

**Factors Involved in the Regulation of
Gastrointestinal Motility, Hormone Release,
Symptoms and Energy Intake in Health and
Patients with Functional Dyspepsia**

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LIST OF ABBREVIATIONS

1.33/50	1.33 kcal/min lipid infusion for 50 min
1.33/150	1.33 kcal/min lipid infusion for 150 min
4/50	4 kcal/min lipid infusion for 50 min
5HT	5-Hydroxy-tryptamine
APD	antropyloroduodenal
ALE	artichoke leaf extract
ANOVA	analysis of variance
AUC	area under the curve
BMI	body mass index
CCK	cholecystokinin
CHO	carbohydrate
CV	coefficient of variation
EAT	Eating Attitudes Test
EPQ	Eysenck Personality Questionnaire
FD	functional dyspepsia
G1	1 kcal/min glucose infusion
G2	2 kcal/min glucose infusion
G4	4 kcal/min glucose infusion
GIP	glucose-dependent insulintropic polypeptide
GIS	gastrointestinal symptom
GLP-1	glucagon-like peptide-1
HAD	Hospital Anxiety and Depression
<i>H pylori</i>	<i>Helicobacter pylori</i>

HS	healthy subject
IBS	irritable bowel syndrome
IPPW	isolated pyloric pressure wave
IL0.25	0.25 kcal/min lipid infusion
IL1.5	1 kcal/min lipid infusion
IL4	4 kcal/min lipid infusion
MI	motility index
MDP	minimal distending pressure
MMC	migrating motor complex
NDI	Nepean Dyspepsia Index
NS	not significant
NWLRC	Northwest Lipid Research Clinic
PWs	pressure waves
PWSs	pressure wave sequences
PYY	peptide tyrosine tyrosine
RMP	resting membrane potential
TFEQ	Three Factor Eating Questionnaire
THL	tetrahydrolipstatin
TMPD	transmucosal potential difference
VAS	visual analogue scale

THESIS SUMMARY

This thesis presents studies relating to effects of different macronutrients, predominantly fat and carbohydrate, on gastrointestinal motility, hormone release/suppression, appetite and energy intake in healthy subjects, and on symptom generation in patients with functional dyspepsia. The three broad areas that have been investigated in these studies are: (i) the effect of load, and duration, of small intestinal nutrient exposure on gastric motility, gastrointestinal hormone release/suppression, appetite and energy intake in healthy subjects, (ii) the dietary factors that may contribute to symptom generation in patients with functional dyspepsia, through analysis of diet diaries and acute nutrient challenges, and (iii) the effects of the herbal medication, Iberogast®, on gastric motility in healthy subjects.

The ingestion of nutrients, triggers a number of gastrointestinal responses, including the modulation of antropyloroduodenal motility, gastrointestinal hormone release/suppression, and the suppression of appetite and energy intake, resulting in a slowing of gastric emptying to an average rate of 1 - 3 kcal/min, which is required for efficient nutrient digestion and absorption. Additionally, the rate at which glucose enters the small intestine influences postprandial glycaemia and incretin responses. These responses have been demonstrated in animals to be dependent on the length, and region, of the small intestine exposed to fat and glucose, however, this has not been directly investigated in humans.

Functional dyspepsia is a clinical condition, characterised by chronic upper abdominal symptoms, such as nausea, bloating and early fullness, without a known cause, which

affects approximately 11 - 29 % of the population. Many studies have reported that disturbed gastric motor activity may be the cause of these symptoms, but patients frequently experience symptoms following ingestion of food, and some patients report to eat smaller meals more frequently and avoid fatty and spicy foods. In addition, laboratory-based studies have indicated that functional dyspepsia patients may be hypersensitive to fat, but not carbohydrate. To date, the treatments used to reduce symptoms are frequently directed at the normalisation of gastroduodenal motility, using prokinetics. However, the beneficial effect of these drugs is relatively small and variable, and their adverse effects can be substantial. Herbal drug preparations have recently received considerable interest as an alternative treatment option in functional dyspepsia. A commercially available herbal preparation, Iberogast® which contains nine plant extracts, has been reported to improve upper abdominal symptoms in functional dyspepsia and to decrease fundic tone, increase antral contractility and decrease afferent nerve sensitivity in experimental animals. The effects of Iberogast® in the human gastrointestinal tract have not been investigated.

The first three studies presented in this thesis have focused on the effects of delivering fat and glucose into the small intestine at different loads (Chapter 5, 6 and 7), lower, comparable to, and higher than gastric emptying normally occurs, and at different durations of infusion (but still at similar caloric loads - Chapter 5, fat only), on gastrointestinal motility, plasma hormone release/suppression, glycaemia, and energy intake in healthy male subjects.

The study in Chapter 5 demonstrated that antral pressure waves and pressure wave sequences were suppressed, and basal pyloric pressure, isolated pyloric pressure waves,

and plasma cholecystokinin and peptide YY stimulated, during both the low (1.33 kcal/min for 50 min: 67 kcal/min), and high (4 kcal/min for 50 min: 200 kcal), loads of lipid. The effect of the 4 kcal/min load was sustained so that the suppression of antral pressure waves and pressure wave sequences and increase in peptide YY remained evident after cessation of the infusion. The prolonged lipid infusion (1.33 kcal/min for 150 min: 200 kcal) suppressed antral pressure waves, stimulated cholecystokinin and peptide YY and basal pyloric pressure and tended to stimulate isolated pyloric pressure waves when compared with saline throughout the entire infusion period. These results indicate that both the load, and duration, of small intestinal lipid have an influence on antropyloroduodenal motility and patterns of cholecystokinin and peptide YY release.

Chapter 6 demonstrated that lipid loads lower than gastric emptying normally occurs (0.25 kcal/min for 50 min: 12.5 kcal) transiently stimulated isolated pyloric pressure waves and cholecystokinin release and suppressed pressure wave sequences and hunger scores. Loads comparable to (1.5 kcal/min for 50 min: 75 kcal) and higher (4 kcal/min for 50 min: 200 kcal), than the normal rate of gastric emptying, were required to stimulate basal pyloric tone and peptide YY release and suppress antral and duodenal pressure waves. Only the 4 kcal/min load suppressed energy intake. The effects of lipid on all parameters, with the exception of hunger, were load-dependent. In addition, there were relationships between antropyloroduodenal motility and cholecystokinin and peptide YY concentrations with energy/food intake.

The study in Chapter 7 demonstrated that loads of glucose lower than (1 kcal/min for 120 min: 120 kcal), comparable to (2 kcal/min for 120 min: 240 kcal) and higher than (4 kcal/min for 120 min: 480 kcal) the rate gastric emptying normally occurs, stimulated

blood glucose, plasma insulin, glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide and cholecystokinin concentrations and suppressed the number of antral pressure waves, 2 and 4 kcal/min loads were required for the suppression of duodenal pressure waves and pressure wave sequences and the stimulation of basal pyloric pressure and suppression of energy intake only after the 4 kcal/min loads. There were also relationships between glucagon-like peptide-1 and glucose-dependent insulinotropic peptide with basal pyloric tone, and food/energy intake with pyloric pressures.

The studies presented in the subsequent three chapters investigated the contribution of dietary factors on the generation of symptoms in patients with functional dyspepsia when compared with healthy subjects (Chapter 8 and 9) and the effect of Iberogast® on motility in the healthy gastrointestinal tract (Chapter 10). The effects of equi-caloric high-carbohydrate vs. high-fat yoghurt preloads on symptom generation, plasma hormone concentrations, antral area and energy intake were compared between functional dyspepsia patients and healthy subjects (Chapter 8). Nausea and pain were greater in patients after the high-fat, when compared with high-carbohydrate and control, preloads and with healthy subjects. Discomfort was greater after all preloads in patients when compared with healthy subjects. Fasting cholecystokinin and stimulation of cholecystokinin by the high-fat preload were greater in patients, while fasting and postprandial peptide YY were lower in patients than in healthy subjects, with no differences in fasting, or postprandial, plasma ghrelin between patients and healthy subjects. Fasting antral area was greater in patients, with no differences postprandially between patients and healthy subjects. There were no differences in energy intake between the two groups. The relationship between the effect of dietary intake and

eating behaviour over a 7-day period on the occurrence and severity of abdominal symptoms was compared between patients and healthy subjects (Chapter 9). The symptoms experienced by the patients included nausea, fullness discomfort, bloating and upper abdominal, and epigastric, pain, of a modest severity, which occurred within 30 min of eating. The number of “meals” ingested was significantly less in functional dyspepsia patients and there was a trend for total energy and fat intake to be less. The occurrence of these symptoms was also statistically related to the ingestion of fat and energy intake. The results of these studies indicate that diet, particularly the ingestion of fat, influences the development of symptoms in a subgroup of patients with functional dyspepsia.

The study in Chapter 10 evaluated the effect of the herbal drug Iberogast® on gastric motility in the gastrointestinal tract. Iberogast® increased proximal gastric volume, increased antral pressure waves without affecting pyloric or duodenal pressures, and slightly increased the retention of liquid in the total stomach, but had no effect on gastric emptying of solids or intragastric distribution. These results demonstrate that Iberogast® affects gastric motility in humans, and the stimulation of gastric relaxation and antral motility may contribute to the reported therapeutic efficacy of Iberogast® in functional dyspepsia.

The studies reported in this thesis provide new information about the regulation of gastric motility, hormone release/suppression, appetite and energy intake, by varying the loads of lipid and glucose infused into the small intestine in healthy subjects, which may have implications in patients with altered gastric motor functions, such as obese, type-2 diabetes and functional dyspepsia patients. In addition, studies in functional

dyspepsia patients revealed that diet, in particular the ingestion of fat, contribute to the cause of their symptoms, and these findings may have important implications for the development of diet-based therapies for the treatment of functional dyspepsia. Furthermore, functional dyspepsia patients with impaired gastric relaxation and antral dysmotility may benefit from the effects of Iberogast® as demonstrated in the healthy gastrointestinal tract.

DECLARATION OF ORIGINALITY

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being made available in the University Library.

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Amelia Pilichiewicz

January 2008

DEDICATION

To all my rocks...

You know who you are...

I am forever grateful...

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The studies reported in this thesis were conducted in the Discipline of Medicine and the Department of Nuclear Medicine, PET and Bone Densitometry at the Royal Adelaide Hospital. While conducting the research reported in this thesis I was supported by a Royal Adelaide Hospital Dawes Postgraduate Scholarship.

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PUBLICATIONS ARISING FROM THIS THESIS

The data presented in this thesis has formed the basis for the publications listed below:

Pilichiewicz, AN, Little, TJ, Brennan, IM, Meyer, JH, Wishart, JM, Otto, B, Horowitz, M, Feinle-Bisset, C 2005, 'Effects of load, and duration, of duodenal lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men', *Am J Physiol*, vol. 290, no. 3, pp. R668-677.

Pilichiewicz, AN, Papadopoulos, P, Brennan, IM, Little, TJ, Meyer, JH, Wishart, JM, Horowitz, M, Feinle-Bisset, C 2007, 'Load-dependent effects of duodenal lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men', *Am J Physiol*, vol. 293, no. 6, pp. R2170-2178.

Pilichiewicz, AN, Chaikomin, C, Brennan, IM, Wishart, JM, Rayner, CK, Jones, KL, Smout, AJPM, Horowitz, H, Feinle-Bisset, C 2007 'Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men', *Am J Physiol*, vol. 293, no. 3, pp. E743-753.

Pilichiewicz, AN, Feltrin, KL, Horowitz, M, Holtmann, G, Wishart, JM, Jones, KL, Talley, NJ, Feinle-Bisset, C 2007, 'In functional dyspepsia oral carbohydrate and fat differentially modulate symptoms, gut hormones and antral area', *Am J Gastroenterol* (submitted).

Pilichiewicz, AN, Horowitz, M, Russo, A, Maddox, AF, Jones, KL, Schemann, M, Holtmann, M, Feinle-Bisset, C 2007, 'Effects of Iberogast on proximal gastric volume, antropyloroduodenal motility and gastric emptying in healthy men', *Am J Gastroenterol*, vol. 102, no. 6, pp. 1276-1283.

Chapter 1

EFFECTS OF NUTRIENTS ON THE GASTROINTESTINAL TRACT, GASTROINTESTINAL HORMONE RELEASE AND ENERGY INTAKE

1.1 INTRODUCTION

It is well established that the presence of nutrients, such as fat and carbohydrate, in the small intestine is associated with a number of gastrointestinal responses, which have been proposed to mediate, at least in part, the associated inhibition of appetite and energy intake. These inter-related responses include the modulation of gastric distension, gastric emptying, gastrointestinal motility, the stimulation of a number of gastrointestinal hormones, such as cholecystokinin (CCK), peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP), and the suppression of ghrelin. The effects of small intestinal feedback on gastric emptying, gastrointestinal motility, gastrointestinal hormone release/suppression, appetite and energy intake are dependent on a number of factors, including the length and region of the small intestine exposed to nutrients, as well as the nutrient load and the duration of nutrient exposure. This chapter provides an overview

of the factors involved in the regulation of these parameters by nutrients, which all impact on the research conducted for this thesis.

1.2 ANATOMY AND FUNCTION OF THE GASTROINTESTINAL TRACT

1.2.1 Stomach

The stomach is a J-shaped sac-like chamber, which lies between the oesophagus and the duodenum, the first part of the small intestine. The stomach can be divided anatomically into the fundus, corpus and antrum (**Figure 1.1**), and may be considered to have two functional regions; (i) the proximal compartment, which includes the fundus and proximal corpus and possesses a thin smooth muscle layer, and acts as a reservoir for ingested food, and (ii) the distal compartment, which includes the antrum and possesses of a thicker musculature than the proximal compartment, and is responsible for the mixing and grinding of solid food.

1.2.2 Pylorus

The pylorus is a short 2 cm region connecting the antrum to the duodenal bulb. The motility patterns which occur in the pylorus include changes in basal pyloric pressure (or tone) and the frequency and amplitude of phasic pyloric pressures, also known as isolated pyloric pressure waves (IPPWs). The primary function of the pylorus is that of a sieve, to regulate outflow of gastric contents, in response to small intestinal feedback (**Figure 1.1**).

1.2.3 Small intestine

The small intestine is a muscular tube, approximately 5 metres in length, which can be divided into three regions; the duodenum (~ 25 cm), the jejunum (~ 2 m long) and, most distally, the ileum (~ 3 m long). The small intestine is the primary organ for nutrient absorption, and triggers feedback signals, which control gastric motility, gastrointestinal hormone release, appetite and energy intake (**Figure 1.1**). A number of factors regulate the effects of small intestinal nutrients and these are discussed in Chapter 1.5.

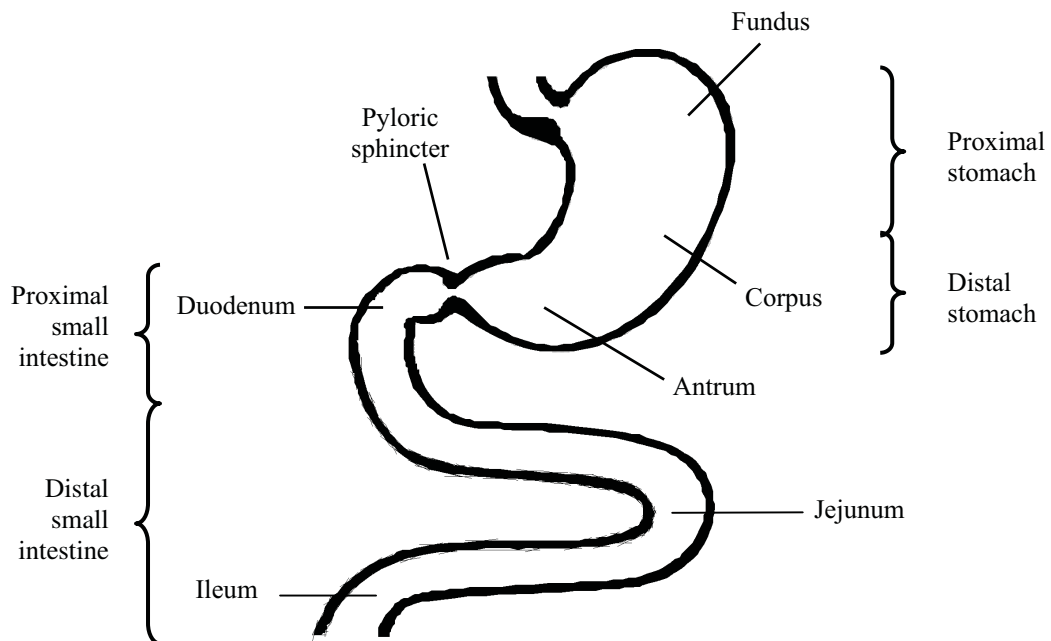


Figure 1.1 Basic anatomy of the stomach and small intestine.

1.3 GASTROINTESTINAL MOTOR FUNCTION AND ITS ROLE IN GASTRIC SENSATIONS, APPETITE AND ENERGY INTAKE

1.3.1 Fasting motor patterns

During fasting, the gastrointestinal tract exhibits cyclical pattern of motility, termed the migrating motor complex (MMC). The MMC comprises three phases; phase I, phase II and phase III (Carlson et al., 1972), which occur over a 90 - 100 min period in humans. Phase I is characterised by little, or no, contractile activity and usually has a duration of 40 - 60 min; phase II is characterised by intermittent contractions whose frequency increases progressively, and has a duration between 20 - 40 min; and phase III is a brief (5 - 10 min) period of contractions which occur at maximal frequency and amplitude, which is 3 in the stomach, and 12 in the duodenum, contractions per minute. The MMC propagates aborally along the small intestine, and serves to “sweep” the lumen of indigestible debris (Sarna and Otterson, 1988) (**Figure 1.2A**).

NOTE: This figure is included on page 5 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.2 Example of manometric recordings during infusion of saline, representing fasting (A) and infusion of glucose (B), in a healthy young subject. Saline infusion did not change antropyloroduodenal motility. During the glucose infusion, basal pyloric pressure and number of isolated pyloric pressure waves increased and antral motility was inhibited. A3 and A4 represent antral channels; S1 and S2 represent channels along the sleeve which straddles the pylorus; D1 and D2 represent duodenal channels. Adapted from (Verhagen et al., 1998).

1.3.2 Fed motor patterns

Following a meal, a complex and coordinated process of the proximal and distal regions of the stomach occurs: (i) ingested food is stored in the proximal stomach – which undergoes “adaptive relaxation” so the meal can be accommodated, without a substantial increase in intragastric pressure (Azpiroz and Malagelada, 1987), (ii) the bolus is mixed with gastric secretions and ground into small particles in the distal

stomach, or antrum, and (iii) “liquefied” gastric contents are delivered into the duodenum, at a rate that allows efficient digestion and absorption (Horowitz et al., 1994). This process is known as gastric emptying. Phasic and tonic pyloric contractions are considered to play a major role in the regulation of gastric emptying by acting as a brake; as emptying can only occur when the pylorus is open - in the fed state there are prolonged periods of closure to allow grinding of food in the antrum (Horowitz et al., 1994).

There are major differences in gastric emptying patterns of digestible and non-digestible solids and nutrient and non-nutrient liquids. Emptying of digestible solids is characterised by a lag phase, before emptying commences, followed by an emptying phase that approximates an overall linear pattern. The lag phase is dependent on the time taken for redistribution of food from the proximal to the distal stomach. Nutrient-containing liquids and liquefied solids empty from the stomach in an overall linear fashion. In contrast, non-nutrient emptying occurs relatively rapidly in a non-linear, mono-exponential fashion (Horowitz et al., 1994).

1.3.2.1 Effects of small intestinal nutrients on gastrointestinal motor function

During the digestion process, nutrients are broken down, absorbed and transported along the gut. These digestive products then interact with the receptors in the small intestine, and in turn, result in feedback inhibition of gastric emptying (Cooke, 1977, Heddle et al., 1989, Hunt, 1963, Hunt and Knox, 1968, Lin et al., 1989, Lin et al., 1990). The motor correlates of the slowing of gastric emptying triggered by the presence of nutrients in the small intestine include the interruption of the MMC (**Figure**

1.2B), relaxation of the proximal stomach (Azpiroz and Malagelada, 1985a), suppression of antral and duodenal motility (Hedde et al., 1988a) and, perhaps most importantly, the stimulation of phasic and tonic contractions localised to the pylorus (Hedde et al., 1988c, Tougas et al., 1992) (**Figure 1.3**). As a result of this feedback inhibition, gastric emptying of nutrients, in humans, is tightly regulated at 1 - 3 kcal/min (Hunt et al., 1985), after an initial phase that may be more rapid (Horowitz et al., 1993). These changes in gastrointestinal motility reflect the interaction of nutrients with the small intestine. The different macronutrients, fat, carbohydrate and protein, may vary in their effects on gastrointestinal motility. For example, long chain triglycerides have been reported to induce proximal gastric relaxation (Azpiroz and Malagelada, 1985a), slow gastric emptying (Kumar et al., 1987) and stimulate phasic and tonic motility (Cook et al., 1997, Kumar et al., 1987), to a greater extent than both carbohydrates and protein.

Small intestinal nutrient infusion of fat, carbohydrate and protein also affect the release of a number of gastrointestinal hormones, which mediate, at least in part, the effects of nutrients on gastrointestinal function, as discussed in Chapter 1.4.

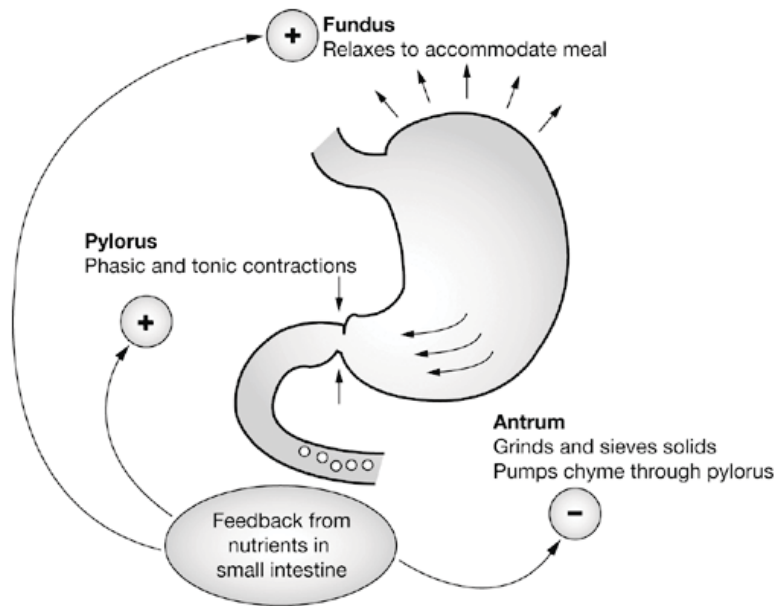


Figure 1.3 Motor patterns associated with normal gastric emptying. Adapted from (Rayner and Horowitz, 2005).

1.3.2.1.1 Effects of fat digestion on gastric emptying/motility

The effects of fat in the small intestine on gastrointestinal motility are dependent on the digestion of triglycerides into free fatty acids by the enzyme lipase. A number of studies have established the importance of fat digestion and the release of free fatty acids, by using a pharmacological agent which blocks fat digestion, tetrahydrolipstatin (THL), also known as orlistat (Borovicka et al., 2000, Feinle et al., 2001b, Feinle et al., 2003). For example, gastric emptying of a mixed, fat-containing, nutrient meal is accelerated by THL (Borovicka et al., 2000), and THL attenuates the effect of intraduodenal fat on proximal gastric relaxation (Feinle et al., 2001b) and antropyloroduodenal (APD) motility (Feinle et al., 2003) (**Figure 1.4**).

NOTE: This figure is included on page 9 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.4 Example of antro-pyloro-duodenal pressure patterns during duodenal infusion of a triglyceride emulsion without (FAT; left) and with (FAT-THL; right) 120 mg of the lipase inhibitor, THL. Left: Infusion of FAT resulted in a “fed” motor pattern, characterised by stimulation of isolated pyloric pressure waves (P) and the inhibition of antral (A) and duodenal (D) pressure waves. Right: In contrast, when THL was co-administered with fat (FAT-THL), there was pronounced propulsive antro-pyloro-duodenal pressure activity. Adapted from (Feinle et al., 2003).

1.3.2.2 *Effects of small intestinal nutrients on appetite and energy intake*

In humans, infusion of nutrients directly into the small intestine in lean and obese humans decreases perceptions of hunger, increases fullness and decreases subsequent energy intake (Chapman et al., 1999, Cook et al., 1997, Lavin et al., 1996, MacIntosh et al., 2001a). Intravenous administration of nutrients, on the other-hand, has little, if any, effect on energy intake (Lavin et al., 1996, Welch et al., 1985), indicating the importance of the interaction of nutrients with small intestinal receptors in the regulation of appetite and energy intake. Fat, carbohydrate and protein, all reduce energy intake when infused directly into the small intestine in humans and animals – fat may be the most potent in increasing fullness and decreasing hunger to a greater extent than isocaloric glucose and protein in humans and animals (Andrews et al., 1998, Burton-Freeman et al., 1997, Chapman et al., 1999, Cook et al., 1997). Small intestinal stimulation of the release, and suppression, of gastrointestinal hormones, mediates, at

least in part, the effects of nutrients on appetite and energy intake, as discussed in Chapter 1.4.

1.3.2.2.1 Effects of fat digestion on appetite and energy intake

Fat digestion is also important in the regulation of appetite and energy (Feltrin et al., 2004). This has again been established through investigations into the effects of inhibition of fat digestion using THL (Feinle et al., 2003, O'Donovan et al., 2003). For example, the inhibitory effect of an oral fat load (70 %) on energy intake is attenuated by THL in healthy lean subjects (O'Donovan et al., 2003) - the increase in energy intake approximates the amount of energy lost due to fat malabsorption. Furthermore, when THL is added to a fat emulsion and infused into the small intestine, energy intake and appetite are greater compared to when the emulsion is administered alone (Feinle et al., 2003). THL also reduces the intensity of fullness, nausea and bloating induced by concurrent gastric distension and duodenal lipid infusion (Feinle et al., 2001b) (**Figure 1.5**). Taken together, these results establish the important role of luminal free fatty acids in the regulation of gastrointestinal motility, appetite and energy intake.

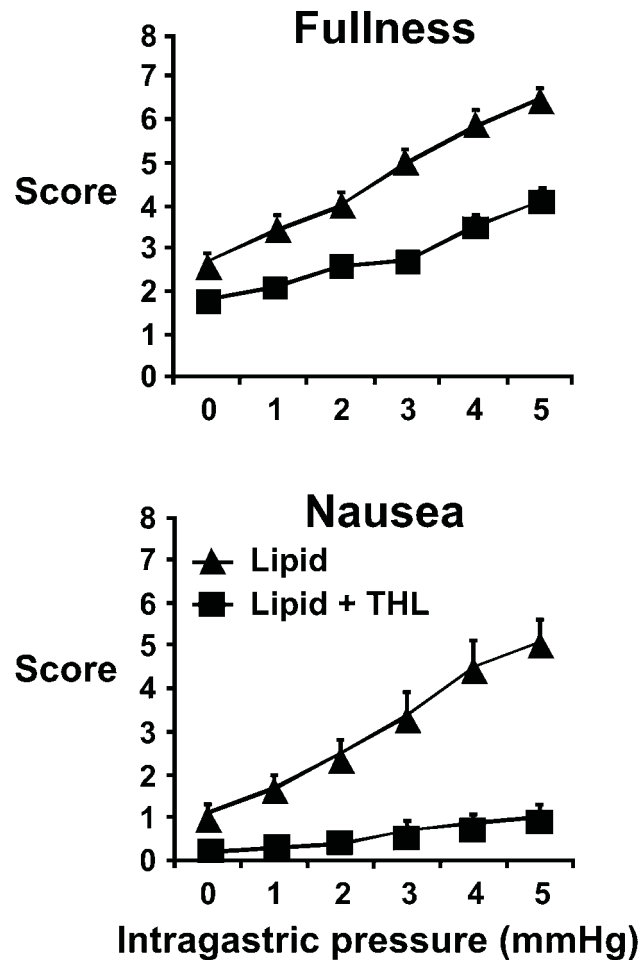


Figure 1.5 Effect of lipase inhibition, THL, on fullness (top) and nausea (bottom), induced by a duodenal lipid infusion during gastric distension in healthy subjects ($n = 14$). Inhibition of fat digestion, by the addition of THL, to the lipid emulsion attenuated fullness ($P < 0.05$) and nausea ($P < 0.05$), compared to the lipid emulsion alone. Adapted from (Feinle et al., 2001b).

1.3.2.3 Relationships between the effects of nutrients on appetite, and energy intake with gastric motor function

There is evidence that the effects of nutrients on appetite and energy intake may potentially result from the concomitant effects on gastric motor function. For example, human studies indicate that gastric distension with intragastric volumes > 400 ml contributes to the generation of postprandial sensations, such as an increase in fullness and a decrease in hunger, desire to eat and energy intake, in both healthy and obese subjects (Geliebter et al., 1988a, Geliebter, 1988b), as a result of the activation of

mechanoreceptors within the wall of the stomach (Feinle et al., 1996). During proximal gastric distension, the sensation of fullness is only induced when nutrients are concurrently infused into the small intestine; e.g. gastric distension alone is perceived as discomfort or pain (Feinle et al., 1997), indicating that feedback signals arising from the small intestine are important. Recent studies have also indicated a relationship of postprandial fullness (Hveem et al., 1996, Jones et al., 1997, Santangelo et al., 1998) and energy intake (Sturm et al., 2004) with the content of the distal stomach, in healthy subjects. For example, the perception of fullness induced by a 350 ml glucose drink is closely related to antral area, while there is no significant relationship between fullness and the content of either the total, or proximal stomach (Jones et al., 1997) (**Figure 1.6**).

NOTE: This figure is included on page 12 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.6 Relationship between antral area and postprandial fullness, after a glucose drink, in healthy subjects ($n = 14$). Fullness is related to antral area ($r = 0.68$, $P < 0.01$). Adapted from (Jones et al., 1997).

In addition, energy intake 60 min after ingestion of a yoghurt preload is inversely related to antral area, such that the larger the antral area the smaller the energy intake (Sturm et al., 2004) (**Figure 1.7**).

NOTE: This figure is included on page 13 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.7 Relationship between energy intake with antral area in healthy young ($n = 12$) and old ($n = 12$) subjects. Energy intake at a meal (kcal) is inversely related to antral area ($r = -0.58$, $P < 0.001$). Adapted from (Sturm et al., 2004).

No study has, to date, attempted to relate the effects of nutrients on appetite and energy intake with those on APD motility (Chapters 6 and 7).

1.4 GASTROINTESTINAL HORMONE RELEASE AND SUPPRESSION AND THEIR EFFECTS ON GASTROINTESTINAL MOTILITY AND ENERGY INTAKE

A number of gastrointestinal hormones, including cholecystokinin (CCK) and glucose-dependent insulintropic polypeptide (GIP), secreted from the proximal small intestine, peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1), secreted from the distal small intestine, and insulin secreted from the pancreas, are released in response to enteral nutrients, while the release of ghrelin, from the stomach, is suppressed. All these gastrointestinal hormones have been investigated for their potential role in the regulation of gastric and intestinal motility and appetite.

