenum. Considering the larger number of 5-HT positive cells, the higher level of SERT expression, and the much higher expression of the 5-HT_4 receptor and the 5-HT_3 receptor subunits in the duodenum, serotonergic signalling and activity at the mucosal level is likely to be highest in the duodenum, followed by the antrum and fundus. These findings can be of importance when developing specific serotonergic agents for enhanced fundic relaxation or gastric emptying.

References

1 Talley NJ, Van Zanten SV, Saez LR, Dukes G, Perschy T, Heath M et al. A dose-ranging, placebo-controlled, randomized trial of alosetron in patients with functional dyspepsia. Aliment Pharmacol Ther 2001; 15(4):525-537.

2 Coleman NS, Marciani L, Blackshaw E, Wright J, Parker M, Yano T et al. Effect of a novel 5-HT3 receptor agonist MKC-733 on upper gastrointestinal motility in humans. Aliment Pharmacol Ther 2003; 18(10):1039-1048.

3 Degen L, Matzinger D, Merz M, Appel-Dingemanse S, Osborne S, Luchinger S et al. Tegaserod, a 5-HT4 receptor partial agonist, accelerates gastric emptying and gastrointestinal transit in healthy male subjects. Aliment Pharmacol Ther 2001; 15(11):1745-1751.

4 Fraser RJ, Horowitz M, Maddox AF, Dent J. Postprandial antropyloroduodenal motility and gastric emptying in gastroparesis--effects of cisapride. Gut 1994; 35(2):172-178.

5 Tack J, Broekaert D, Coulie B, Fischler B, Janssens J. Influence of the selective serotonin re-uptake inhibitor, paroxetine, on gastric sensorimotor function in humans. Aliment Pharmacol Ther 2003; 17(4):603-608.

6 van Nieuwenhoven MA, Valks SD, Sobczak S, Riedel WJ, Brummer RJ. Acute tryptophan depletion slows gastric emptying in females. Br J Nutr 2004; 91(3):351-355.

7 Gershon MD. Review article: serotonin receptors and transporters - roles in normal and abnormal gastrointestinal motility. Aliment Pharmacol Ther 2004; 20 Suppl 7:3-14.

8 Bulbring E, Crema A. The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. J Physiol 1959; 146(1):18-28.

9 Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 2003; 299(5603):76.

10 Cote F, Thevenot E, Fligny C, Fromes Y, Darmon M, Ripoche MA et al. Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. Proc Natl Acad Sci U S A 2003; 100(23):13525-13530.

11 Foxx-Orenstein AE, Kuemmerle JF, Grider JR. Distinct 5-HT receptors mediate the peristaltic reflex induced by mucosal stimuli in human and guinea pig intestine. Gastroenterology 1996; 111(5):1281-1290.

12 Grider JR, Foxx-Orenstein AE, Jin JG. 5-Hydroxytryptamine4 receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. Gastroenterology 1998; 115(2):370-380.

13 Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG et al. The 5-HT3B subunit is a major determinant of serotonin-receptor function. Nature 1999; 397(6717):359-363.

14 Niesler B, Frank B, Kapeller J, Rappold GA. Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. Gene 2003; 310:101-111.

15 Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D. Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science 1991; 254(5030):432-437.

16 Stewart A, Davies PA, Kirkness EF, Safa P, Hales TG. Introduction of the 5-HT3B subunit alters the functional properties of 5-HT3 receptors native to neuroblastoma cells. Neuropharmacology 2003; 44(2):214-223.

17 Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC et al. The pharmacological and functional characteristics of the serotonin 5-HT(3A) receptor are specifically modified by a 5-HT(3B) receptor subunit. J Biol Chem 1999; 274(43):30799-30810.

18 Wade PR, Chen J, Jaffe B, Kassem IS, Blakely RD, Gershon MD. Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract. J Neurosci 1996; 16(7):2352-2364.

19 Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H et al. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 2004; 126(7):1657-1664.

20 Horowitz M, Dent J, Fraser R, Sun W, Hebbard G. Role and integration of mechanisms controlling gastric emptying. Dig Dis Sci 1994; 39(12 Suppl):7S-13S.

21 Azpiroz F, Malagelada JR. Intestinal control of gastric tone. Am J Physiol 1985; 249(4 Pt 1):G501-G509.

22 Takemura K, Takada K, Mameya S, Kaibara M, Taniyama K. Regional and functional differences of 5-hydroxytryptamine-receptor subtypes in guinea pig stomach. Jpn J Pharmacol 1999; 79(1):41-49.

23 Chen JX, Pan H, Rothman TP, Wade PR, Gershon MD. Guinea pig 5-HT transporter: cloning, expression, distribution, and function in intestinal sensory reception. Am J Physiol 1998; 275(3):G433-48.

24 Kidd M, Modlin IM, Eick GN, Champaneria MC. Isolation, functional characterization, and transcriptome of Mastomys ileal enterochromaffin cells. Am J Physiol 2006; 291(5):G778-91.

25 Gebauer A, Merger M, Kilbinger H. Modulation by 5-HT3 and 5-HT4 receptors of the release of 5-hydroxytryptamine from the guinea-pig small intestine. Naunyn Schmiedebergs Arch Pharmacol 1993; 347(2):137-140.

26 Racke K, Reimann A, Schworer H, Kilbinger H. Regulation of 5-HT release from enterochromaffin cells. Behav Brain Res 1996; 73(1-2):83-87.

27 Schworer H, Ramadori G. Autoreceptors can modulate 5-hydroxytryptamine release from porcine and human small intestine in vitro. Naunyn Schmiedebergs Arch Pharmacol 1998; 357(5):548-552.

28 Borman RA, Burleigh DE. Human colonic mucosa possesses a mixed population of 5-HT receptors. Eur J Pharmacol 1996; 309(3):271-274.

29 Tuo BG, Sellers Z, Paulus P, Barrett KE, Isenberg JI. 5-HT induces duodenal mucosal bicarbonate secretion via cAMP- and Ca2+-dependent signaling pathways and 5-HT4 receptors in mice. Am J Physiol Gastrointest Liver Physiol 2004; 286(3): G444-G451.

30 Day J, King B, Haque SM, Kellum JM. A nonneuronal 5-hydroxytryptamine receptor 3 induces chloride secretion in the rat distal colonic mucosa. Am J Surg 2005; 190(5):736-738.

Chapter 07

Serotonergic signalling in the stomach and duodenum of patients with gastroparesis

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Submitted

Abstract

Background and aim

Serotonin (5-HT) is involved in the regulation of motoric and sensory functions of the upper gastrointestinal tract. The aim of the current study was to determine whether serotonergic signalling is altered in patients with idiopathic gastroparesis.

Methods

Mucosal biopsy specimens were collected from the duodenum, antrum, and fundus of 11 patients with idiopathic gastroparesis and 11 healthy controls. Neuroendocrine cells, specifically 5-HT producing cells, were counted after immunohistochemistry, and non-neuronal mRNA expression levels of TPH-1, SERT, 5-HT_3 and 5-HT_4 receptor were quantified by real time RT-PCR.

Results

The number of 5-HT producing cells was comparable between patients and controls. No difference in expression of TPH-1 (rate limiting enzyme in 5-HT biosynthetic pathway) and SERT (responsible for 5-HT uptake) was found between patients and controls ($P > 0.05$). In the duodenum, the expression of the 5-HT3 receptor subunits and the 5-HT_4 receptor was comparable between both groups. However, the 5-HT_4(c) splice variant was expressed more abundantly in healthy controls compared to patients ($P = 0.015$).

Conclusions

This study suggests that the delayed gastric emptying and upper abdominal symptoms in idiopathic gastroparesis do not result from altered mucosal 5-HT biosynthetic and uptake capacity.

Introduction

Idiopathic gastroparesis refers to a symptomatic disorder of gastric emptying without an organic abnormality present. The pathophysiological basis of idiopathic gastroparesis is poorly understood. Abnormal intragastric transport and/or impaired coordination of antropyloroduodenal pressure waves has been shown to play an essential role in delayed gastric emptying.[1] Symptoms are variable and include early satiety, nausea, vomiting, bloating, and upper abdominal discomfort.[2] Studies using 5-HT receptor agonists and antagonists, selective serotonergic reuptake inhibitors (SSRIs), and tryptophan depletion, have revealed that serotonergic signalling is involved in the regulation of gastric emptying and, potentially, in the generation of upper abdominal symptoms.[1;3-7]

5-HT is synthesized in mucosal neuroendocrine (enterochromaffin) cells, and released in response to intraluminal stimuli.[8] Tryptophan hydroxylase (TPH) catalyses the reaction of tryptophan to 5-hydroxy-L-tryptophan, which is the rate limiting step of the biosynthetic pathway of 5-HT. The expression level of TPH can therefore be considered as a marker for serotonin synthesis. Two genes encoding TPH have been identified; TPH-1 and TPH-2.[9] TPH-1 is responsible for serotonin synthesis in non-neuronal cells such as neuroendocrine cells, whereas TPH-2 is expressed in neurons of the raphe nuclei and of the myenteric plexus.[10]

The 5-HT receptors, which are situated on afferent nerve endings in the lamina propria, carry information to submucosal or myenteric neurons of the enteric nervous system or to the CNS upon stimulation by 5-HT.[11] Secondly, 5-HT receptors are situated on the neuroendocrine mucosal cells, modulating 5-HT release by autoregulatory mechanisms.[12-14] 5-HT has to be removed rapidly from the neuroendocrine cell-sensory nerve junction to terminate responses and to prevent desensitisation of the receptors. The 5-HT transport protein (SERT), localized to enterocytes, is responsible for this uptake, thereby controlling 5-HT availability.[15]

There are seven classes of 5-HT receptors. Of these, the 5-HT_3 and 5-HT_4 receptors play an important role in gastrointestinal sensory and motor functions.[11] The 5-HT_3 receptor is a pentameric complex. Five different subunits have been identified (termed A – E).[16;17] The subunit composition influences the pharmacological and biophysical properties of the receptor, thereby determining excitability and receptor-mediated current.[16;18-20]

Seven 5-HT_4 receptor variants that differ in their C-termini due to alternative splicing have been identified.[21-23] Functional diversity, including level of constitutive activity, exists among the 5-HT_4 splice variants.[22;24;25] Thus, the distribution and expression levels of 5-HT_4 receptor splice variants affects 5-HT_4 mediated response.

We hypothesized that an altered expression of genes encoding components of serotonergic signalling at the mucosal level may be involved in delayed gastric emptying and the generation of symptoms. Therefore, we conducted a study, comparing the number of neuroendocrine cells, and particularly 5-HT producing neuroendocrine cells, and the mRNA expression levels of TPH-1, SERT, the 5-HT_4 receptor, 5-HT_4 receptor splice-variants and 5-HT_3 receptor subunits in mucosal biopsy specimens of the duodenum, antrum, and fundus from patients with gastroparesis and healthy controls.