1.4.1 Cholecystokinin (CCK)

CCK is synthesised in the “I” cells of the duodenum and jejunal mucosa and released in response to the digestive products of fat, protein (Larsson and Rehfeld, 1978, Liddle et al., 1985, Lieverse et al., 1994a) and, to a lesser extent, carbohydrate (Parker et al., 2005). CCK is also present in enteric vagal afferent neurones (Moran et al., 1987), and some areas of the central nervous system, including the thalamus, hypothalamus, basal ganglia and dorsal hindbrain (Moran and Kinzig, 2004). CCK occurs in a number of forms, i.e. CCK-5, -8, -22, -33, -39, -54 and 58 (Rehfeld, 1981, Eberlein et al., 1992). CCK-8 is the most abundant form of CCK in the human brain, while in the human intestine and circulation CCK-58, CCK-33, CCK-22 and CCK-8 are all present in significant amounts (Eberlein et al., 1988, Rehfeld et al., 2001). Following its release, CCK elicits a number of biological effects on the gastrointestinal system, including the regulation of gut motility (Brennan et al., 2005, Fraser et al., 1993, Rayner et al., 2000a), contraction of the gallbladder (Liddle et al., 1985), pancreatic enzyme secretion (Harper and Raper, 1943), slowing of gastric emptying (Liddle et al., 1986), and suppression of energy intake (Kissileff et al., 1981).

1.4.1.1 Effects on gastric and intestinal motility

Exogenous administration of CCK slows gastric emptying in both animals (Moran and McHugh, 1988, McHugh and Moran, 1986) and humans (Liddle et al., 1986). Intravenous CCK-8 also reduces proximal gastric tone and increases proximal gastric compliance (Straathof et al., 1998), stimulates IPPWs and pyloric tone (Fraser et al., 1993, Brennan et al., 2005), and suppresses antral and duodenal motility (Fraser et al., 1993), in fasting healthy humans. The inhibitory effects of fat on gastric emptying

(Fried et al., 1984), proximal gastric compliance (Feinle et al., 1996), and gastric motility (Schwizer et al., 1997b), are attenuated by administration of the CCK1 receptor antagonist, loxiglumide, indicating that the inhibitory effects of fat on gastric and intestinal motility are mediated, at least in part, by CCK.

1.4.1.2 Effects on appetite and energy intake

CCK is the most studied satiety hormone. Central and peripheral administration of CCK-8 in rats (Gibbs et al., 1973), dogs (Pappas et al., 1985) and rhesus monkeys (Gibbs et al., 1976), suppresses food intake. In rats these effects are dose-dependent, and only apparent when administered close to the start of a meal - if administered 15 min before eating CCK-8 has no effect on meal size (Gibbs et al., 1973). In healthy young (Lieverse et al., 1995, Kissileff et al., 1981), older (MacIntosh et al., 2001b) and obese (Pi-Sunyer et al., 1982), individuals, intravenous infusions of CCK-8, in doses that result in circulating CCK concentrations within the post-prandial range i.e. 5 - 15 pmol/l (Lieverse et al., 1995), decrease food intake. In addition intravenous CCK-8 and CCK-33 increase the perception of fullness and decrease hunger in healthy humans (Lieverse et al., 1994b, MacIntosh et al., 2001b).

Only a small number of studies have evaluated the role of endogenous CCK in the regulation of energy intake, using the CCK1 receptor antagonist, loxiglumide. Concurrent administration of loxiglumide attenuated the inhibitory effects of an intraduodenal lipid infusion on energy intake (Lieverse et al., 1994a, Matzinger et al., 2000). In addition, intravenous infusion of loxiglumide for one hour prior to, and during, ingestion of a meal, increased energy intake and hunger when compared with

saline infusion (Beglinger et al., 2001). It should be recognised that in these studies, the effects of loxiglumide were modest at best, i.e. the reduction in energy intake was only 10 % (Lieverse et al., 1994a, Matzinger et al., 2000, Beglinger et al., 2001), whereas the suppression induced by exogenous CCK approximates 20 % (Lieverse et al., 1995, Kissileff et al., 1981).

It has been suggested that the effects of CCK to suppress appetite and energy intake are mediated, in part, by its actions on the gastrointestinal tract (Brennan et al., 2005). The association between nutrient-induced secretion of CCK with gastrointestinal motility and energy intake has hitherto not been assessed in humans (Chapters 6 and 7).

1.4.2 Peptide Tyrosine Tyrosine (PYY)

PYY is a 36 amino acid peptide synthesised by endocrine “L” cells, predominantly located in the ileum and large small intestine (Adrian et al., 1985). PYY is secreted as PYY(1-36) and degraded to PYY(3-36) by dipeptidyl peptidase IV, and PYY(3-36) is thought to be the circulating active peptide (Grandt et al., 1994). Receptors that mediate the effects of PYY belong to the NPY receptor family and include Y1, Y2, Y4 and Y5. PYY(1-36) is an agonist at the Y1 and Y2 receptors (Grandt et al., 1994) and once PYY(3-36) is formed, it has an affinity for the Y2 receptor (Batterham et al., 2002). The secretion of PYY from the gut is proportional to the caloric density of ingested nutrients (Ekblad and Sundler, 2002), with lipids and carbohydrates being the most important nutrients (Adrian et al., 1985, Greeley et al., 1989), and fatty acids are the most potent stimulants (Onaga et al., 2002). Plasma PYY levels begin to rise within 15 min after meal ingestion, plateau within approximately 90 min, and remain elevated

for up to 6 hours (Ueno et al., 2008). The release of PYY does not depend solely on direct exposure of the distal gut to fat; studies in dogs have demonstrated that PYY may also be released indirectly by fat in the proximal gut (Lin et al., 2000) secondary to the stimulation of CCK secretion (Lin et al., 2000, Kuvshinoff et al., 1990, McFadden et al., 1992).

1.4.2.1 Effects on gastric and intestinal motility

PYY(3-36), when infused intramuscularly in rhesus monkey, dose-dependently decreased liquid gastric emptying of saline (Moran et al., 2005). In humans, intravenous infusion of PYY(3-36) dose-dependently slows mouth-to-caecum transit and gastric emptying (Savage et al., 1987). As cells for PYY are located in the distal small intestine and secretion of PYY correlates with fat-induced inhibition of distal gut motility, it has been suggested that PYY also acts as the primary mediator of a fat-induced “ileal-break” (Lin et al., 1997).

1.4.2.2 Effects on appetite and energy intake

There are conflicting observations regarding the role of PYY as a regulator of appetite and energy intake. When PYY is administered centrally in rats, it stimulates feeding (Hagan, 2002). In contrast, in a recent study in obese humans subcutaneous injections of PYY(1-36) and PYY(3-36) had no effect on energy intake, but PYY(3-36) dose-dependently increased ratings of satiety and decreased ratings of hunger, thirst, and prospective food consumption (Sloth et al., 2007). It is clear that when administered peripherally, in supraphysiological doses, PYY(3-36) reduces food intake in rodents, primates and lean and obese humans (Batterham et al., 2002, Batterham et al., 2003a,

Batterham et al., 2003b). It has been suggested that the effect of PYY on food intake is mediated through its interaction with the Y2 receptor. Y2 receptors are located in the hypothalamic arcuate nucleus which is a major channel for feeding-related signals (Woods et al., 1998, Schwartz et al., 2000, Seeley and Woods, 2003, Cone et al., 2001), and circulating PYY gains access to the brain, as it freely crosses the blood brain barrier (Nonaka et al., 2003).

When these observations are considered together, it is evident that the role of PYY in the regulation of appetite and energy intake in humans remains uncertain. The relationship between nutrient-induced secretion of PYY with gastrointestinal motility and energy intake has hitherto not been assessed in humans (Chapter 6). Studies employing a specific receptor antagonist(s) to evaluate the role of endogenous PYY represent a priority.

1.4.3 Ghrelin

Ghrelin was identified in 1999, as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). Ghrelin is a small 28 amino acid peptide with an acyl side chain that is essential for its biological action (Kojima et al., 1999). Ghrelin mRNA is expressed predominantly in the stomach (Kojima et al., 1999, Date et al., 2000), primarily by the oxyntic cells of the fundic mucosa (Kojima et al., 1999). Unlike the other peptides which have been described, ghrelin is suppressed after enteral nutrients, rather than stimulated - so that in humans plasma ghrelin rises during fasting and falls rapidly in response to food ingestion (Cummings et al., 2001). The suppression of ghrelin occurs in response to administration of nutrients in the stomach,

duodenum or jejunum, and carbohydrate and protein appear to be more potent suppressors of ghrelin than fat (Overduin et al., 2005, Parker et al., 2005).

1.4.3.1 Effects on gastric and intestinal motility

Ghrelin, when administered intravenously in rats, stimulates gastric acid secretion and gastric motility in a dose-dependent manner (Kamegai et al., 2001, Nakazato et al., 2001). These effects are inhibited by vagotomy and administration of atropine, but not by a histamine H₂ receptor antagonist, suggesting that ghrelin affects gastric function via the vagus nerve (Masuda et al., 2000). In healthy humans exogenous ghrelin when infused intravenously at 10 pmol/kg/min accelerates gastric emptying (Levin et al., 2006), but not at 5 pmol/kg/min (Wren et al., 2001), when compared with saline. Ghrelin also has a prokinetic effect in patients with gastroparesis (Murray et al., 2005, Tack et al., 2005). Intravenous ghrelin has also been reported to induce premature phase III activity and increase the tone of the proximal stomach in healthy humans (Tack et al., 2006). Taken together these observations suggest that ghrelin increases gastric motility/emptying at doses greater than 5 pmol/kg/min, although studies using specific ghrelin antagonists are required. The effect of meals high in fat or carbohydrate, in patients with impaired gastric motility, such as those with functional dyspepsia (see Chapter 2) on ghrelin secretion has not been evaluated (Chapter 8).

1.4.3.2 Effects on appetite and energy intake

Intravenous ghrelin stimulates food intake in humans. In a study by Wren and colleagues (Wren et al., 2001), there was a 28 % increase in energy intake from a buffet meal, and an increase in subjective hunger when compared with saline, in healthy

subjects. The effects of endogenous ghrelin on appetite and energy intake in humans has not been investigated, therefore, the physiological role of ghrelin in feeding behaviour has not been established.

1.4.4 Glucagon-like peptide-1 (GLP-1)

GLP-1, a 33 amino acid peptide hormone product of the glucagon gene, is secreted from the “L” cells, predominantly located in the distal small intestinal mucosa in response to carbohydrate (Näslund et al., 1998), protein (Herrmann et al., 1995) and fat (Feinle et al., 2003). GLP-1 plays an important role as an incretin hormone (Kreymann et al., 1987), enhancing insulin secretion, suppressing glucagon release and stimulating insulin-independent glucose disposal in peripheral tissues (Gutniak et al., 1992, D'Alessio et al., 1994), thereby decreasing glycaemia (Nauck et al., 1998).

1.4.4.1 Effects on gastric and intestinal motility

A number of studies have demonstrated that intravenous GLP-1 relaxes the proximal stomach (Schirra et al., 2002, Delgado-Aros et al., 2002), suppresses antral and duodenal motility (Schirra et al., 2000) and stimulates pyloric pressures (Schirra et al., 2000) in humans, which is associated with slowing of gastric emptying (Nauck et al., 1997, Delgado-Aros et al., 2002, Little et al., 2006b). For example, a recent study demonstrated that GLP-1 at 0.3 (“physiological” dose) and 0.9 (“supraphysiological” dose) pmol/kg/min slowed gastric emptying of both solids and liquids (to induce “gastroparesis” in about half the cohort), and increased meal retention in the distal stomach (Little et al., 2006b). This inhibitory effect of GLP-1 has been reported in both healthy (Little et al., 2006b), type-2 diabetes (Meier et al., 2003) and obese (Flint et al.,

2001, Näslund et al., 1998) individuals, and may well be the major mechanism by which GLP-1 reduces postprandial glycaemic excursions (Horowitz and Nauck, 2006). In contrast, in another study a “supraphysiological” dose of GLP-1 surprisingly had no effect on IPPWs or basal pyloric tone, but decreased antral and duodenal pressure waves (PWs) in healthy human subjects (Brennan et al., 2005). The effect of endogenous GLP-1 on gastropyloroduodenal motility has been evaluated using its specific receptor antagonist exendin(9-39). Exendin(9-39) has been reported to block the effects of GLP-1 on gastric emptying in rats (Tolessa et al., 1998), as well as fasting, and the effects of small intestinal glucose on APD motility, in humans (Schirra et al., 2006).

1.4.4.2 Effects on appetite and energy intake

Data relating to the role of GLP-1 in appetite and energy intake regulation are inconsistent. While most studies have demonstrated that intravenous GLP-1 inhibits energy intake and/or increases the perception of fullness and decreases hunger in animals (Turton et al., 1996) in lean, overweight and type-2 diabetes subjects (Näslund et al., 1998, Verdich et al., 2001a, Gutzwiller et al., 1999a), a few studies have failed to demonstrate an effect (Brennan et al., 2005, Long et al., 1999). The reduction of food intake may result from the effects of GLP-1 on gastric motility and gastric emptying, and appears to be mediated by vagal mechanisms (Delgado-Aros et al., 2002). Exendin(9-39), has been reported to attenuate the inhibitory effects of GLP-1 on energy intake in rats (Turton et al., 1996); comparable studies have not been performed in humans.

The potential relationship between endogenous GLP-1 release by nutrients with gastrointestinal motility and energy intake in healthy human subjects, has not been evaluated (Chapter 7).

1.4.5 Glucose-dependent insulintropic polypeptide (GIP)

GIP is a 42 amino acid peptide that inhibits gastric acid secretion. It is synthesised, and released from intestinal “K” cells, the majority of which are located in the duodenum and proximal jejunum (Fehmann et al., 1995). In humans, GIP is secreted in response to carbohydrate (Rayner et al., 2000b) and fat (Falko et al., 1975). GIP, like GLP-1, acts as an incretin, as this hormone also stimulates insulin release during hyperglycaemia (Morgan, 1996).

1.4.5.1 Effects on gastric and intestinal motility

The limited information that is available indicates that GIP has no effect on gastric emptying/motility (Meier et al., 2004, Miki et al., 2005). For example, there was no difference in gastric emptying rates or emptying half time of a solid meal after treatment with intravenous GIP at 2 pmol/kg/min or placebo in healthy humans (Meier et al., 2004). In mice, subcutaneous injection of 100 µg human GIP did not affect gastrointestinal transit of an orally ingested barium sulphate meal (Miki et al., 2005).

1.4.5.2 Effects on appetite and energy intake

The role of GIP on appetite and energy intake is uncertain. When administered to rats, GIP had no effect on food intake (Garlicki et al., 1990). A few studies, however, have

reported that appetite and subsequent energy intake after intraduodenal glucose infusions (Lavin et al., 1998), or a meal (Verdich et al., 2001b) are related to postprandial GIP responses in lean and obese humans. As these studies have not evaluated the direct effect of GIP on appetite and energy intake, it is possible that any effect of GIP may be due to insulin.

1.4.6 Insulin

Insulin is a peptide hormone synthesised in the pancreas within the β -cells of the islet of Langerhans. Its primary role relates to glucose homeostasis. The magnitude of insulin secretion in response to carbohydrate is dependent on the release of the incretin hormones, GLP-1 and GIP.

1.4.6.1 Effects on gastric and intestinal motility

Intravenous infusion of insulin in dogs has reported to enhance gastric motility in a number of studies (Quigley and Templeton, 1930, Regan, 1933, Nelsen et al., 1966), possibly due to vagal stimulation induced by hypoglycaemia (Walker et al., 1974). The effect of insulin on gastric emptying and motility, however, has produced conflicting results in humans. Hyperinsulinaemia under euglycaemic conditions may slow solid and liquid gastric emptying, slightly, but significantly, in healthy humans (Kong et al., 1998, Eliasson et al., 1995), but has little, if any, effect in type-1 or type-2 diabetes patients (Kong et al., 1999a). In regards to antroduodenal motility, hyperinsulinaemia may inhibit antral PWs (Gielkens et al., 1997), attenuate antral phase III, and shorten duodenal phase III, activity (Bjornsson et al., 1995) and inhibits fed jejunal motility,

therefore delaying small intestinal transit time (Kong et al., 1998). However a study by Hasler and colleagues, found no effect (Hasler et al., 1995). The direct effect of insulin on gastric emptying/motility has not been determined. Another study investigated the effect of hypoglycaemia on gastrointestinal motility, induced by intravenous infusions of insulin in healthy humans and reported that there was no significant difference in the number of antral, pyloric or duodenal PWs or basal pyloric pressure in the 45 min after insulin injection (hypoglycaemia) when compared with the 45 min after saline injection (euglycaemia) (Fraser et al., 1991a). The relationship between the small intestinal glucose load and insulinaemia is poorly defined. No study has evaluated the effects of intraduodenal infusions of glucose, at rates lower than, comparable to, and higher than gastric emptying occurs on the secretion of insulin and incretin hormones (Chapter 7).

1.4.6.2 Effect on appetite and energy intake

The role of insulin in the regulation of appetite is unclear and controversial. In animals, in the absence of hypoglycaemia, elevated levels of circulating insulin appears to decrease food intake (Nicolaidis and Rowland, 1976, VanderWeele et al., 1980, Woods et al., 1984), however, in humans infusion of insulin under euglycaemic conditions has been shown not to alter appetite, suggesting that insulin does not enhance satiety under these circumstances (Chapman et al., 1998). In contrast, endogenous increases in insulin, in response to a meal, have been reported to be related inversely to subsequent energy intake in lean, but not obese, human subjects (Verdich et al., 2001b, Speechly and Buffenstein, 2000). No study has evaluated the effect of infusions of glucose, at

rates lower than, comparable to and higher than gastric emptying occurs on the secretion of insulin and related these to effects on subsequent energy intake (Chapter 7).

1.5 FACTORS THAT MODULATE THE EFFECTS OF NUTRIENTS ON GASTRIC EMPTYING, MOTILITY, HORMONE RELEASE/SUPPRESSION, APPETITE AND ENERGY INTAKE

A number of factors may influence the effects of small intestinal nutrients on gastrointestinal motility, hormone release/suppression, appetite and energy intake. These include; the length and region of small intestine exposed to nutrient, the load of nutrient delivered into the small intestine, and the duration of exposure of the small intestine to the nutrient. The influence of length and region have not been extensively evaluated in humans, however, the effects of different loads (g or kcal/min) and durations (Castiglione et al., 1998) of small intestinal nutrient infusions on gastrointestinal motility, hormone release/suppression, appetite and energy intake have been explored, thereby assessing the effects of length, or region, of exposure indirectly. It is reasonable to assume that with greater nutritional loads, a greater length of small intestine would be exposed to the nutrient. For example, in both dogs (Lin et al., 1996) and rats (Meyer et al., 1998a) ingestion of meals with increasing fat and/or glucose content results in increasing loads of triglyceride or glucose emptying from the stomach into the duodenum, associated with increased spread of both fat and glucose to, and along, the ileum. This caudal spread of digestion and absorption occurs when the digestive capacity of nutrients, as well as the absorptive capacities for their products per cm of gut, are exceeded (Meyer et al., 1998a, Meyer et al., 1998c).

1.5.1 Effects of length of small intestinal exposure to nutrient

1.5.1.1 Effects on gastric and intestinal motility

In animals, the effects of nutrients on gastric emptying are dependent on the length of small intestine exposed to that nutrient, and these effects are also nutrient-specific (Cooke, 1977, Lin et al., 1989, Lin et al., 1990). In dogs, glucose and fat delayed gastric emptying when perfused at the duodenojejunal junction, but had no effect when infused only into the first 5 cm of the duodenum (Cooke, 1977). In this same study, perfusion of 0.1 M HCl or L-tryptophan into the first 5 cm of the duodenum inhibited gastric emptying, to the same extent, as perfusion of the entire duodenum and proximal jejunum (Cooke, 1977). This study, therefore, indicated that exposure of > 5 cm of the gut to glucose/fat is required to inhibit gastric emptying. Two studies by Lin and colleagues investigated the effect of exposing various lengths (15, 65, 150 cm and all), to glucose (at 0.25, 0.5 and 1.0 M) and the fatty acid oleate (at 3, 9 and 27 mM), in dogs (Lin et al., 1990, Lin et al., 1989). For glucose, no inhibition of gastric emptying was observed, at any load, when confined to the first 15 cm of the duodenum (Lin et al., 1989). By contrast, maximal inhibition of gastric emptying was achieved during glucose perfusions involving 150 cm, and all, of the small intestine. When 65 cm of the small intestine was exposed to glucose, gastric emptying was inhibited by 50 - 60 % (Lin et al., 1989). Similar, inhibitory effects on gastric emptying were observed for fat (oleate), in a dose-dependent manner (Lin et al., 1990) (**Figure 1.8**). These observations establish that the length of the small intestine exposed to nutrient is a determinant of gastric emptying in animals.

NOTE: This figure is included on page 27 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.8 The inhibition index ($= 1 - \text{AUC}_{\text{oleate}}/\text{AUC}_{\text{buffer}}$) for gastric emptying (1 = maximal inhibition, 0 = minimal inhibition) of 3, 9, 27 mM loads of sodium oleate in carbonate buffer expressed as a function of length of small intestine exposed to nutrient in dogs implanted with small intestinal fistula. Adapted from (Lin et al., 1990).

Less information is available regarding the effect of length of small intestinal exposure to nutrients on gastric motility in humans. More recently, a study performed in humans compared the effects of infusing glucose into an isolated 60 cm segment of the proximal intestine (“short segment”), with an infusion that had access to the entire small intestine (“long segment”) (Little et al., 2006a). The number of antral PWs was suppressed during the “long”, not the “short”, segment infusion while pyloric pressures were stimulated by both infusions (Little et al., 2006a). Taken together, these studies in animals and humans indicate that feedback from the distal small intestine is an important regulator in gastric emptying - as more receptors are recruited along the small intestine, the greater the feedback occurs. The effects of exposing different lengths of the small intestine, by increasing loads of small intestinal infusions of fat and glucose, on APD motility, in humans has not been evaluated (Chapters 6 and 7).

1.5.1.2 Effects on gastrointestinal hormone release

As discussed in Chapter 1.4, gastrointestinal hormone release in response to nutrients occurs in differing regions of the small intestine, depending on the location of the hormone-producing cells. Accordingly, their stimulation or suppression is dependent on the length of exposure of small intestine exposed to nutrients.

In a recent study, referred to above, the release of insulin, GLP-1, GIP, CCK and ghrelin was demonstrated to be dependent on the length of small intestine exposed to glucose in humans (Little et al., 2006a). Plasma GIP and CCK increased similarly after both “short segment” and “long segment” infusions, however, in contrast plasma GLP-1 only increased and ghrelin suppressed, when > 60 cm of small intestine was exposed to glucose. Rises in insulin were greater during the long-, than during the short-segment infusion (Little et al., 2006a). The effect of exposing different lengths of the small intestine, by increasing loads of small intestinal infusions of fat and glucose, on gastrointestinal hormone release has not been evaluated (Chapters 5, 6 and 7).

1.5.1.3 Effects on appetite and energy intake

A number of studies have suggested that infusion of either fat and/or carbohydrate into the duodenum, jejunum or ileum decreases appetite and/or subsequent energy intake in humans (Chapman et al., 1999, Welch et al., 1988). The extent of this suppression may be dependent on the length exposed to the nutrient. In rats, studies have been conducted to assess the effect of length of nutrient exposure on the regulation of appetite and energy intake directly, and these have demonstrated that when lactose or oleate were confined to a 35 cm segment of the jejunum, there was no suppression, but, when

allowed access to the entire length of the small intestine, there was potent suppression, of energy intake (Meyer et al., 1998c). The effect of exposing different lengths of the small intestine to nutrients on appetite and energy intake in humans have hitherto, not been investigated.

1.5.2 Effects of region of small intestinal exposure to nutrient

1.5.2.1 Effects on gastric and intestinal motility

Less information is available about the effect of the region of the small intestine exposed to nutrients on gastric and intestinal motility, probably reflecting the technical challenges in performing these studies. For several decades, it was believed that nutrients inhibited gastric emptying by triggering nutrient sensors confined to the duodenum (Hunt, 1963), however subsequent studies established that there were sensors in the terminal human ileum that inhibited jejunal motility when the ileum was perfused with fat (Spiller et al., 1984, Read et al., 1984). More recently a study by Azpiroz and Malagelada, compared the effects of proximal vs. distal small intestinal nutrients on gastric motility and reported that the inhibition of fundic tone in dogs, occurred when the proximal, but not the distal, one-third of the intestine was perfused with fat, however this effect was the opposite for maltose (Azpiroz and Malagelada, 1985a). In relation to gastric emptying, a study by Lin and colleagues investigated region-specific differences in the intestinal inhibition of solid emptying, by infusing glucose into the first, second, third and fourth quarter of the small intestine, in dogs. They reported that gastric emptying of solids, but not liquids, was ~ three times more potently inhibited by glucose in the fourth quarter vs. the first and second quarter of the small intestine, suggesting that signals arising from the distal small intestine have more potent effects to

inhibit gastric emptying (Lin et al., 1992). The potential region-dependent effect of nutrients on motility in humans has not been evaluated.

1.5.2.2 Effects on gastrointestinal hormone release

Gastrointestinal hormone release, or suppression, occurs as a result of exposure of nutrients to different regions of the small intestine, depending on the location of the hormone-producing cells, as discussed in Chapter 1.4.

1.5.2.3 Effects on appetite and energy intake

The extent of suppression of appetite and energy intake by nutrients has been shown to be dependent on the region of small intestine exposed to nutrients. In humans, Welch and colleagues demonstrated that infusion of a 50 % corn oil emulsion into the jejunum or ileum reduced the period of eating and quantity of food consumed at a buffet meal, when compared with saline (Welch et al., 1988). However suppression of hunger before the meal was only evident after the jejunal infusion (Welch et al., 1988). In a more recent study in humans, glucose was infused into either the proximal (duodenal) or distal (mid-jejunal), small intestine and hunger scores and energy intake was higher with mid-jejunal infusions and lower with duodenal infusion (unpublished data). Taken together these studies indicate differences in the regional effects of lipid and glucose on appetite and energy intake.

1.5.3 Effect of load

1.5.3.1 Effects on gastric and intestinal motility

A few studies have evaluated the effects of different small intestinal nutrient loads on gastrointestinal motility. For example, at loads of 0.25, 0.5 and 1.5 kcal/min, duodenal lipid caused a load-related suppression of duodenal pressure wave sequences (PWSs) (Andrews et al., 2001). These loads reflect rates of gastric emptying at the lower range (Edelbroek et al., 1992b, Kunz et al., 2005, Little et al., 2007, Meyer et al., 1996) and the effects on the antral and pyloric motility were not evaluated. Another study using intraduodenal lipid at increasing loads of 1, 2 and 3 kcal/min demonstrated that all loads were capable of causing an increase in gastric volume, indicative of gastric relaxation (as measured using a barostat), when compared with saline infusion, with no difference between the three infusions, indicating that the 1 kcal/min load caused maximal gastric relaxation of the fundus (Feinle et al., 2000).

In regards to glucose, a study by Heddle and colleagues reported that infusions at 2.4 and 4 kcal/min for 10 min stimulated IPPWs, with the effect of 4 kcal/min being greater, indicative of load-dependence (Heddle et al., 1988c). Increasing oral glucose loads (50 g vs. 100 g loads) also prolonged the rate of gastric emptying by 27 % (Schirra et al., 1996). The effect of increasing loads of glucose on antral and duodenal pressures has not been assessed, nor the effects of intraduodenal lipid or glucose at loads lower than, comparable to, and greater than, the normal rate of gastric emptying on APD motility (Chapters 6 and 7).

1.5.3.2 *Effects on gastrointestinal hormone release*

Only a handful of studies have evaluated the effects of small intestinal load of fat and/or glucose on the secretion of gastrointestinal hormones. A study by Feinle and colleagues demonstrated that duodenal lipid at 1, 2 and 3 kcal/min, for 30 min, increased plasma CCK and PYY. Only the secretion of CCK was observed to be load-dependent (Feinle et al., 2000), which was postulated to reflect the short duration period and/or small load of lipid infused. - As CCK is secreted from cells located in the proximal small intestine (Buffa et al., 1976), at the site of infusion, all loads of lipid would have caused a secretion of CCK, whereas the lipolytic products of the lowest loads (i.e. 1 kcal/min) may not have reached the distal part of the small intestine before being digested and absorbed, and accordingly, failed to stimulate PYY (Adrian et al., 1985). It is not known how small intestinal lipid loads that are substantially less than average gastric emptying, e.g. as would occur when gastric emptying is slowed, affect gut hormone secretion (Chapter 6).

In regards to glucose, it has been suggested that a threshold of small intestinal glucose delivery of about 1.8 kcal/min needs to be exceeded in order to stimulate GLP-1 secretion (Schirra et al., 1996). This threshold was suggested as a 1.1 kcal/min intraduodenal glucose load failed to increase GLP-1 levels, whereas a 2.2 kcal/min load brought about a steady GLP-1 release (Schirra et al., 1996). However, this observation is inconsistent with two recent studies which demonstrated that duodenal glucose infusions at 1 kcal/min were sufficient to stimulate GLP-1, at least transiently, in healthy humans (Chaikomin et al., 2005, O'Donovan et al., 2004) and type-2 diabetes patients (O'Donovan et al., 2004). In contrast, the release of GIP has been shown to be load-dependent, occurring in response to loads as small as 1 kcal/min (Schirra et al.,

1996, Chaikomin et al., 2005, O'Donovan et al., 2004), and eliciting a constant release with a plateau achieved after ~ 30 min (Schirra et al., 1996). The load that elicits a maximal GIP response is unknown and no study has evaluated the effect of increasing duodenal glucose loads on CCK release (Chapter 7).

1.5.3.3 Effects on appetite and energy intake

The effects of lipid and glucose on appetite and energy intake are also dependent on the nutrient load administered, however, there are differences between fat and glucose. For lipid, loads as low as 2 kcal/min for 45 min (Castiglione et al., 1998) up to 2.9 kcal/min for 120 min (MacIntosh et al., 2001a, Chapman et al., 1999) have demonstrated to reduce energy intake, in healthy young, old and obese individuals. In healthy subjects lipid, at these loads, also reduced hunger and increased fullness (MacIntosh et al., 2001a, Chapman et al., 1999). For glucose, intraduodenal loads between 2 (Rayner et al., 2000b) and 2.9 (Chapman et al., 1999, MacIntosh et al., 2001a) kcal/min for 120 min (corresponding to loads of 240 kcal and 339 kcal, respectively) failed to reduce energy intake, when compared with saline, in healthy young males. There was, however, an increase in fullness (MacIntosh et al., 2001a, Chapman et al., 1999). To date, the only load of glucose that has been shown to reduce energy intake is 3.2 kcal/min for 90 min (288 kcal/min) (Lavin et al., 1998). The effects of loads of lipid/glucose lower than, comparable to, and greater than the normal rate of gastric emptying on appetite and energy intake have not been evaluated (Chapters 6 and 7).

1.5.4 Effects of duration of exposure of the small intestine to nutrient

1.5.4.1 Effects on gastric and intestinal motility

Little information is available about the effect of the duration of small intestinal nutrient exposure on gastrointestinal function. One study, by Edelbroek and colleagues, demonstrated that glucose infusions, at 2.4 kcal/min, for a long period (i.e. $t = 120$ min) and two short periods (i.e. for 20 min at $t = 0 - 20$ min and $t = 80 - 100$ min) increased the number of IPPWs and basal pyloric pressure during the first 20 min, but after this time there was a decrease, with a subsequent return to baseline. In addition the second short 20 min infusion of glucose at $t = 80 - 100$ min increased pyloric pressures, but at a smaller magnitude when compared with the first 20 min infusion (Edelbroek et al., 1992a) (**Figure 1.9**). In this study, antral PWs were suppressed for the entire 120 min infusion period. This suggests that there may be an “adaptation” in the pyloric motor response. It would therefore be of interest to assess the effects of “short” and “long” durations of nutrient infusion on gastrointestinal motility to determine whether there is an adaptive response (Chapter 5).