Methods

Participants

At the outpatients clinic in Utrecht, the standard workup for patients with upper abdominal symptoms includes a gastric emptying test. Patients fulfilling the ROME II criteria for dyspepsia and with a delayed gastric emptying seen were asked to participate in the study.[26] A total of 18 patients were screened, of which eleven patients fulfilled the inclusion criteria and agreed to participate in the study (2 male, mean age 46.5 ± 4.0). All patients underwent clinical evaluation, laboratory testing, upper abdominal ultrasound, and an upper GI endoscopy (see section study protocol) in order to rule out a structural cause for gastroparesis. None of the patients had diabetes. The rate of gastric emptying was established within 6 months prior to inclusion by the ^{13}C-octanoic breathtest.[27] The mean (range) half emptying time of the solid meal (294 kcal) in the patient group was 138 (124 – 168) minutes (cut of point: 120 minutes), and the mean (range) retention 120 minutes after meal ingestion was 55 (45 – 76)% (cut of point: 40%).[28]

Prior to inclusion, patients were asked to fill out a validated questionnaire, scoring six different symptoms (pain or discomfort centered in the upper abdomen, early satiety, bloating in the upper abdomen, fullness, nausea, and vomiting). For inclusion, two symptoms had to be scored three or higher on a scale of 0 – 5: mild (1-2); tolerated without interference with usual

Table 1
Frequency of severity grading of each of six upper abdominal symptoms in 11 patients with gastroparesis

	0 (absent)	1-2 (mild)	3 (moderate)	4-5 (severe)
UA Pain	0 (0)	1 (9)	4 (36)	6 (55)
Early sat	0 (0)	3 (27)	6 (55)	2 (18)
Bloating	0 (0)	3 (27)	5 (45)	3 (27)
Fullness	0 (0)	2 (18)	5 (45)	4 (36)
Nausea	2 (18)	5 (45)	3 (27)	1 (9)
Vomiting	8 (73)	1 (9)	0 (0)	2 (18)

NOTE. Numbers in parentheses represent row percentages.

activity), moderate (3); enough to cause some interference with usual activity), and severe (4-5); incapacitating with inability to work or do usual activity).[29-31] Symptoms needed to be present for at least 12 weeks, not necessary consecutive, in the preceding 12 months. Table 1 summarizes the grading of upper gastrointestinal symptoms of the patients. Upper abdominal pain, early satiety, bloating, fullness, and nausea were scored three or higher in 91%, 73%, 72%, 81%, and 36% of patients respectively. Two patients reported severe vomiting (18%).

Eleven healthy controls (1 male, mean age 46.3 ± 2.5) were recruited through advertisement. The presence of gastrointestinal symptoms or a history of gastrointestinal disease was ruled out by clinical interview. None of the participants had a history of gastrointestinal surgery. Serious co-morbidity (including neurologic or psychiatric illness) was considered an exclusion criterion. The use of narcotics, anticholinergic medication, serotonergic medication (including selective serotonin reuptake inhibitors), and antidepressants was considered an exclusion criterion. None of the participants were on NSAID therapy.

Study protocol

All participants underwent upper GI endoscopy. All patients were asked to discontinue any medication known to influence gastrointestinal motility for at least seven days prior to upper GI endoscopy. If no abnormalities were seen during upper GI endoscopy, mucosal biopsy specimens of the duodenum, antrum, and fundus were obtained. Biopsies were either immediately snap frozen in liquid nitrogen and subsequently stored at -80°C for mRNA expression analysis or fixed in 4% formalin for histopathology and immunohistochemistry. The study was approved by the medical ethics committee of the University Medical Center Utrecht and written informed consent was obtained from all participants.

Histopathology and immunohistochemistry

The number of chromogranin-immunoreactive (neuroendocrine) cells and 5-HT immunoreactive cells per gastric pit or crypt/villous complex were evaluated in 24 biopsy specimens obtained from four patients and four healthy subjects (duodenum, antrum, and fundus). After routine fixation and dehydration procedures the biopsy specimens were cut in serial sections of 5 mu, stained with haematoxylin-eosin (HE) and immunohistochemistry staining with monoclonal antibodies for chromogranin (DAKO A430, rabbit-anti-human, 1/500) and serotonin (Eurodiagnostica, rabbit-anti-human, PSE 1/200). By microscopic evaluation, the total amount of chromogranin- and serotonin positive cells in the mucous membrane were counted in a blinded way in at least three serial sections per biopsy specimen.

mRNA expression analysis

Total RNA isolation from biopsies was performed using the Qiagen RNeasy micro kit. Spectrophotometric quantification of total RNA was performed and A260/A280 ratios were within normal range. Subsequently, the integrity and size distribution of total RNA was checked by denaturing agarose gel electrophoresis. First strand cDNA synthesis from total RNA was performed using the BioRad iScript cDNA synthesis kit.

Expression analysis was performed by quantitative real time RT-PCR, using the iCycler iQ system (BioRad, Hercules, CA, USA). Prior to real-time PCR analysis, cDNA samples were diluted 1:10 (duodenum) or 1:2.5 (antrum and fundus) with RNAse free water. The PCR reactions were set up in a volume of 25 μl, containing 5 μl of the diluted cDNA and 12.5 μl of 2x iQ SYBR

Table 2
Oligonucleotides (5′ – 3′) and thermal cycling conditions used for mRNA expression analysis.

Gene	Forward primer Reverse primer TaqMan MGB-probe	PCR product	Amplification		
SERT	tggttctatggcatcactcagttc gttgtggcgggctcatcag	148 bp	15 sec 95°C	30 sec 60° C	30 sec 72°C
TPH-1	tgcaaaggagaagatgagagaatttac ctggttatgctcttggtgtctttc	114 bp	15 sec 95°C	30 sec 60° C	30 sec 72°C
HTR4	caaggctggaataacattggcata gttgaccatgaagacacagtacg	93 bp	15 sec 95°C	30 sec 58° C	30 sec 72°C
HTR4(a)	cgctaccgaagaccttccattc tgtgcagaacggtgtaccttag cagactgtcccttgttc	97 bp	15 sec 95°C	1 min 60°C	
HTR4(b)	tggcgggtgacactgactc gactgtcccttgttcaaccaca ccaccacactccactg	100 bp	15 sec 95°C	1 min 60°C	
HTR4(c)	cgctaccgaagaccttccattc cggtttcagttccagaacttagtac cagactgtcccttgttc	97 bp	15 sec 95°C	1 min 60°C	
HTR3C	acacttctgctgggctacaac tgaccaccatcagggacagg	115 bp	10 sec 95°C	30 sec 60°C	
HTR3E	aacgctcctgctgggctac agggcgaagtagacaccgatg	93 bp	10 sec 95°C	30 sec 60°C	

Green Supermix (BioRad, Hercules, CA, USA). Specific primers and reaction conditions for amplification are listed in table 2. The primers were mRNA/cDNA specific (designed on intron/exon boundaries or flanking an intron) to prevent signal formation from contaminating genomic DNA. All protocols consisted of a 3 min 95°C initial denaturation and enzyme-activating step. All cDNA samples were analysed in duplicate.

The expression of SERT, TPH-1, HTR4, HTR3C, and HTR3E was quantified by

SYBR green-based detection. The amplification was followed by a melting curve analysis; performed by increasing the temperature in 0.5°C increments from 55°C to 95°C and measuring fluorescence at each temperature for a period of 10 seconds. TaqMan MGB-probe based detection was used for quantification of mRNA levels of the 5-HT_4 splice variants: HTR4(a), HTR4(b), and HTR4(c).

In every run, a relative standard curve was included. The standard curve allows comparison of the expression levels across runs and takes differences in PCR efficiency into account. cDNA synthesised from total RNA extracted from full thickness jejunum resection material was used to generate the relative standard curve. Because of the low mRNA expression level of the HTR4 splice variants, HTR3C, and HTR3E in stomach and duodenum, we used purified PCR product to generate the relative standard curve. Expression levels in the various biopsy specimens were quantified by calculating initial target concentrations using the obtained threshold cycle values and the relative standard curve.

Expression levels of all genes were normalized against the endogenous reference gene porphobilinogen deaminase (PBGD). Quantification of PBGD was carried out using 5 μl of diluted cDNA, 12.5 μl 2x iQ Supermix (BioRad, Hercules, CA, USA), 1.25 μl 20x Assays-on-demand gene expression assay mix Hs00609297 (Applied Biosystems, Foster City, CA, USA) in a total volume of 25 μl. $MgCl_2$ was added to obtain a final concentration of 4mM. Amplification consisted of a 3 min 95°C initial denaturation step, followed by 40 cycles of 15 sec denaturation at 95°C and 1 min annealing and extension at 60°C.

Statistics

The averaged expression of a gene was calculated and divided by the expression of PBGD in each participant, giving the normalized expression (given as median, and the 25^{th} – 75^{th} percentiles. Normality was tested using Kolmogorov-Smirnov test. The normalized mRNA expression of the various genes was compared between patients and healthy controls using the Mann-Whitney U test.

Furthermore, we have analyzed the differences in mRNA expression between the three regions using data of all participants (n = 22). In order to obtain normally distributed data for evaluation of differences between the three regions, the normalized mRNA levels were transformed by taking the

natural logarithm. The mRNA expression data of the three regions were analyzed using univariate analysis of variance (one-way Anova) and a post-hoc test (Bonferroni correction).The relative differences between the regions are expressed as a fold change (95% confidence interval).
The neuroendocrine cell numbers were compared between patients and controls in the three regions using ANOVA repeated measures. A $P < 0.05$ was considered significant. All statistical analysis was performed using commercially available software (SPSS 11.0 for Microsoft windows).

Results

Biopsy specimens were obtained without any complications. Macroscopically, no evidence of inflammation was observed in any of the subjects. In the patients and healthy subjects for which histopathology was performed the absence of inflammation was confirmed at the various biopsy locations. For all SYBR Green-based assays, amplification yielded a single product which size was equivalent to that predicted from the relevant sequence. PCR efficiency was comparable between runs. The biopsies examined in the current study consist of the mucosal layer (epithelium and lamina propria) as shown by HE staining. Most of the biopsies just reached the muscularis mucosae, however none of the biopsies contained submucosal tissue. Therefore the biopsy specimens do not contain neuronal mRNA.

Neuroendocrine cell count

The number of chromogranin- and serotonin positive cells was comparable between patients and healthy controls ($P > 0.05$) (figure 1). In all three regions, approximately 50% of all neuroendocrine cells were positively stained with the serotonin antibody. The number of chromogranin- and serotonin positive cells was larger in the duodenum compared to the stomach ($P < 0.001$), however comparable between the antrum and the fundus.

Expression analysis

The level of expression of PBGD, the reference gene used for normalization of the mRNA expression, was comparable between the duodenum, the antrum, and the fundus and between patients and controls (all $P > 0.05$).

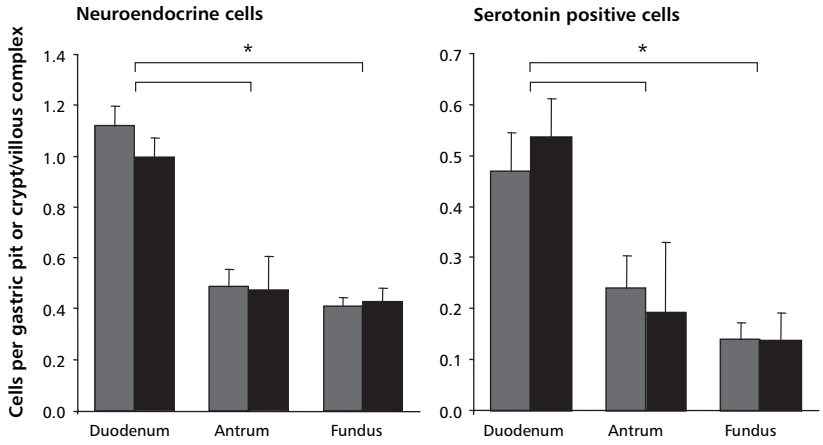

Figure 1
The number of neuroendocrine cells (chromogranin positive) and serotonin positive cells in duodenum and stomach was comparable between patients (black) and healthy controls (grey) ($P > 0.05$). The number of chromogranin- and serotonin positive cells was larger in the duodenum compared to the stomach (* $P < 0.001$), however comparable between the antrum and the fundus.

TPH-1

In duodenum, the expression of TPH-1 was lower compared to antrum and fundus (all $P < 0.001$). The relative differences of TPH-1 expression were -3.0 (-1.8 - -4.9) (duodenum versus antrum) and -4.2 (-2.6 - -6.8) (duodenum versus fundus). No differences in mRNA expression of TPH-1 were observed between antrum and fundus. TPH-1 expression was comparable between gastroparesis patients and healthy controls in the duodenum, antrum or fundus ($P > 0.05$) (table 3).