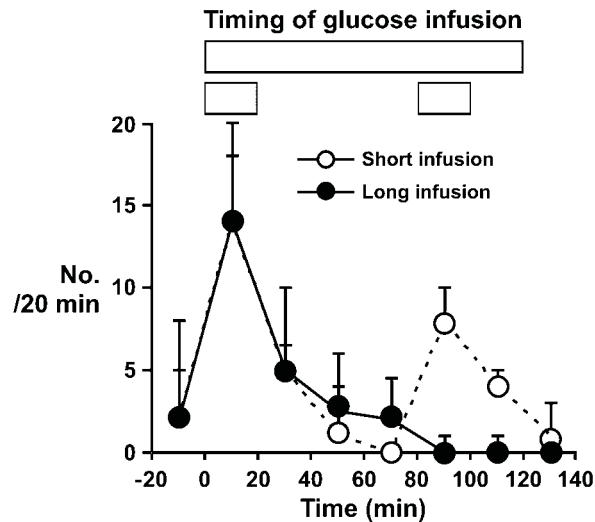


Figure 1.9 Number of isolated pyloric pressure waves during the 120 min (●) and two 20 min (○) intraduodenal glucose infusion. There was a stimulation of isolated pyloric pressure waves during the first 20 min of the 120 min infusion ($P < 0.05$). Both 20 min infusions were associated with stimulation of isolated pyloric pressure waves ($P < 0.05$). The response to the second infusion was less ($P < 0.05$) than the first. Data are median values and interquartile ranges. Adapted from (Edelbroek et al., 1992a).

1.5.4.2 *Effects on gastrointestinal hormone release*

The effect of duration of exposure of the intestine to nutrients on the stimulation of gastrointestinal hormones has not been assessed in humans (Chapter 5). However, it can be anticipated that their release would differ depending on the site of secretion of the hormone and the distribution of digestive products along the gut, which would also be dependent on the load of the nutrient (Chapter 5).

1.5.4.3 *Effects on appetite and energy intake*

The duration of nutrient infusions, as well as the time period between the ingestion of a “preload” and ad libitum food intake has been reported to influence energy intake (Castiglione et al., 1998, Rolls et al., 1991). For example, Castiglione and colleagues investigated the effects of lipid infused into the small intestine at a constant rate (2

kcal/min) over different durations, i.e. 15 min, 45 min and 90 min, on subsequent energy intake (Castiglione et al., 1998). The 45 min and 90 min infusions reduced energy intake, in a duration-dependent fashion, when compared with a saline, and the 15 min, infusion. In addition, there was a trend for the 90 min infusion to decrease hunger and prospective consumption and increase fullness when compared with saline (Castiglione et al., 1998). These apparent duration-dependent effects to reduce food intake are likely reflect a combination of factors, including the number of calories, duration of exposure of any one segment of the small intestine and the length of intestine exposure. As mentioned in Chapter 1.5.1, the inhibition of gastric emptying by the presence of nutrients in the small intestine is proportional to the length of the gut exposed to the nutrient, therefore, the longer the nutrient infusion, the more intestinal receptors would be recruited thereby a bigger effect on energy intake. Therefore, the observations by Castiglione and colleagues for the 15 min infusion are indicative of the delivery of insufficient calories, or possibly an insufficient period of stimulation to elicit feedback response from the small intestine to inhibit energy intake (Castiglione et al., 1998).

A study by Rolls and colleagues demonstrated that the duration between a preload and ad libitum food intake may also affect energy intake in humans (Rolls et al., 1991). Equi-caloric and equi-volume high-fat or high-carbohydrate preloads were consumed at three different intervals, i.e. 30, 90 and 180 min, before a self-selected lunch was served. For the 30 min delayed condition subjects accurately compensated for the calories in the preload compared with a no preload condition, and the compensation was less precise as the interval increased (Rolls et al., 1991). A limitation of the two studies mentioned above is that too many variables may have influenced their observations on

energy intake, such as increasing caloric loads of the preload/infusion as a result of longer durations. No study has evaluated the effects of small intestinal infusion of nutrients over different durations, but at the same caloric load, on appetite or energy intake (Chapter 5).

1.6 GASTROINTESTINAL MOTILITY DISORDERS

A number of disorders of the gastrointestinal tract result in altered gastrointestinal function, hormone release/suppression, appetite and energy intake. These disorders include obesity, type-2 diabetes mellitus and functional dyspepsia. Abnormalities of gastrointestinal function and diet in functional dyspepsia have been described in detail in Chapter 2.

1.7 SUMMARY

This chapter has reviewed the potential factors involved in the regulation of gastric emptying, gastrointestinal motility, hormone release/suppression, appetite and energy intake. The studies described in the subsequent chapters of this thesis address the following hypotheses:

- (i) the load and/or duration of intraduodenal lipid affect APD motility, plasma CCK and PYY and energy intake (Chapters 5 and 6)
- (ii) the load of intraduodenal glucose affect glycaemia, gastrointestinal hormones, APD motility and energy intake (Chapter 7)
- (iii) there are relationships between the effects of intraduodenal lipid and/or glucose on APD motility, gastrointestinal hormone release and energy intake (Chapters 6 and 7).

Chapter 2

FUNCTIONAL DYSPEPSIA

2.1 INTRODUCTION

Functional dyspepsia (FD) is a clinical syndrome characterised by chronic, or recurrent, upper gastrointestinal symptoms without a known cause that is identifiable by conventional diagnostic means. While FD is not life-threatening, it affects quality of life negatively and accounts for a substantial number of attendances to doctors (Meineche-Schmidt et al., 1999). Hence, FD represents a substantial source of morbidity, as well as a considerable financial burden to the health care system.

FD is clearly a heterogenous disorder, and the pathophysiology and aetiology of FD remain poorly defined, despite considerable research interest. Patients with FD characteristically report symptoms such as abdominal bloating and discomfort, fullness, nausea, vomiting and epigastric pain and are often unable to finish normal-sized meals. Attempts to correlate these symptoms with functional abnormalities have generally been unsuccessful. Symptoms have been related to a number of gastrointestinal abnormalities in subgroups of patients. These include disordered gastric emptying and gastrointestinal motility, *Helicobacter pylori* infection, visceral hypersensitivity, functional abnormalities in the central nervous system, and psychological and cognitive

factors (Talley, 1995). Current treatments are, accordingly, targeted at normalisation of gastric emptying and motility using prokinetic drugs, *Helicobacter pylori* eradication and acid suppression. However, the efficacy of all therapies is marginal at best (see Chapter 3). Current treatments also fail to address the fact that symptoms are frequently induced, or exacerbated, by the ingestion of food, moreover, there is limited information about the relationship between symptoms, disordered gastric motor function and patterns of nutrient intake. This chapter provides an overview of the potential roles of gastric emptying and motility, nutrient intake, gastrointestinal hormones and cognitive factors to the pathophysiology of FD. The limitations of previous studies of FD patients, such as inconsistencies and inadequacies in the methods used, is also discussed.

2.2 DEFINITION OF FUNCTIONAL DYSPEPSIA

Until recently, the international definition of FD, as adopted by the Rome II working party, of “discomfort”, or “pain” centred in the upper abdomen, has been most widely applied (Talley et al., 1999a). FD has been defined by the Rome II international working party, by, (i) persistent or recurrent dyspepsia (discomfort or pain centred in the upper abdomen), lasting for at least 12 weeks, which need not be consecutive, within the preceding 12 months, (ii) no evidence of organic disease (as evaluated by upper endoscopy and/or ultrasound) that is likely to explain the symptoms, and (iii) no evidence that dyspepsia is exclusively relieved by defecation or associated with the onset of a change in stool frequency or stool form (i.e. not irritable bowel syndrome (IBS) (Talley et al., 1999a)). Patients with FD have been subdivided into three groups, based on the predominant symptom(s); “dysmotility-like” dyspepsia, if the

predominant symptom is of discomfort (upper abdominal fullness, bloating, belching or nausea), “ulcer-like” dyspepsia, if the predominant symptom is that of pain, and “unspecified” (non-specific) dyspepsia, if the predominant symptom(s) fails to meet one of the two previous descriptions. In 2006, the Rome III criteria were introduced, which are more user-friendly for both clinical and research settings than the Rome II criteria. Under the Rome III criteria, FD is divided into two subgroups; meal-induced dyspeptic symptoms (“postprandial distress syndrome”) and epigastric pain (“epigastric pain syndrome”) (Drossman and Dumitrascu, 2006). At the time the studies presented in Chapters 8 and 9 were performed, the Rome III criteria were not available.

2.3 PREVALENCE AND COSTS OF FUNCTIONAL DYSPEPSIA

The prevalence of FD varies considerably between different populations. Although this may reflect genuine epidemiological differences, it is also clear that the varying definitions used in different studies may well have contributed to discrepancies. In a recent survey, using the Rome II criteria, and where symptoms of reflux and IBS were excluded, the prevalence varied between 11 - 29 % around the world (Li et al., 2002, Westbrook and Talley, 2002). Population-based studies have also attempted to identify epidemiological risk factors for FD. These studies have indicated that; FD does not appear to be related to age, however, FD occurs more frequently in those who are less than 59 years of age in Japan (Hirakawa et al., 1999), female adults with FD significantly outnumber males in Australia (Koloski et al., 2002), while ethnicity has not been investigated (as surveys have only evaluated single/similar ethnic groups) (Mahadeva and Goh, 2006). There is a strong direct relationship between lower household income and larger household membership and the incidence in FD in the

USA (Drossman et al., 1993). Psychological disturbances have also been associated with FD. For example, in the American adult population, sexual, emotional and verbal abuse, either in childhood or adulthood, are associated with FD (Talley et al., 1994), and in an Australian survey, levels of anxiety and depression were higher in adult FD patients (Koloski et al., 2002). Smoking (Bernersen et al., 1996) and regular alcohol (Mahadeva and Goh, 2006) intake are apparently not risk factors.

FD has substantial implications for medical workload and the economy. In two recent community surveys of several European and North American populations, 20 % of people with dyspeptic symptoms had consulted either primary care physicians or hospital specialists, more than 50 % of sufferers were on medication, and approximately 30 % reported taking days of work or school because of their symptoms (Haycox et al., 1999, Moayyedi and Mason, 2002). This medical attention may also involve extended and repeated investigations, plus high prescription costs and follow-up rates, which together result in considerable direct costs (Agreus, 1993, Jones and Lydeard, 1992). Only one study has attempted to estimate the costs of FD, in regards to in- and out-patient care, as well as costs to society for loss of production - a Swedish study in 8 million people (Nyren et al., 1985). The annual expenditure for in-patient care was estimated to be 10.3 million Swedish Krona (SEK) (~ 1.7 million AUS), and for out-patient care and drug costs, as determined from a nation-wide sample survey, 197.1 million SEK (~ 32 million AUS) and 60 million SEK (~ 10 million AUS), respectively. The most dominant item among social costs was loss of production due to short-term sick leave, which amounts to 2496 SEK (~ 406 million AUS) (Nyren et al., 1985). No recent studies have evaluated the impact of FD on social and economic trends in other countries. However, gastroenterologists are now showing increased interest in the

investigation of the pathophysiology of FD and this is likely to promote studies to clarify its causation, natural history and socio-economic impact, all of which are currently poorly defined (Tebaldi and Heading, 1998).

2.4 PATHOPHYSIOLOGY OF FUNCTIONAL DYSPEPSIA - RELATIONSHIP TO GASTROINTESTINAL FUNCTION

A number of gastrointestinal factors have been investigated as potential causes of symptoms in FD, including *Helicobacter pylori* infection, hypersensitivity to gastric acid secretion, delayed gastric emptying, disordered intragastric meal distribution, impaired gastric relaxation, dysfunction of the antrum and visceral hypersensitivity.

2.4.1 *Helicobacter pylori* and acid secretion

It is well established that *Helicobacter pylori* (*H pylori*) infection is the main cause of peptic ulcer disease (NIH Consensus Conference, 1994), but its role in the aetiology of FD is unclear and controversial. Results from a number of therapeutic studies in FD have demonstrated results in favour of, or against, *H pylori* eradication therapy for symptom improvement (Talley, 1994). Gastric inflammation, caused by *H pylori*, has been shown to alter gastric function and may thereby cause symptoms (Czinn et al., 1991, Tytgat et al., 1991). However, a study which compared the symptom profile of patients with and without *H pylori* and investigated potential underlying mechanisms including disturbed gastric emptying, gastric hypersensitivity and gastric accommodation, failed to demonstrate any association between the prevalence of symptoms or gastric sensory motor functions in those with or without *H pylori* (Sarnelli et al., 2003).

Increased gastric acid secretion and duodenal acid overload have been suggested as causes of FD in patients with abdominal pain in the fasted state (Nyren et al., 1987), however, basal and stimulated gastric acid secretion have been reported to be comparable to that in asymptomatic controls regardless of the subgroup of FD (Nyren et al., 1987). Another possibility is that FD patients are hypersensitive to acid. Samson and colleagues reported that the duodenal bulb is hypersensitive to acid infusion, which induced nausea and decreased duodenal motility in FD patients, but not in controls (Samsom et al., 1999). However, therapies, such as acid suppressants and antacids (see Chapter 3.2.3), have had little, if any, effect on symptoms in patients with FD. Accordingly, the role of acid secretion in the induction of symptom in FD, remains uncertain.

2.4.2 Delayed gastric emptying

Slowing of gastric emptying in response to nutrients, particularly fat, is caused by the combined effects of relaxation of the proximal stomach (Azpiroz and Malagelada, 1985b), decreased contractility of the antrum and small intestine (Heddle et al., 1988a) and stimulation of isolated pyloric pressure waves (Heddle et al., 1988b, Andrews et al., 1998) (see Chapter 1.3.2). Delayed gastric emptying has long been considered the main pathogenic mechanism underlying the generation of symptoms in FD. Delayed gastric emptying has been reported in 20 - 60 % of FD patients (Cuomo et al., 2001, Duan et al., 1993, Jian et al., 1989, Mittal et al., 1997, Stanghellini et al., 1996, Wegener et al., 1989). This substantial variability in the prevalence of delayed gastric emptying is likely to reflect inconsistencies in study design, particularly test meals and methods used to measure gastric emptying, and the inclusion criteria for FD patients. In

addition, previous studies have failed to demonstrate a meaningful relationship between the presence of dyspeptic symptoms with a delay in solid/liquid gastric emptying (Gilja et al., 1996, Talley et al., 2001) or have not evaluated symptoms concurrently with gastric emptying (Cuomo et al., 2001, Duan et al., 1993, Stanghellini et al., 1996). For example, a study which measured gastric emptying of a solid meal using radioisotopic techniques in 343 patients with FD, showed a significant, albeit weak, correlation between postprandial fullness and vomiting and gastric emptying, suggesting that patients with delayed gastric emptying are more likely to experience dyspeptic symptoms (Stanghellini et al., 1996). However, symptoms were evaluated as an average symptom score, and were not assessed concurrently with gastric emptying. Moreover, patients were recruited from a tertiary referral centre, hence, the observations may have been influenced by selection bias, as both symptoms and disturbances in gastric emptying may have been more severe than in dyspeptic patients recruited from the general population. In contrast to these findings, a study by Gilja and colleagues showed no correlation between symptoms experienced after ingestion of a soup meal and the rate of gastric emptying, despite FD patients reporting significantly higher symptom scores than healthy control subjects (Gilja et al., 1996). A further study also failed to demonstrate any association between individual symptoms and the delay in gastric emptying (as assessed by C13 octanoic acid breath test) in patients with FD (Talley et al., 2001). Furthermore, Hausken and colleagues reported that symptoms characteristically occur within minutes of food consumption (approximately 3 min) (Hausken et al., 1998), while a delay in gastric emptying is characteristically only quantified much later, e.g. at 120 - 180 min. Therefore, it seems unlikely that dyspeptic symptoms result from delayed gastric emptying, per se.

Symptoms in FD patients have also been suggested to be the result of accelerated emptying in the “early phase”, particularly that of fat (Lin et al., 1999). A study by Lin and colleagues demonstrated that in patients with fat intolerance (as diagnosed by symptoms of postprandial bloating, early satiety, nausea and pain), gastric emptying of a fat meal containing 15 or 60 g of fat was faster in the first 15 min, but not at 60 min, when compared with healthy subjects (Lin et al., 1999). Symptoms, however, were not evaluated in this study. No study has, to date, evaluated the “early phase” gastric emptying of fat, and its relationship with symptoms, in patients with FD.

The studies summarised above, therefore, indicate that while delayed gastric emptying is prevalent in FD, it is unlikely to be a direct cause of symptoms, in at least the majority of cases, and the potential effect of accelerated “early phase” gastric emptying has not been evaluated. The lack of a significant contribution of delayed gastric emptying to dyspeptic symptoms is supported by the fact that in a large proportion of FD patients gastric emptying is normal.

2.4.3 Intra-gastric meal distribution

In the healthy stomach, ingested food is stored in the proximal stomach and gradually transported into the distal stomach where it is “ground” and “mixed” with gastric secretions and then emptied into the small intestine. In approximately 30 - 40 % of patients with FD intra-gastric meal distribution is disturbed (Troncon et al., 1994). Studies, using scintigraphic (Troncon et al., 1994) and ultrasound (Gilja et al., 1996) techniques, have demonstrated that in many patients with FD intra-gastric distribution is abnormal, such that the relative amount of food present in the distal stomach (or

antrum) is increased. Troncon and colleagues reported that after ingestion of a liquid meal, the amount of the meal which remained in the proximal half of the stomach was less in FD patients compared with healthy subjects with a greater proportion in the distal half of the stomach (Troncon et al., 1994) (**Figure 2.1**). - Overall gastric emptying rates were comparable between FD patients and healthy subjects. The disturbance in the intragastric meal distribution may reflect impaired postprandial relaxation of the proximal stomach (Tack et al., 1998), and/or dysfunction of the distal stomach (Hausken and Berstad, 1992b).

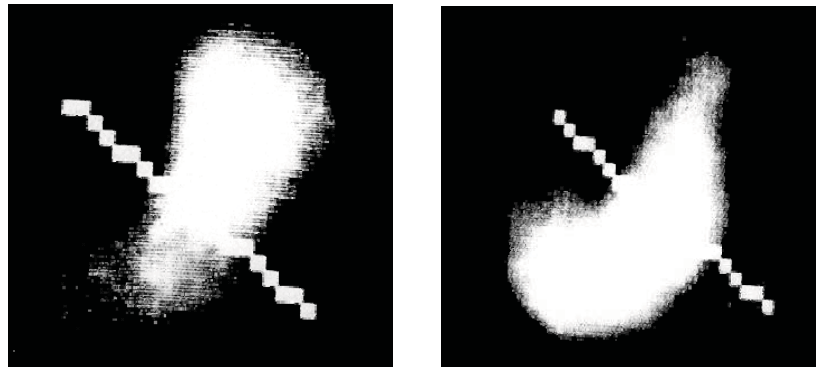


Figure 2.1 Representative gamma camera images taken immediately after ingestion of a liquid meal in a healthy subject (left) and a FD patient (right). Images have been divided into proximal and distal gastric regions. In the FD patient relatively more of the meal is in the distal stomach. Adapted from (Troncon et al., 1994).

2.4.4 Impaired gastric relaxation

Gastric relaxation allows the proximal stomach to accommodate a large volume without a significant increase in intragastric pressure (Blackshaw et al., 1987). The relaxatory response of the proximal stomach to a meal is impaired in approximately 40 % of patients with FD (Gilja et al., 1996, Tack et al., 1998, Feinle et al., 2001a, Kim et al., 2001) (**Figure 2.2**) and it has been suggested that patients with FD who have impaired proximal gastric accommodation experience more postprandial symptoms, as assessed

by a global symptom score (Gilja et al., 1996). However, such an association remains controversial, as investigations into the effects of pharmacological improvements in gastric accommodation on dyspeptic symptoms have yielded conflicting outcomes (Tack et al., 1998, Boeckxstaens et al., 2001, Boeckxstaens et al., 2002). A study by Tack and colleagues suggested that impaired proximal gastric accommodation is associated with “early satiety”, in that the volume of a nutrient-containing liquid drink which could be consumed, was less in FD patients when compared with healthy subjects (Tack et al., 1998). In turn, restoration of proximal gastric accommodation in patients with FD induced by the fundus-relaxing 5HT-1 (5-Hydroxy-tryptamine-1) agonist, sumatriptan, improved “early satiety”, in that a greater volume of nutrient-containing liquid could be consumed (Tack et al., 1998). However, a more recent study reported that treatment with sumatriptan had no effect on the maximal volume ingested, or postprandial symptoms when compared with placebo in either FD patients or healthy subjects (Boeckxstaens et al., 2002). Thus, while there is no doubt that impaired proximal gastric accommodation is present in a subgroup of patients with FD, its role in the genesis of symptoms in FD remains uncertain.

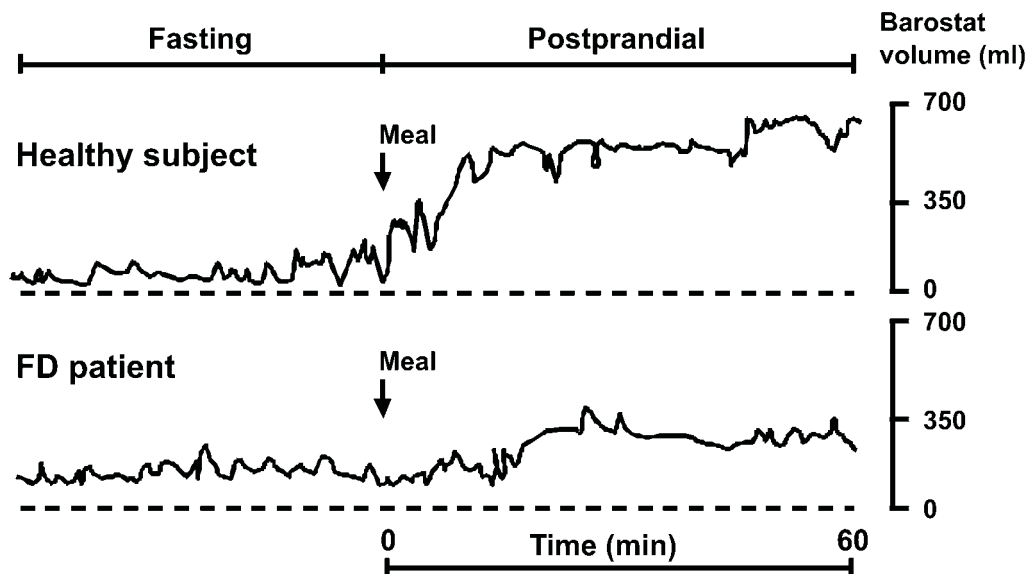


Figure 2.2 Mean intragastric volume as measured by a gastric barostat in a healthy subject and a FD patient before and after administration of liquid meal. Ingestion of the meal induced a rapid and sustained increase in intragastric volume in the healthy subject, reflecting a relaxation of the gastric fundus. The mean balloon volume, the average increase in intragastric volume, and the maximum volume increase after the meal, were all significantly less in the FD patient when compared with the healthy the subject ($P < 0.05$), indicating that the patient had an impaired gastric accommodation. Adapted from (Tack et al., 1998).

2.4.5 Dysfunction of the antrum

Antral dysfunction occurs in ~ 35 % of FD patients (Hausken and Berstad, 1992b), and there is some, albeit limited, evidence that this may contribute to the generation of symptoms in a subgroup of patients. Both fasting and postprandial antral volumes are larger in many FD patients when compared with healthy subjects (Hausken and Berstad, 1992b) (Figure 2.3).

NOTE: This figure is included on page 49 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2.3 Fasting and postprandial antral volume in healthy subjects (n = 13) and patients with FD (n = 13) quantified by two-dimensional ultrasound. A proportion (~ 50 %) of FD patients have greater fasting (* P < 0.05) and postprandial (** P < 0.01) antral sizes when compared with healthy subjects. Adapted from (Hausken and Berstad, 1992b).

Studies in healthy subjects suggest antral volume plays an important role in the regulation of appetite and energy intake (Jones et al., 1997, Santangelo et al., 1998, Hveem et al., 1996, Sturm et al., 2004). For example, the perception of postprandial fullness is strongly related to antral area after ingestion of a glucose drink (Jones et al., 1997) and a homogenised meal (Santangelo et al., 1998) in healthy subjects (see Chapter 1.3.2.3 and **Figure 1.6**). In addition, subsequent food intake is related inversely to antral size in healthy young and old subjects (Sturm et al., 2004) (see Chapter 1.3.2.3 and **Figure 1.7**). Abnormal intragastric filling may lead to increased distension of the distal stomach. As discussed, it has been suggested that increased distal gastric distension, secondary to changes in intragastric distribution, may contribute to symptoms in patients with FD (Gilja et al., 1996, Troncon et al., 1994). In healthy subjects, distal, but not proximal, gastric distension, apparently induce more dyspeptic symptoms (Ladabaum et al., 1998) - activation of mechano-receptors of the distal stomach by gastric distension induce more bloating and pain compared with the distension of the proximal stomach (Ladabaum et al., 1998). It can, therefore, be

speculated that abnormal distribution of food to a more sensitive distal stomach plays a role in symptom generation in at least some patients with FD. Taken together, these studies suggest that an increase in antral size may contribute to the development of gastrointestinal symptoms, “appetite-related” sensations and a decrease in energy intake. However, the effects of antral volume/area on dyspeptic symptoms and energy intake in patients with FD are unknown.

2.4.6 Integration of proximal and distal gastric function

As discussed above, studies evaluating the effects of proximal and distal stomach on symptom generation in FD have focussed on their separate contribution(s). However, their functions are known to be closely related, and, accordingly, assumptions as to the effects that modulation of the function of one compartment may have on the other often inappropriate (Troncon et al., 1994). The interrelationship between proximal and distal gastric function, and its contribution to symptoms, has been evaluated in a study by Caldarella and colleagues, with the hypothesis that a multiple sensory-reflex dysfunction affects both regions of the stomach and disrupts their coordinated functions, i.e. impaired gastric relaxation may increase antral distension, which is predominantly responsible for the symptoms (Caldarella et al., 2003). In this study antral distension was associated with impaired reflex relaxation of the fundus, and it was suggested that impaired fundic accommodation is associated with the transfer of an excessive volume of chyme into a hypersensitive antrum and, in this way, causes symptoms (Caldarella et al., 2003).

2.4.7 Visceral hypersensitivity

A more recent area of interest in the investigation of gastrointestinal factors underlying dyspeptic symptoms has been that of visceral hypersensitivity. As food is ingested, mechanoreceptors located in the gastric wall are stimulated, the extent of which is dependent on the amount eaten. When the meal empties to the small intestine, the chemical/nutritional composition of the meal assumes primary importance in the regulation of gastric emptying, stimulation of gastrointestinal hormones and suppression of hunger and subsequent food intake, as a result of exposure of receptors located in the small intestinal mucosa to nutrients. In patients with FD, these factors, i.e. luminal physiological stimuli, both mechanical and chemical, may be perceived as unpleasant, or painful. The effects of mechanical stimulation (distension) and small intestinal chemosensitivity (nutrient infusions) have both been investigated and shown to cause altered symptom perception in patients with FD as discussed below.

2.4.7.1 *Hypersensitivity to mechanical stimulation*

Approximately 35 - 48 % of patients with FD are hypersensitive to gastric distension. A number of independent groups demonstrated that patients with FD are more sensitive to mechanical stimulation (distension) of the stomach than healthy subjects (Bradette et al., 1991, Mearin et al., 1991, Lemann et al., 1991) - during gastric balloon/barostat distension, patients with FD report both first perception and discomfort at lower distension volumes or pressures than healthy controls (**Figure 2.4**). Hypersensitivity to mechanical distension is also evident in the distal stomach (Caldarella et al., 2003, Ladabaum et al., 1998) (see Chapter 2.4.5).

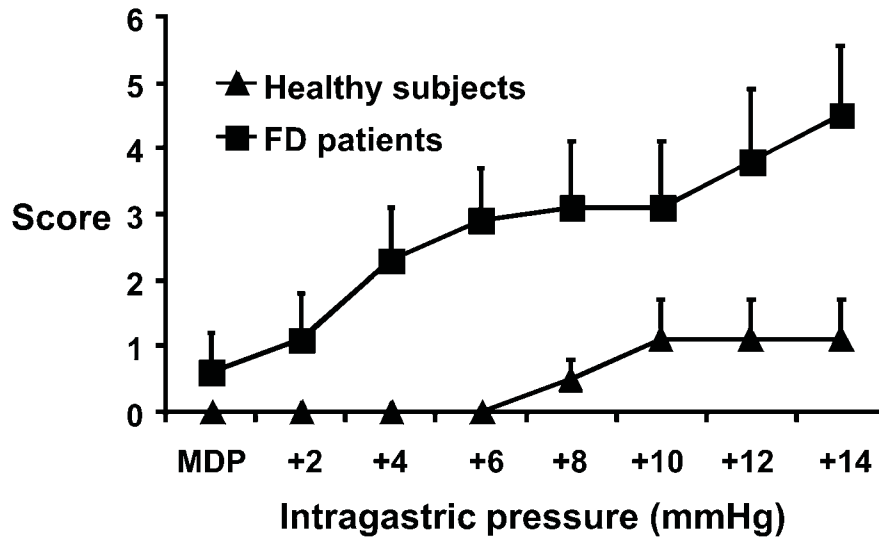


Figure 2.4 Intensity of abdominal symptoms during stepwise distension of the proximal stomach in patients with FD (n = 10) and healthy subjects (n = 10). FD patients reported greater scores of discomfort at lower pressures than healthy controls. Adapted from (Mearin et al., 1991).

2.4.7.2 *Hypersensitivity to nutrient stimulation*

Reports of FD patients that their symptoms are induced, or exacerbated, by food, has led to the hypothesis that a meal has the capacity to induce symptoms indirectly, by inducing abnormal gastrointestinal motor function. There has, however, been little interest in the possible, direct, role of gastrointestinal hypersensitivity to nutrients, as a pathophysiological mechanism. Although the number of studies is small, it appears that 56 - 100 % of FD patients, particularly women (Houghton et al., 1993), are hypersensitive to oral and/or small intestinal fat, as will be discussed in Chapter 2.5.3.

2.5 **ROLE OF DIET**

The majority of studies investigating the pathophysiology of FD have focussed on the role of gastrointestinal motor/sensory dysfunction and have largely ignored the potential effect of food intake and individual macronutrients on dyspeptic symptoms.

2.5.1 Food intolerance

Patients with FD frequently report that their symptoms are related to, or exacerbated by, food, particularly “rich” and fatty foods (Taggart and Billington, 1966, Kaess et al., 1988). There is also evidence that patients with FD identify the foods which they believe aggravate their symptoms, and, therefore, avoid eating them (Kearney et al., 1989). Attempts have also been made to identify specific foods which induce symptoms: onions, peppers, citrus fruits, spices, as well as fatty foods have all been reported to induce, or exacerbate, symptoms in FD (Kearney et al., 1989). In another study FD patients reported to be intolerant of mayonnaise (80 % of patients), nuts (70 % of patients), fish (60 % of patients) and chocolate (62 % of patients); while the aim of this study was to assess overall dietary behaviour and not intolerance to specific macronutrients, it is interesting to note that three of these four foods have a high fat content (Kaess et al., 1988). The results of these studies are consistent with acute studies which have identified a role for dietary fat in the induction of symptoms in FD (Barbera et al., 1995b, Barbera et al., 1995a, Feinle et al., 1997). Studies assessing the temporal relationship between the ingestion of specific foods and the occurrence and severity of dyspeptic symptoms in patients with FD are, however, lacking.