SERT

The expression of SERT was found to be highest in the duodenum ($P < 0.001$ compared to antrum and fundus) and lowest in the fundus ($P < 0.001$ compared to antrum). The relative differences in SERT expression were 19 (9.6 – 36) (duodenum versus antrum), 23 (12– 45) (antrum versus fundus), and 433 (224 – 812) (duodenum versus fundus). SERT expression was comparable between gastroparesis patients and healthy controls in the duodenum, antrum, or fundus ($P > 0.05$) (table 3).

Table 3
mRNA expression of TPH-1, SERT, and the 5-HT$_4$ receptor in patients and healthy controls, normalized to PBGD.

		Duodenum	Antrum	Fundus
TPH-1	Patients	0.19 (0.12 – 0.31)	0.85 (0.38 – 1.23)	0.82 (0.52 – 1.85)
	Controls	0.24 (0.13 – 0.29)	0.47 (0.21 – 0.89)	0.82 (0.48 – 1.11)
SERT	Patients	4.1 (3.6 – 5.3)	0.44 (0.09 – 0.56)	0.012 (0.004 – 0.026)
	Controls	4.4 (3.3 – 5.3)	0.17 (0.12 – 0.39)	0.009 (0.007 – 0.012)
HTR4	Patients	1.8 (1.3 – 2.3)	0.03 (0.01 – 0.058)	0.017 (0.013 – 0.032)
	Controls	1.7 (1.5 – 2.0)	0.028 (0.01 – 0.043)	0.020 (0.016 – 0.030)

All values represent median (25^{th} – 75^{th} percentiles). No differences in expression were found between patients and healthy controls ($P > 0.05$).

5-HT$_4$ receptor

The total expression of the 5-HT$_4$ receptor in the duodenum was higher compared to the antrum and the fundus, with relative differences of 87 (51 – 147) and 85 (50 – 144) respectively (all $P < 0.001$). No differences were observed between antrum and fundus. The expression of the 5-HT$_4$ receptor was comparable in the three regions between patients and controls ($P > 0.05$) (table 3).

The mRNA expression of the 5-HT$_4$ splice variants in the antrum and fundus was too low for reliable quantification. Furthermore, we have focused on the three most prevalent forms of the 5-HT$_4$ splice variants. In the small intestine, the 5-HT$_{4(a)}$, 5-HT$_{4(b)}$, and 5-HT$_{4(c)}$ splice-variants constitute the largest part of total 5-HT$_4$ expression, previously described by Medhurst et al. and confirmed in our own laboratory.[32] In the duodenum, the expression of the 5-HT$_{4(c)}$ splice variant was lower in patients compared to healthy controls ($P = 0.015$). No differences were found in the expression of the 5-HT$_{4(a)}$) and the 5-HT$_{4(b)}$ splice variants ($P > 0.05$) (figure 2).

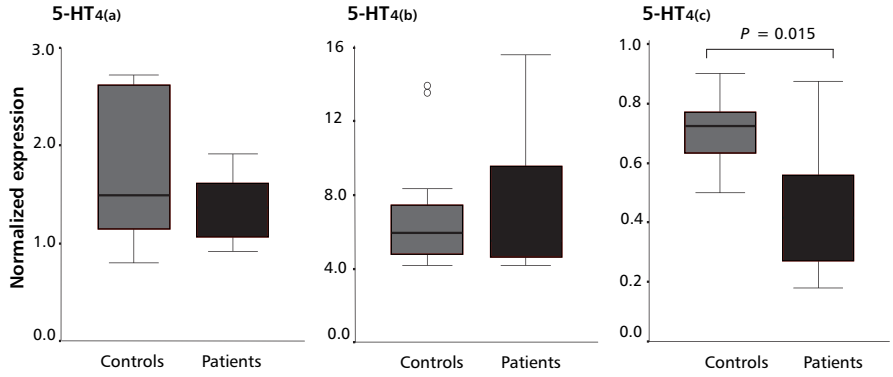

Figure 2
The normalized mRNA expression of 5-HT$_4$ splice variants in the duodenum of 11 patients with gastroparesis and 11 healthy controls. The expression of 5-HT$_{4(c)}$ was lower in patients with gastroparesis compared to healthy controls ($P = 0.015$), whereas no differences were found in 5-HT$_{4(a)}$ and 5-HT$_{4(b)}$ between both groups ($P > 0.05$).

5-HT$_3$ receptor

The expression of the 5-HT$_3$ subunits in the antrum and fundus was too low for reliable quantification. Furthermore, we found very low expression of the 5-HT$_{3A}$ and 5-HT$_{3B}$ receptor subunits in the duodenum at the mucosal level. Since 5-HT$_{3D}$ is reportedly not expressed in the stomach and intestine[17], we have focused on the mRNA expression levels of 5-HT$_{3C}$ and the 5-HT$_{3E}$ receptor subunits in the duodenum. The expression of the 5-HT$_{3C}$ and the 5-HT$_{3E}$ subunits in the duodenum was comparable between patients and controls ($P > 0.05$) (table 4).

Table 4
mRNA expression of the 5-HT$_3$ receptor subunits in patients and healthy controls, normalized to PBGD.

Duodenum	HTR3C	HTR3E
Patients	0.47 (0.20 – 0.69)	0.17 (0.07 – 0.25)
Controls	0.36 (0.25 – 0.75)	0.11 (0.09 – 0.26)

All values represent median (25th – 75th percentiles). No differences in expression of the 5-HT$_3$ subunits were found between patients and healthy controls ($P > 0.05$).

Discussion

The main findings of the current study are that the total number of neuroendocrine cells, specifically the 5-HT producing cells, and the mRNA expression levels of TPH-1 (the rate-limiting enzyme of 5-HT synthesis) and SERT (responsible for the high affinity uptake of 5-HT thereby controlling the level of 5-HT available for receptor stimulation) in mucosal biopsy specimens of fundus, antrum, and duodenum were comparable between patients with idiopathic gastroparesis and healthy controls. Post-hoc analysis revealed that a 1.5 - fold relative difference in TPH-1 and SERT mRNA expression was detectable with at least 80% power given the number of participants and observed standard deviations ($\alpha = 0.05$).

This study has allowed more insight into the putative role of aberrant serotonergic signalling in idiopathic gastroparesis. Delayed gastric emptying can be explained by a reduced availability of 5-HT at 5-HT receptors on intrinsic afferents in stomach and duodenum as a consequence of decreased biosynthesis (reduced TPH-1 expression or a reduced number of 5-HT producing neuroendocrine cells) and/or increased uptake (enhanced SERT expression). Besides, an increased availability of 5-HT at 5-HT_3 receptors on extrinsic afferents in the duodenum is expected to cause delayed gastric emptying and enhanced genesis of upper gastrointestinal symptoms. This study revealed that mucosal 5-HT biosynthesis and uptake capacity are not altered in the stomach and duodenum of patients with idiopathic gastroparesis.

However, it cannot be ruled out that mucosal 5-HT availability is altered in idiopathic gastroparesis due to abnormal release of 5-HT from neuroendocrine cells. It has been shown that 5-HT synthesis does not always correlate with 5-HT release, presumably due to the large storage facility of 5-HT by the neuroendocrine cells.[33] We have recently postulated that SERT expression, and not so much TPH-1, is a marker for the activity of serotonergic signalling (5-HT activity and/or 5-HT release.[34] In view of equal SERT expression, we can speculate that 5-HT availability is comparable between patients and controls.

The release of 5-HT from the neuroendocrine cells in the gastrointestinal mucosa is modulated by 5-HT_3 and 5-HT_4 receptors located in their surface membrane. We found no differences in the expression levels of the 5-HT_{3C} and the 5-HT_{3E} receptor subunits, indicating that 5-HT_3 autoreceptors are equally expressed in patients and controls. The total expression of the 5-HT_4

receptor was also comparable in all three regions between patients and controls. However, the expression of the 5-$HT_{4(c)}$ splice variant in the duodenum of gastroparesis patients was lower compared to healthy controls. The expression levels of the 5-$HT_{4(a)}$ and the 5-$HT_{4(b)}$ splice variants did not differ. Activation of 5-HT_4 autoreceptors triggers a negative feedback mechanism leading to a reduction of 5-HT release.[14] The 5-$HT_{4(c)}$ splice variant exhibits a higher constitutive activity,[22] suggesting that the inhibitory action on basal 5-HT release is less in the duodenum of patients with idiopathic gastroparesis. On the other hand, 5-$HT_{4(c)}$ has a higher number of putative phosphorylation sites.[22] Phosphorylation in the C terminus of G protein coupled receptors has been shown to induce receptor desensitisation [35]. Thus, in patients with idiopathic gastroparesis the negative autoregulatory action on 5-HT release may be prolonged. Anyhow, altered expression of the 5-$HT_{4(c)}$ splice variant may exert a modest effect on 5-HT availability.

The biopsy specimens used in the current study were taken from the mucosa, implying that our data give insight into 5-HT synthesis by neuroendocrine cells, 5-HT uptake capacity by SERT expressed by epithelial cells, and 5-HT receptors expressed by neuroendocrine cells. The 5-HT synthesis and uptake capacity by serotonergic neurons, which are situated within the myenteric plexus were not assessed in the current study. It should be emphasized that 5-HT is situated mainly (>90%) in neuroendocrine cells.[11] Expression of 5-HT receptors present on afferent nerve endings in the lamina propria could not be studied, since mRNA for these receptors is situated in the neuronal cell bodies and is therefore not acquired in a mucosal biopsy. Differential distribution or expression of 5-HT_3 receptor subtypes and 5-HT_4 receptor splice variants on afferent nerve endings may play a role in the pathophysiology of idiopathic gastroparesis. For instance, expression of 5-HT_4 receptors with a higher tendency to desensitise or 5-HT_3 receptors more sensitive to 5-HT would exert the gastric motor and visceral sensitivity effects respectively observed in idiopathic gastroparesis. Stimulatory effects of serotonin on gastrointestinal motility are also mediated by activation of 5-HT receptors on myenteric neurons. These receptors are stimulated by serotonin originating from myenteric neurons. Therefore, differences in serotonin biosynthesis, release, or inactivation by serotonergic neurons in the myenteric plexus may account for the abnormal intragastric transport and impaired antropyloroduodenal coordination.

In summary, this study suggests that the delayed gastric emptying and

upper abdominal symptoms in idiopathic gastroparesis do not result from altered mucosal 5-HT biosynthetic and uptake capacity. To determine the role of reduced mucosal 5-$HT_{4(c)}$ splice variant expression in patients with idiopathic gastroparesis, its effect on 5-HT_4 receptor function and 5-HT release needs to be examined in future studies.

References

1 Fraser RJ, Horowitz M, Maddox AF, Dent J. Postprandial antropyloroduodenal motility and gastric emptying in gastroparesis--effects of cisapride. Gut 1994; 35(2):172-178.

2 Parkman HP, Hasler WL, Fisher RS. American Gastroenterological Association technical review on the diagnosis and treatment of gastroparesis. Gastroenterology 2004; 127(5):1592-1622.

3 Talley NJ, Van Zanten SV, Saez LR, Dukes G, Perschy T, Heath M et al. A dose-ranging, placebo-controlled, randomized trial of alosetron in patients with functional dyspepsia. Aliment Pharmacol Ther 2001; 15(4):525-537.

4 Coleman NS, Marciani L, Blackshaw E, Wright J, Parker M, Yano T et al. Effect of a novel 5-HT3 receptor agonist MKC-733 on upper gastrointestinal motility in humans. Aliment Pharmacol Ther 2003; 18(10):1039-1048.

5 Degen L, Matzinger D, Merz M, Appel-Dingemanse S, Osborne S, Luchinger S et al. Tegaserod, a 5-HT4 receptor partial agonist, accelerates gastric emptying and gastrointestinal transit in healthy male subjects. Aliment Pharmacol Ther 2001; 15(11):1745-1751.

6 Tack J, Broekaert D, Coulie B, Fischler B, Janssens J. Influence of the selective serotonin re-uptake inhibitor, paroxetine, on gastric sensorimotor function in humans. Aliment Pharmacol Ther 2003; 17(4):603-608.

7 van Nieuwenhoven MA, Valks SD, Sobczak S, Riedel WJ, Brummer RJ. Acute tryptophan depletion slows gastric emptying in females. Br J Nutr 2004; 91(3):351-355.

8 Bulbring E, Crema A. The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. J Physiol 1959; 146(1):18-28.

9 Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 2003; 299(5603):76.

10 Cote F, Thevenot E, Fligny C, Fromes Y, Darmon M, Ripoche MA et al. Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. Proc Natl Acad Sci U S A 2003; 100(23):13525-13530.

11 Gershon MD. Review article: serotonin receptors and transporters - roles in normal and abnormal gastrointestinal motility. Aliment Pharmacol Ther 2004; 20 Suppl 7:3-14.

12 Gebauer A, Merger M, Kilbinger H. Modulation by 5-HT3 and 5-HT4 receptors of the release of 5-hydroxytryptamine from the guinea-pig small intestine. Naunyn Schmiedebergs Arch Pharmacol 1993; 347(2):137-140.

13 Racke K, Reimann A, Schworer H, Kilbinger H. Regulation of 5-HT release from enterochromaffin cells. Behav Brain Res 1996; 73(1-2):83-87.

14 Schworer H, Ramadori G. Autoreceptors can modulate 5-hydroxytryptamine release from porcine and human small intestine in vitro. Naunyn Schmiedebergs Arch Pharmacol 1998; 357(5):548-552.

15 Wade PR, Chen J, Jaffe B, Kassem IS, Blakely RD, Gershon MD. Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract. J Neurosci 1996; 16(7):2352-2364.

16 Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG et al. The 5-HT3B subunit is a major determinant of serotonin-receptor function. Nature 1999; 397(6717):359-363.

17 Niesler B, Frank B, Kapeller J, Rappold GA. Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. Gene 2003; 310:101-111.

18 Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC et al. The pharmacological and functional characteristics of the serotonin 5-HT(3A) receptor are specifically modified by a 5-HT(3B) receptor subunit. J Biol Chem 1999; 274(43):30799-30810.

19 Brady CA, Stanford IM, Ali I, Lin L, Williams JM, Dubin AE et al. Pharmacological comparison of human homomeric 5-HT3A receptors versus heteromeric 5-HT3A/3B receptors. Neuropharmacology 2001; 41(2):282-284.

20 Hapfelmeier G, Tredt C, Haseneder R, Zieglgansberger W, Eisensamer B, Rupprecht R et al. Co-expression of the 5-HT3B serotonin receptor subunit alters the biophysics of the 5-HT3 receptor. Biophys J 2003; 84(3):1720-1733.

21 Gerald C, Adham N, Kao HT, Olsen MA, Laz TM, Schechter LE et al. The 5-HT4 receptor: molecular cloning and pharmacological characterization of two splice variants. EMBO J 1995; 14(12):2806-2815.

22 Blondel O, Gastineau M, Dahmoune Y, Langlois M, Fischmeister R. Cloning, expression, and pharmacology of four human 5-hydroxytryptamine 4 receptor isoforms produced by alternative splicing in the carboxyl terminus. J Neurochem 1998; 70(6):2252-2261.

23 Bender E, Pindon A, van O, I, Zhang YB, Gommeren W, Verhasselt P et al. Structure of the human serotonin 5-HT4 receptor gene and cloning of a novel 5-HT4 splice variant. J Neurochem 2000; 74(2):478-489.

24 Mialet J, Berque-Bestel I, Sicsic S, Langlois M, Fischmeister R, Lezoualc'h F. Pharmacological characterization of the human 5-HT(4(d)) receptor splice variant stably expressed in Chinese hamster ovary cells. Br J Pharmacol 2000; 131(4):827-835.

25 Pindon A, van Hecke G, van Gompel P, Lesage AS, Leysen JE, Jurzak M. Differences in signal transduction of two 5-HT4 receptor splice variants: compound specificity and dual coupling with Galphas- and Galphai/o-proteins. Mol Pharmacol 2002; 61(1):85-96.

26 Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR, Tytgat GN. Functional gastroduodenal disorders. Gut 1999; 45 Suppl 2:II37-II42.

27 Minderhoud IM, Mundt MW, Roelofs JM, Samsom M. Gastric emptying of a solid meal starts during meal ingestion: combined study using 13C-octanoic acid breath test and Doppler ultrasonography. Absence of a lag phase in 13C-octanoic acid breath test. Digestion 2004; 70(1):55-60.

28 Samsom M, Vermeijden JR, Smout AJ, Van Doorn E, Roelofs J, Van Dam PS et al. Prevalence of delayed gastric emptying in diabetic patients and relationship to dyspeptic symptoms: a prospective study in unselected diabetic patients. Diabetes Care 2003; 26(11):3116-3122.

29 Stanghellini V, Tosetti C, Paternic inverted question mA, Barbara G, Morselli-Labate AM, Monetti N et al. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. Gastroenterology 1996; 110(4):1036-1042.

30 Tucci A, Corinaldesi R, Stanghellini V, Tosetti C, Di Febo G, Paparo GF et al. Helicobacter pylori infection and gastric function in patients with chronic idiopathic dyspepsia. Gastroenterology 1992; 103(3):768-774.

31 Cuomo R, Sarnelli G, Grasso R, Bruzzese D, Pumpo R, Salomone M et al. Functional dyspepsia symptoms, gastric emptying and satiety provocative test: analysis of relationships. Scand J Gastroenterol 2001; 36(10):1030-1036.

32 Medhurst AD, Lezoualc'h F, Fischmeister R, Middlemiss DN, Sanger GJ. Quantitative mRNA analysis of five C-terminal splice variants of the human 5-HT4 receptor in the central nervous system by TaqMan real time RT-PCR. Brain Res Mol Brain Res 2001; 90(2):125-134.

33 Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H et al. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 2004; 126(7):1657-1664.

34 van Lelyveld N, Ter Linde J, Schipper MEI, Samsom M. Regional differences in serotonergic activity in the mucosa of the human stomach and duodenum. Neurogastroenterol Motil 2007;In press.

35 Dohlman HG, Thorner J, Caron MG, Lefkowitz RJ. Model systems for the study of seven-transmembrane-segment receptors. Annu Rev Biochem 1991; 60:653-688.

Chapter 08

Candidate genotypes associated with functional dyspepsia

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Submitted

Abstract

Background and aim

There is accumulating evidence of a genetic predisposition for developing a functional gastrointestinal disorder. Identification of the genetic factors may improve understanding of underlying pathophysiologic mechanisms. We aimed to test the association of functional polymorphisms in genes involved in serotonergic signalling and G-protein mediated signal transduction, both affecting gastroduodenal sensory and motor function, with functional dyspepsia (FD).

Materials and Methods

FD patients, send to our tertiary referral center, were studied (n = 112). Healthy controls (n = 336) free of gastrointestinal symptoms were matched 1:3 for age and gender. Polymorphisms in genes encoding the serotonin receptor type three A subunit (HTR3A), the serotonin transporter (SERT), and the G protein β3 subunit (GNB3) were analyzed.

Results

The FD patients displayed a higher prevalence of the T allele of the GNB3 C825T polymorphism compared to healthy controls (OR = 1.60, 95% CI 1.03 – 2.49, P = 0.038). No association between functional dyspepsia and the genotype of the insertion/deletion polymorphism in the promoter of SERT (SERT-P) or HTR3A C178T polymorphism was observed.

Conclusion

Tertiary referral functional dyspepsia is associated with the 825T allele of the GNB3 gene. The increased signal transduction associated with this allele may contribute to the abnormalities in gastroduodenal sensory and motor function observed in FD.

Introduction

Functional dyspepsia (FD) is defined as the presence of symptoms thought to originate in the gastroduodenal region, in the absence of any organic, systemic, or metabolic disease that is likely to explain the symptoms.[1] Three pathophysiologic mechanisms have been described as possible etiologic factors; a delayed gastric emptying,[2] impaired proximal gastric accommodation,[3] and visceral hypersensitivity.[4] Potential risk factors for FD include age, gender, *H Pylori* infection, the use of NSAID's, heavy smoking, unemployment, and psychological disturbances.[5-7] There is increasing evidence that susceptibility to functional gastrointestinal disorders is also influenced by hereditary factors.[8-10] In the current study, we have performed a candidate gene approach, to identify genetic factors that may contribute to the manifestation of abnormalities in gastroduodenal sensory and motor function as observed in FD.

Since serotonin (5-HT) is a key signalling molecule affecting gastrointestinal motor and sensory functions, genes of the serotonergic system are critical candidates in assessing the role of genetic determinants in FD. 5-HT_3 receptors are present on central and enteric neurons and the mucosal terminals of extrinsic primary afferents. Gastroduodenal motility and visceral sensitivity are modulated by 5-HT release and subsequent action on 5-HT_3 receptors.[11] The 5-HT_3 receptor is a ligand-gated ion channel, structured as a pentameric complex containing 1 or more of 5 different subunits, termed A – E.[12-14] A functional polymorphism has been identified in HTR3A, the gene coding for the 5-HT_3 receptor A subunit. The 5' untranslated region (UTR) of HTR3A contains two upstream open reading frames (uORFs).[15] Peptides encoded by uORFs may affect the translation rate of the downstream major transcript.[16;17] A single nucleotide polymorphism, C178T, changing Pro^{16} to Ser, resides in the second uORF.[18] It has been shown in vitro that the T allele is related to increased HTR3A protein levels.[18] Changes in the subunit composition of the 5-HT_3 receptor affect 5-HT affinity and desensitization rate and hence lead to altered response to 5-HT.[19]

The action of 5-HT is terminated, peripherally and centrally, by 5-HT transporter (SERT) mediated uptake, thereby determining 5-HT availability at the receptors.[20] A 44 bp insertion/deletion polymorphism is present in the 5' flanking region of the SERT gene, creating a long (L) and short (S) allelic variant.[21] The presence of the short allele results in reduced SERT expression

and 5-HT uptake.[22] The SS genotype of the SERT promoter polymorphism has been reported to be associated with diarrhea predominant IBS.[23] Moreover, the SERT-P polymorphism influences the response to serotonergic intervention in diarrhea predominant IBS.[24]
The third polymorphism studied in our candidate gene approach is located in exon 10 (C825T) of the gene encoding the G protein β3 subunit (GNB3). G-proteins mediate the response to the release of serotonin and several other neurotransmitters modulating gastroduodenal sensory and motor function. The 825T allele is associated with alternative splicing of the gene, and an increased intracellular signal transduction.[25] In recent publications, an association with functional gastrointestinal disorders has been suggested.[26;27]

Methods

Subjects

Patients with dyspeptic symptoms visiting our tertiary referral centre were asked to participate in the study. Before inclusion, each patient completed a validated symptom questionnaire.[2;3;28] Patients were asked to score six different symptoms (pain or discomfort centred in the upper abdomen, early satiety, bloating in the upper abdomen, fullness, nausea and vomiting) from 0-5 (0=none, 1=very mild; awareness of symptoms but easily tolerated, 2=mild; tolerated without interference with usual activity, 3=moderate; enough to cause some interference with usual activity, 4=severe; enough to cause significant interference with usual activity, 5=very severe; incapacitating with inability to work or do usual activity). For inclusion, two of these symptoms had to be scored as moderate, severe or very severe and these symptoms needed to be present for at least 12 weeks, not necessary consecutive, in the preceding 12 months.
All FD patients presented at the outpatients clinic of the University Medical Centre Utrecht undergo an extensive workup, including upper GI endoscopy, assessment of gastric emptying rate by ^{13}C octanoic breathtest, assessment of proximal gastric relaxation by three-dimensional ultrasound, laboratory testing, and a clinical evaluation. Patients with abnormalities seen during upper GI endoscopy were excluded. One hundred and twelve FD patients were included. All patients were classified as having idiopathic dys-

pepsia, with no identifiable explanation for their symptoms.[1] None had a history of gastrointestinal surgery or concomitant illnesses.
Healthy controls were recruited through advertisement. Respondents with upper or lower gastrointestinal symptoms, or a history of gastrointestinal disease or surgery were excluded. Patients and healthy controls were matched 1:3 according to sex and age.
All participants were asked to fill out a questionnaire concerning gastrointestinal symptoms based on the ROME II criteria and part of the Nepean dyspepsia index).[29] For the patients, the outcome of the questionnaire was used to assess the presence of concomitant symptoms of gastroesophageal reflux disease (GERD) and irritable bowel syndrome (IBS). Coexistence of GERD symptoms was defined as having acid regurgitation and/or heartburn for at least 12 weeks in the past 12 months (not necessarily consecutive). Patients with predominant heartburn and/or acid regurgitation were excluded. Concomitant IBS symptoms was defined as pain or discomfort in the lower intestine for at least 12 weeks in the past 12 months (not necessarily consecutive), relieved by defecation and a change in frequency or consistency of stool in relation to abdominal pain.
All patients and controls were Caucasian. The study was approved by the medical ethics committee of the University Medical Centre Utrecht and written informed consent was obtained from all participants.