2.5.2 Patterns of food ingestion

Very few studies have, to date, investigated whether eating patterns, such as meal frequency, size and composition, and total energy intake, differ between FD patients and healthy subjects (Cuperus et al., 1996, Mullan et al., 1994). Snacking was reported to be greater (by about 9 %), and the number of larger meals lower (by about 24 %) in FD patients compared with healthy subjects, although these differences were not

statistically significant (Mullan et al., 1994). This study also reported that only 55 % of patients ate three meals per day compared with 80 % of healthy subjects. In contrast, a study using food frequency diaries reported no difference in eating patterns, such as breakfast intake, time spent fasting, meal size, and eating speed between FD patients and healthy controls (Cuperus et al., 1996). The inconsistencies between these two studies are likely to reflect discrepancies in the techniques used to assess food intake and symptoms; and in these studies, the latter were not evaluated concurrently. Therefore, the impact of eating patterns in patients with FD on, and its contribution to the development of, dyspeptic symptoms remains uncertain. No studies have evaluated the effect of dietary modification, presumably the reduction of dietary fat content on, dyspeptic symptoms. Investigations into the effects of different diet regimes are needed to establish their effects on the improvement of dyspeptic symptoms in FD.

2.5.3 Influence of individual macronutrients on symptoms

Considering the reports by FD patients that fatty foods exacerbate dyspeptic symptoms, it is not surprising that a number of laboratory-based studies have investigated the effects of individual macronutrients, especially fat, on gastric function, food intake, and their potential role in symptom generation. The incorporation of fat into a soup meal has been shown to induce more dyspeptic symptoms in FD patients compared to a bland soup, when no fat was added (Houghton et al., 1993). Other studies have reported that intraduodenal infusion of lipid, but not glucose, exacerbates dyspeptic symptoms in FD patients, both before and during gastric distension, when compared with healthy subjects (Barbera et al., 1995b, Barbera et al., 1995a, Feinle et al., 1997) (**Figure 2.5**).

The effect of protein on the generation of symptoms in FD patients, has not been assessed.

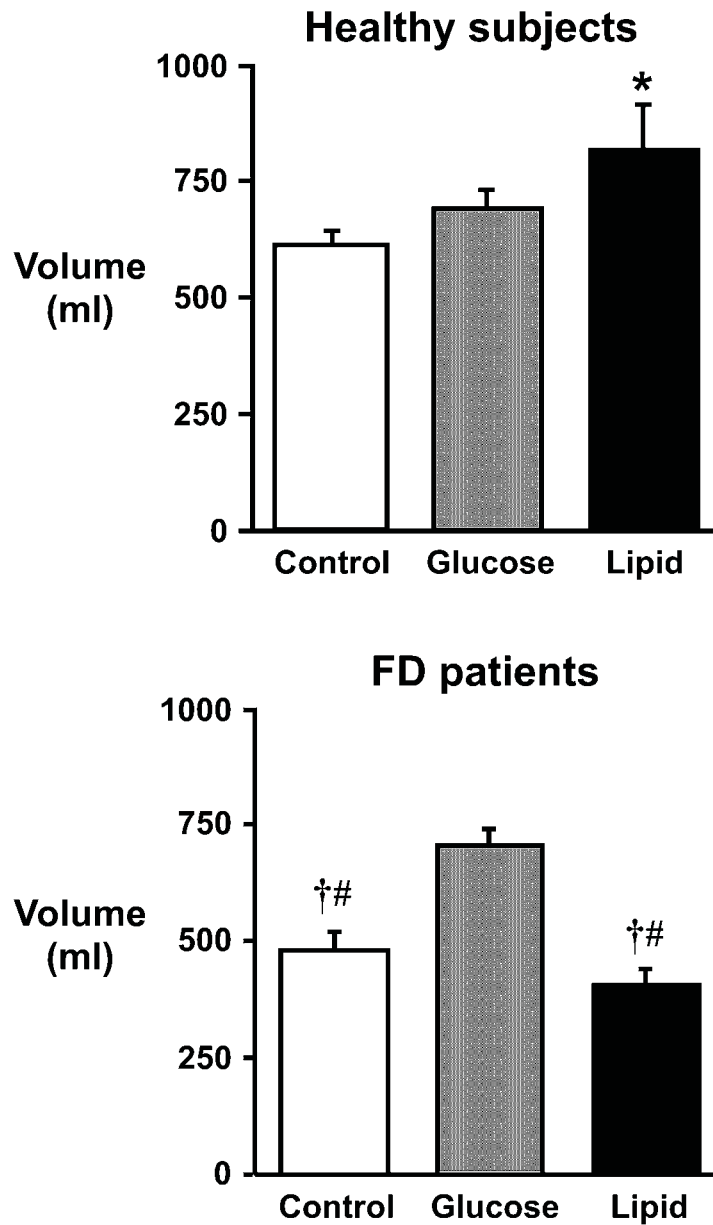


Figure 2.5 The volume at which discomfort was perceived during gastric distension and intraduodenal infusions of saline (control), glucose or lipid in healthy subjects ($n = 9$) (top) and FD patients ($n = 9$) (bottom). In healthy subjects, discomfort was perceived at higher volumes when lipid was infused into the duodenum compared with saline (* vs. saline; $P < 0.05$). In FD patients, the gastric volumes at which discomfort occurred were lower during the saline and lipid infusions when compared with glucose († vs. glucose; $P < 0.05$). In addition, in FD patients discomfort was perceived at lower volumes during the saline and lipid infusions when compared with healthy subjects (# vs. healthy subjects; $P < 0.001$). Adapted from (Barbera et al., 1995b).

A further study showed that infusion of lipid into the small intestine increased gastric compliance and reduced gastric phasic contractility in both FD patients and healthy subject (Barbera et al., 1995a). However, lipid increased the mechano-sensitivity of the stomach in FD patients, so that higher scores of abdominal discomfort were perceived. In addition increasing the caloric rate of lipid administration (i.e. 1 vs. 2 kcal/min) increased the severity of symptoms, without a further change in gastric relaxation (Feinle et al., 2001a). Accordingly, these observations suggest that input from the small intestine plays an important, and direct, role, which may reflect altered visceral perception, rather than a primary gastric motor dysfunction, in the induction of symptoms. The above studies have, however, only investigated the effects of liquid meals or intraduodenal infusion on symptoms, and no studies have evaluated the effects of orally ingested semi-solid meals containing a high proportion of fat or carbohydrate on symptoms.

The effects of fat in inducing symptoms in FD may be attenuated by inhibiting fat digestion. For example, in healthy subjects, acute inhibition of fat digestion by orlistat, reduced the intensity of the “dyspeptic symptoms”, fullness, nausea and bloating induced by gastric distension and duodenal lipid infusion (Feinle et al., 2001b) (see Chapter 1.3.2.2.1 and **Figure 1.5**). These observations, support the concept that orlistat (THL) may have a therapeutic role in reducing “meal-associated” dyspeptic symptoms after the ingestion of fat in FD. – However, orlistat is associated with a number of side-effects in the gut, such as fatty stools, diarrhoea and flatulence due to its inhibition of fat digestion, therefore this treatment for patients with digestive symptoms is unideal.

2.6 GASTROINTESTINAL HORMONE RELEASE AND SUPPRESSION

Gastrointestinal hormones may potentially mediate the effects of nutrients, particularly fat, on dyspeptic symptoms. The limited number of studies which have investigated the effects of gastrointestinal hormones on symptom induction FD, have indicated a role of CCK. An initial study in healthy subjects reported that intravenous CCK-8 induced nausea when given at high doses (Miaskiewicz et al., 1989). A further study reported that CCK-8 infusion resulted in dyspeptic symptoms, including; abdominal bloating, nausea and fullness, which were greater in patients with FD than healthy subjects (Chua et al., 1994). However, it was not clarified how symptoms were assessed in this study, and the high dose of CCK-8 would have resulted in supraphysiological plasma levels (MacIntosh et al., 2001b). The most convincing evidence that there is a role for CCK in the induction of dyspeptic symptoms, is derived from one study, which used the CCK1 receptor antagonist, dexloxiglumide (Feinle et al., 2001a). In this study symptoms of fullness, bloating and nausea, induced by duodenal lipid infusion, were reduced by concurrent administration of dexloxiglumide in patients with FD (Feinle et al., 2001a). Since data from a small number of FD patients indicate that CCK secretion in response to duodenal lipid does not differ between FD patients and healthy subjects (Feinle et al., 2001a), these observations suggest that FD may be associated with an increased sensitivity to CCK, however, this has not been evaluated.

The effects of GLP-1 and PYY on appetite suppression and gastric motor function are similar to those of CCK, suggesting that these peptides may play a role in the induction of dyspeptic symptoms. Intravenous infusion of increasing doses of synthetic human GLP-1 and PYY dose-dependently reduce energy intake (Gutzwiller et al., 1999b,

Batterham et al., 2002), and induce nausea (Verdich et al., 2001a, Degen et al., 2001), in healthy subjects. The effects of intravenous GLP-1 and PYY on dyspeptic symptoms and food intake have not been evaluated in FD. The role of GLP-1 may also be clarified by assessing the effects of its receptor antagonist, exendin(9-39) (Turton et al., 1996). A receptor antagonist, for PYY, that is suitable for use in humans is not currently available.

It has been reported that fasting ghrelin concentrations (active and total) are greater in patients with FD when compared with healthy subjects (Lanzini et al., 2006, Nishizawa et al., 2006), however, this result conflicts with an earlier study which showed no difference in fasting ghrelin concentrations (Shinomiya et al., 2005). Unlike CCK, GLP-1 and PYY, ghrelin is suppressed by enteral nutrients (Cummings et al., 2001). The potential effect of nutrient-induced suppression of ghrelin on symptoms in patients with FD has not yet been investigated.

2.7 COGNITIVE INFLUENCES

There is evidence from a small number of studies that cognitive factors contribute to the development of symptoms after food ingestion in FD. Cognitive factors influence eating behaviour in healthy subjects (Cecil et al., 1998). For example a nutrient-containing soup induces greater perceptions of fullness when subjects are informed of the nature of the soup when compared with the control condition, in which the subjects are not explicitly given this information (Cecil et al., 1998). The route by which a meal is introduced i.e. orally vs. intragastrically, also influences symptoms. For example, when a high-fat soup was given orally, greater sensations of fullness resulted when

compared to the same high-fat meal given intragastrically. Intragastric administration of the meal may have by-passed cognitive influences and oro-sensory mechanisms (such as sight, smell and taste) which may modulate appetite sensations (Cecil et al., 1999). It is, therefore, conceivable that symptoms in patients with FD in response to certain foods may be influenced by previous negative learning experiences or information they received. A recent study evaluated whether information given about the fat content of an appetising yoghurt would influence symptom development in patients with FD (Feinle-Bisset et al., 2003). When subjects were given the high-fat yoghurts, scores of bloating and fullness increased regardless on the information they received about the nature of the yoghurt. More interestingly, when the patients were given the low-fat yoghurt and told it was high-fat, symptom scores were similar to those of the high-fat yoghurt, indicating that foods low in fat may have the capacity to exacerbate symptoms, when patients with FD believe that the food is high in fat (Feinle-Bisset et al., 2003) (**Figure 2.6**).

It is also interesting that the placebo arms of clinical trials in FD patients show a strikingly high response rate, of the order of 30 - 50 % (Talley and Phillips, 1988). The mechanism of the apparent therapeutic benefit of placebo in patients with FD, i.e. if the patient believes in something, this appears to affect symptoms, remains unclear. Paying attention to the gut may also magnify perceptions of abdominal symptoms in patients with FD (Accarino et al., 1997). For example, perception of gastric distension is increased when the patients anticipate the distension. In contrast, distraction during gut distension, by performing a mental task (Accarino et al., 1997) or by applying a painless somatic stimulus (Coffin et al., 1994), decreases perception of gastric distension. It has been speculated that the activation of the endogenous opioid-

mediated analgesia system, reduction of anxiety and modification in patient expectations may be relevant (Mearin et al., 1999).

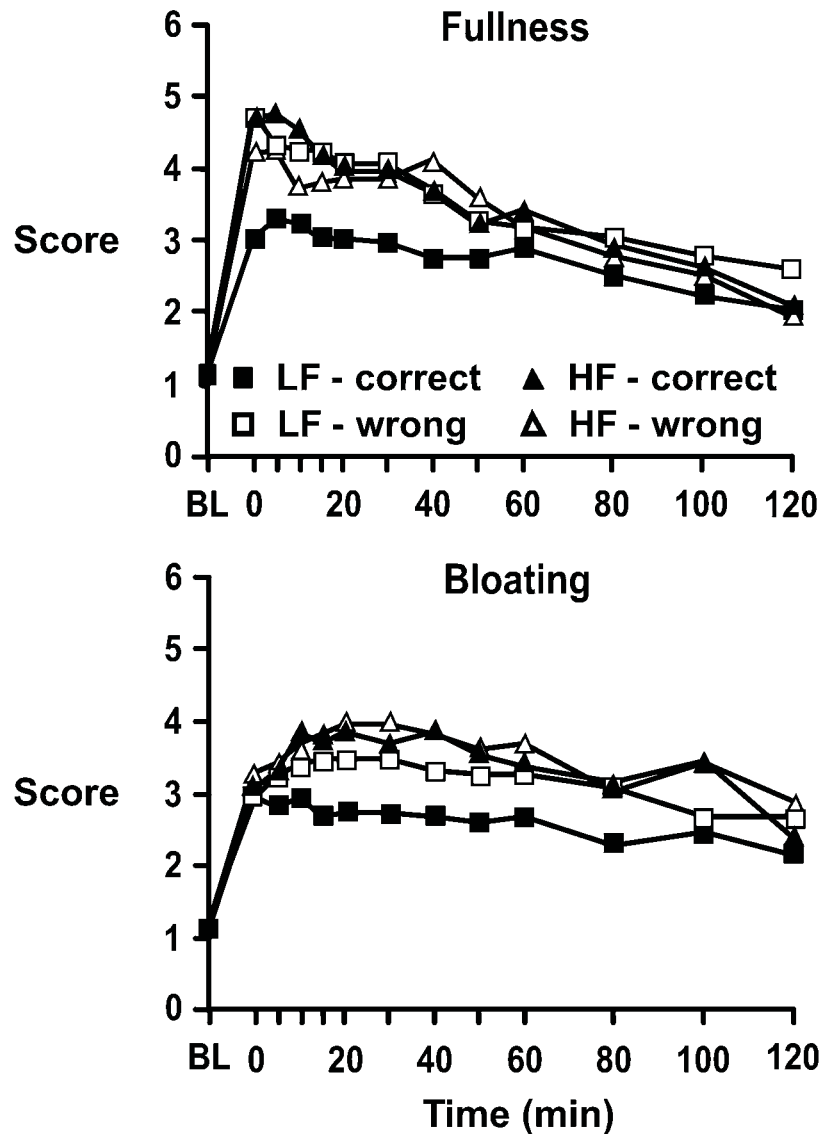


Figure 2.6 Effect of cognitive factors on fullness (top) and bloating (bottom) after high-fat or low-fat yoghurts in FD patients (n = 15). The high-fat and low-fat yoghurts were consumed by FD patients on four occasions. On one occasion the patient was given a high-fat yoghurt and informed that it was high-fat (HF - correct) and on the other occasion, a high-fat yoghurt and informed that it was low-fat (HF- wrong). The same applied to the low-fat yoghurts (LF - correct and LF - wrong). Scores for fullness and bloating increased significantly on both occasions when the high-fat yoghurt was given, regardless of the information patients received about the nature of the yoghurt, compared to the low-fat yoghurt (fullness; $P < 0.05$, bloating; $P < 0.05$). On the occasion when the low-fat yoghurt was given and patients were informed that it was high-fat (LF - wrong), scores of bloating and fullness were similar to those after the high-fat yoghurt and significantly greater than the low-fat yoghurt (fullness; $P < 0.05$, bloating; $P = 0.05$). Adapted from (Feinle-Bisset et al., 2003).

2.8 SUMMARY

This chapter has discussed the potential factors, including gastric emptying, intragastric distribution, the proximal and distal stomach, visceral hypersensitivity, diet and food intake, gastrointestinal hormones and cognitive factors, responsible for symptoms in FD. Many studies evaluating dietary behaviour and food intake in FD are inconclusive, or have failed to establish a meaningful association between the occurrence of symptoms and disturbances in gastric motor function. However, a small number of laboratory-based, acute studies and anecdotal reports from FD patients indicate that dietary fat may be an important factor in the induction of dyspeptic symptoms, and this factor has largely been ignored. A study evaluating the comparative effects of oral carbohydrate and fat on gastrointestinal symptoms, gastrointestinal hormones and antral area in FD is described in Chapter 8, with the hypothesis that high-fat, but not high-carbohydrate, meals will induce dyspeptic symptoms and that the latter will be related to changes in gut hormone responses and antral area. Chapter 9 reports a study which evaluated the relationship between symptom occurrence and severity and dietary habits using a 7-day diet/symptom diary, with the hypothesis that patients with FD would consume smaller meals, experience more meal-associated symptoms, eat more frequently and that symptoms would be related to the amount of ingested fat when compared with healthy individuals.

Chapter 3

TREATMENT OPTIONS FOR FUNCTIONAL DYSPEPSIA

3.1. INTRODUCTION

A number of different therapies have been, or are being, developed for the treatment of FD. The rationale for the development of these therapies is based on normalising the mechanisms that are disturbed and believed to contribute to the development of symptoms. As outlined in Chapter 2, the pathophysiology of FD is poorly defined, therefore, it is not surprising that the therapies used to treat the symptoms in FD have produced poor, and variable, outcomes. Furthermore, because FD is a heterogenous disease, as discussed in Chapter 2, a uniform response to drug treatment is unlikely to be achieved. This chapter reviews present and emerging therapies for the treatment of FD, including, prokinetic drugs that accelerate gastric emptying, drugs designed to modulate proximal gastric relaxation and gastroduodenal sensory function, suppress acid secretion, *H pylori* eradication, antidepressants, as well as non-pharmacological treatments, including acupuncture and hypnotherapy, and herbal medications (**Table 3.1**).

Table 3.1 Common therapies used for the treatment of symptoms in functional dyspepsia.

<i>PHARMACOLOGICAL</i>	
Prokinetics (3.2.1)	Cisapride Dopaminergic receptor blockers (Metoclopramide, domperidone, itopride) Motilin Agonists (erythromycin, ABT-229)
Fundus relaxing agents (3.2.2)	Sumatriptan
Antisecretory (3.2.3)	Histamine type-2 receptor antagonists (cimetidine, ranitidine) Proton pump inhibitors (omeprazole)
Antacids (3.2.3)	
Helicobacter pylori eradication (3.2.4)	
Antidepressants (3.2.5)	Tricyclic antidepressants Serotonin reuptake inhibitors
<i>NON PHARMALOGICIAL</i>	
Acupuncture (3.3.1)	
Hypnotherapy (3.3.2)	
<i>HERBAL PREPARATIONS</i>	
Mono-therapies (3.4.2)	Banana Artichoke leaf extract Tumeric Capsaicin
Combination preparations (3.4.3 and 3.3.4)	Peppermint/caraway oil Lomatol® Peppermint oil/ginger Iberogast®

3.2 COMMON PHARMACOTHERAPIES USED FOR THE TREATMENT OF FUNCTIONAL DYSPEPSIA

3.2.1 Prokinetics

Prokinetic drugs accelerate gastric emptying by modulating gastrointestinal motility, through acting on receptors in the gut that enhance the release of acetylcholine, dopamine, motilin and/or serotonin. Given that a subgroup of patients with FD have delayed gastric emptying the use of prokinetics has been suggested to be beneficial in the improvement of symptoms. However, the mechanism(s) by which these drugs reduce symptoms are unknown, particularly as the relationship between acceleration of gastric emptying and symptom improvement is inconsistent.

3.2.1.1 *Cisapride*

Cisapride stimulates motility of the digestive tract by selectively enhancing acetylcholine release in the myenteric plexus of the gut, probably via acting as a partial serotonin agonist on the 5-HT₄ receptor (McCallum et al., 1988). Cisapride has been shown to increase lower oesophageal sphincter tone, increase oesophageal motility and accelerate both gastric emptying and small intestinal transit (Ceccatelli et al., 1988, Gilbert et al., 1987, Madsen, 1990, Smout et al., 1985). In addition to its effects on gastric motility, cisapride has also been shown to improve dyspeptic symptoms. As described in a recent meta-analysis, cisapride, when given at doses between 5 - 20 mg t.i.d for 2 - 6 weeks, improved global symptom scores and scores for individual symptoms, including, epigastric pain, early satiety, discomfort, abdominal distension and nausea, when compared with placebo (Veldhuyzen van Zanten et al., 2001).

However, as suggested by this meta-analysis, these studies need to be interpreted with caution, due to suboptimal study design (i.e. inconsistent symptom assessment), unclear/inconsistent inclusion criteria (e.g. inclusion of patients with gastroesophageal reflux disease), short duration of follow-up, and only a modest improvement of symptoms (Veldhuyzen van Zanten et al., 2001).

Only two studies have, hitherto, assessed whether the improvement of symptoms is related to the acceleration of gastric emptying by cisapride (Kellow et al., 1995, Jian et al., 1989). Kellow and colleagues evaluated solid and liquid gastric emptying before a 4 week treatment of cisapride, and, surprisingly, found that bloating improved in the patients with normal, but not those with delayed, gastric emptying (Kellow et al., 1995), and an equal reduction of total symptom scores for both cisapride and placebo. However, this study did not measure gastric emptying concomitantly during treatment with cisapride and evaluation of symptoms, and, therefore, the relationship between gastric emptying and symptoms generation was not assessed optimally. Jian and colleagues demonstrated an improvement in solid and liquid gastric emptying, which correlated with an improvement in symptoms, however, the total number of patients with delayed gastric emptying was very small, and in this group, symptom reduction did not reach statistical significance (Jian et al., 1989). Taken together, these studies suggest that while delayed gastric emptying is evident in FD patients, it is probably not the primary cause of their symptoms (see Chapter 2.4.2), as those with normal gastric emptying benefit, at least as much, from the use of cisapride. A major disadvantage in the use of cisapride is that it has interactions with other drugs and serious, albeit rare, cardiac side effects with prolonged QT times and ventricular tachycardia. This has led

to its withdrawal from the market in the USA, and markedly limited availability in other countries. Hence, the use of cisapride cannot be recommended.

3.2.1.2 Dopaminergic receptor blockers

Receptors for the ligand, dopamine, are located both centrally, in the brain (Vallone et al., 2000), and peripherally, predominantly in the gastric and duodenal mucosa (Hernandez et al., 1987). Dopamine reduces gastric tone and intragastric pressure and inhibition of antroduodenal contractions, partly through the activation of dopamine D₂ receptors (Willems et al., 1985). Blockade of these results would, therefore, result in a gastroprokinetic effect.

3.2.1.2.1 Metoclopramide

Metoclopramide acts both centrally and peripherally with different effects and mechanisms. The central effect of metoclopramide result in a decrease in nausea via blockade of the dopamine (D₂), and 5-HT₃, receptors, whereas the peripheral effects enhance gastric motility by its agonistic action on the 5-HT₄ receptor and increase in acetylcholine release. Only a small number of studies have evaluated the efficacy of metoclopramide and concluded that it improves symptoms in FD, however the magnitude of improvement is modest (Johnson, 1971, Perkel et al., 1979, Archimandritis et al., 1992, Fumagalli and Hammer, 1994). To date, no studies have evaluated the relationships between the effects of metoclopramide on changes in gastric motility/emptying and symptoms. Due to the ability of metoclopramide to cross the blood brain barrier, there is a high prevalence of unwanted central nervous system effects including drowsiness, anxiety and extrapyramidal symptoms (Talley, 1995).

Hence, although efficacy has been demonstrated, its unfavourable side-effect profile generally makes it unsuitable for long-term use.

3.2.1.2.2. Domperidone

Domperidone is a peripheral dopamine (D₂) antagonist, and its actions include the stimulation of oesophageal, gastric and small intestinal motility (Weihrauch et al., 1979). Domperidone has also been shown to improve symptoms in FD patients when given at doses between 10 mg t.i.d - 10 mg q.i.d for 2 - 4 weeks compared with placebo (Veldhuyzen van Zanten et al., 2001). Only two studies have observed both, an acceleration in gastric emptying, and improvement symptoms using domperidone. Sarin and colleagues reported that the rate of gastric emptying of a potato curry meal was faster after 2 weeks of 10 mg domperidone t.d.s, compared with placebo in 44 FD patients. In this study there was also an improvement in symptoms of belching, fullness, postprandial abdominal distension and heartburn after domperidone (Sarin et al., 1986). In the second study, Duan and colleagues reported that domperidone, in a dose of 20 mg t.i.d for 7 days, accelerated gastric emptying of a “Chinese” meal as measured by real-time ultrasonography and improved symptoms of bloating and early satiety in 29 FD patients with delayed gastric emptying (Duan et al., 1993). These two studies, however, did not indicate whether there was relationship between the acceleration in gastric emptying and symptom improvement by domperidone.

In female FD patients, domperidone increased the volumes at which symptoms were perceived during distension of the proximal stomach, indicating that domperidone may also act by altering visceral hypersensitivity (Bradette et al., 1991).

Domperidone does not cross the blood brain barrier in significant quantities, hence central side-effects are unusual, and is arguably a more appropriate prokinetic than metoclopramide, however, it is not licensed in the USA. Reported adverse effects of domperidone include galactorrhoea and breast tenderness from hyperprolactinaemia and extra-pyramidal movement disorders (Tonini et al., 2004).

3.2.1.2.3 Itopride

Itopride, a dopamine (D₂) antagonist with acetylcholinesterase inhibitory actions, has been reported to stimulate gastric motility in dogs (Iwanaga et al., 1996), accelerate gastric emptying in patients with chronic gastritis (Harasawa and Miwa, 1993), and reduce total postprandial gastric volume, without affecting gastric emptying in healthy humans (Choung et al., 2007). Itopride hydrochloride has also been reported to increase plasma levels of somatostatin and motilin-immunoreactive substances and decrease plasma levels of CCK-immunoreactive substances or adrenocorticotrophic hormone-reactive substances under stress in healthy humans (Katagiri et al., 2006). These actions may prove beneficial in FD patients with impaired proximal gastric relaxation. The effect of itopride on symptom improvement in FD has produced conflicting results (Holtmann et al., 2006, Talley et al., 2007). Holtmann and colleagues evaluated the effects of itopride at 50, 100 and 200 mg t.i.d for 8 weeks in FD patients and reported that all doses improved symptom scores and quality of life when compared with placebo (Holtmann et al., 2006). However, a more recent study indicated that itopride at 100 mg t.i.d for 8 weeks, was not superior to placebo for the treatment of FD (Talley et al., 2007). Differences in the two studies may include heterogeneity of FD, patient selection, suboptimal evaluation of symptom intensities or lack of a true efficacy of the

drug (Talley et al., 2007). The relationship between the acceleration in gastric motility and associated reduction in symptoms has not been evaluated.

3.2.1.3 *Motilin agonists*

Motilin is a 22 amino acid peptide hormone expressed throughout the gastrointestinal tract, which acts to increase smooth muscle contractions, therefore, enhancing gastric emptying, increasing antral contractions and antroduodenal coordination, but decreasing fundic volume and compliance (Feighner et al., 1999, Galligan and Vanner, 2005). The antibiotic, erythromycin, is a motilin agonist, and has been shown to accelerate gastric emptying in healthy subjects, as well as patients with diabetic or post-vagotomy gastroparesis (Peeters, 1993, Kendall et al., 1997, DiBaise and Quigley, 1999). In patients with FD and delayed gastric emptying, treatment with intravenous erythromycin enhanced both liquid and solid emptying, when compared with intravenous saline. In this study, treatment with erythromycin only improved meal-induced bloating, but had no effect on postprandial fullness, epigastric pain, early satiety, nausea, vomiting, belching or epigastric burning (Arts et al., 2005). Another potent motilin agonist, ABT-229, failed to relieve symptoms in patients with or without delayed gastric emptying, after 4 weeks of treatment at any of four doses (1.25, 2.5, 5 and 10 mg b.d.) compared with placebo (Talley et al., 2000). Results from these studies suggest that the therapeutic effect of the currently available motilin receptor agonists in the treatment of FD is modest, at best.

3.2.2 Fundus-relaxing agents

While prokinetics accelerate gastric emptying, which physiologically is associated with an increase in the tonus of the proximal stomach, fundus-relaxing agents decrease the tone of the fundus, thus, causing proximal gastric relaxation. As discussed in Chapter 2.4.4, impaired proximal gastric relaxation/accommodation is evident in a substantial subgroup of patients with FD (Gilja et al., 1996, Tack et al., 1998, Feinle et al., 2001a, Kim et al., 2001), and, therefore, normalisation of this impairment, with the use of fundus-relaxing agents, may prove beneficial in the relief of symptoms. Treatment with sumatriptan, a 5-HT₁ receptor agonist, has produced conflicting results. Sumatriptan, relaxes the gastric fundus and reduces pain perception in response to gastric distension in healthy volunteers (Tack et al., 2000). In patients with FD, restoration of proximal gastric relaxation with sumatriptan was associated with the ability to consume a greater volume of a nutrient-containing liquid (Tack et al., 1998). In contrast, a more recent study failed to establish any relationship between the restoration of proximal gastric relaxation with sumatriptan and the amount of water or nutrient drink ingested by FD patients when compared with healthy subjects (Boeckxstaens et al., 2002). To add to the difficulties in interpreting this information, sumatriptan is known to also increase antral volume (Vingerhagen et al., 2000) and slow gastric emptying (Coulie et al., 1997, Houghton et al., 1992) in healthy subjects; as given that increased antral volume and delayed gastric emptying occur frequently in patients with FD, improvement in symptoms may potentially only occur in patients without these abnormalities.

Sumatriptan has also been reported to relieve symptoms of vomiting and nausea in patients with cyclic vomiting (Benson et al., 1995). It is possible that sumatriptan may

be effective in the treatment of these specific symptoms, if they are dominant in patients with FD, however, this has not been evaluated.

3.2.3 Antisecretory drugs and antacids

Although at most, only a small number of patients with FD have mild to moderate hypersensitivity to, or hypersecretion of, gastric acid (Collen and Loebenberg, 1989), acid suppression treatment is frequently used in an attempt to relieve symptoms in FD. There are two approaches for gastric acid suppression, antisecretory drugs (histamine type 2 receptor antagonists and proton pump inhibitors) and antacids. A number of studies have evaluated the effects of antisecretory drugs and antacids in the treatment of FD, and the magnitude of symptom improvement has been comparable to that of placebo.

3.2.3.1 Histamine type 2 receptor antagonists

Histamine type 2 receptor antagonists block the action of histamine on the parietal cells in the stomach, thereby decreasing acid production. The use of histamine type 2 receptor antagonists for symptom relief in FD has produced conflicting results. Agents such as cimetidine and ranitidine were commonly prescribed histamine type 2 receptor antagonists in the 1980's, and controlled studies have suggested that their therapeutic effect is both positive (Talley et al., 1986b, Saunders et al., 1986) and negative (Nyren et al., 1986, Olubuyide et al., 1986), possibly as a result of different subject selection criteria. For example, those studies which included FD patients without acid-predominant symptoms, failed to show any clear benefits. Furthermore, in a meta-analysis, histamine type 2 receptor antagonists improved the symptom of epigastric

pain, but not global symptoms, in FD (Redstone et al., 2001). Therefore, patients with “ulcer-like” dyspepsia or reflux disease, in whom epigastric pain is the predominant symptom, are more likely to respond to this treatment. Histamine type 2 receptor antagonists are relatively inexpensive, well tolerated and have few adverse effects, however, are not a cost-effective treatment because their overall effect on symptoms is negligible.

3.2.3.2 *Proton pump inhibitors*

Proton pump inhibitors block the secretion of hydrogen ions in the gastric lumen and inhibit acid secretion and are more potent inhibitors of acid secretion than histamine type 2 receptor antagonists. Two large, randomised, double-blind, placebo-controlled studies, known as the BOND and OPERA studies, assessed the effects of the proton pump inhibitor, omeprazole (10 or 20 mg), for 4 weeks in 1262 FD patients (Talley et al., 1998). Complete symptom relief was observed in 38 % of patients on 20 mg omeprazole, compared with 36 % on 10 mg omeprazole and 28 % on placebo. It is important, however, to note the high response of FD patients to placebo (see Chapter 2.7). The BOND and OPERA studies demonstrated that the response to proton pump inhibitors was greater in patients with “ulcer-like” and “reflux-like” dyspepsia, respectively - in 40 % and 54 % of the 20 mg omeprazole group ($P = 0.05$) and in 35 % and 45 % in the 10 mg omeprazole group ($P = 0.08$ and $P = 0.05$, respectively), compared with 27 % and 23 % in the placebo group. In contrast, in patients with “dysmotility-like” dyspepsia, there was no significant benefit of omeprazole on symptoms in either 10 mg (32 %) or 20 mg (37 %) over placebo (31 %) (Talley et al.,

1998). This indicates that acid suppression therapy has no benefit in patients with symptoms relating to “dysmotility-like” FD, only “ulcer-like” FD.

3.2.3.3 *Antacids*

Antacids are commonly used, inexpensive, over-the-counter medications, which patients tend to purchase before they visit their doctor. To date, there are only four studies which have assessed the efficacy of antacids (Gotthard et al., 1988, Nyren et al., 1986, Norrelund et al., 1980, Weberg and Berstad, 1988), and all have failed to demonstrate a significant improvement in dyspeptic symptoms compared with placebo. Therefore, there is no evidence that antacids have any beneficial therapeutic effects in FD. This is not surprising given their modest effect on gastric acidity when administered in conventional doses.

3.2.4 *Helicobacter pylori eradication*

H pylori infection is the most important cause of peptic ulcers (NIH Consensus Conference, 1994). The role of *H pylori* infection and eradication in FD, however, remains controversial. Two recent trials have concluded that *H pylori* eradication improves quality of life (Suzuki et al., 2005) and provides symptom relief (Malfertheiner et al., 2003) in FD. A recent meta-analysis and Cochrane Database systematic review showed that there is a small, but significant, benefit of *H pylori* reduction in patients with FD (Moayyedi et al., 2005). Other studies, however, have failed to demonstrate any benefit (Veldhuyzen van Zanten et al., 2003, Greenberg and Cello, 1999). Another meta-analysis has indicated that differences in the definition of FD among studies may have influenced study outcomes (Laine et al., 2001). When

patients with heartburn as the predominant symptom were excluded, there was no evidence of benefit with *H pylori* therapy (Laine et al., 2001). *H pylori* eradication therapy has also been associated with the development of gastro-oesophageal reflux (Labenz et al., 1997). Based on current evidence this therapy is not recommended for FD.

3.2.5 Tricyclic antidepressants

The use of antidepressants for the treatment of symptoms in FD has been employed with the rationale that the brain-gut connection is the mechanism that links the psycho-emotional state with gastrointestinal dysfunction (Wood et al., 1999). For example, stress is known to affect the gastrointestinal tract by stimulating the release of neuropeptides and neurotransmitters, triggering various gastrointestinal responses (Wood et al., 1999, McMillin et al., 1999). When tricyclic antidepressants are used to treat FD patients, lower doses are necessary than when treating depression. In a small, randomised trial, the tricyclic antidepressant, amitriptyline, was found to be more effective in improving symptoms compared with placebo in a small number of FD patients (n = 7) after 4 weeks of treatment (Mertz et al., 1998a). The clinical benefit of amitriptyline has been found not to correlate with changes in perception of gastric balloon distension, suggesting that the analgesic effect is likely to be mediated centrally, perhaps through effects on the cortical processing of painful visceral sensations (Fioramonti and Bueno, 2002, Gorelick et al., 1998). In contrast, another randomised trial which evaluated the efficacy of the tricyclic antidepressant, desipramine, demonstrated only a slight benefit of desipramine over placebo in the improvement of symptoms (desipramine: 60 % vs. placebo: 47 %) (Drossman et al., 2003) - however 28

% of patients did not complete the trial due to adverse side effects, or were non-compliant. The most frequent adverse effects of desipramine relate to the anticholinergic and antihistaminic effects of the drug, i.e. dry mouth, sleep disturbances, dizziness and constipation. When the patients who did not complete the study, and those who were non-compliant, were excluded from the analysis, the desipramine group demonstrated a greater efficacy than placebo (desipramine: 73 % vs. placebo: 49 %). This study confirms that not all patients are able to tolerate antidepressants, but indicate that those who can are likely to experience symptomatic benefit (Drossman et al., 2003). It is important to note that the relief of symptoms by antidepressants is apparently not due to their antidepressant effects, given that appear to be just as effective in patients without psychiatric disorders and psycho-morbidity.

Selective serotonin reuptake inhibitors are another form of antidepressant; they increase the availability of 5-HT released from synapses in the central and enteric nervous systems by blocking its transporting protein. To date, there have been no randomised, placebo-controlled trials, which have evaluated the effect of selective serotonin reuptake inhibitors in FD. A recent study (Tack et al., 2003) indicates that the selective serotonin reuptake inhibitor, paroxetine, alters gastric accommodation in healthy subjects, and that this drug warrants further investigation in patients with FD.

The use of antidepressants has an attached social stigma and, in some cases, a narrow therapeutic window, hence, their use should be reserved for patients with persistent severe symptoms who have failed to improve with other forms of therapy.

3.3 NON-PHARMACOLOGICAL THERAPY AND ALTERNATIVE MEDICINES

Besides the well characterised pharmaceuticals which are commonly prescribed, a number of non-pharmacological therapies, such as acupuncture and hypnotherapy, as well as alternative medicines, such as herbal preparations, have been employed in the treatment of FD.

3.3.1 Acupuncture

Acupuncture has been used to treat gastrointestinal diseases in China for thousands of years. During the last decade, a number of studies have been performed to examine the effects of acupuncture on gastric and myoelectrical activity in animals and humans (Ouyang and Chen, 2004, Sato et al., 1993, Takahashi, 2006, Xu et al., 2004). Acupuncture is known to have beneficial effects on nausea and vomiting (Gan, 2002, Hu et al., 1992). There is little information about the effects of acupuncture in the treatment of FD.

Two acupoints have been identified that influence gastric motility; PC6 is located in the groove caudal to the flexor carpi radialis and cranial to the superficial digital flexor muscle, and ST36 is located at the proximal one-fifth of the craniolateral surface of the leg, distal to the head of the tibia in a depression between the muscle of the cranial tibia and long digital extensor (Takahashi, 2006). The effects of acupuncture on the gastrointestinal tract include inhibition of gastric motility when applied to the abdomen and excitation of gastric motility when applied to the limbs, in rats (Sato et al., 1993). Acupuncture has also been shown to stimulate vagal activity and reduce both

postoperative nausea and vomiting (Gan, 2002) and motion sickness (Hu et al., 1992) in healthy humans. In one study acupuncture effectively reduced symptoms, mainly epigastric pain, in 95 % of FD patients, however, no details were provided in regard to effects on other dyspeptic symptoms (Zhang et al., 1994). Two recent studies reported that acupuncture in FD patients at the ST36 and PC6 points for 2 weeks accelerated delayed gastric emptying (Xu et al., 2004, Xu et al., 2006), and relieved symptoms of early satiety, distension, pain, belching and nausea, but only in those patients with normal gastric emptying (Xu et al., 2006). Taken together, these studies are suggestive of a beneficial effect of acupuncture on symptom relief in FD, and that this effect may be unrelated to changes in gastric emptying and/or motility.

3.3.2 Hypnotherapy

Hypnotherapy has proven to be an effective, long-term, treatment for symptoms of IBS and also improves quality of life (Whorwell et al., 1984, Houghton et al., 1996). Only one trial has, to date, assessed the efficacy of hypnotherapy, compared with supportive therapy coupled with placebo medication, or with medical management in the short (16 weeks) and long-term (56 weeks) treatment of 126 FD patients (Calvert et al., 2002). During the hypnotherapy sessions (12 x 30 min sessions), the FD patients were put under hypnosis by a qualified therapist using eye fixation and closure, followed by progressive muscular relaxations. Suggestions of disease improvement were made using tactile and imagery techniques. The patient was asked to place a hand on their abdomen and imagine a reduction in all symptoms. Suggestions of positive changes in motor activity, sensitivity and secretion of acid and mucus were also introduced (Calvert et al., 2002). Supportive therapy (12 x 30 min sessions) was performed with a clinical

research assistant who provided general supportive advice and listened to patients' concerns about their condition, with no psychological intervention. The medication used for the supportive therapy or medical management groups was ranitidine (dosage not indicated) and the patients were told that they were receiving either placebo, or an active form of a drug that might help their condition. Short- and long-term symptom scores improved more in the hypnotherapy group when compared with the supportive or medical treatment. Hypnotherapy also benefited short and long-term quality of life compared with the other two treatments (Calvert et al., 2002). During a 40 week follow-up, approximately 82 % of patients in the supportive therapy group and 90 % in the medical group resorted to some form of medication for relief of their symptoms compared with 0 % in the hypnotherapy group. Additionally, the number of general practitioner or physician consultations was lower after hypnotherapy compared with the other two treatment groups. The long-term benefits and reduction in medication use and consultation rate suggest major beneficial economic and psychological effects of hypnotherapy as a treatment for FD and argue for its more widespread use.

3.4 HERBAL PREPARATIONS

Recently, there has been considerable interest in the use of herbal medications for the treatment of self-managed, chronic gastrointestinal disorders. In accordance with the European Scientific Co-operative on Phytotherapy, the definition of a herbal drug is "any medicinal product containing as active ingredients only plants, parts of plants or plant materials or combinations thereof, whether in the crude or processed state". The herbal preparations discussed in this chapter affect gastrointestinal function, either by inducing gastric tonic or spasmolytic and anti-ulcerogenic actions, or by increasing

acid neutralising capacity or flow of bile. Considering subgroups of FD patients have gastric motor abnormalities and, thus, may benefit from these actions, these herbal preparations are appealing as potential treatments. Herbal preparations have been advocated for the relief of symptoms in patients with FD, either alone or in combination with other herbal drugs (**Table 3.1**), however, their mechanism(s) of action are poorly understood and the correlation between any improvement in symptoms and their effects on gastrointestinal tract has not been investigated.

3.4.1 Clinical trials of mono-preparations

Several single herbal medicinal products have been recommended for the treatment of symptoms in FD. Only a handful of these products, however, have undergone an assessment of their safety and efficacy in randomised, placebo-controlled or equivalent clinical trials.

3.4.1.1 *Banana (Musa sapientum)*

The use of banana for the treatment of various ailments is traditional in India. It has been speculated that a flavonoid in unripe banana pulp has anti-ulcerogenic activity as well as acid neutralising capacity (Lewis et al., 1999, Best et al., 1984). Only one controlled trial has been conducted assessing the effects of banana powder (“Musapep” (Reckitt and Coleman, no other information provided)), on gastrointestinal symptoms in patients with FD (Arora and Sharma, 1990). 22 FD patients received 2 capsules t.i.d of Musapep (unknown dose) for 8 weeks, and 24 control patients did not receive any treatment. Gastrointestinal symptoms were partly relieved in 25 %, or completely relieved in 50 %, of the treatment group and in only 20 % in the untreated group.

However, this apparent improvement by Musapep may simply represent a placebo effect, given that the untreated group received nothing. While this preparation is safe, cheap and readily available (Arora and Sharma, 1990), further studies are required to establish efficacy and, if this is demonstrated, define potential mechanisms of action.

3.4.1.2 *Artichoke leaf (Cynara scolymus)*

Artichoke leaf extracts (ALE) have been shown to have antioxidant and antispasmodic effects on the gastrointestinal tract (Brown and Rice-Evans, 1998, Perez-Garcia et al., 2000, Rechner et al., 2001). A study in 516 healthy self-reported FD patients demonstrated a 40 % improvement in dyspeptic symptoms after 2 months treatment with 320 or 640 mg of ALE (Marakis et al., 2002). In a recent placebo-controlled, double-blind clinical trial, 247 FD patients were treated with either ALE preparation (3 x 230 mg plant extract t.d.s) or placebo for 6 weeks (Holtmann et al., 2003). The magnitude of the improvement in symptoms, specifically fullness (sum of difference to baseline: ALE, 6.6 ± 5.6 vs. placebo, 4.5 ± 5.8 ; $P < 0.01$) and early satiety (sum of difference to baseline: ALE, 5.3 ± 5.9 vs. placebo 3.1 ± 5.8 ; $P < 0.01$) was greater during treatment with ALE compared with placebo. There was an improvement of all symptoms (fullness, flatulence, early satiety, nausea, vomiting, epigastric pain) in all subjects with both treatments, although more FD patients responded to the treatment than the placebo. It is important to note that there was great variability in the responses to ALE which explain the large standard error (Holtmann et al., 2003). This extract was also superior to placebo in improving disease-specific quality of life as assessed by the Nepean Dyspeptic Index (see Chapter 4.5.6.1 and **Appendix 4.7**). The efficacy of ALE treatment was rated as “satisfactory”, “good” or “very good” in 85 % of patients

compared with 70 % in the placebo group. Similar to other studies investigating treatments of functional gastrointestinal disorders, there was a remarkable improvement in symptoms during the placebo treatment. The difference in the global response between ALE and placebo, however, was 15 %, which is considered within the range of a therapeutic gain (Dobrilla et al., 1989). The tolerability of both treatments was regarded as good or very good (ALE vs. placebo, 94 % vs. 96 %), and this treatment appears to be not associated with any serious adverse effects (Holtmann et al., 2003). While the above information suggest that ALE is an effective therapy for patients with FD, further studies are required to confirm this.

3.4.1.3 Tumeric (*cucuma longa*)

The active component of tumeric, curcumin, has antispasmodic effects on the gastrointestinal tract and also stimulates bile flow (Thamlikitkul et al., 1989). In a randomised, double-blind clinical trial in Thailand, 116 patients with so-called acid, flatulent or atonic dyspepsia received 2 g/day capsules of tumeric or placebo for 7 days. 53 % of patients responded to the placebo, whereas 87 % responded to tumeric. The efficacy of tumeric was said to be greater and clinically important when compared with placebo (no other information provided). Nine patients in the tumeric group experienced adverse events such as nausea, diarrhoea, headache, tiredness and sleepiness and 10 patients in the placebo group reported nausea, diarrhoea, headache, constipation, anorexia and tiredness. These adverse effects were described as mild (Thamlikitkul et al., 1989). This study did not attempt to discriminate the effects between the three different patient groups; accordingly therefore the effects of tumeric in patients with FD alone, still has not been identified.

3.4.1.4 *Capsaicin*

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the active component of red pepper (capsicum) powder, reduces the activity of nociceptive C-type fibres, which transmit pain sensations to the central nervous system (Lynn, 1990, Holzer, 1991, Mayer and Gebhart, 1994). Therefore, capsaicin may be a possible target treatment for visceral hypersensitivity. A study by Bortolotti and colleagues investigated whether ingestion of 2.5 g/day of red pepper powder, for 5 weeks, would lead to symptom improvement in patients with FD (Bortolotti et al., 2002). After 3 weeks of treatment with capsaicin, the overall symptom score and scores for epigastric pain, fullness and nausea were reduced (60 %) when compared with placebo (30 %) (Bortolotti et al., 2002). While the therapeutic actions of capsaicin are unclear, it has been suggested that there is a “desensitisation” effect, which renders the nociceptive C-type fibres, located in the gastrointestinal tract, less responsive to stimuli that give rise to dyspeptic symptoms (Bortolotti et al., 2002). Another study indicated that capsaicin alters gastrointestinal motility, by decreasing gastric tone, inhibiting phasic contractility and increasing the compliance of the proximal stomach (Lee et al., 2004), in healthy humans. No significant adverse effects have been observed with the use of capsaicin. Larger scale studies focussing on the long term effects of capsaicin on the gastrointestinal tract, including symptoms, have not been conducted.

3.4.2 *Clinical trials of combination preparations*

The several herbal medicinal products, which are suggested to have beneficial effects on symptoms, have been evaluated in combination as two or more herbal extracts. It is unclear which of the herbs is effective, or if they all play a role in alleviating symptoms,

however, combination preparations are popular in herbal medicine as traditional herbalists believe that synergistic interactions can occur between the different components, thereby eliciting a larger effect than would result from individual constituents alone (Thompson Coon and Ernst, 2002).

3.4.2.1 *Peppermint oil and caraway oil*

When given alone, peppermint oil has calcium antagonistic properties, therefore, its activity is primarily spasmolytic (Hills and Aaronson, 1991), whereas caraway oil increases muscular tone (May et al., 2000). Considering subgroups of FD patients have gastric motor abnormalities which may benefit from the spasmolytic and tonic actions of these separate oils, a fixed combination of these two herbs is appealing as potential treatments. To date, there have been four randomised clinical trials assessing the effects of peppermint oil and caraway oil on symptoms in FD.

The first study assessed the effect of a fixed combination of peppermint and caraway oils (Enteroplant, Dr Willmar Schwabe GmbH, Karlsruhe, Germany; 270 mg/day peppermint oil and 150 mg/day caraway oil), in 45 FD patients for 4 weeks, and reported a significant improvement in epigastric pain scores when compared with placebo (May et al., 1996). A second, similarly designed, study was performed by the same group using a lower dosage (180 mg/day peppermint oil and 100 mg/day caraway oil), in 96 FD for 4 weeks (May et al., 2000). There was a statistically significant reduction in pain intensity (40 % vs. 22 %) and sensations of pressure, heaviness and fullness (44 % vs. 22 %) compared with placebo, with an overall rating of “much improved” during the herbal combination and “minimally improved” for the placebo

group. Only five subjects reported adverse events during the peppermint and caraway oil treatment. These adverse events were similar complaints experienced by the patient before inclusion into the trial, and therefore were unrelated to the study (May et al., 2000). A further study compared the effects of the fixed combination of peppermint and caraway oil (180 mg/day peppermint oil and 100 mg/day caraway oil) and the prokinetic drug, cisapride (30 mg/day), for 4 weeks, in 120 FD patients (Madisch et al., 1999). Both treatments showed similar improvements in the occurrence and severity of epigastric pain and discomfort. Taken together, these four studies indicate that there is good tolerability and lack of adverse effects. However, a shortcoming is that predominantly only “pain”, and no other dyspeptic symptoms, appears to be relieved.

3.4.2.2 *Lomatol®*

Lomatol® (Lomapharm, Emmerthal, Germany) contains a combination of extracts of peppermint leaves, caraway fruit, fennel fruit and wormwood herbs. In a controlled, randomised, double-blind study, 60 patients complaining of at least one of the following symptoms; upper abdominal pain, gastrospasms, belching, nausea, retching, heartburn or loss of appetite, were treated with Lomatol® (25 drops in water t.i.d) for 14 days and the results compared to treatment with metoclopramide (dose not given). Results indicated that Lomatol® was superior to metoclopramide in terms of efficacy and tolerability, and no adverse events were reported (Westphal et al., 1996). This study, however did not evaluate the effects of Lomatol® in patients characterised with FD only, therefore, future studies are required to establish the effects of Lomatol® in this group.

3.4.2.3 *Peppermint oil and ginger*

In the only randomised, double-blind, placebo-controlled study using peppermint oil and ginger extract (180 mg and 25 mg/day, respectively), as described in a review of herbal medicinal products for non-ulcer dyspepsia (Thompson Coon and Ernst, 2002), there was a reported improvement in gastrointestinal symptom scores (not identified) in 74 % of FD patient compared with 30 % in the placebo group. Further studies are required before recommendation can be made regarding the use of this preparation.

3.4.3 **Iberogast®**

Iberogast® (Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany), is a herbal preparation, which has been used in Europe for the treatment of gastrointestinal diseases for more than 40 years and was launched on the Australian market in 2004. Iberogast® is a complex herbal preparation, containing nine constituents, including fresh plant extract of *Iberis amara* (bitter candy tuft) and the extracts of eight other dried herbal drugs, including; *Angelicae radix* (angelica roots), *Matricariae flos* (camomile flowers), *Carvi fructus* (caraway fruit), *Cardui mariae fructus* (St. Mary's thistle fruit), *Melissae folium* (balm leaves), *Menthae peperitae folium* (peppermint leaves), *Chelidonii herba* (greater celandine), *Liquiritiae radix* (liquorice roots) in 30.9 % ethanol (see **Table 4.3**). There is evidence that all nine constituents have pharmacological effects on the gastrointestinal tract (Saller et al., 2002). Arguably the main active ingredient, the extract of *Iberis amara*, has a predominantly tonic effect on the gastrointestinal tract, whereas the properties of the other components have been described as being spasmolytic (Ammon, 1986). During the last 15 years, the effects and efficacy of Iberogast® have been evaluated. There has been a number of double-blind,

randomised, clinical trials comparing the effects of Iberogast® with placebo and other pharmacological agents commonly used to treat symptoms in FD. The efficacy of Iberogast® and other treatments has been determined by the improvement of individual symptoms or the gastrointestinal symptom (GIS) sum score. The latter contains ten gastrointestinal symptoms typical of FD, including nausea, retching, vomiting, early sensation of satiety, lack of appetite, heartburn, retrosternal paresthesia, abdominal spasms, epigastric pain and flatulence. Patients rated the intensity of these symptoms on validated four- or five-point Likert scales, ranging from 0 (none) to 3 (pronounced) or 4 (very pronounced). The use of Iberogast® was not associated with any adverse effects or interactions with other drugs, so that tolerability was excellent. Only a handful of studies have investigated the mechanism(s) of action of Iberogast® on the gastrointestinal tract, and these have been performed mainly in animals, as will be discussed in Chapter 3.4.4.3.

3.4.3.1 Comparative effects of Iberogast® and placebo in functional dyspepsia

Studies which have compared the effects of Iberogast® with placebo have used the recommended dose of 20 drops (1.1 ml), three times a day, and indicate that Iberogast® is superior to placebo in improving dyspeptic symptoms (Nicolay, 1984, Buchert, 1994, Madisch et al., 2001, Rösch et al., 2002). For example, a study by Buchert (1994) investigated, in a placebo-controlled, double-blind, parallel, two-arm study, the efficacy and tolerability of Iberogast® in 243 FD patients (Buchert, 1994) who were treated for 4 weeks with the recommended dose. Efficacy was evaluated on the basis of a GIS score, which was measured before, and 4 weeks after, initial drug treatment. GIS sum scores decreased significantly more in the Iberogast®, compared with the placebo, group

(Buchert, 1994) (**Figure 3.1**). At the final visit, 52 out of 83 patients treated with Iberogast®, compared with 14 out of 80 patients treated with placebo, reported their most bothersome symptom as “mild” or “absent”. The analysis of individual symptoms revealed that Iberogast® reduced the score for all symptoms, except abdominal cramps, when compared with placebo. Tolerability was rated as “very good” or “good” by 89 % of patients in the Iberogast® group, but only by 60 % of patients in the placebo group. A more recent study compared the effects of Iberogast® and STW5-S (an experimental preparation without bitter candy tuft), with placebo for 4 weeks at the recommended dose, and demonstrated that both herbal preparations produced a statistically significant improvement in GIS sum score in 60 FD patients after 2 and 4 weeks of treatment ($P < 0.001$). There was no overall difference between the two herbal preparations, however, there was a more rapid improvement in symptoms in the Iberogast® group, which suggests that the inclusion of bitter candy-tuft (*Iberis amara*) in the preparation may be important (Madisch et al., 2001).

NOTE: This figure is included on page 88 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.1 Gastrointestinal symptom scores measured at baseline and 4 weeks during treatment with placebo or 1.1 ml Iberogast®, taken three times per day, in 243 FD patients. Iberogast® improved symptoms after 4 weeks of treatment compared with baseline scores (* $P < 0.0001$). The magnitude of improvement during placebo was less marked (Buchert, 1994).

3.4.3.2 *Comparative effects of Iberogast® and prokinetic agents in functional dyspepsia*

Studies have also been performed to compare the effects of Iberogast® with prokinetic agents commonly used to treat symptoms of FD, including metoclopramide (Nicolay, 1984) and cisapride (Rösch et al., 2002). In an early, single-blind clinical trial, 77 FD patients were treated with 20 drops of Iberogast®, three times a day for 2 weeks, and efficacy and tolerability were compared with metoclopramide (10 mg t.i.d) (Nicolay, 1984). In both groups, there were comparable improvements in all symptoms (**Figure 3.2**), with approximately 50 % of patients reporting resolution of symptoms.

NOTE: This figure is included on page 89 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.2 Fullness scores at baseline and at 3, 7 and 14 days of treatment with 10 mg metoclopramide or 1.1 ml of Iberogast®, taken three times per day in 77 FD patients. Fullness scores decreased similarly in both groups (Nicolay, 1984).

However, the tolerability of Iberogast® was predictably superior to that of metoclopramide. A more recent study compared the effects of Iberogast® with cisapride in 124 FD patients (Rösch et al., 2002). After 4 weeks, gastrointestinal symptoms decreased similarly in both treatment groups (**Figure 3.3**). The tolerability of the two treatments was also similar (Rösch et al., 2002), however, as discussed (Chapter 3.2.1.1), cisapride is not generally available anymore. An advantage of Iberogast® is that it targets only the gastrointestinal tract and the enteric nervous system, but not the central nervous system, like cisapride and metoclopramide, and, therefore, is essentially free of side-effects. The results from these studies indicate that Iberogast® is an effective, well tolerated and safe treatment option in FD.

NOTE: This figure is included on page 90 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.3 Gastrointestinal symptom scores measured at baseline and 4 weeks during treatment with 10 mg cisapride or 1.1 ml Iberogast®, taken three times per day, in 183 FD patients. Symptom scores decreased similarly with both treatments compared with baseline, with no difference between them (* $P < 0.0001$) (Rösch et al., 2002).

3.4.3.3 *Effects of Iberogast® on gastrointestinal motility and sensitivity*

While the above studies have demonstrated improvement in symptoms, they have not investigated potential mechanism(s) of action of Iberogast®. A limited number of recent studies have evaluated the mechanisms underlying the effects of Iberogast® in animal models and more recently in human gastrointestinal tissue. In these studies, animal (and human) gastric tissues were treated with Iberogast® and its effects on relaxation, contractile force, resting membrane potential, slow wave frequency and small intestinal sensitivity were determined. The effect of Iberogast® on gastric emptying, in vivo, has not yet been investigated.

3.4.3.3.1 Proximal and distal gastric motility

One of the first studies to evaluate the effects of Iberogast® on gastric motility demonstrated a dual action of this herbal preparation, which was region-specific

(Hohenester et al., 2004). Isometric muscle tension activity from guinea pig gastric fundus, corpus and antrum was measured in response to several concentrations (32, 64, 128, 256 and 512 $\mu\text{g/ml}$) of Iberogast®. Iberogast® evoked a sustained, and reversible, relaxation of both circular and longitudinal gastric fundus and corpus muscles, in a concentration-dependent fashion, but had no effect on antral tone (**Figure 3.4**).

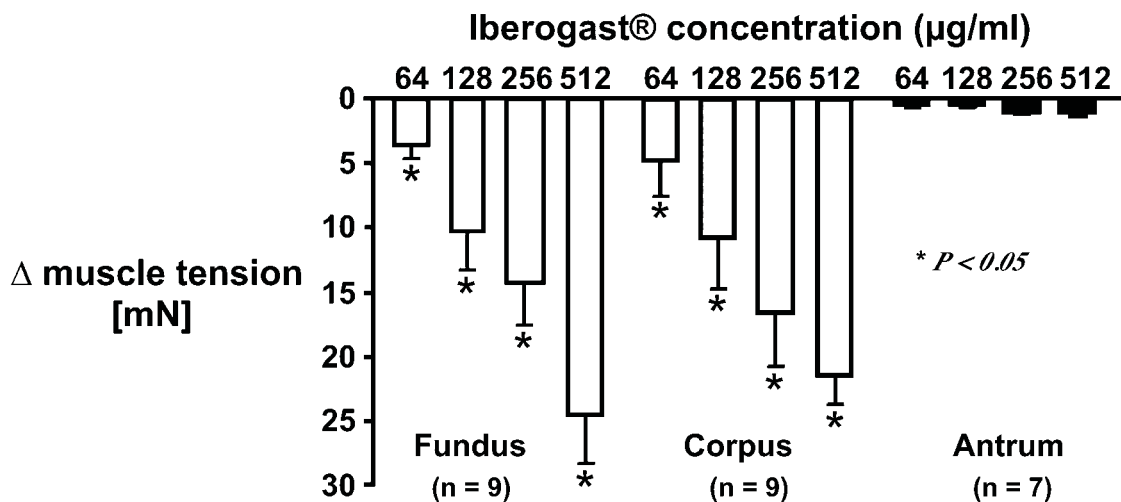


Figure 3.4 Effect of Iberogast® at 64, 128, 256 and 512 $\mu\text{g/ml}$ on the muscle tension (tone) in muscle strips from guinea pig fundus, corpus and antrum. All concentrations of Iberogast® reduced muscle tension in the fundus and corpus, but not the antrum, when compared with basal muscle tone (* $P < 0.05$) (Hohenester et al., 2004).

In contrast, both the circular and longitudinal muscles from the antrum responded with an immediate, and long-lasting, increase in contractile force, also in a concentration-dependent fashion (Hohenester et al., 2004) (**Figure 3.5**). A preliminary study by the same group suggested that these observations are also applicable to human gastric motility. - Muscle preparations from human proximal stomach revealed that Iberogast® has a strong relaxing effect, corresponding to a reduction in tone (Schemann et al., 2006). These effects of Iberogast® on gastric motility were further investigated by determining the pathway(s) involved in its action. - Guinea pig gastric tissue was incubated with four inhibitors of enteric neural pathways. The effects of Iberogast® on

gastric motility were resistant to blockade of nerve conduction by tetrodotoxin, blockade of synaptic transmission by the N-type Ca_v -channel blocker ω -conotoxin GVIA, defunctionalisation of capsaicin-sensitive primary afferents by capsaicin and blockade of nitric oxide synthesis by the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester. Moreover, the Iberogast®-induced increase in the contractile effect of the antrum could not be blocked by the muscarinic antagonist, atropine. In addition, the relaxatory effect could not be blocked by the nitric oxide synthase inhibitor, N-nitro-L-arginine methyl ester. Taken together, these observations indicate that the action(s) of Iberogast® on proximal gastric relaxation and antral contractility are not nerve-mediated or involve the nitric oxide pathway, but reflect a direct effect on smooth muscle cells (Hohenester et al., 2004).