Genotyping

Genomic DNA was isolated from whole blood using the QIAamp DNA blood minikit (Qiagen Inc., Valencia, California, USA). For the HTR3A C178T (rs1062613) and GNB3 C825T (rs5443) polymorphisms genotyping was performed by molecular beacon assay, using the iCycler iQ system (BioRad, Hercules, CA, USA). The assays were carried out in a volume of 25 μl, containing 50 ng of genomic DNA, 12.5 μl of 2x iQ Supermix (BioRad, Hercules, CA, USA), 1000 nM forward primer, and 250 nM reverse primer. For HTR3A C178T the primers were 5′-GCAGCCTCAGAAGGTGTG-3′ (forward) and 5′-CCACAGCAGCATAGCGAG-3′ (reverse). $MgCl_2$ was added to obtain a final concentration of 4mM. 400 nM of the following beacons were used; 5′-FAM-CGGACCAGTGCTCAGGGCGAGGCGGTCCG-DABCYL-3′ (C-allele specific) and 5′-TXR-CGCGACCGAGTGCTCAGGACGAGGCGTCGCG-DABCYL-3′ (T-allele specific). For GNβ3 C825T the primers were 5′-TGCCGCTTGTTTGACCTG-3′ (forward) and 5′-CAGTTGAAGTCGTCGTAGCC-3′

(reverse) and $MgCl_2$ was added to obtain a final concentration of 5mM. 200 nM of the C-allele specific molecular beacon 5′-FAM-CGGCTCGAAGGCCAC-GGACGTGATGGAGCCG-DABCYL-3′ and 400 nM of the molecular beacon specific for the T-allele 5′-TXR-CGGCTCAGAAGGCCACAGACGTGATG-GAGCCG-DABCYL-3′ were used.

The PCR protocols consisted of a 3 min 95°C initial denaturation and enzyme-activating step followed by 40 cycles of 95°C for 30 sec, at annealing temperature for 1 min and 72°C for 45 sec. The HTR3A C178T and GNB3 C825T assays were run at an annealing temperature of 60°C and 63°C respectively. In each run, representative samples from each genotype were inserted.

To validate genotyping of GNB3 C825T by molecular beacon assay, PCR-based restriction fragment length polymorphism analysis was performed in a set of randomly chosen patients. For this purpose the PCR fragments were digested with BsaJ1 overnight at 60°C and separated by 2.5% agarose gel electrophoresis. The C allele yielded DNA fragments of 77 and 57 bp, whereas the T allele PCR product remained uncut. Concordance was 100%. The genotyping of HTR3A C178T was validated by sequencing.

The SERT-P polymorphism was genotyped by PCR followed by agarose gel electrophoresis. PCR was performed using the primers and reaction conditions described by Camilleri et al., generating 528- and/or 572-base-pair fragments.[24] However, we performed PCR amplification in a total volume of 25 μl containing 50 ng genomic DNA. The size of the amplified fragments was determined by electrophoresis on a 2.5% low range ultra agarose gel (Biorad, Hercules, CA, USA) stained with ethidium bromide; 572 bp and 528 bp products were typed as long (L) and short (S) alleles respectively.

Data Analysis

In vitro studies have revealed that both the heterozygous (LS) and homozygous S genotypes of SERT-P result in reduced SERT protein expression and uptake of serotonin.[22] Moreover, a greater response to alosetron (a 5-HT_3 receptor antagonist) in slowing transit time in diarrhea predominant IBS patients with the LL genotype compared with the heterozygous genotype indicates that the S allele is dominant.[24] Therefore, for SERT-P the association of the combined LS and SS genotypes versus LL with functional dyspepsia was assessed.

Data from in vitro experiments demonstrate that the presence of the

T allele, whether homozygous or heterozygous, is associated with increased intracellular signal transduction.[25;30;31] Therefore, we analyzed T allele carriers versus subjects with the CC genotype.
The C178T variant is associated with increased translation of the downstream HTR3A transcript.(18) The significant difference in amygdaloidal activity in subjects with CC and CT genotypes suggests a dominant effect of the T allele.[18;32] Therefore, we have analysed the CC genotype versus the combined homozygous and heterozygous T genotype.

Sample size

We considered an OR of 2.0 for assessing the necessary sample size. This odds ratio holds for the odds of cases with respect to the odds of controls in an unmatched study. It can be used to estimate the probability of discordance in a case-control study with 1:1 matching,[33] varying the prevalence of the putative genotype in the controls between 0.20 and 0.70. In the present study, we match 3 controls to each case. The sample size based on 1:1 matching can therefore be reduced by a factor of 2/3.[34] With a level of significance of 5% and a power of 80%, this leads to a necessary sample size of 100 cases and 300 matched controls.
The genotype distribution in patients and controls for the various polymorphisms was tested for Hardy-Weinberg equilibrium using the Chi square test. To take the matching between cases and controls into account, conditional logistic regression analysis was performed to calculate odds ratios. BMI was included as a covariate in the analysis. A $P < 0.05$ was considered significant. All statistical analysis was performed using commercially available software (SPSS 12.0 for Microsoft windows).

Results

A total of 448 unrelated subjects consisting of 112 functional dyspeptic patients and 336 sex- and age-matched controls were included in the study. The mean age (± SEM) of the FD patients was 42.3 ± 1 year versus 41.9 ± 1 year of the healthy controls. In both the FD group and the control group, 72% was female. No statistical difference was found in BMI between the two groups; mean BMI of FD patients 22.9 ± 0.4 kg/m^2 versus 24.2 ± 0.2 kg/m^2 of the healthy controls. Symptoms suggestive of gastroesophageal reflux disease were present in 45% of patients, and approximately 30% of patients reported lower abdominal symptoms suggestive for IBS (table 1).
The genotype distributions of SERT-P, HTR3A C178T and GNB3 C825T are depicted in table 2. There was no statistical deviation from Hardy-Weinberg equilibrium for any of the three polymorphisms in the control group. Furthermore, the genotype distributions of the polymorphisms in the controls were similar to those previously reported for Caucasian populations.[35-37] In the FD group the genotype distribution of each polymorphism was in concordance with Hardy-Weinberg equilibrium. Odds ratios for the associations between the three polymorphisms and functional dyspepsia are shown in figure 1. The presence of GNB3 825T was associated with an increased OR (95% CI) for FD versus controls. There was no significant association between SERT-P or HTR3A C178T polymorphisms and functional dyspepsia.

Table 1
Functional characteristics and co-morbidity in tertiary referral FD patients

	FD patients (n = 112)
Delayed gastric emptying	26%
Impaired proximal gastric relaxation	29%
IBS symptoms	30%
GERD symptoms	45%

Table 2
Genotype distributions in controls and FD patients

Polymorphism	FD patients (n = 112)	Controls (n = 336)
SERT-P		
LL	37 (33.0)	108 (32.1)
LS	50 (44.6)	170 (50.6)
SS	25 (22.3)	58 (17.3)
HTR3A C178T		
CC	66 (58.9)	218 (64.9)
CT	42 (37.5)	105 (31.3)
TT	4 (3.6)	13 (3.9)
GNB3 C825T		
CC	48 (42.9)	180 (53.6)
CT	54 (48.2)	126 (37.5)
TT	10 (8.9)	30 (8.9)

The genotype distributions are depicted as number (%).

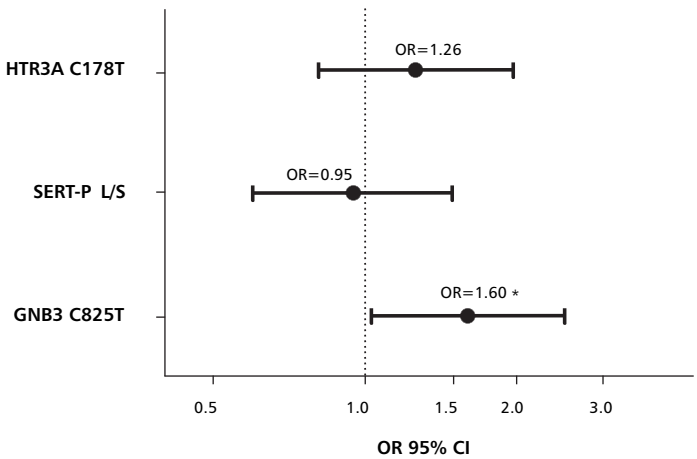

Figure 1
Odds ratios with 95% confidence intervals for tertiary referral FD patients versus healthy controls in GNB3 825T carriers, SERT-P S carriers, and HTR3A 178T carriers relative to respective homozygous wild-type genotypes (* $P = 0.038$).

Discussion

The genetic factors contributing to the manifestation of FD are largely unknown. We have detected an association between the T allele of the GNB3 C825T polymorphism and tertiary referral functional dyspepsia. No association was found between SERT-P or HTR3A C178T and functional dyspepsia.

Recently, it has been reported that homozygous GNB3 825C carrier status is associated with unexplained upper abdominal symptoms.[26] This contrasting observation may be explained by different genotype distributions in the control sample. The higher frequency of the homozygous C genotype compared to the TC genotype in our control sample is in accordance with the genotype distribution found in a large population sample of Caucasians.[35] The unusual low frequencies of homozygous C carriers in the control groups of the studies conducted by Holtmann et al. may have led to the significant odds ratio for the CC genotype. Furthermore, differences in sample selection may serve as an explanation for the contrasting results. We have included subjects with longstanding dyspeptic symptoms, examined by upper GI endoscopy and diagnosed as having functional dyspepsia at a tertiary referral centre, whereas blood donors reporting upper abdominal symptoms were part of the case sample in one of the studies described by Holtmann et al. Moreover, in their second study, the control sample consisted of blood donors for which no data regarding symptoms related to the gastrointestinal tract are available. The control subjects enrolled in our study are free of any gastrointestinal symptoms.

Uninvestigated dyspepsia has also been reported to be associated with both the homozygous GNB3 825 T and C genotypes.[27] Although it is biologically plausible that either the 825 C or T allele is associated with functional dyspepsia or upper abdominal symptoms in general, it is unlikely that subjects belonging to the same dyspepsia phenotype group reveal an association with both alleles. Furthermore, in the latter study the genotype distribution in the control group was atypical as well and the relatively small sample size does not preclude the effect of a type I error.