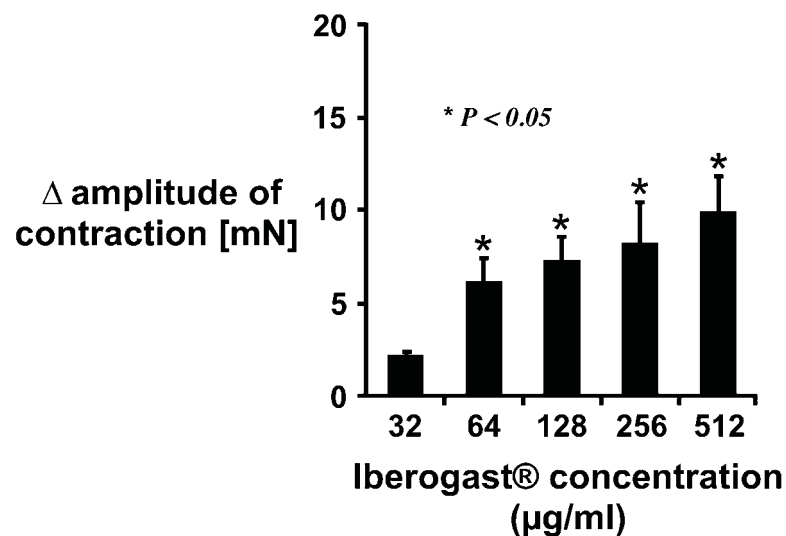


Figure 3.5 Effect of Iberogast® at 32, 64, 128, 256 and 512 µg/ml on the contraction amplitudes in muscle strips from guinea pig antrum. All concentrations of Iberogast® increased the amplitude of contraction of the antrum when compared with basal antral tone (* $P < 0.05$) (Hohenester et al., 2004).

A recent study reported the effects of the individual components of Iberogast® on proximal and distal gastric motility in guinea pig gastric tissue (Schemann et al., 2006).

The extracts of angelica root, chamomile flower and liquorice root induced a concentration-dependent relaxation of both the circular and longitudinal muscle strips from gastric fundus and corpus and a decrease in muscle tone, which was similar to that of Iberogast®. On the other hand, extracts of greater celandine herb, Melissa leaf, caraway fruit and bitter candy tuft failed to induce relaxation, but evoked contractions, in the gastric fundus. No consistent effects were observed for peppermint leaf and milk thistle fruit extracts. All extracts, except peppermint leaf and milk thistle fruit extracts, exerted a contractile response in the antrum, mimicking the excitatory effects of Iberogast® on antral motility. These observations have, therefore, identified plant extracts within the Iberogast® preparation with purely contractile effects and extracts with both contractile and relaxatory effects, but none with a sole relaxatory effect. The observations that some individual extracts have discrepant actions on the proximal, and the distal, stomach, may not indicate that individual extracts have different region-specific effects in the proximal and distal stomach as first suggested (Hohenester et al., 2004), but reflect the differences in smooth muscle physiology, specifically in the calcium-handling properties of gastric fundus vs. antral smooth muscle (Kong et al., 1986). This may indicate that the site of action of Iberogast® is exerted on ion channels and/or signal transduction pathways that are expressed in the proximal and distal stomach (Schemann et al., 2006). The effects of Iberogast® on gastric and pyloric motility and gastric emptying in humans have not been evaluated.

3.4.3.3.2 Small intestinal motility

Two recent studies have evaluated the effects of Iberogast® and its individual components on small intestinal motility; one on the spasmolytic and tonic effects in

intestinal smooth muscle of the guinea pig (Ammon et al., 2006) and the other on intracellular recordings of smooth muscle cells of the circular muscle layer of mouse small intestine, specifically the resting membrane potential (RMP) and slow wave frequency and amplitude, which are the electrophysiological basis of smooth muscle contractility and motility of the intestine (Storr et al., 2004).

Similar to the effect in the proximal stomach, Iberogast® and the individual components, chamomile flower and liquorice root, but also peppermint leaf, were able to decrease acetylcholine- and histamine-induced contractions of guinea pig ileum, in a dose-dependant fashion (Ammon et al., 2006). These spasmolytic effects were also observed in the duodenum, jejunum and colon. Extracts from bitter candy tuft did not have a spasmolytic effect. However, both Iberogast® and bitter candy tuft increased basal resting tone and contractions of the ileum. These observations reinforce the “dual-action” effect of Iberogast® (Ammon et al., 2006).

Iberogast® dose-dependently depolarises the RMP of smooth muscle cells, which influences smooth muscle excitability. The mode of action of Iberogast® on changes in smooth muscle RMP and slow wave rhythmicity is proposed to be by way of blockade of large conductance calcium channels (Storr et al., 2004). When the individual components of Iberogast® were investigated, angelica root, chamomile flowers and greater celandine caused the depolarisation. There was also a dose-dependent reduction in the amplitude and frequency of slow waves after treatment with Iberogast®. As for the RMP, the different individual herbal extracts had different effects on slow waves; angelica root and chamomile flowers blocked slow wave activity, whereas bitter candy tuft increased the frequency and amplitude. Greater celandine reduced the frequency

and amplitude, peppermint leaves only reduced the frequency, and liquorice root and milk thistle and balm leaves had no effect. No study has evaluated the effects of Iberogast® on motility in the human small intestine.

3.4.3.3.3 Small intestinal sensitivity

Iberogast® not only exhibits effects on gastrointestinal motility, it also modulates neuronal activity. Iberogast® decreases afferent nerve discharge at low and high pressure distensions (10 - 60 cm H₂O) compared with control (30.8 % ethanol) in male Wistar rats (**Figure 3.6**). Additionally, Iberogast® attenuated a dose-dependent increase in afferent discharge by pharmacological agents 5-HT and bradykinin. These observations suggest that Iberogast® decreases the sensitivity of vagal (5-HT sensitive) and spinal (bradykinin) afferents to low and high pressure distension which reflect a “desensitising” effect of Iberogast® on the mesenteric intestinal afferent nerve fibres to chemical (5-HT and bradykinin) and mechanical stimuli (Liu et al., 2004). It is not possible to determine which components of Iberogast® were responsible for the decrease in intestinal afferent sensitivity, therefore, future studies are required to identify these extracts and their particular effects. Furthermore, it would be of interest to investigate the effects of Iberogast® on the sensitivity to gastric distension and motility in patients with FD, specifically in response to a meal.

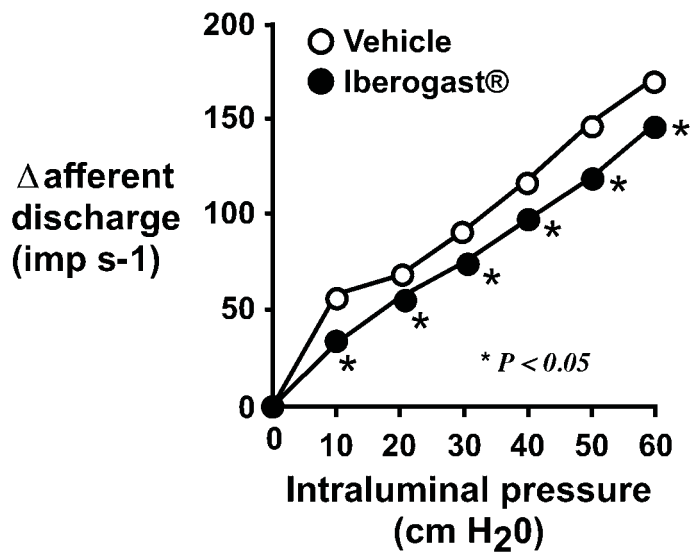


Figure 3.6 Afferent nerve responses of the jejunum of Wistar rats, at different levels of intraluminal pressure following Iberogast® or vehicle (30.8 % ethanol) pre-treatment. Afferent discharge was reduced at all distension pressures following Iberogast® pre-treatment when compared with vehicle (* $P < 0.05$) (Liu et al., 2004).

3.5 SUMMARY

Treatments used for symptom relief in patients with FD have in general produced modest and inconsistent effects, and in regards to pharmacotherapies, a number of adverse events. The use of herbal medications has received substantial attention during last decade and while it appears clear that a number of these medications improve symptoms in FD, with few or no adverse effects, their mechanism(s) of action are poorly defined. One of the latest herbal preparations on the market in Australia, Iberogast®, improves symptoms in FD and is well tolerated. In animal studies, treatment with Iberogast®; increases proximal gastric relaxation and contractions of the antrum, while decreasing vagal sensitivity, which are all logical targets for the management of FD. The effects of Iberogast® in the healthy human gastrointestinal tract, specifically proximal gastric volume, contractions in the antrum, pylorus and

duodenum and gastric emptying and intragastric distribution, have been investigated and these results are reported in Chapter 10.

Chapter 4

COMMON METHODOLOGIES

4.1 INTRODUCTION

The methods presented in this chapter are used in the studies described in Chapters 5 - 10. All the techniques have been validated and are well established for the assessment of gastric emptying, gastroduodenal motility, gastrointestinal hormone release/suppression, appetite and energy intake.

4.2 SUBJECTS

4.2.1 Subject recruitment

4.2.1.1 *Healthy subjects*

Healthy male (Chapters 5 - 7 and 9 - 10) and female (Chapters 8 and 9) subjects were recruited through advertisements in the local newspapers, flyers throughout the Universities of Adelaide and South Australia, Flinders University, the Royal Adelaide Hospital and from a list of potential volunteers available within our Department.

4.2.1.2 *Functional dyspepsia patients*

Patients with FD (Chapters 8 and 9) were recruited from the endoscopy list and outpatients of the Department of Gastroenterology, Hepatology and General Medicine at the Royal Adelaide Hospital. Patients were also recruited through the combined use of advertisements placed in the local newspapers, flyers located within the Royal Adelaide Hospital and National and Chem-Mart Pharmacies. Gastroenterologists at the Royal Adelaide Hospital, Queen Elizabeth Hospital and Repatriation Hospital were informed of the studies and asked to refer suitable patients. FD patients were recruited based on the criteria set down by the Rome II working party (see Chapter 2.2), and had to have experienced symptoms for at least 3 months prior to entry into the study. Additionally, patients were included if the severity of their symptoms was at least moderate in nature. This was assessed by asking the patients to rate the severity of the symptoms, upper abdominal pain, bloating, the inability to finish a normal-sized meal and nausea, on a scale of 0 - 3, where 0 represented “symptom not experienced”, 1 “slight symptoms, but can be ignored”, 2 “moderate symptoms, but not impairing daily activities” or 3 “severe symptoms, impairing daily activities”. If the total score was ≥ 3 or the score for one symptom was ≥ 2 , the patient was invited to participate in the studies described in Chapters 8 and 9.

Signed informed consent was obtained from each subject prior to participation in a study. All subjects understood that their participation was voluntary and that they were free to withdraw from the study at any time and that their medical and nursing care would not be affected. All subjects were offered an honorarium for their participation.

4.2.2 Common exclusion criteria

Prior to enrolment, each healthy subject and FD patient underwent a screening visit in person or was questioned over the telephone to exclude individuals who; had a history of gastrointestinal surgery, took medication which affected gastrointestinal motor function, body weight or appetite, had any significant illnesses (i.e. diabetes, cardiovascular or respiratory complications etc), evidence of drug abuse, consumption of greater than two standard (20 g alcohol) alcoholic drinks per day, smoked more than ten cigarettes per day, or scored greater than twelve for the eating restraint component (Factor I) of the “Three-Factor Eating Questionnaire”, for healthy subjects only in Chapters 5 - 7 (**Chapter 4.5.5.4**).

4.2.3 Additional exclusion criteria for functional dyspepsia patients

Additional exclusion criteria in patients with FD were;

- an organic cause of dyspepsia (e.g. gallstones, peptic ulcers) as assessed by endoscopy and/or ultrasound within 24 months prior to study entry
- heartburn or lower bowel symptoms as the predominant symptom
- positive *H pylori* status as assessed by biopsy with urease test, histological examination and microbial culture.
- positive clinically significant results on laboratory test (biochemistry [urea, electrolytes, calcium, glucose, liver function tests] and haematology [complete blood examination, erythrocyte sedimentation rate])
- lactose intolerance as assessed by breath test

4.3 ETHICS COMMITTEE APPROVAL

All protocols were approved by the Royal Adelaide Hospital Research Ethics Committee, and, where necessary, the Royal Adelaide Hospital Investigational Drug Sub-Committee (Chapter 10).

4.4 STUDY TREATMENTS

Study treatments were given via two different routes, orally or intraduodenally, to assess the effects on gastrointestinal motility, hormone release, appetite and energy intake. Orally administered treatments, so-called preloads, assess the effect of nutrients incorporated into a meal, therefore factors such as taste, gastric filling and emptying influence the results. Preloads were consumed within 10 min. Treatments given directly into the small intestine via an intraduodenal catheter, are able to assess the direct effect of nutrients without the influence of gastric distension and gastric emptying. Intraduodenal treatments were infused over a longer period of time (i.e. 50 - 150 min) at constant rates.

4.4.1 Yoghurt preloads

The aim of a well-designed preload is to mask all sensory aspects by ensuring that the sight, smell, taste and texture of the foods consumed are kept constant (Rolls et al., 1991). Three yoghurt preloads were used in the study described in Chapter 8, one high in carbohydrate, one high in fat and a low-nutrient volume control. All yoghurts had the same sensory properties so that any differences in their effects would be due to the post-ingestive physiological effects of the nutrients.

The recipes were based on those used previously in studies conducted in the Department (Vozzo et al., 2003). The yoghurts were prepared in the Departmental research kitchen using commercially available ingredients. The base ingredients were natural yoghurt (Farmers Union Natural European Style yoghurt, National Foods Ltd, Melbourne, VIC, Australia), or fat-free yoghurt (Jalna Dairy foods Pty Ltd, Colac, VIC, Australia), and mangos (Admiral sliced mangos in natural juice, Riviana Foods Pty Ltd, Rowville, VIC, Australia). The final macronutrient composition was modified with respect to; (i) total fat content, with the addition of double-thickened cream and full-fat milk, and (ii) total carbohydrate content, with the addition of glucose and maltodextrin. All three yoghurts had the same protein content. The sensory characteristics (sight and taste) were also standardised with addition of gelatine, artificial sweetener, food colouring and fruit sauce and were indistinguishable from each other (**Table 4.1**). A pilot study was conducted to standardise the sensory characteristics of the yoghurts in 19 healthy subjects. Each subject received each of the three yoghurts and was asked to rate taste, sweetness, fattiness, colour, bitterness and sourness on a visual analogue scales (Chapter 4.5.5.2, **Appendix 3**). There were no significant differences in these characteristics between the three yoghurts (**Table 4.2**). The yoghurts were prepared in the afternoon before the study and allowed to set in the fridge at 4°C overnight.

Table 4.1 Composition of yoghurt preloads.

	Low-energy	High-carbohydrate	High-fat
kcal/100 g	45.0	125.5	125.5
Yoghurt (natural) (g)	-	46.8	42.5
Low-fat yoghurt (g)	23.8	-	-
Mango (g)	19.5	26.0	25.0
Mango topping (g)	3.5	10.6	6.5
Gelatine (g)	1.3	1.8	1.0
Maltodextrin (g)	-	9.4	-
Glucose (g)	-	3.1	-
Cream (> 35 g fat) (g)	-	-	14.0
Milk (full-cream) (g)	-	-	6.9
Water (g)	52.4	8.0	9.9
Low-fat cream (g)	4.3	-	-
Artificial sweetener (g)	0.4	0.1	0.3
Colouring (g)	0.1	0.1	0.2
Salt (pinches)	2.0	-	-
kJ from fat (%)	10.8	10.2	55.5
kJ from CHO (%)	55.7	73.8	31.4
kJ from protein (%)	22.5	11.7	10.4

CHO, carbohydrate.

Table 4.2 Scores of taste, sweetness, colour, bitterness, fattiness and sourness of low-energy, high-carbohydrate and high-fat yoghurt preloads.

	Low-energy ("control")	High-carbohydrate ("high-CHO")	High-fat ("high-FAT")	<i>P</i> value
Taste (mm)	63 ± 5	72 ± 5	70 ± 6	NS
Sweetness (mm)	56 ± 5	63 ± 6	60 ± 5	NS
Colour (mm)	53 ± 5	64 ± 5	62 ± 5	NS
Bitterness (mm)	23 ± 5	16 ± 5	17 ± 3	NS
Fattiness (mm)	42 ± 6	35 ± 6	43 ± 5	NS
Sourness (mm)	25 ± 4	17 ± 4	26 ± 4	NS

NS, not significant.

4.4.2 Intraduodenal infusions

4.4.2.1 Triglyceride emulsion

A commercially available lipid emulsion, Intralipid® (10 %, 300 mOsmol/kg, 1.1 kcal/ml, Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia), which consists predominantly of long-chain triglycerides extracted from soy bean oil (50 g/500 ml), egg phospholipids (1.2 g/500 ml) and glycerol anhydrous (2.25 g/500 ml), was used as the nutrient infusion in Chapters 5 and 6. Intralipid® was diluted with isotonic saline (0.9 %) to achieve the specific loads required, as specified in these chapters. All lipid emulsions were administered intraduodenally at a rate of 4 ml/min for 150 min in Chapter 5 so the total volume infused in all study conditions was 600 ml, and for 50 min in Chapter 6 so that the total volume infused in all study conditions was 200 ml. Intralipid® was selected as it has been used in the majority of studies evaluating the

effects of lipid on gastrointestinal function and appetite (Feinle et al., 2000, Heddle et al., 1988a, Castiglione et al., 1998, Andrews et al., 2001, Chapman et al., 1999).

4.4.2.2 *Glucose solution*

The intraduodenal glucose solutions, used in Chapter 7, were prepared from a 25 % glucose stock solution, which contained 250 g of glucose powder (Glucodin, Boots Healthcare, North Ryde, NSW, Australia) dissolved in distilled water to make up 1000 ml of solution, with an osmolarity of 1390 mosmol/l, and administered at: (i) 1 kcal/min, (ii) 2 kcal/min and (iii) 4 kcal/min. This stock solution was used for the 4 kcal/min treatment. For the 2 kcal/min treatment, the 25 % glucose solution was diluted with iso-osmotic saline (i.e. 1390 mosmol/l) at a 1:1 ratio. For the 1 kcal/min treatment, the 25 % glucose solution was diluted with iso-osmotic saline at a 1:3 ratio. On the remaining study day hypertonic saline (4.2 %, 1390 mosmol/l) was given. Accordingly, all solutions had an osmolarity of 1390 mosmol/l, and they were delivered into the small intestine at a constant rate of 4 ml/min for 120 min, so that the total volume in all study conditions was 480 ml.

4.4.3 *Iberogast®*

Iberogast®, used in the studies described in Chapter 10, is a complex herbal preparation used for the treatment of abdominal symptoms in patients with FD and irritable bowel syndrome. It contains nine constituents, including fresh plant extract of *Iberis amara* (bitter candy tuft) and the extracts of eight other dried herbal drugs, including; *Angelicae radix* (angelica roots), *Matricariae flos* (camomile flowers), *Carvi fructus* (caraway fruit), *Cardui mariae fructus* (St. Mary's thistle fruit), *Melissae folium* (balm

leaves), *Menthae piperitae folium* (peppermint leaves), *Chelidonii herba* (greater celandine), *Liquiritiae radix* (liquorice roots), in a 30.9 % ethanol solution (**Table 4.3**). The recommended dose of Iberogast® is 20 drops (1.1 ml), three times per day.

Table 4.3 Content of Iberogast® per 1.1 ml dose.

Medicinal plant or drug	Common name	mg
Iberis amara totalis (FP)	bitter candy tuft fresh plant	75.0
Carvi fructus (DR)	caraway fruits	33.3
Liquiritiae radix (DR)	liquorice root	33.3
Menthae piperitae folium (DR)	peppermint leaves	16.7
Melissae folium (DR)	balm leaves	33.3
Matricariae flos (DR)	chamomile flowers	66.7
Chelidonii herba (DR)	greater celandine	33.3
Cardui mariae fructus (DR)	milk thistle fruits	33.3
Angelicae radix (DR)	garden angelica root	33.3
Alcohol (31 % by volume)		34.0

DR, dry drug; FP, fresh plant.

4.5 TECHNIQUES

4.5.1 High-resolution manometry

High-resolution perfusion manometry is a technique used to measure pressures in the gastrointestinal tract (Heddle et al., 1988c, Dent, 1976), and relies on the occurrence of luminal-occlusive contractions. This technique employs external transducers linked to a multi-lumen manometric catheter perfused with water through side-holes spaced along

the tube. These transducers allow concurrent recordings of pressures at multiple points along the regions of interest.

Subjects were intubated with the silicone rubber manometric catheter (3.5 mm outer diameter; Dentsleeve, Adelaide, Australia (Chapters 5 and 6) or Dentsleeve International Ltd. Mui Scientific, Ontario, Canada (Chapters 7 and 10)), via an anaesthetised (Lignocaine 5 %, ORION Laboratories Pty Ltd, Calcatta, WA, Australia) nostril, and allowed to pass through the stomach and into the duodenum by peristalsis (Heddle et al., 1989). The catheter contained six side-holes (0.1 mm diameter: channels 1 - 6), which were positioned in the antrum, and seven side-holes (0.1 mm diameter: channels 10 - 16) positioned in the duodenum. All side-holes were spaced at 1.5 cm intervals. The catheter also incorporated a sleeve sensor to measure pressures in the pylorus, which is a mobile and narrow structure (Heddle et al., 1988b), accurately. The sleeve sensor (channel 7) was 4.5 cm in length, with two side-holes on the back of the sleeve (0.1 mm diameter: channels 8 and 9) (Dent, 1976). An infusion channel (0.4 ml diameter) was located 11.75 cm distal to the end of the sleeve sensor (i.e. ~ 14.5 cm from the pylorus), and used for the delivery of lipid and glucose solutions (Chapters 5 - 7). The correct positioning of the catheter, so that the sleeve sensor straddled the pylorus, was monitored by continuous measurement of the transmucosal potential difference (TMPD) between the most distal antral (channel 6) (~ -40 mV), and the most proximal duodenal (channel 10) (~ 0 mV), channel (Heddle et al., 1989). For this purpose, an intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode (Heddle et al., 1989). All manometric channels were perfused with degassed, distilled water, except for the two TMPD

channels, which were perfused with degassed 0.9 % saline (Heddle et al., 1989) (**Figure 4.1**).

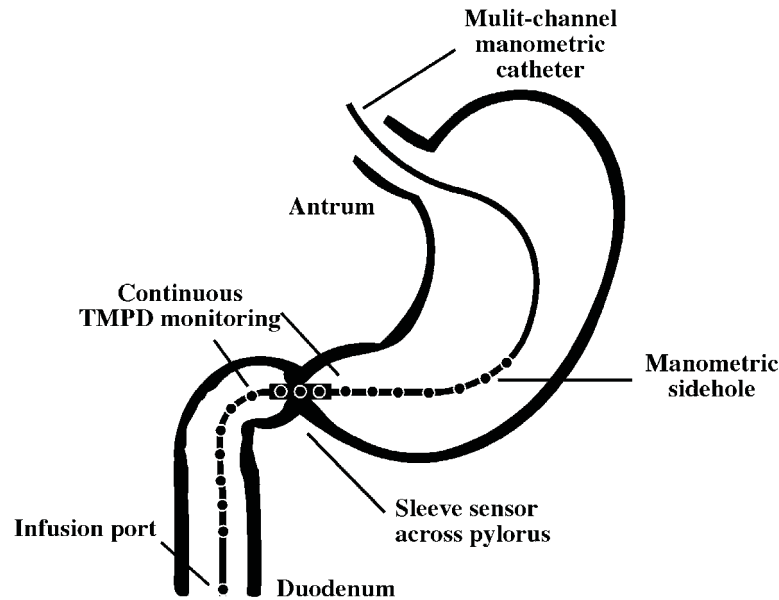


Figure 4.1 Schematic representation of manometric catheter incorporating six antral and seven duodenal side-holes, spaced 1.5 cm apart, a pyloric sleeve sensor and duodenal infusion port.

Manometric pressures were digitised and recorded on a computer-based system running commercially available software (Flexisoft®, Version 3, Oakfield Instruments, Assoc Prof GS Hebbard, Royal Melbourne Hospital, Melbourne, Australia, written in Labview 3.1.1 (National Instruments)), and stored for subsequent analysis. APD pressures were analysed for the (i) number and amplitude of antral and duodenal PWS, (ii) basal pyloric pressure, (iii) number and amplitude of IPPWS, and (iv) number of PWSs in the APD region. Phasic PWS in the antrum and IPPWS were defined by an amplitude of ≥ 10 mmHg, with a minimum interval between peaks of 15 s (Samsom et al., 1998). Basal pyloric pressure (“tone”) was calculated for each minute by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side-hole from the mean basal pressure recorded at the sleeve (Heddle et al., 1988b), using custom-

written software (MAD, Prof C-H Malbert, Institut National de la Recherche Agronomique, Rennes, France). Phasic duodenal PWs were defined by an amplitude of ≥ 10 mmHg, with a minimum interval of 3 s between peaks (Samsom et al., 1998). PWSs were defined as two or more temporally related PWs with onsets within ± 5 s (in the antrum), or ± 3 s (in the duodenum), of each other (Samsom et al., 1998). PWSs were characterised according to the distance traveled i.e. over two (1.5 - < 3 cm), three (3 - < 4.5 cm), four (4.5 - < 6 cm), ... , fifteen (21 - < 22.5 cm) channels, and expressed as the total number of waves, using custom-written software (Gastrointestinal Motility Unit, University Hospital Utrecht, Utrecht, Netherlands), modified to our requirements.

4.5.2 Barostat

The electronic barostat device is an instrument used to measure intragastric pressure and volume. The barostat consists of a strain-gauge linked by an electronic relay to an air aspiration / injection system (**Figure 4.2**). Both the strain-gauge and injection system are connected to an ultra-thin, polyethylene bag by means of a single-lumen polyvinyl catheter. When the stomach contracts, the barostat aspirates air from the bag to maintain the pre-set pressure within the bag; when the stomach relaxes, air is injected into the bag (Azpiroz and Malagelada, 1985a). In addition to maintaining a specified pressure within the bag, the barostat is also capable of performing distensions of the stomach.

Subjects swallowed the orogastric catheter (4 mm outer diameter, 2 mm inner diameter; Tygon® tubing, Saint Gobain Performance Plastics, OH, USA), which had an ultrathin, flaccid, polyethylene bag (capacity 1200 ml) tightly wrapped around its distal end

(Feinle et al., 2000). The catheter was initially advanced to ~ 50 cm. The proximal end of the catheter was then connected via a three-way tap to the measurement and balloon ports of a gastric barostat (Distender Series IITM, G & J Electronics Inc, Toronto, Ontario, Canada). To correctly position the bag in the fundus of the stomach, the bag was unfolded by manually inflating it with 400 ml then pulling it back gently until its passage was restricted by the lower oesophageal sphincter and then pushing it aborally by 2 cm (Feinle et al., 2000). The bag was then deflated. The minimal distending pressure (MDP) was determined by increasing the pressure in 1 mmHg increments. The MDP is defined as the lowest pressure level that provides a mean intra-bag volume of 30 ml (Azpiroz and Malagelada, 1990) and is necessary to overcome intra-abdominal pressure. The pressure was set at 2 mmHg above MDP and the corresponding volume monitored until stable. After a baseline period the treatment was given, in this case, Iberogast®, as described in Chapter 10. Changes in intra-bag volume were then recorded as a measure of gastric relaxation.

Intra-bag pressures and volumes were digitised and recorded on a computer-based system running commercially available software (Protocol PlusTM, G&J Electronics, Toronto, Ontario, Canada), and stored for subsequent analysis.

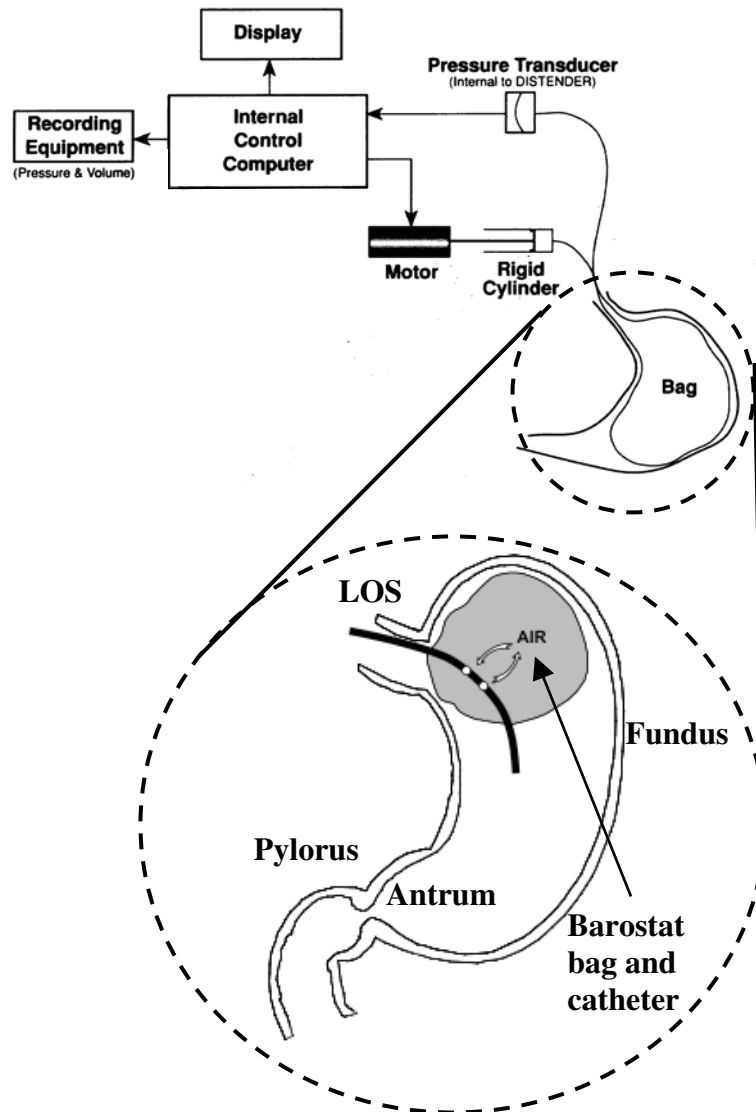


Figure 4.2 Schematic representation of the barostat bag and recording equipment used for assessment of proximal gastric volume.

4.5.3 Scintigraphy

Scintigraphy is the gold standard and most commonly applied method for the evaluation of gastric emptying and intragastric distribution and quantifies the rate at which a radiolabelled meal empties from the stomach, by acquiring images on a computer, via a gamma camera (Collins et al., 1983).

A dual isotope technique was used to label the solid and liquid components of a meal. The solid phase consisted of a 100 g ground beef patty (270 kcal, 25 g protein, 21 g fat) and was labelled with 20 MBq ^{99m}Tc -sulfur colloid chicken liver. The liquid phase consisted of a 150 ml drink containing 10 % dextrose (60 kcal) and was labelled with 6 MBq ^{67}Ga -EDTA (Little et al., 2006b). ^{99m}Tc has a half-life of 6 hours and total body radiation exposure is approximately 0.48 mSv. ^{67}Ga has a half-life of 78 hours and total body radiation exposure is approximately 1.8 mSv.

Scintigraphic images were acquired in 1 min frames for the first 60 min and thereafter in 3 min frames. The counts detected by the gamma camera were corrected for subject movement, radionuclide decay and gamma-ray attenuation, as previously described (Collins et al., 1983). Regions-of-interest were drawn for total, proximal and distal gastric regions, with the proximal region corresponding to the fundus and proximal corpus, and the distal region corresponding to the distal corpus and antrum. The counts plotted over time represented emptying of the meal from each region and were expressed as % retention over time (Collins et al., 1983). The lag phases for solid and liquid meal components were determined as the time period between meal completion and the appearance of radioactivity in the proximal small intestine (Collins et al., 1983). The amount of solid remaining in the stomach at $t = 100$ min and the time for 50 % of the liquid to empty (T50) were also calculated (Collins et al., 1983). Gastric emptying was classified as delayed when the solid % retention at $t = 100$ min was $> 61\%$ and/or liquid T50 was > 31 min, based on an established normal range (Jones et al., 2002).

4.5.4 Ultrasonography

Ultrasonography is a non-invasive technique, which does not expose the subject/patient to radiation. Two or three-dimensional ultrasound can be used to measure gastric emptying, intragastric distribution and proximal and antral motility (Holt et al., 1986, Hausken and Berstad, 1992a, Gentilcore et al., 2006b). Ultrasonography correlates well with scintigraphy, in that after ingestion of a low and high caloric drink, the time for the distal stomach content, as measured by scintigraphy, to decrease to 50 % of the maximum is similar to the ultrasound T50 (time taken for antral area to decrease to half its maximum) (Hveem et al., 1996). Two-dimensional ultrasonography was used for the measurement of fasting and postprandial antral area, using an Aloka SSD-650 CL Ultrasound Machine (ALOKA Co., LTD. Japan) with a 3.5 MHz sector transducer. To optimise precision, the transducer was positioned vertically to obtain a parasagittal image of the antrum with the superior mesenteric vein and the abdominal aorta in the longitudinal section and measurements performed at the end of expiration, as described previously (Hausken and Berstad, 1992b). The outer profile of the areas was outlined, and the width calculated automatically with the built-in calliper and the calculation program of the ultrasound apparatus (**Figure 4.3**).

NOTE: This figure is included on page 114 of the print copy of the thesis held in the University of Adelaide Library.

Figure 4.3 Schematic diagram of a two-dimensional ultrasonographic vertical scan through the antrum, superior mesenteric vein (Mv) and aorta (A) (Hausken and Berstad, 1992b).

4.5.5 Assessment of eating behaviour and perception

4.5.5.1 Diet / symptom diaries

Diet diaries are a commonly used tool for evaluating short-term dietary intake. 7-day diet diaries (**Appendix 1**) were used in the study reported in Chapter 9 to quantify food intake in healthy subjects and FD patients. For this purpose subjects and patients recorded everything they ate or drank, and the time of the eating or drinking episode, over a complete 7-day period (i.e. five week days and two weekend days) (Karvetti and Knuts, 1992). Subjects were asked to weigh as many foods as practical, or, alternatively, to use cup or spoon measures or common serves e.g. slice of bread. They were instructed to be as specific as possible, e.g. specify the type of bread (white/wholemeal), the degree of fat trimming of meat, type of margarine or oil, the type of milk (whole fat or skim) and the type of cooking method (fried, boiled or

roasted). If a recipe was followed the subject was instructed to also record it in the food diary.

The diary also included a symptom diary (**Appendix 1**), where all subjects recorded any symptoms experienced (abdominal pain, cramps, bloating, nausea, uncomfortable fullness after meals), its severity on a scale out of 10 (with 0 representing “symptom not present” and 10 “symptom most severe”) and the time at which these symptoms occurred.

All individual food and beverage items from the diet diaries were entered and the energy intake (in kcal or kJ), weight (in g or kg), and macronutrient content (absolute and % of total fat, carbohydrate, protein and alcohol) calculated using commercially available software (Foodworks Version 2.10, Xyris Software (Australia) Pty Ltd, Highgate Hill, QLD, Australia). In addition, the frequency of eating, periods between meals, total number of meal, snack and beverage episodes, were quantified.

4.5.5.2 *Visual analogue scale questionnaire*

The most common tool used to measure subjective appetite perceptions (specifically hunger and fullness) during or after a feeding challenge are validated visual analogue scales (VAS) (Parker et al., 2004, Flint et al., 2000, Sepple and Read, 1989). The VAS was also used to quantify sensory aspects (i.e. taste, sweetness, fattiness, colour, bitterness and sourness) of the preloads described in Chapter 4.4.1. The VAS used comprised of 100 mm horizontal lines with strengths of perceptions anchored at each end, describing the two different extremes (e.g. 0 mm “I am not hungry”, 100 mm “I am

extremely hungry”). Subjects were asked to place a vertical mark along the line corresponding to the strength of the perception at that time point at which the VAS was administered (**Appendices 2 and 3**). Quantification of each perception was achieved by measuring the distance from the left end of the line to the vertical mark. Symptoms of nausea, bloating (Chapter 5 -7), discomfort and pain (Chapters 5 - 7 and 8) were assessed using VAS. Two pre-treatment VAS were administered and each perception was averaged to obtain a baseline value. Subsequent VAS were given at different time intervals throughout the studies, as outlined in Chapters 5 - 7 and 8.

4.5.5.3 *Food intake*

The most commonly used method to quantify how much food a subject is able to consume after a specific treatment is ad libitum food intake at a buffet meal (Lavin et al., 1998, Feltrin et al., 2004). The buffet meal was presented at a fixed time interval, after a test meal was consumed (Chapter 8), or immediately after the cessation of intraduodenal infusions (Chapters 5 - 7). The buffet meal contained a variety of different food items as described in detail in **Table 4.4**, at quantities in excess of what the subject was expected to consume (Lavin et al., 1998, Feltrin et al., 2004). The subject was asked to eat until they were comfortably full or was given up to 30 min. All food items were weighed before and after presentation to the subject. Subsequently, energy consumption (kcal or kJ), amount eaten (g or kg) and macronutrient intake of fat, carbohydrate and protein (absolute (g) and % of total) were calculated using commercially available software (Foodworks Version 3.01, Xyris Software (Australia) Pty Ltd, Highgate Hill, QLD, Australia) (Feltrin et al., 2004).

Table 4.4 Composition of the buffet meal.

Food items	Amount served g	Energy content kJ	Fat g	Carbohydrate g	Protein g
Wholemeal bread, 4 slices ^a	125	1,304	3.6	50.0	12.6
White bread, 4 slices ^a	125	1,295	2.9	56.4	11.8
Ham, sliced ^b	100	453	3.6	0	18.8
Chicken, sliced ^c	100	677	7.0	0	24.6
Cheese, sliced ^d	85	1,436	28.3	0.9	21.9
Tomato, sliced	100	56	0.1	1.9	1.0
Lettuce	100	27	0	0.4	0.9
Cucumber, sliced	100	44	0.1	1.	0.5
*Strawberry yoghurt ^e	200	966	6.2	33.8	9.4
Fruit salad ^f	140	343	0.1	19.3	0.6
Chocolate custard ^g	150	622	5.3	22.7	4.8
[#] Milky Way®, 2 bars ^h	35	606	6.0	21.9	1.8
Apple	170	359	0.2	21.3	0.5
Banana	190	680	0.2	37.8	3.2
Orange juice, unsweetened ⁱ	500	800	5.0	42.5	5.0
Iced coffee ^l	600	1,788	10.2	61.8	21.0
Water	600	0	0	0	0
Margarine ^k	20	609	16.4	0.1	0.1
Mayonnaise ^l	20	310	6.5	4.0	0.2
Total	3,260* or 3,425 [#]	11,448* or 11,808 [#]	95.5* or 95.7 [#]	342.7* or 354.6 [#]	129.0* or 136.9 [#]

^aSunblest, Tiptop, Australia; ^bDeli leg ham, Woolworths, Australia; ^cVirginian chicken, Woolworths, Australia; ^dCoon Tasty Cheese slices, Australian Cooperative Foods Ltd., Australia; ^eYoplait, National Foods Ltd., Australia; ^fGoulburn Valley, SPC Ardmona Operations Ltd., Australia; ^gYogo, National Foods Ltd., Australia; ^hMars Inc, Virginia, USA; ⁱDaily Juice company, Australia; ^jFarmers Union, Balemar Pty. Ltd., Australia; ^kFlora, Unilever Australasia, Australia; ^lKraft, Kraft Foods Ltd., Australia. Please note: * used studies described in Chapters 5 - 7 only, [#] used in study described in Chapter 8 only.

4.5.5.4 *Three-factor eating questionnaire*

Because most studies in this thesis (Chapters 5 - 9) evaluated the effects of specific treatments on appetite and energy intake, it was important to identify and exclude individuals with “abnormal” eating behaviour. The three factor eating questionnaire, constructed by Stunkard and Messick (1985) (Stunkard and Messick, 1985), assesses three dimensions of human eating behaviour; (i) cognitive restraint of eating, (ii) disinhibition of eating and (iii) hunger. The questionnaire contains fifty one items; twenty one for factor I, sixteen for factor II and fourteen for factor III (see **Appendix 4** for scoring and distribution of factors of this questionnaire). The factor relevant to the studies reported in Chapters 5 - 7 of this thesis is “restrained eating” (Factor I), which is described as the tendency of individual to restrict their food intake in order to control their body weight (Herman and Mack, 1975). A score of ≤ 12 for factor I on this questionnaire was used as a cut off - healthy subjects were excluded from participating in these studies, if their score was higher than 12, as this classified them as restrained eaters. For studies 8 and 9, FD patients were not excluded based on their scores, as they were expected to have some degree of eating restraint.

4.5.5.5 *Eating attitudes test*

The eating attitudes test (EAT) was developed for evaluating a broad range of behaviours and attitudes frequently observed in anorexia nervosa or those who are at a high risk of developing this disorder (Garner and Garfinkel, 1979). This scale contains forty items which reflect a range of reported “anorexic” behaviours and attitudes, and subjects were required to judge whether these items applied to them quantitatively as; “always”, “very often”, “often”, “sometimes”, “rarely”, or “never” (see **Appendix 5** for

scoring of this questionnaire). If the subject answered an item which was marked with an “x” (**Appendix 5**), it was regarded as the most “symptomatic” response and, therefore, received a score of three points, and the adjacent alternatives were weighted as two points and one point, respectively. A minimum cut off score of 30 on this test was used to differentiate individuals who are “normal” from those with anorexic behaviour (Garner and Garfinkel, 1979). The EAT was administered to both healthy controls and FD patients, in the study described in Chapter 9.

4.5.5.6 *The Northwest Lipid Research Clinic fat intake scale*

The Northwest Lipid Research Clinic fat intake scale provides a reliable and valid estimate of fat, saturated fat, and cholesterol intake, when compared with 4 day food records (Retzlaff et al., 1997). This scale is brief, self-administered, hand-scored in under 3 minutes, with good reproducibility. It contains twelve items, which describe how many, how much and what type of foods and cooking methods used (**Appendix 6**). The maximum score is 44, and a score of less than 24 indicates the diet is moderate to low in fat and cholesterol. This instrument was administered in both healthy subjects and patients with FD, in the study described in Chapter 9.

4.5.6 Assessment of symptoms, quality of life, personality and psychological distress

4.5.6.1 *Nepean Dyspepsia Index*

The Nepean Dyspepsia Index (NDI) is a multidimensional disease-specific quality of life instrument, which discriminates dyspepsia from health (Talley et al., 1999b). It can

also be used to assess changes in symptoms. This questionnaire contains two sections; a symptom checklist and a disease-specific quality of life measure (**Appendix 7**). The symptom checklist contains fifteen upper abdominal symptoms. Subjects indicated, on a 5-point scale, the frequency, intensity and bothersomeness of each symptom in the last 2 weeks, and these scores were added together to give a total symptom score. The quality of life section contains twenty five items, divided into four sub-scales with questions relating to; (i) interference, or difficulty, with activities of daily living or work because of dyspepsia, combined with impaired enjoyment of life and emotional well-being (questions 1, 9 - 16, 19, 20, 21, 25 (maximum score: 65)), (ii) lack of knowledge of, and control over, the illness (questions 2, 3, 17, 18, 22 - 24 (maximum score: 35)), (iii) disturbances in eating or drinking (questions 4 - 6 (maximum score: 15)) and (iv) sleep disturbances (questions 7 and 8 (maximum score 10)), on a 5-point scale (Talley et al., 1999b) (**Appendix 7**). The NDI was administered to both healthy controls and FD patients, in the study described in Chapter 9.

4.5.6.2 Eysenck Personality Questionnaire

Eysenck Personality Questionnaire is a tool used to measure two traits of personality, extraversion and neuroticism. The questionnaire contains twenty four items, where the subject was to answer yes or no to behaviours relating to extraversion (sociable, dominant, active, impulsive) and neuroticism (anxiety, moodiness, obsessive, guilt) (Eysenck and Eysenck, 1964) (**Appendix 8**).

4.5.6.3 *The Hospital Anxiety and Depression Scale*

The Hospital Anxiety and Depression (HAD) Scale (**Appendix 9**) was developed to assess anxiety disorders and depression among patients in non-psychiatric hospitals (Zigmond and Snaith, 1983). The HAD scale is also a valid measure of severity of emotional disorders or neurosis which may coexist with a physical illness causing the patient to be more distressed by the symptoms of the illness which may lead to a complicated clinical presentation and a poor response to treatment (Zigmond and Snaith, 1983). This self-assessed questionnaire determines changes in the patients emotional state. It is subdivided into an anxiety and a depression subscale both containing seven items. Each item has a choice of four responses, i.e. not at all, occasionally, quite often, very often. Optimal cut-off scores for depression and anxiety have not been clearly identified for those with gastrointestinal disorders, but it has been suggested that scores of ≥ 11 for each subscale should be used (Zigmond and Snaith, 1983). The HAD scale was used in the study described in Chapter 9.

4.5.6.4 *Zung self-rating depression scale*

The Zung questionnaire was developed to quantify the symptoms of depression in patients whose primary diagnoses were that of a depressive disorder, but has also proved useful in patients with other psychiatric disorders, such as; anxiety reactions, personality disturbances, and psychophysiological disturbances (Zung, 1965). In this questionnaire, each subject is asked to rate each of the twenty items as to how it applies to them at the time of testing, in the following four quantitative terms: “a little of the time”, “some of the time”, “good part of the time”, or “most of the time” (See **Appendix 10** for the scoring of each item). The items included are divided into the

following; pervasive affect (depression, crying spells (questions 1 and 3)), physiological equivalents (diurnal variations, sleep, appetite, sex, weight loss, gastrointestinal, cardiovascular and musculoskeletal disturbances (questions 2, 4 - 10 respectively)), or psychological equivalents (confusion, retardation, agitation, hopelessness, irritability, indecisiveness, personal devaluation, emptiness, suicidal rumination and dissatisfaction (questions 11 - 20 respectively)). The maximum score which can be obtained is 80. A score of ≤ 50 is considered “normal”, i.e. not depressed (Zung, 1965). The Zung questionnaire was administered to both healthy controls and FD patients, in the study described in Chapter 9.

4.5.7 Blood sampling

In the studies described in Chapter 5 - 8 an intravenous cannula was inserted into an antecubital vein for blood sampling. Venous blood samples (~ 10 ml) were taken during the studies at specified time points as described in individual chapters for determination of blood glucose and plasma gastrointestinal hormone concentrations. Blood samples were collected in ice-chilled dipotassium EDTA tubes containing 480 μ l / 10 ml whole blood of the protease inhibitor, aprotinin (Trasylol®; Bayer Australia Ltd, Pymble, Australia). Plasma was separated by centrifugation (3200 rpm, 15 min, 4°C) within 30 min of collection and stored at 70°C until assayed.

4.5.8 Biochemical analysis

4.5.8.1 Blood glucose

Venous blood glucose concentrations (mmol/l) were determined immediately by the glucose oxidase method, using a portable glucose meter (Medisense Precision QID, Abbott Laboratories, Bedford, MA, USA). This technique has a coefficient of variation (CV) of 2.1 - 5.6 %. The accuracy of this method has been confirmed in our laboratory using the hexokinase technique (Horowitz et al., 1991).

4.5.8.2 Plasma insulin

Plasma insulin was measured by Enzyme-Linked ImmunoSorbent Assay (Diagnostics Systems Laboratories Inc, Webster, Texas, USA). The intra-assay CV was 2.6 % and the inter-assay CV was 6.2 %, with a detection limit of 2.6 mU/l (O'Donovan et al., 2004).

4.5.8.3 Plasma cholecystokinin

Plasma CCK concentrations (pmol/l) were determined after ethanol extraction using a previously described radioimmunoassay (MacIntosh et al., 2001b). A commercially available antibody (C258, Lot 105H4852, Sigma Chemical, St Louis, MO, USA) raised in rabbits against synthetic sulphated CCK-8 was employed. This antibody binds to all CCK peptides containing the sulphated tyrosine residue in position 7, shows a 26 % cross-reactivity with unsulphated CCK-8, less than 2 % cross-reactivity with human gastrin (0.2 % with gastrin I and 1 % with Big gastrin), and does not bind to structurally

unrelated peptides. The intra-assay CV was 9 % and the inter-assay CV was 27 %, with a detection limit of 2.5 pmol/l.

4.5.8.4 Plasma peptide YY

Plasma PYY concentrations (pmol/l) were measured by radioimmunoassay using an antiserum (kindly donated by Dr B Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) raised in rabbits against human PYY(1-36) (Sigma-Aldrich, St Louis, MO, USA). This antiserum showed < 0.001 % cross-reactivity with human pancreatic polypeptide and sulphated CCK-8, and 0.0025 % cross-reactivity with human neuropeptide Y. Tracer (purchased from Prosearch International Pty Ltd, Malvern, VIC, Australia) was prepared by radiolabeling synthetic human PYY(1-36) (Auspep, Parkville, VIC, Australia) using the lactoperoxidase method. Monoiodo-tyrosine-PYY was separated from free ¹²⁵I-iodine, diiodo- and unlabelled PYY by reverse phase HPLC (Phenomenex Jupiter C4 300A 5u column cat. no. 00B-4167-EO 250 x 4.6 mm). An elution gradient from 27 - 40 % acetonitrile in triethylamine phosphoric acid (pH 3.0) yielded 4 peaks of labeled PYY of which peak 3 demonstrated the highest specific binding and was used in the assays. Standards (1.6 - 50 fmol/tube) or samples (200 µl plasma) were incubated in assay buffer (0.05 M phosphate containing 0.5 % bovine serum albumin (BSA) and 0.02 % azide (pH 7.4)) with 100 µl antiserum at a final dilution of 1:10,000 for 20 - 24 hours at 4°C, 100 µl iodinated PYY (10,000 cpm) was then added and the incubation continued for another 20 - 24 hours. Separation of the antibody-bound tracer from free tracer was achieved by addition of 200 µl of dextran-coated charcoal containing gelatin (0.015 g gelatin, 0.09 g dextran, 0.15 g charcoal/30 ml assay buffer), incubated at 4°C for 20 min, and then

centrifuged at 4°C for 25 min. Radioactivity of the bound fraction was determined by counting the supernatants in a gamma counter. The intra-assay CV was 12.3 %, the inter-assay CV was 16.6 %, with a detection limit of 4 pmol/l.

4.5.8.5 *Plasma glucagon-like peptide-1*

Plasma GLP-1(7-36) amide concentrations (pmol/l) were measured by radioimmunoassay (Wishart et al., 1998). Ethanol extraction of plasma samples was undertaken using an antibody supplied by Professor SR Bloom (Hammersmith Hospital, London) that did not cross-react with glucagon, gastric inhibitory polypeptide, or other gut or pancreatic peptides and had been demonstrated by chromatography to measure intact GLP-1(7-36). The intra-assay CV was 17 %, the inter-assay CV was 18 %, with a detection limit of 1.5 pmol/l.

4.5.8.6 *Plasma glucose-dependent insulinotropic polypeptide (GIP)*

Plasma GIP was measured by radioimmunoassay (Wishart et al., 1992). The standard curve was prepared in buffer rather than extracted charcoal-stripped serum and the radioiodinated label was supplied by ProSearch International (Victoria, Australia). Both the intra- and inter-assay CVs were 15 %, with a detection limit of 2 pmol/l.

4.5.8.7 *Plasma ghrelin*

Plasma total ghrelin concentrations (pmol/l) were measured by radioimmunoassay (Parker et al., 2005), using an antiserum (RAST-4745, Bachem, CA, USA) that does not cross-react with human secretin, orexin, motilin, galantam or vasoactive intestinal

peptide. The intra-assay CV was 5 %, the inter-assay CV 18 %, with a detection limit of 40 pg/l.

4.6 STATISTICAL ANALYSIS

A P value of < 0.05 was considered significant. Detailed descriptions of the data and statistical analysis are provided in the individual chapters.

Chapter 5

EFFECTS OF LOAD, AND DURATION, OF DUODENAL LIPID ON GUT MOTILITY, PLASMA CCK AND PYY AND ENERGY INTAKE IN HEALTHY MEN

5.1 SUMMARY

Enterally administered lipid modulates APD motility, gut hormone release, appetite and energy intake. The aim of this study was to determine whether these effects would be dependent on both the load, and duration, of small intestinal exposure to lipid. 11 healthy men were studied on four occasions in double-blind, randomised, fashion. APD motility, plasma CCK and PYY concentrations and appetite perceptions were measured during intraduodenal infusion of lipid (Intralipid®) at (i) 1.33 kcal/min for 50 min, (ii) 4 kcal/min for 50 min and (iii) 1.33 kcal/min for 150 min, or (iv) saline for 150 min. Immediately after the infusions (i.e. t = 150 min) energy intake was quantified. PWSs were suppressed, and basal pyloric pressure, IPPWs, plasma CCK and PYY stimulated (all $P < 0.05$), during the first 50 min of lipid infusion, in a load-dependent fashion. The effect of the 4 kcal/min infusion was sustained so that the suppression of antral PWSs and PWSs and increase in PYY remained evident after cessation of the infusion (all $P < 0.05$). The prolonged lipid infusion (1.33 kcal/min for 150 min) suppressed antral PWSs,

stimulated CCK and PYY and basal pyloric pressure (all $P < 0.05$) and tended to stimulate IPPWs when compared with saline throughout the entire infusion period. There was no significant effect of any of the lipid infusions on appetite or energy intake, although nausea was slightly higher ($P < 0.05$) with the 4 kcal/min infusion. In conclusion, both the load, and duration, of small intestinal lipid influence APD motility and patterns of CCK and PYY release.

5.2 INTRODUCTION

Ingestion of fat triggers a number of gastrointestinal responses (Heddle et al., 1988a, Feinle et al., 2000, Chapman et al., 1999, Matzinger et al., 1999, Cook et al., 1997) as a result of the interaction of lipolytic products (principally fatty acids) with small intestinal receptors (Feinle et al., 2003, Feltrin et al., 2004, Malagelada et al., 1976, Matzinger et al., 2000). Studies in animals indicate that fat-induced small intestinal feedback on both gastric emptying, intestinal transit and appetite is dependent on both the length (Lin et al., 1990, Meyer et al., 1998c, Meyer et al., 1998b), and region (Meyer et al., 1998c, Lin et al., 1997, Meyer et al., 1998b), of small intestine exposed to fat. The effects of exposing different lengths, or regions, of the small intestine to fat on gastric emptying, APD motility, gut hormone release or appetite have not been directly evaluated in humans (Layer et al., 1990, Spiller et al., 1984, Welch et al., 1988); however, the effects of different loads (g, or kcal, per min) (Feinle et al., 2000, Andrews et al., 2001) and durations (Castiglione et al., 1998) of small intestinal lipid infusions on some of these parameters have been explored, indirectly assessing the effects of length, or region, of exposure. It seems reasonable to assume that with greater lipid loads, a greater length of small intestine would be exposed to lipid. For example, in both dogs

(Lin et al., 1996) and rats (Meyer et al., 1998a) ingestion of meals with an increasing fat content results in an increasing load of triglyceride emptying from the stomach into the duodenum, associated with an increasing spread of both lipolysis and absorption of fat to, and along, the ileum. This caudal spread of digestion and absorption results from exceeding the digestive capacity of lipase, as well as the absorptive capacities for lipolytic products per cm of gut (Meyer et al., 1998c, Meyer et al., 1998a).

Much less is known about how the duration of small intestinal fat exposure might affect gastrointestinal responses. Theoretically, this could have two important effects, i.e. (i) depending on the rate of intestinal transit, the duration of infusion could affect the distribution of lipolytic products along the gut, and (ii) at any given rate of infusion, the duration could affect the total amount of fat digested and absorbed. - Duodenal infusion of a high lipid load (which exceeds proximal gut lipolytic and absorptive capacities) for a prolonged period would ultimately result in the release of lipolytic products in both the proximal, and distal, small intestine, i.e. a long length of contact, while a shorter infusion at the same rate would limit spread of contact initially to the proximal small intestine, and then, after cessation of the infusion (as the bolus of fat and lipase moves distally), to contact in the distal, but no longer the proximal, small intestine. By contrast, the duration of infusion would not be expected to affect the distribution along the gut of a low lipid load, because, irrespective of the time period over which the fat was infused, it would be digested and absorbed efficiently within the proximal small intestine. The effects of the duration of exposure of the small intestine to lipid on the stimulation of CCK and PYY have also not been assessed in humans. It would, however, be anticipated that the patterns of release of these hormones would differ in response to changes in the load, and duration, of lipid infusions, because these affect the

distributions of lipolytic products along the gut, and CCK-secreting cells are located in the proximal small intestine (Buffa et al., 1976), while those which release PYY are located more distally (Adrian et al., 1985).

The aims of this study were to evaluate the acute effects of load, and duration, of intraduodenal lipid exposure on APD motor, plasma CCK and PYY, appetite and energy intake responses in healthy subjects. Accordingly, the effects of “low” (1.33 kcal/min, total: 67 kcal) and “high” (4 kcal/min, total: 200 kcal) lipid loads, infused intraduodenally for 50 min were quantified. The responses to these 50 min infusions were compared with those observed in response to infusions of lipid (1.33 kcal/min, total: 200 kcal) and saline (control), both given over 150 min. Fat empties initially from the human stomach at rates that vary with the fat content of the meal and the physical state of fat within the stomach, so that 1.33 kcal/min is a moderate rate typical of a meal containing 15 - 20 g of oil; whereas 4 kcal/min is a rapid rate typical of a meal containing 60 g of fat emulsified in a relatively large volume of water, as in a cream soup or milkshake (Meyer et al., 1999, Meyer et al., 1996). Since transit through the jejunum is known to take 60 min or less (Williams et al., 1984), it was postulated that infusion of the 4 kcal/min load for 50 min would result in the release of lipolytic products in the jejunum between 0 - 50 min, followed by further release of lipolytic products in the ileum between 50 - 150 min; whereas, by contrast, with the 1.33 kcal/min loads, whether given for 50 or 150 min, lipolytic products would be confined to the jejunum. If so, the plasma time-courses of CCK and PYY release would be anticipated to differ fundamentally after the “low” and “high” fat loads.

5.3 SUBJECTS AND METHODS

5.3.1 Subjects

11 healthy male subjects (age: 25 ± 2 yrs; body mass index (BMI): 23.4 ± 0.7 kg/m²) were recruited according to guidelines described in Chapter 4.2.

5.3.2 Study outline

Each subject was studied on four occasions, separated by 3 - 10 days, on which they received, in randomised, double-blind fashion, an intraduodenal infusion of a lipid emulsion, at (i) 1.33 kcal/min for 50 min (“1.33/50”), (ii) 4 kcal/min for 50 min (“4/50”) or (iii) 1.33 kcal/min for 150 min (“1.33/150”), or (iv) intraduodenal isotonic saline (“control”) for 150 min. APD pressures, plasma CCK and PYY concentrations, appetite and energy intake were evaluated.

5.3.3 Intraduodenal infusions

The intraduodenal lipid emulsion (10 % Intralipid®, 300 mOsmol/kg, 1.1 kcal/ml, Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia) was administered as: (i) 1.33 kcal/min for 50 min, (ii) 4 kcal/min for 50 min and (iii) 1.33 kcal/min for 150 min. On the remaining study day (iv), isotonic saline was given for 150 min. Conditions (i) and (ii) were designed to assess the effects of lipid load, resulting in total energy deliveries of 67 kcal and 200 kcal, respectively, over 50 min. Conditions (ii) and (iii) allowed evaluation of the effects of identical intraduodenal lipid loads (200 kcal), given over different time periods (50 min vs. 150 min). In conditions (i) and (ii), the intraduodenal lipid infusion was followed by an intraduodenal saline infusion between

50 - 150 min, so that the infusion duration was identical in all four study conditions. Lipid solutions were diluted with isotonic saline to achieve the specific loads, and all infusions were administered at a rate of 4 ml/min, so that the total volume infused in all study conditions was 600 ml (see Chapter 4.4.2.1). The infusions were prepared by an investigator who was not otherwise involved in either the performance of the studies or data analysis. During studies the infusion apparatus was covered at all times.

5.3.4 Protocol

Each subject attended the laboratory in the Discipline of Medicine at 0830 h after an overnight fast (14 hours for solids, 12 hours for liquids). A manometric catheter was positioned, as described in Chapter 4.5.1. An intravenous cannula was placed in a forearm vein for blood sampling.

Once the manometric catheter was positioned correctly, fasting motility was monitored until the occurrence of a phase III of the interdigestive MMC (Feltrin et al., 2004). Immediately after cessation of the phase III (at $t = -10$ min), a baseline blood sample was taken, and a VAS questionnaire, assessing appetite-related sensations and gastrointestinal symptoms (Parker et al., 2004), was completed. At $t = 0$ min, during phase I of the MMC, the duodenal infusion of lipid or saline was commenced. APD pressures were monitored throughout the infusion period; VASs were completed every 15 min from $t = 0 - 150$ min and blood samples taken every 15 min from $t = 0 - 30$ min and every 30 min from $t = 30 - 150$ min. At $t = 150$ min, the infusion was terminated, the subject immediately extubated, and then offered a cold, buffet-style, meal (Feltrin et al., 2004). Each subject was given 30 min (i.e. $t = 150 - 180$ min) to consume the meal

and instructed to eat until comfortably full. After the meal, the intravenous cannula was removed, and the subject allowed to leave the laboratory.

5.3.5 Measurements

5.3.5.1 *Antropyloroduodenal pressures*

APD pressures were assessed, and analysed for (i) number and amplitude of antral and duodenal PWs, (ii) number and amplitude of IPPWs, (iii) basal pyloric pressure and (iv) number and length of PWSs, as described in Chapter 4.5.1.

5.3.5.2 *Plasma cholecystokinin and peptide YY concentrations*

Blood sample collection and analysis of plasma CCK and PYY were performed as described in Chapters 4.5.7 and 4.5.8.

5.3.5.3 *Appetite perceptions*

Appetite perceptions (desire to eat, hunger, fullness, prospective consumption ('how much would you eat if given a meal now?')) were rated on VAS, as described in Chapter 4.5.5.2 (Parker et al., 2004). Nausea and bloating were also assessed (Chapter 4.5.5.2).

5.3.5.4 *Energy intake*

Assessment of energy intake is described in Chapter 4.5.5.3.

5.3.6 Data and statistical analyses

Baseline (“0”) values were calculated as the means of values obtained between $t = -10 - 0$ min for the number and amplitude of IPPWs and antral and duodenal PWs, basal pyloric pressure and PWSs, and at $t = -10$ and 0 min for plasma hormone concentrations and VAS scores. Antral and duodenal PWs and PWSs were analysed in three periods, i.e. from $t = 0 - 50$ min (the duration of the intraduodenal lipid infusion in conditions (i) and (ii)), $t = 50 - 100$ min and $t = 100 - 150$ min (to evaluate the effect of the more prolonged infusion in condition (iii)). Antral and duodenal motility indices (MI) were derived using the equation; natural logarithm [(sum of amplitudes x number of phasic pressure waves) + 1] (Camilleri and Malagelada, 1984). Basal pyloric pressures and the number and amplitude of IPPWs were expressed as the mean of 10 min periods over the 150 min infusion period. PWSs were expressed as the total number of waves spanning over two ($1.5 < 3$ cm), three ($3 < 4.5$ cm), four ($4.5 < 6$ cm), ... , fifteen ($21 < 22.5$ cm) channels. Mean values for plasma CCK and PYY concentrations and VAS scores were calculated at each time point from $t = 0 - 150$ min. All motility and VAS data were expressed as changes from baseline, while plasma CCK and PYY were expressed as absolute values.