A polymorphism of a G protein may lead to a wide number of pathophysiologic effects, as many hormones, neurotransmitters, chemokines, local mediators, and sensory stimuli exert their effects on cells by binding to G – protein coupled receptors (GPCRs). The cholecystokinin (CCK)-1 receptor

has been implicated in the generation of dyspeptic symptoms and belongs to the GPCR family.[38] CCK is released postprandially from neuroendocrine cells in the duodenal mucosa and delays gastric emptying and promotes sensations of satiety. There is evidence of CCK hyperresponsiveness in FD patients.[39] It is conceivable that in carriers of the GNB3 825T allele the response via the CCK-1 receptor, situated on vagal afferents in the duodenum, is enhanced. In this way the higher prevalence of the GNB3 825T may contribute to the abnormalities in gastroduodenal function and symptoms encountered in FD patients.

A high percentage of the FD patients in our study have symptoms associated with GERD and/or IBS. Considering the large overlap between these gastrointestinal disorders, a common pathophysiology of increased visceral sensitivity underlying the generation of symptoms is conceivable.[40-42]. We have recently shown that GNB3 825T carriership is more prevalent in GERD patients relative to healthy controls and that the association was stronger in the subgroup of patients with physiologic acid exposure and a positive symptom association score.[43] The findings of the current study support the hypothesis that the presence of the T allele of the GNB3 polymorphism predisposes to visceral hypersensitivity in the gastrointestinal tract.

In summary, we have observed an association between tertiary referral functional dyspepsia and the 825T allele of the GNB3 gene. The higher prevalence of the 825T allele, which is related to enhanced signal transduction upon GPCR activation, may underlie the pathophysiologic mechanisms or the generation of symptoms in functional dyspepsia. Further research is required to elucidate the specific signal transduction pathways affected by this genetic susceptibility factor.

References

1 Tack J, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR et al. Functional gastroduodenal disorders. Gastroenterology 2006; 130(5):1466-1479.

2 Stanghellini V, Tosetti C, Paternic inverted question mA, Barbara G, Morselli-Labate AM, Monetti N et al. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. Gastroenterology 1996; 110(4):1036-1042.

3 Tack J, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998; 115(6):1346-1352.

4 Salet GA, Samsom M, Roelofs JM, Berge Henegouwen GP, Smout AJ, Akkermans LM. Responses to gastric distension in functional dyspepsia. Gut 1998; 42(6):823-829.

5 Mahadeva S, Goh KL. Epidemiology of functional dyspepsia: a global perspective. World J Gastroenterol 2006; 12(17):2661-2666.

6 Wildner-Christensen M, Hansen JM, De Muckadell OB. Risk factors for dyspepsia in a general population: non-steroidal anti-inflammatory drugs, cigarette smoking and unemployment are more important than Helicobacter pylori infection. Scand J Gastroenterol 2006; 41(2):149-154.

7 Locke GR, III, Weaver AL, Melton LJ, III, Talley NJ. Psychosocial factors are linked to functional gastrointestinal disorders: a population based nested case-control study. Am J Gastroenterol 2004; 99(2):350-357.

8 Morris-Yates A, Talley NJ, Boyce PM, Nandurkar S, Andrews G. Evidence of a genetic contribution to functional bowel disorder. Am J Gastroenterol 1998;(8):1311-7.

9 Levy RL, Jones KR, Whitehead WE, Feld SI, Talley NJ, Corey LA. Irritable bowel syndrome in twins: heredity and social learning both contribute to etiology. Gastroenterology 2001; 121(4):799-804.

10 Kalantar JS, Locke GR, III, Zinsmeister AR, Beighley CM, Talley NJ. Familial aggregation of irritable bowel syndrome: a prospective study. Gut 2003; 52(12):1703-7.

11 Hansen MB. Neurohumoral control of gastrointestinal motility. Physiol Res 2003; 52(1):1-30.

12 Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG et al. The 5-HT3B subunit is a major determinant of serotonin-receptor function. Nature 1999; 397(6717):359-363.

13 Niesler B, Frank B, Kapeller J, Rappold GA. Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. Gene 2003; 310:101-111.

14 Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D. Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science 1991; 254(5030):432-437.

15 Miyake A, Mochizuki S, Takemoto Y, Akuzawa S. Molecular cloning of human 5-hydroxytryptamine3 receptor: heterogeneity in distribution and function among species. Mol Pharmacol 1995; 48(3):407-416.

16 Morris DR, Geballe AP. Upstream open reading frames as regulators of mRNA translation. Mol Cell Biol 2000; 20(23):8635-8642.

17 Cazzola M, Skoda RC. Translational pathophysiology: a novel molecular mechanism of human disease. Blood 2000; 95(11):3280-3288.

18 Niesler B, Flohr T, Nothen MM, Fischer C, Rietschel M, Franzek E et al. Association between the 5' UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics 2001; 11(6):471-475.

19 Liu MT, Rayport S, Jiang Y, Murphy DL, Gershon MD. Expression and function of 5-HT3 receptors in the enteric neurons of mice lacking the serotonin transporter. Am J Physiol Gastrointest Liver Physiol 2002; 283(6):G1398-411.

20 Wade PR, Chen J, Jaffe B, Kassem IS, Blakely RD, Gershon MD. Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract. J Neurosci 1996; 16(7):2352-2364.

21 Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996; 66(6):2621-2624.

22 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996; 274(5292):1527-1531.

23 Yeo A, Boyd P, Lumsden S, Saunders T, Handley A, Stubbins M et al. Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. Gut 2004; 53(10):1452-1458.

24 Camilleri M, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE et al. Serotonin-transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. Gastroenterology 2002; 123(2):425-432.

25 Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R et al. Association of a human G-protein beta3 subunit variant with hypertension. Nat Genet 1998; 18(1):45-48.

26 Holtmann G, Siffert W, Haag S, Mueller N, Langkafel M, Senf W et al. G-protein beta 3 subunit 825 CC genotype is associated with unexplained (functional) dyspepsia. Gastroenterology 2004; 126(4):971-979.

27 Camilleri CE, Carlson PJ, Camilleri M, Castillo EJ, Locke GR, III, Geno DM et al. A study of candidate genotypes associated with dyspepsia in a U.S. community. Am J Gastroenterol 2006; 101(3):581-592.

28 Tucci A, Corinaldesi R, Stanghellini V, Tosetti C, Di Febo G, Paparo GF et al. Helicobacter pylori infection and gastric function in patients with chronic idiopathic dyspepsia. Gastroenterology 1992; 103(3):768-774.

29 Talley NJ, Verlinden M, Jones M. Validity of a new quality of life scale for functional dyspepsia: a United States multicenter trial of the Nepean Dyspepsia Index. Am J Gastroenterol 1999; 94(9):2390-2397.

30 Virchow S, Ansorge N, Rubben H, Siffert G, Siffert W. Enhanced fMLP-stimulated chemotaxis in human neutrophils from individuals carrying the G protein beta3 subunit 825 T-allele. FEBS Lett 1998; 436(2):155-8.

31 Lindemann M, Virchow S, Ramann F, Barsegian V, Kreuzfelder E, Siffert W et al. The G protein beta3 subunit 825T allele is a genetic marker for enhanced T cell response. FEBS Lett 2001; 495(1-2):82-6.

32 Iidaka T, Ozaki N, Matsumoto A, Nogawa J, Kinoshita Y, Suzuki T et al. A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala. J Neurosci 2005; 25(27):6460-6.

33 Lachenburg PA. On the sample size for studies based upon McNemar's test. Statistics in Medicine 1992; 11:1521-25.

34 Ury HK. Efficiency of case-control studies with multiple controls per case: continuous or dichotomous data. Biometrics 1992; 31:643-49.

35 Andresen V, Camilleri M, Kim HJ, Stephens DA, Carlson PJ, Talley NJ et al. Is there an association between GNbeta3-C825T genotype and lower functional gastrointestinal disorders? Gastroenterology 2006; 130(7):1985-94.

36 Gelernter J, Cubells JF, Kidd JR, Pakstis AJ, Kidd KK. Population studies of polymorphisms of the serotonin transporter protein gene. Am J Med Genet 1999; 88(1):61-6.

37 Melke J, Westberg L, Nilsson S, Landen M, Soderstrom H, Baghaei F et al. A polymorphism in the serotonin receptor 3A (HTR3A) gene and its association with harm avoidance in women. Arch Gen Psychiatry 2003; 60(10):1017-23.

38 Feinle C, Meier O, Otto B, D'Amato M, Fried M. Role of duodenal lipid and cholecystokinin A receptors in the pathophysiology of functional dyspepsia. Gut 2001; 48(3):347-355.

39 Chua AS, Keeling PW. Cholecystokinin hyperresponsiveness in functional dyspepsia. World J Gastroenterol 2006; 7;12(17):2688-93.

40 Agreus L, Svardsudd K, Nyren O, Tibblin G. Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. Gastroenterology 1995; 109(3):671-680.

41 Talley NJ, Dennis EH, Schettler-Duncan VA, Lacy BE, Olden KW, Crowell MD. Overlapping upper and lower gastrointestinal symptoms in irritable bowel syndrome patients with constipation or diarrhea. Am J Gastroenterol 2003; 98(11):2454-2459.

42 Quigley EM. Functional dyspepsia (FD) and non-erosive reflux disease (NERD): overlapping or discrete entities? Best Pract Res Clin Gastroenterol 2004; 18(4):695-706.

43 De Vries D.R., Ter Linde J, Van Herwaarden M.A., Smout A.J.P.M., Samsom M. G protein B3 subunit 825 T allele is associated with disease susceptibility and visceral hypersensitivity in GERD. Neurogastroenterol Motil 2006; 18:A157.

Chapter 09

Summary

Nederlandse samenvatting

Dankwoord

Curriculum Vitae

Summary

In the introduction (**chapter 1),** the gastric motor and sensory abnormalities identified in patients with functional dyspepsia are described. Next, the current knowledge about the relationship between these abnormalities and upper abdominal symptoms is reviewed. Furthermore, the action of the mucosal serotonergic signalling system is explained, and its proposed role in disturbed gastroduodenal function and upper abdominal symptom generation is laid out. Finally, the aims of this thesis are presented, which have been laid out in the following chapters.

In **chapter 2**, the change in proximal and distal gastric volumes after ingestion of a nutrient drink, analyzed by three-dimensional ultrasonography (3D-US), and its relationship with postprandial upper abdominal sensations is described. At present, the barostat technique is the gold standard for estimation of meal induced relaxation of the proximal stomach. An important disadvantage of this technique is the presence of a balloon in the stomach, which influences the physiologic response of the stomach after meal ingestion. 3D-US is a non-invasive and patient friendly alternative. This technique has shown excellent in vitro and in vivo accuracy in volume estimation of the stomach and a low inter observer variation. We analyzed patients with functional dyspepsia (FD), patients with gastroesophageal reflux disease (GERD), and healthy controls. FD patients and GERD patients show remarkable differences in partial gastric volumes compared to healthy controls; FD patients exhibit a smaller proximal gastric volume, whereas GERD patients have a larger proximal gastric volume compared to controls after ingestion of a nutrient drink. Interestingly, FD patients with impaired proximal relaxation show a larger distal gastric volume and score higher fullness sensations compared to FD patients with normal proximal gastric relaxation. A strong positive relationship between the increase in distal gastric volume and fullness was found in healthy controls and in GERD patients, whereas no such relationship was observed between proximal gastric volume and fullness. This finding indicates that the distal stomach is important in the regulation of the sensation fullness under physiological conditions. Interestingly, in FD patients, the relationship between distal gastric volume and fullness was lacking. In our opinion, these observations somewhat downplay the role of impaired proximal gastric relaxation (and

an overextended gastric antrum) in the symptom generation in functional dyspepsia, implying that other causes than altered meal distribution are involved in the excessive generation of fullness, such as increased visceral sensitivity.

In **chapter 3**, the relationship between gastric function and chronic upper abdominal sensations is investigated. Secondly, we evaluated the relationship between the main outcome parameters of the gastric function tests. Gastric function was assessed by the ^{13}C octanoic gastric emptying test, three-dimensional ultrasonography (proximal and distal gastric volume), and the nutrient drinktest (maximum drinking capacity). Impaired proximal gastric relaxation (23%) and a delayed gastric emptying (33%) are highly prevalent in the studied group of FD patients, however only a small overlap exists between these two pathophysiologic mechanisms (5%). No relationship was found between chronic upper abdominal symptoms and gastric function (proximal gastric relaxation, gastric emptying rate, or drinking capacity). Finally, the maximum drinking capacity of FD patients, seen at a tertiary referral center is not influenced by gastric emptying rate or proximal gastric relaxation. The lack of relationship between chronic upper abdominal sensations and gastric function questions the role of these pathophysiologic mechanisms in the generation of symptoms, and limited effect on symptoms may therefore be expected when targeting these specific mechanisms.

Functional dyspeptic patients complain about a variety of symptoms, which are mostly related to food intake. In order to reduce postprandial symptoms, many patients reduce the energy content of a single meal, however the rational of this is not fully understood and not evidence based. Therefore, we evaluated the influence of the energy content of a nutrient drink on postprandial abdominal sensations and total and partial gastric volumes in patients with FD (**chapter 4**). Proximal and distal gastric volumes were significantly larger after the high-energy nutrient drink compared to the low energy nutrient drink. However, postprandially, no differences in fullness, nausea, and upper abdominal pain were observed between the high-energy and low-energy nutrient drink. Furthermore, no relationship between

distal gastric volume and fullness was found, either after the low-energy and the high-energy drink. Therefore, although the energy content of a nutrient drink influenced gastric motility, no effect on visceral perception was present. Complementary to chapter 2, we showed that the relationship between distal gastric volume and fullness was not restored after ingestion of a low-energy drink, suggesting that the disturbance between the perception of abdominal sensations and distal gastric volume in FD patients persists, irrespective of the energy content of a meal.

5-HT_4 agonists are known for their prokinetic properties, whereas the effect of 5-HT_4 antagonists on upper gastrointestinal functions is largely unknown. **Chapter 5** describes the first study in which the effect of a 5-HT_4 receptor antagonist (R216073) on gastric relaxation and visceral sensitivity is evaluated in patients with functional dyspepsia. A double blind, randomised, placebo controlled, 2-period crossover study was performed in 20 FD patients. The effect of a single dose of 2000 mg R216073 was tested using three-dimensional ultrasonography and a nutrient drinktest. We observed that the 5-HT_4 receptor antagonist had no effect on proximal or distal gastric relaxation, maximum drinking capacity, or postprandial upper abdominal symptoms in patients with functional dyspepsia. Although high plasma concentrations of R216073 were reached, the current study suggests that R216073 has no therapeutic potential in FD patients.

Studies using agents intervening in serotonergic signalling, such as serotonin receptor agonists and antagonists, selective serotonin reuptake inhibitors (SSRIs), and tryptophan depletion, demonstrate that serotonergic signalling is involved in the process of regulating the orderly and controlled delivery of nutrients to the small intestine. In **chapter 6,** we aimed to increase the understanding of the role of serotonergic signalling in normal gastroduodenal function at a molecular level. For this purpose, mucosal biopsy specimens were collected from the fundus, antrum, and duodenum of 11 healthy subjects. Serotonin positive cells were counted and the mRNA levels of tryptophan hydroxylase (TPH)-1, serotonin transporter (SERT), 5-HT_4 receptor, and 5-HT_3 receptor subunits were quantified by real time RT-PCR. This study shows large regional differences in mRNA expression levels of the various proteins involved in serotonergic signalling. SERT mRNA expression level was found to be highest in the duodenum, lower in the

antrum, and lowest in the fundus. The expression of the 5-HT_3 receptor subunits and the 5-HT_4 receptor was higher in the duodenum compared to both regions of the stomach. TPH-1 (5-HT synthesis) and SERT (5-HT uptake) mRNA expression do not correlate. Considering the larger number of 5-HT positive cells, the higher level of SERT expression, and the much higher expression of the 5-HT_4 receptor and the 5-HT_3 receptor subunits in the duodenum, serotonergic signalling and activity at the mucosal level is likely to be highest in the duodenum, followed by the antrum and fundus. These findings can be of importance when developing specific serotonergic agents for enhanced fundic relaxation or gastric emptying.

In **chapter 7** we aimed to determine whether serotonergic signalling is altered in patients with a delayed gastric emptying. In order to study this, we collected mucosal biopsy specimens from the duodenum, antrum, and fundus of patients with idiopathic gastroparesis and healthy controls. Neuroendocrine cells, specifically 5-HT producing cells, were counted after using immunohistochemistry, and mRNA expression levels of TPH-1, SERT, 5-HT_3 and 5-HT_4 receptor were quantified by real time RT-PCR. The main findings of the current study are that the total number of neuroendocrine cells, specifically the 5-HT producing cells, and the mRNA expression levels of TPH-1 (the rate-limiting enzyme of 5-HT synthesis) and SERT (responsible for the high affinity uptake of 5-HT, thereby controlling the level of 5-HT available for receptor stimulation) in mucosal biopsy specimens of fundus, antrum, and duodenum were comparable between patients with idiopathic gastroparesis and healthy controls. In addition we found that the 5-$HT_{4(c)}$ splice variant was expressed more abundantly in healthy controls compared to patients. This study indicates that the delayed gastric emptying and upper abdominal symptoms in idiopathic gastroparesis do not result from altered mucosal 5-HT biosynthetic and uptake capacity. To value the pathogenic role of reduced 5-$HT_{4(c)}$ splice variant expression in patients with idiopathic gastroparesis, its effect on 5-HT_4 receptor function and 5-HT release needs to be examined in future studies.

Genetic variants may affect the response to the release of neurotransmitters, such as serotonin. Therefore, we evaluated the association of functional polymorphisms in several genes involved in serotonergic signalling and G-protein mediated signal transduction with FD (**chapter 8**). 112 FD

patients, send to our tertiary referral center, were studied. Healthy controls (n = 336) were matched 1:3 for age and gender. Polymorphisms in genes encoding the serotonin receptor type three A subunit (HTR3A), the serotonin transporter (SERT), and the G protein β3 subunit (GNB3) were analyzed. Tertiary referral FD patients have a higher prevalence of the T allele of the GNB3 C825T polymorphism compared to healthy controls. No association between functional dyspepsia and the genotype of the insertion/deletion polymorphism in the promoter of SERT (SERT-P) or HTR3A C178T polymorphism was observed. The increased signal transduction, associated with the T allele of the GNB3 C825T polymorphism might contribute to the manifestation of FD.

The aims of this thesis, formulated in chapter 1, should be addressed as follows:

1 Although impaired proximal gastric relaxation and delayed gastric emptying are highly prevalent in this patient group, we were not able to establish a relationship between gastric (dys)function and upper abdominal sensations experienced in daily life. The results of this thesis question the role of these pathophysiologic mechanisms in the generation of dyspeptic symptoms. Therefore, we have concluded that gastric function does not serve as a clear marker for the symptoms experienced by FD patients in daily life, and limited effect on symptoms may be expected when targeting these specific mechanisms.

2 Several components of mucosal serotonergic signalling are higher in the duodenum compared to the stomach. Therefore we conclude that serotonin, released by EC cells, predominantly contributes to gastroduodenal function at the level of the duodenum. Examination of serotonergic signalling components in patients with idiopathic gastroparesis revealed that delayed gastric emptying and upper abdominal symptoms do not result from altered mucosal 5-HT biosynthetic and uptake capacity. Decreased expression of the $5\text{-HT}_{4(c)}$ splice variant in the duodenum of FD patients may exert a modest effect on 5-HT availability, and be of importance in the pathogenesis of gastroparesis. No association between functional dyspepsia and the genotype of the insertion/deletion polymorphism in the promoter of SERT (SERT-P) or HTR3A C178T polymorphism was observed. Interestingly, tertiary referral FD patients have a higher prevalence of the T allele of the GNB3 C825T polymorphism compared to healthy controls. Therefore, a second messenger abnormality may be one of the molecular factors underlying the gastric motor and sensory dysfunction and upper abdominal symptoms observed in FD.

Nederlandse samenvatting

In de introductie **(hoofdstuk 1)** worden de gestoorde motorische en sensorische mechanismen van de maag in patiënten met functionele dyspepsie beschreven. Er wordt een overzicht gegeven van de laatste inzichten over de relatie tussen deze pathofysiologische mechanismen en bovenbuik klachten. De signaal transductie van serotonine in het enterische zenuwstelsel wordt beschreven, samen met de veronderstelde rol van serotonine in een gestoorde gastroduodenale functie en de generatie van bovenbuik klachten. Tenslotte worden de doelstellingen van dit proefschrift gepresenteerd.

In **hoofdstuk 2** worden de veranderingen van het proximale en het distale maagvolume en de relatie met het ontstaan van bovenbuik klachten na inname van een vloeibare maaltijd beschreven. Het maagvolume wordt bepaald met 3D echografie. Momenteel is de barostat techniek de gouden standaard voor het bepalen van de relaxatie van de maag na inname van een maaltijd. De aanwezigheid van een ballon in de proximale maag kan echter de fysiologie van de maag na inname van de maaltijd beïnvloeden, wat een belangrijk nadeel is van deze techniek. 3D echografie is een non-invasief en patiënt vriendelijk alternatief. In vitro en in vivo studies hebben een goede nauwkeurigheid van de metingen van het maagvolume aangetoond met een lage inter-observer variatie. In deze studie zijn drie groepen geanalyseerd: patiënten met functionele dyspepsie (FD), patiënten met gastro-oesofageale reflux (GERD), en gezonde vrijwilligers. FD patiënten en GERD patiënten hebben opmerkelijk verschillen in partiele volumina vergeleken met gezonde vrijwilligers; na inname van de vloeibare maaltijd hebben FD patiënten een kleiner proximaal maagvolume, terwijl GERD patiënten juist een groter proximaal maagvolume hebben vergeleken met gezonde vrijwilligers. FD patiënten met een afgenomen proximale relaxatie hebben een groter distaal maagvolume en geven meer klachten van een vol gevoel aan vergeleken met FD patiënten met een normale proximale relaxatie. Er bestaat een sterke positieve relatie tussen het distale maagvolume en de sensatie van een vol gevoel in gezonde vrijwilligers en GERD patiënten. Deze relatie wordt niet aangetoond tussen het proximale maagvolume en een vol gevoel. Dit geeft aan dat de distale maag voor een belangrijk deel verantwoordelijk is voor de sensatie van een vol gevoel in een fysiologische situatie. Opmerkelijk is dat deze relatie tussen het distale volume en een vol

gevoel niet aangetoond wordt bij patiënten met functionele dyspepsie. Deze gegevens geven aan dat de rol van een gestoorde proximale relaxatie (en een uitgerekt antrum) als oorzaak van het ontstaan van bovenbuik klachten in FD wellicht overschat is. Waarschijnlijk zijn er andere factoren die een belangrijke rol spelen zoals een verhoogde viscerale sensibiliteit.

In **hoofdstuk 3** wordt de relatie tussen de functie van de maag en de aanwezigheid van chronische bovenbuik klachten onderzocht. Vervolgens is de mogelijke relatie tussen de uitkomsten van de maagfunctie testen bestudeerd. De functie van de maag werd geanalyseerd met gebruik van de ^{13}C octaanzuur ademtest (maagledigingstest), 3D echografie (proximaal en distaal maagvolume), en een drinktest (maximale drink capaciteit). Een afgenomen proximale relaxatie (23%) en een vertraagde maaglediging (33%) komen frequent voor in de bestudeerde groep FD patiënten, echter er bestaat slechts een kleine overlap tussen deze twee pathofysiologische mechanismen (5%). Er werd geen relatie aangetoond tussen chronische bovenbuik klachten en de functie van de maag (proximale relaxatie van de maag, snelheid van de maaglediging, of de maximale drinkcapaciteit). Ten slotte werd de maximale drink capaciteit van deze groep FD patiënten (tertiair centrum) niet beïnvloed door de snelheid van de maaglediging of de proximale relaxatie van de maag. Aangezien er geen duidelijke relatie bestaat tussen chronische bovenbuik klachten en de functie van de maag, is de rol van deze pathofysiologische mechanismen van de maag in het ontstaan van klachten twijfelachtig. Om deze reden valt slechts een beperkt effect te verwachten van een behandeling gericht op deze pathofysiologische mechanismen in het onderdrukken van klachten

Patiënten met functionele dyspepsie ervaren een verscheidenheid aan symptomen, welke in de meeste gevallen gerelateerd zijn aan de maaltijd. Om de postprandiale klachten te reduceren, gebruiken veel patiënten calorie arme maaltijden. Echter de reden waarom het eten van (kleine) calorie arme maaltijden de postprandiale klachten verlicht is niet duidelijk en is niet wetenschappelijke onderzocht. In deze studie hebben we het effect van de calorische inhoud van een maaltijd op postprandiale symptomen en totale en partiele maagvolumina geanalyseerd in patiënten met FD (**hoofdstuk 4**).

Het proximale en het distale maagvolume waren groter na inname van de calorie rijke drank vergeleken met de calorie arme drank. Echter, er werd geen verschil gemeten in de klachten die patiënten aangaven na inname van een calorie rijke of een calorie arme drank (vol gevoel, misselijkheid, of pijn in de bovenbuik). Zowel na de calorierijke als de caloriearme drank werd geen relatie tussen het distale maagvolume en een vol gevoel aangetoond. Ondanks dat de calorische samenstelling van invloed is op de motiliteit, werd er geen verschil in viscerale perceptie aangetoond. Als aanvulling op hoofdstuk 2 hebben wij laten zien dat de relatie tussen het distale maagvolume en een vol gevoel niet hersteld wordt bij inname van een laag calorische maaltijd. De gestoorde relatie die is aangetoond tussen de perceptie van sensaties na inname van de maaltijd en het distale maagvolume blijft bestaan, onafhankelijk van de calorische inhoud van de maaltijd.

In tegenstelling tot het prokinetische effect van 5-HT_4 receptor agonisten, is het effect van een 5-HT_4 receptor antagonist onbekend. In **hoofdstuk 5** wordt de eerste studie beschreven waarbij het effect van een 5-HT_4 antagonist (R216073) op maagrelaxatie en viscerale sensitiviteit wordt bestudeerd in patiënten met functionele dyspepsie. Een dubbel blind, gerandomiseerde, placebo gecontroleerde, cross-over studie werd uitgevoerd in 20 FD patiënten. Het effect van een enkele dosis van 2000 mg R216073 werd getest met behulp van 3D echografie en een drinktest. De 5-HT_4 receptor antagonist had geen effect op het proximale of het distale maagvolume, de maximale drinkcapaciteit, of de postprandiale klachten in patiënten met functionele dyspepsie. Ondanks dat er hoge plasma spiegels bereikt werden, geven de resultaten van deze studie aan dat R216073 geen therapeutische waarde heeft in FD patiënten.

Uit interventie studies waarbij serotonerge medicatie zoals serotonine receptor agonisten en antagonisten, serotonine heropname remmers (SSRI's), en tryptophan depletie is gebruikt, blijkt dat de signaal transductie van serotonine een rol speelt bij het voedsel transport van de maag naar de dunne darm. Het doel van de studie die wordt beschreven in **hoofdstuk 6** is om de rol van serotonine in de normale gastroduodenale functie te verduidelijken op een moleculair niveau. Hiervoor werden mucosale biopten genomen van het duodenum, het antrum, en de fundus van 11 gezonde

vrijwilligers. Cellen die positief aankleurde na serotonine kleuring werden geteld. De mRNA expressie van tryptophan hydroxylase (TPH)-1, het serotonine transport eiwit (SERT), de 5-HT_4 receptor, en de 5-HT_3 receptor subunits werden gekwantificeerd met behulp van real time RT-PCR. Uit deze studie blijken grote verschillen te bestaan in de mRNA expressie van de verscheidene eiwitten die betrokken zijn bij signaal transductie van serotonine tussen de regio's. De mRNA expressie van SERT was het hoogste in het duodenum, gevolgd door het antrum en de fundus. De expressie van de 5-HT_3 receptor subunits en de 5-HT_4 receptor was hoger in het duodenum vergeleken met beide regio's van de maag. De mRNA expressie van TPH-1 (5-HT synthese) and SERT (5-HT opname) correleren niet. Gezien het grote aantal 5-HT positieve cellen, het hogere niveau van mRNA expressie van SERT, de hogere expressie van de 5-HT_4 receptor en de 5-HT_3 receptor subunits in het duodenum, kunnen we stellen dat de signaal transductie en de activiteit van serotonine op mucosaal niveau het hoogste is in het duodenum, gevolgd door het antrum en de fundus. Deze bevindingen kunnen van belang zijn bij het ontwikkelen van specifieke serotonerge medicatie om relaxatie van de fundus van de maag of de snelheid van de maaglediging te beïnvloeden.

Het doel van **hoofdstuk 7** was om te bepalen of de signaal transductie van serotonine verstoord is in patiënten met een vertraagde maaglediging. Hiervoor werden mucosale biopten genomen van het duodenum, het antrum, en de fundus van patiënten met idiopathische gastroparese en gezonde vrijwilligers. De neuroendocriene cellen, en in het bijzonder de 5-HT producerende cellen werden gekwantificeerd na immunohistochemie. Vervolgens werd het mRNA expressie niveau van TPH-1, SERT, de 5-HT_3 receptor en de 5-HT_4 receptor bepaald met RT-PCR. De belangrijkste bevindingen van deze studie waren dat het totaal aan neuroendocriene cellen, met in het bijzonder 5-HT producerende cellen, en het mRNA expressie niveau van TPH-1 (het enzym van de snelheidsbepalende stap in de synthese van serotonine) en SERT (verantwoordelijk voor de opname van serotonine en de beschikbaarheid van serotonine voor stimulatie van serotonine receptoren) in mucosale biopten van het duodenum, het antrum, en de fundus vergelijkbaar was tussen patiënten met idiopathische gastroparese en gezonde controles. De mRNA expressie van de 5-$HT_{4(c)}$ splice variant was hoger in gezonde controles vergeleken met patiënten. Uit deze studie blijkt dat een vertraagde maaglediging en de aanwezigheid van bovenbuik klachten, zoals

gezien wordt in patiënten met idiopathische gastroparese niet het gevolg is van een veranderde mucosale synthese of opname capaciteit van 5-HT. Om de betekenis van een afgenomen mRNA expressie van de 5-$HT_{4(c)}$ splice variant in patiënten met idiopathische gastroparese te bepalen, zal er meer onderzoek gedaan moeten worden naar het effect hiervan op 5-HT receptor functie en 5-HT secretie.

Genetische variaties kunnen van invloed zijn op het effect van het vrijkomen van neurotransmitters zoals serotonine. Om die reden hebben wij de relatie bepaald tussen functionele polymorfismen in verscheidene genen betrokken bij de serotonerge signaal transductie en bij de G-protein gemedieerde signaal transductie en functionele dyspepsie (**hoofdstuk 8**). 112 FD patiënten, allen doorgestuurd naar ons tertiaire centrum, werden bestudeerd. Gezonde controles (n = 336) werden in de verhouding 1:3 gematched op leeftijd en geslacht. De polymorfismen in de genen die coderen voor het serotonine receptor type drie A subunit (HTR3A), het serotonine transport eiwit (SERT), en het G-protein β3 subunit (GNB3) werden bestudeerd. Tertiair doorverwezen FD patiënten hebben een hogere prevalentie van het T allel van het GNB3 C825T polymorfisme vergeleken met gezonde controles. Er werd geen relatie gevonden tussen functionele dyspepsie en het genotype van het insertie/deletie polymorfisme in de promotor regio van SERT (SERT-P) of het HTR3A C178T polymorfisme. De toegenomen signaal transductie, geassocieerd met het T allel van het GNB3 C825T polymorfisme, zou mogelijk kunnen bijdragen aan de manifestatie van FD.

De doelstellingen van dit proefschrift, welke geformuleerd werden in hoofdstuk 1, kunnen als volgt beantwoord worden:

1 Ondanks dat een afgenomen proximale relaxatie van de maag en een vertraagde maaglediging veel voorkomen in deze patiënten groep, hebben we geen relatie kunnen aantonen tussen de (abnormale) functie van de maag en het ontstaan van de bovenbuik klachten welke in het dagelijkse leven worden ervaren. De resultaten van dit proefschrift zetten vraagtekens bij de veronderstelde rol van deze pathofysiologische mechanismen als veroorzaker van dyspeptische symptomen. Om die reden hebben wij geconcludeerd dat de functie van de maag geen duidelijke indicator is voor de klachten die FD patiënten in het dagelijkse leven ervaren, en dat het effect van medicamenteuze therapieën gericht op deze mechanismen teleurstellend zal zijn.

2 Verscheidene componenten van mucosale serotonerge signaal transductie zijn in grotere mate aanwezig in het duodenum vergeleken met de maag. Om die reden concluderen wij dat serotonine welke wordt uitgescheiden door enterochromaffine cellen (EC cellen) voornamelijk bijdraagt aan gastroduodenale functie op het niveau van het duodenum. Uit onderzoek naar onderdelen van serotonerge signaal transductie in patiënten met idiopathische gastroparese blijkt dat een vertraagde maaglediging en bovenbuik klachten niet het resultaat zijn van een veranderde mucosale 5-HT synthese of opname capaciteit. Een afgenomen expressie van de $5\text{-HT}_{4(c)}$ splice variant in het duodenum van FD patiënten zou de beschikbaarheid van 5-HT kunnen beïnvloeden, en van belang kunnen zijn in de pathogenese van gastroparese. Er werd geen associatie tussen functionele dyspepsie en het genotype van het insertie/deletie polymorfisme in the promotor van SERT (SERT-P) of het HTR3A C178T polymorfisme aangetoond. Tertiair doorverwezen FD patiënten hebben een hogere prevalentie van het T allel van het GNB3 C825T polymorfisme vergeleken met gezonde vrijwilligers. Dit zou kunnen betekenen dat een abnormaliteit in het "second messaging" systeem een van de onderliggende moleculaire factoren is die bijdraagt aan de gestoorde motorische en sensibele functie en aan het ontstaan van bovenbuik klachten in functionele dyspepsie.

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Curriculum vitae

Niels van Lelyveld werd geboren op 20 juli 1976 in Groningen. Na het behalen van het VWO diploma heeft hij een jaar door Australië gereisd, alvorens hij begon met de studie geneeskunde aan de Universiteit van Utrecht. Tijdens de studie heeft hij voor een periode van drie maanden meegewerkt aan een ontwikkelingsproject in de sloppenwijken van Brazilië. Na het behalen van het doctoraal geneeskunde deed hij voor een periode van vier maanden onderzoek naar groeifactoren bij patiënten met het Sotos syndroom aan de universiteit van Portland (Oregon Health Science University) onder leiding van Prof. dr. Wit (LUMC) en Prof. dr. Rosenveld (OHSU). In maart 2003 deed hij als laatste co-schap onderzoek op de afdeling gastroenterologie. Na het afronden van de studie in juli 2003 werd hij aangesteld als arts onderzoeker in het UMC Utrecht onder begeleiding van Prof. dr. M. Samsom en Dr. J. ter linde. Op 1 juli 2006 is hij gestart met de vooropleiding interne geneeskunde in het Meander Medisch Centrum (opleider: Dr. C.A.J.M. Gaillard). Vanaf 2010 zal hij zijn opleiding tot gastroenteroloog voortzetten in het UMC Utrecht (opleider Prof. Dr. M. Samsom).

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