Basal pyloric pressure, the number and amplitude of IPPWs, plasma concentrations of CCK and PYY and VAS scores were analysed by repeated measures analysis of variance (ANOVA), with time and treatment as factors. The total number of PWSs was analysed by repeated measures ANOVA, between $t = 0 - 50$ min, $t = 50 - 100$ min and $t = 100 - 150$ min, with number and length (cm) as factors. The number, amplitude and MI of antral and duodenal PWs, between $t = 0 - 50$ min, $t = 50 - 100$ min and $t = 100 -$

150 min, and energy intake were analysed by one-way ANOVA. Statistical significance was accepted at $P < 0.05$, and data are presented as means \pm SEM.

5.4 RESULTS

All subjects tolerated the studies well. Baseline values for motility and VAS data are given in **Table 5.1**. There were no differences between the four experimental conditions.

5.4.1 Antropyloroduodenal pressures

5.4.1.1 *Antral pressures waves*

5.4.1.1.1 Period 1: 0 - 50 min

There was no effect of treatment on the number, or amplitude, of antral PWs (data not shown). There was, however, an effect of treatment on the MI of antral PWs ($P < 0.05$) (**Figure 5.1A**); 4/50 ($P < 0.01$) decreased, and 1.33/50 ($P = 0.07$) tended to decrease, the MI when compared with saline, with no difference between saline and 1.33/150 or between 1.33/50, 1.33/150 and 4/50.

5.4.1.1.2 Period 2: 50 - 100 min

There was no effect of treatment on the number of antral PWs (data not shown). There was, however, a significant effect of treatment on the amplitude of antral PWs ($P < 0.05$; saline: 15 ± 8 mmHg, 1.33/50: 16 ± 6 mmHg, 1.33/150: 0 ± 5 mmHg, 4/50: -6 ± 5 mmHg (the negative value reflects the correction for baseline)); 4/50 decreased the

amplitude when compared with saline and 1.33/50 ($P < 0.05$ for both). There was a trend for 1.33/150 to decrease the amplitude when compared with saline ($P = 0.09$) and 1.33/50 ($P = 0.07$). There was no difference between 1.33/150 and 4/50. There was also an effect of treatment on the MI of antral PWs ($P < 0.05$) (**Figure 5.1B**); 1.33/150 and 4/50 decreased the MI when compared with saline and 1.33/50 ($P < 0.05$ for all), with no difference between saline and 1.33/50, or between 1.33/150 and 4/50.

5.4.1.1.3 Period 3: 100 - 150 min

There was an effect of treatment on the number of antral PWs ($P < 0.01$; saline: 62 ± 13 , 1.33/50: 32 ± 10 , 1.33/150: 2 ± 1 , 4/50: 29 ± 13); 1.33/50 ($P < 0.05$), 1.33/150 ($P < 0.001$) and 4/50 ($P < 0.05$) decreased the number of antral PWs when compared with saline; 1.33/150 decreased the number of antral PWs when compared with 1.33/50 and 4/50 ($P < 0.05$ for both), with no difference between 1.33/50 and 4/50. There was an effect of treatment on the amplitude of antral PWs ($P < 0.05$; saline: 21 ± 5 mmHg, 1.33/50: 30 ± 9 mmHg, 1.33/150: 4 ± 6 mmHg, 4/50: -5 ± 8 mmHg); 4/50 decreased the amplitude when compared with saline ($P < 0.05$) and 1.33/50 ($P < 0.01$); 1.33/150 decreased the amplitude when compared with 1.33/50 ($P < 0.05$), while there was no difference between 1.33/150 and 4/50. There was an effect of treatment on the MI for antral PWs ($P < 0.05$) (**Figure 5.1C**); 1.33/150 ($P < 0.01$) and 4/50 ($P < 0.05$) decreased the MI when compared with saline; 1.33/150 decreased the MI when compared with 1.33/50 ($P < 0.05$), with no differences between 1.33/50 and saline or 4/50, or between 1.33/150 and 4/50.

5.4.1.2 Pyloric pressures

5.4.1.2.1 Basal pressures

There was a treatment by time interaction for basal pyloric pressure ($P < 0.01$) (**Figure 5.2A**); 1.33/50, 1.33/150 and 4/50 increased basal pyloric pressure when compared with saline; 1.33/50 between 0 - 20 min, 1.33/150 between 0 - 20 min and 120 - 140 min and 4/50 between 20 - 40 min ($P < 0.05$ for all). 4/50 increased basal pressure when compared with 1.33/50 between 20 - 60 min and 1.33/150 between 20 - 40 min ($P < 0.01$ for both). 1.33/150 increased basal pressure when compared with 1.33/50 between 60 - 70 min and 120 - 150 min ($P < 0.05$), while there was no difference between 1.33/150 and 4/50 between 40 - 150 min.

5.4.1.2.2 Phasic pressures

There was a treatment by time interaction for the number of IPPWs ($P < 0.001$) (**Figure 5.2B**); 1.33/50, 1.33/150 and 4/50 increased the number of IPPWs when compared with saline, 1.33/50 between 0 - 60 min and 1.33/150 and 4/50 between 0 - 70 min ($P < 0.05$ for all). There was a trend for 1.33/150 to increase IPPWs between 90 - 120 min ($P = 0.08$) when compared with saline. 4/50 increased the number of IPPWs more than 1.33/50 between 30 - 70 min and 1.33/150 between 30 - 50 min ($P < 0.05$ for both). There was no difference between 1.33/50 and 1.33/150 between 0 - 60 min, however, between 70 - 110 min the number of IPPWs was greater for 1.33/150 when compared with 1.33/50 ($P < 0.05$). There was no difference between 1.33/150 and 4/50 after 70 min, although the mean values for 1.33/150 were higher. There was no effect of treatment on the amplitude of IPPWs (data not shown).

5.4.1.3 Duodenal pressures waves

5.4.1.3.1 Period 1: (0 - 50 min)

There was a trend for an effect of treatment on the number of duodenal PWs ($P = 0.08$; saline: 204 ± 24 , 1.33/50: 172 ± 34 , 1.33/150: 204 ± 35 , 4/50: 111 ± 34); the number of duodenal PWs was less with 4/50 when compared with saline and 1.33/150 ($P < 0.05$ for both), with no difference between saline, 1.33/50 and 1.33/150. There was no effect of treatment on the amplitude of duodenal PWs (data not shown). There was an effect of treatment on the MI of duodenal PWs ($P = 0.05$) (**Figure 5.3A**); 4/50 decreased the MI when compared with saline, 1.33/50 and 1.33/150 ($P < 0.05$ for all), with no difference between saline, 1.33/50 and 1.33/150.

5.4.1.3.2 Period 2: (50 - 100 min)

There was no effect of treatment on the number, or amplitude, of duodenal PWs (data not shown). There was, however, an effect of treatment on the MI of duodenal PWs ($P < 0.05$) (**Figure 5.3B**); the MI during 1.33/50 was greater when compared with saline, 1.33/150 and 4/50 ($P < 0.05$ for all), while there was no difference between saline, 1.33/150 and 4/50.

5.4.1.3.3 Period 3: (100 - 150 min)

There was an effect of treatment on the number of duodenal PWs ($P < 0.01$; saline: 277 ± 21 , 1.33/50: 289 ± 45 , 1.33/150: 167 ± 27 , 4/50: 350 ± 34); the number of duodenal PWs was less for 1.33/150 when compared with saline ($P < 0.05$), 1.33/50 ($P < 0.05$) and 4/50 ($P < 0.001$), with no differences between saline, 1.33/50 and 4/50. There was

no effect of treatment on the amplitude of duodenal PWs (data not shown), but there was a trend for an effect of treatment on the MI of duodenal PWs ($P = 0.07$) (**Figure 5.3C**); the MI during 1.33/50 ($P = 0.07$) and 4/50 ($P < 0.01$) was greater when compared with 1.33/150, with no difference between saline, 1.33/50 and 4/50.

5.4.1.4 *Pressure wave sequences*

Only PWSs which spanned over 2 - 6 channels (i.e. 1.5 - 9 cm) were analysed statistically, as PWSs spanning over 7 - 15 channels were infrequent (0 - 50 min: saline, 3 ± 0 ; 1.33/50, 2 ± 0 ; 1.33/150, 2 ± 0 ; 4/50, 5 ± 1 ; 50 - 100 min: saline, 6 ± 0 ; 1.33/50, 6 ± 1 ; 1.33/150, 3 ± 0 ; 4/50, 4 ± 1 ; 100 - 150 min: saline, 6 ± 0 ; 1.33/50, 3 ± 0 ; 1.33/150, 3 ± 0 ; 4/50, 7 ± 1).

5.4.1.4.1 Period 1: (0 - 50 min)

There was a treatment by length interaction for the number of PWSs ($P < 0.05$) (**Figure 5.4A**); both 1.33/50 and 1.33/150 decreased the number of PWSs that spanned over two and three channels when compared with saline ($P < 0.05$ for all). 4/50 decreased the number of PWSs that spanned over two, three and four channels when compared with saline ($P < 0.05$), and the number of PWSs that spanned over two channels when compared with 1.33/50 and 1.33/150 ($P < 0.01$). There was no difference between 1.33/50 and 1.33/150.

5.4.1.4.2 Period 2: (50 - 100 min)

There was a treatment by length interaction for the number of PWSs ($P < 0.05$) (**Figure 5.4B**); 4/50 decreased the number of waves that spanned over two channels when compared with saline ($P < 0.05$). The number of waves that spanned over two and three channels was greater during 1.33/50 when compared with 1.33/150 and 4/50 ($P < 0.05$ for both), while there was no difference between 1.33/150 and 4/50.

5.4.1.4.3 Period 3: (100 - 150 min)

There was a treatment by length interaction for the number of PWSs ($P < 0.05$) (**Figure 5.4C**); 1.33/150 decreased the number of waves that spanned over two channels when compared with saline, 1.33/50 and 4/50 ($P < 0.01$ for all).

5.4.2 Plasma hormone concentrations

5.4.2.1 *Cholecystinin*

There was a treatment by time interaction for plasma CCK concentrations ($P < 0.001$) (**Figure 5.5A**). Plasma CCK concentrations reached a maximum at 15 min during all lipid treatments (1.33/50: 9.3 ± 1.1 pmol/l, 1.33/150: 9.2 ± 1.1 pmol/l, 4/50, 11.8 ± 1.4 pmol/l). During 1.33/50 and 1.33/150, plasma CCK declined after 15 min, returning to baseline values for 1.33/50 by 90 min, while for 1.33/150 levels gradually decreased, but were still higher than baseline at 150 min ($P < 0.01$). During 4/50, CCK concentrations plateaued between 15 - 60 min, and thereafter progressively decreased to be just above baseline at 90 min. 1.33/50, 1.33/150 and 4/50 increased plasma CCK when compared with saline during the first 60 min of the infusion ($P < 0.01$ for all) and

the effect of 4/50 was greater when compared with 1.33/50 and 1.33/150 ($P < 0.01$ for both), with no difference between 1.33/50 and 1.33/150. During 1.33/150, plasma CCK concentrations were greater when compared with saline, 1.33/50 and 4/50 between 90 - 150 min ($P < 0.05$ for all), with no difference between saline, 1.33/50 and 4/50.

5.4.2.2 Peptide YY

There was a treatment by time interaction for plasma PYY concentrations ($P < 0.001$) (**Figure 5.5B**). With both 1.33/50 and 1.33/150, PYY concentrations reached a maximum (1.33/50: 17.3 ± 1.4 pmol/l, 1.33/150: 18.7 ± 2.2 pmol/l) at 30 min, after which levels for 1.33/50 gradually declined, while for 1.33/150 levels remained constant until 150 min. In response to 4/50, there was a marked stimulation of PYY concentrations with a peak at 60 min (36.7 ± 3.8 pmol/l) and a decline after that time, although levels were still higher than baseline at 150 min ($P < 0.001$). 1.33/50, 1.33/150 and 4/50 increased plasma PYY when compared with saline over the entire infusion period ($P < 0.05$ for all), and the effect of 4/50 was greater when compared with 1.33/50 between 30 - 150 min, and with 1.33/150 between 30 - 120 min ($P < 0.05$ for all). There was no difference between 1.33/50 and 1.33/150 during the first 90 min of infusion, while PYY was higher during 1.33/150 between 120 - 150 min ($P < 0.01$).

5.4.3 Appetite perceptions

While there was no effect of treatment on appetite perceptions, mean scores for prospective consumption were less during 4/50 when compared with the other infusions between 0 - 105 min ($P = 0.28$) (**Figure 5.6A**). There was an effect of treatment on scores for nausea ($P < 0.05$) (**Figure 5.6B**); although mean scores were low, they were

greater during 4/50 when compared with saline, 1.33/50 and 1.33/150 ($P < 0.05$ for all) between 0 - 60 min, with no difference between saline, 1.33/50 and 1.33/150.

5.4.4 Energy intake

There was no effect of treatment on energy intake, the amount eaten, or macronutrient distribution of food eaten at the buffet meal, however, mean energy intake was lower following 1.33/150 ($P = 0.3$) (**Table 5.2**).

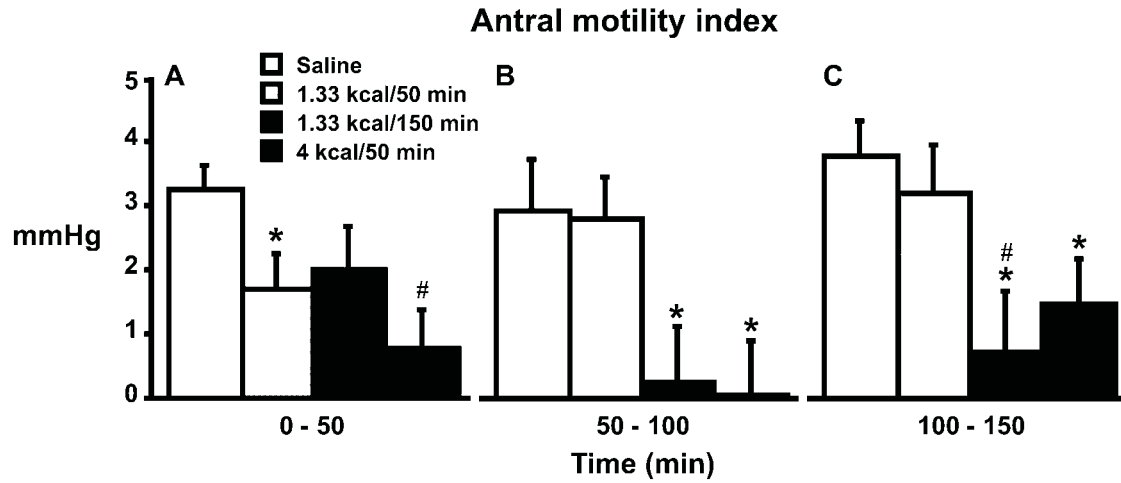


Figure 5.1 Motility index for antral pressure waves between (A) 0 - 50 min, (B) 50 - 100 min and (C) 100 - 150 min, during intraduodenal infusion of 10 % Intralipid® at 1.33 kcal/min for 50 min, 1.33 kcal/min for 150 min and 4 kcal/min for 50 min, or saline for 150 min. (A) * vs. saline; $P = 0.07$, # vs. saline; $P < 0.01$. (B) * vs. saline and 1.33/50; $P < 0.05$. (C) * vs. saline; $P < 0.05$, # vs. 1.33/50; $P < 0.05$. Data are means \pm SEM ($n = 11$).

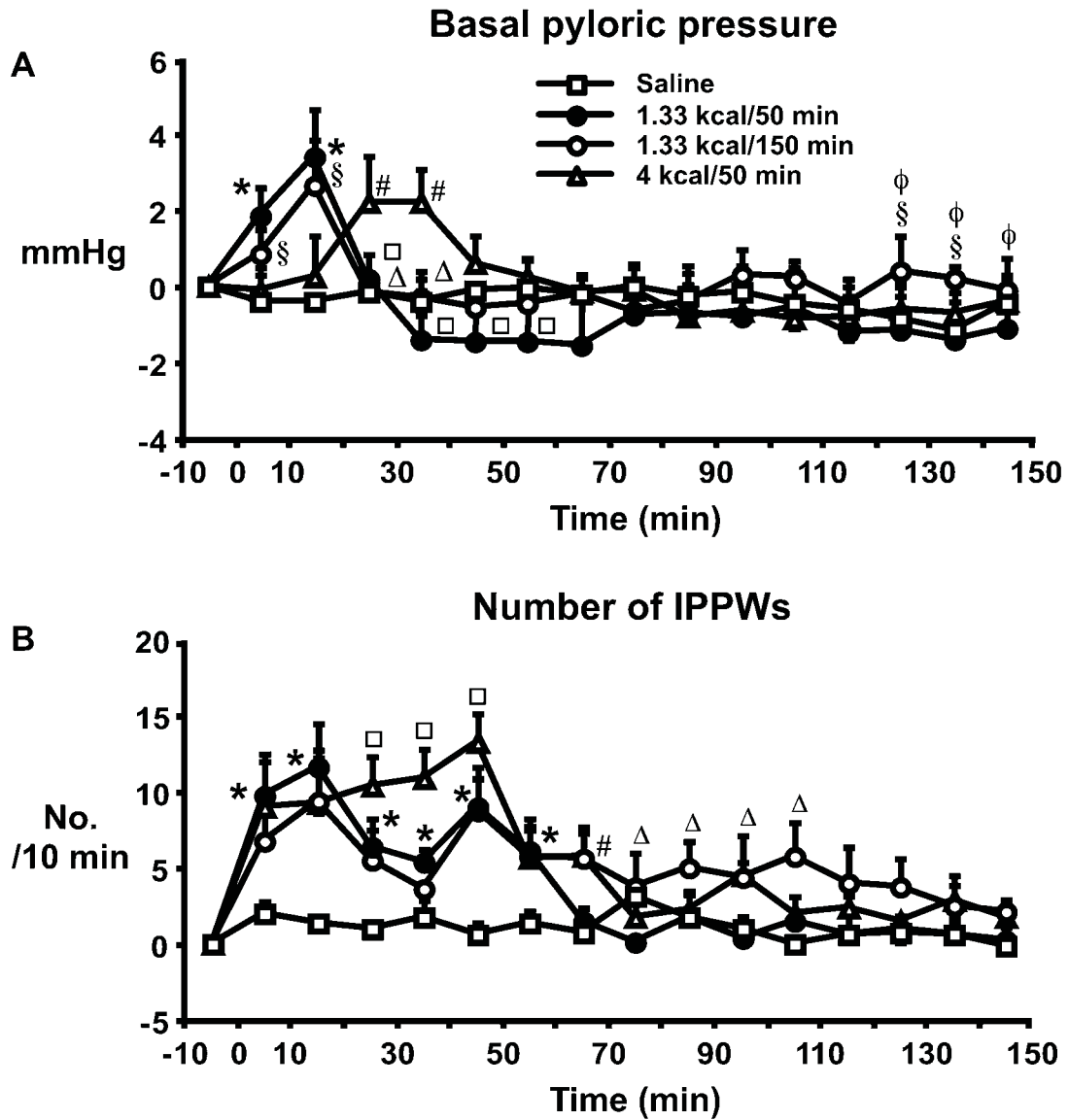


Figure 5.2 (A) Basal pyloric pressure and (B) number of isolated pyloric pressure waves (IPPWs) during intraduodenal infusion of 10 % Intralipid® at 1.33 kcal/min for 50 min (●), 1.33 kcal/min for 150 min (○) and 4 kcal/min for 50 min (△), or saline for 150 min (□). (A) * 1.33/50 vs. saline; $P < 0.05$, # 4/50 vs. saline; $P < 0.05$, § 1.33/150 vs. saline; $P < 0.05$, □ 1.33/50 vs. 4/50; $P < 0.01$; Δ 1.33/150 vs. 4/50; $P < 0.01$, φ 1.33/150 vs. 1.33/50; $P < 0.05$. (B) * 1.33/50, 1.33/150 and 4/50 vs. saline; $P < 0.05$, # 1.33/150 and 4/50 vs. saline and 1.33/50; $P < 0.05$, □ 4/50 vs. 1.33/50 and 1.33/150; $P < 0.05$, Δ 1.33/150 vs. 1.33/50; $P < 0.05$. Data are means \pm SEM (n = 11).

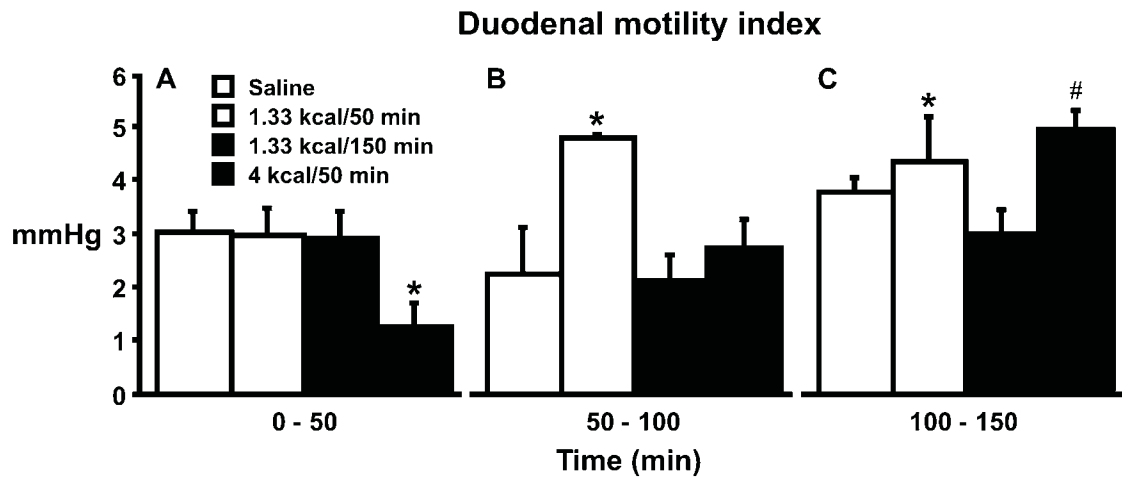


Figure 5.3 Motility index for duodenal pressure waves between (A) 0 - 50 min, (B) 50 - 100 min and (C) 100 - 150 min, during intraduodenal infusion of 10 % Intralipid at 1.33 kcal/min for 50 min, 1.33 kcal/min for 150 min, and 4 kcal/min for 50 min, or saline for 150 min. (A) * vs. saline, 1.33/50 and 1.33/150; $P < 0.05$. (B) * vs. saline, 1.33/150 and 4/50; $P < 0.05$. (C) * vs. 1.33/150; $P = 0.07$, # vs. 1.33/150; $P < 0.01$. Data are means \pm SEM ($n = 11$).

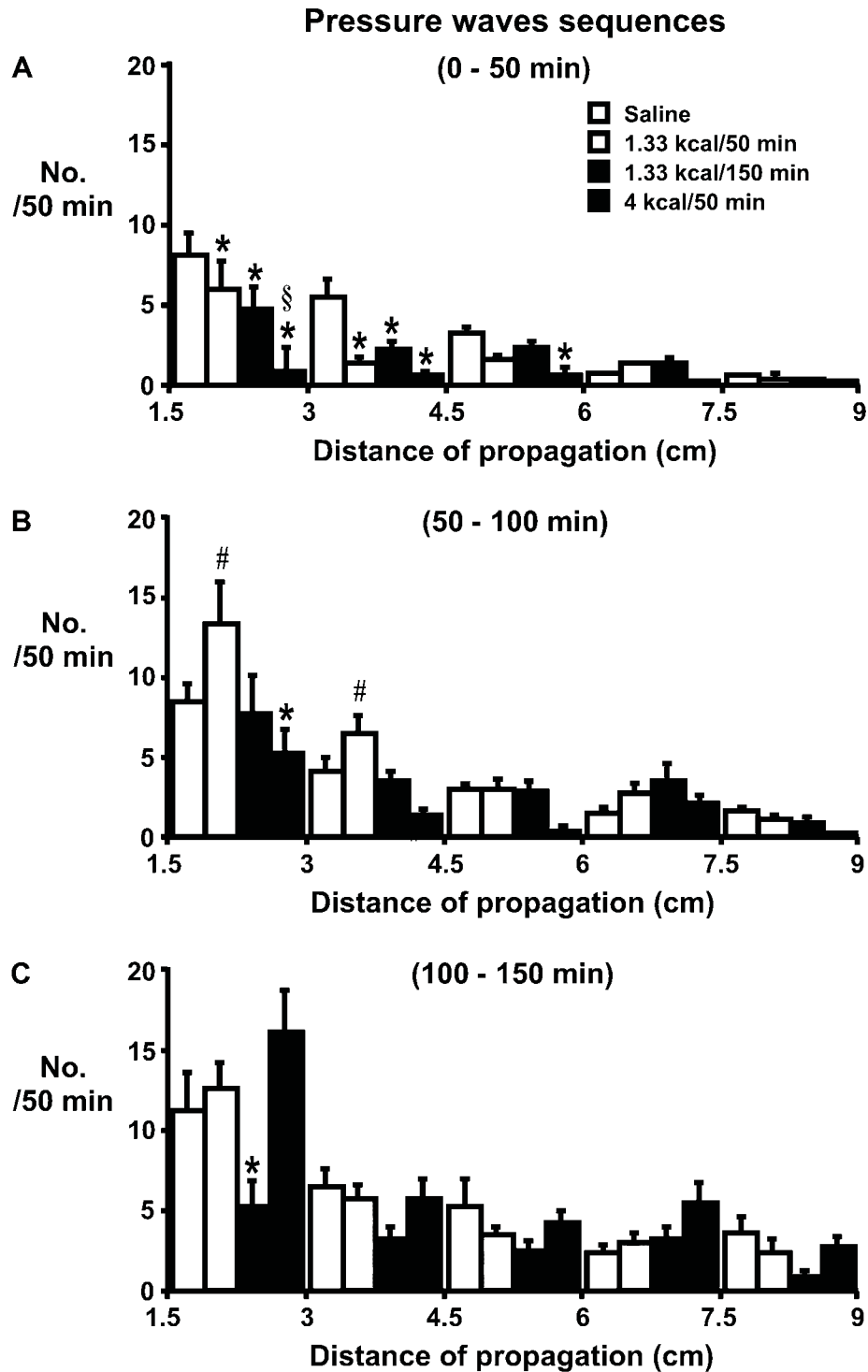
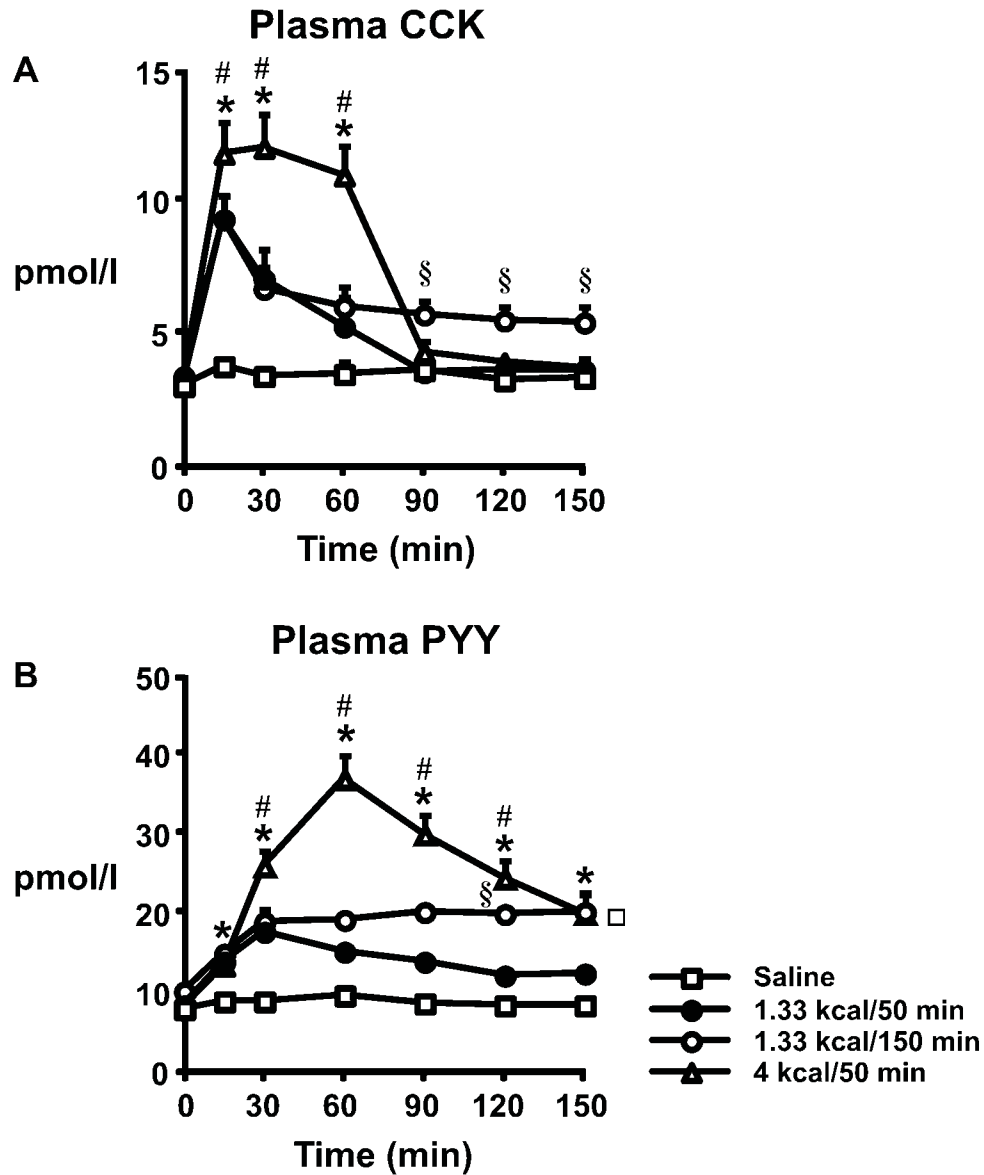


Figure 5.4 Pressure wave sequences between (A) 0 - 50 min, (B) 50 - 100 min and (C) 100 - 150 min, during intraduodenal infusion of 10 % Intralipid® at 1.33 kcal/min for 50 min, 1.33 kcal/min for 150 min and 4 kcal/min for 50 min, or saline for 150 min. (A) * vs. saline; $P < 0.05$, § vs. 1.33/50 and 1.33/150; $P < 0.01$. (B) * vs. saline; $P < 0.05$, # vs. 1.33/150 and 4/50; $P < 0.05$. (C) * vs. saline, 1.33/50 and 4/50; $P < 0.01$. Data are means \pm SEM ($n = 11$).

**Figure 5.5**

Plasma concentrations of (A) cholecystokinin (CCK) and (B) peptide YY (PYY) during intraduodenal infusion of 10 % Intralipid® at 1.33 kcal/min for 50 min (●), 1.33 kcal/min for 150 min (○) and 4 kcal/min for 50 min (△), or saline for 150 min (□). (A) * 1.33/50, 1.33/150 and 4/50 vs. saline; $P < 0.01$, # 4/50 vs. 1.33/50 and 1.33/150; $P < 0.01$, § vs. saline, 1.33/50 and 4/50; $P < 0.01$. (B) * 1.33/50, 1.33/150 and 4/50 vs. saline; $P < 0.05$, # 4/50 vs. 1.33/50 and 1.33/150; $P < 0.05$, § 1.33/150 vs. 1.33/50; $P < 0.01$, □ 1.33/150 and 4/50 vs. 1.33/50; $P < 0.05$. Data are means \pm SEM ($n = 11$).

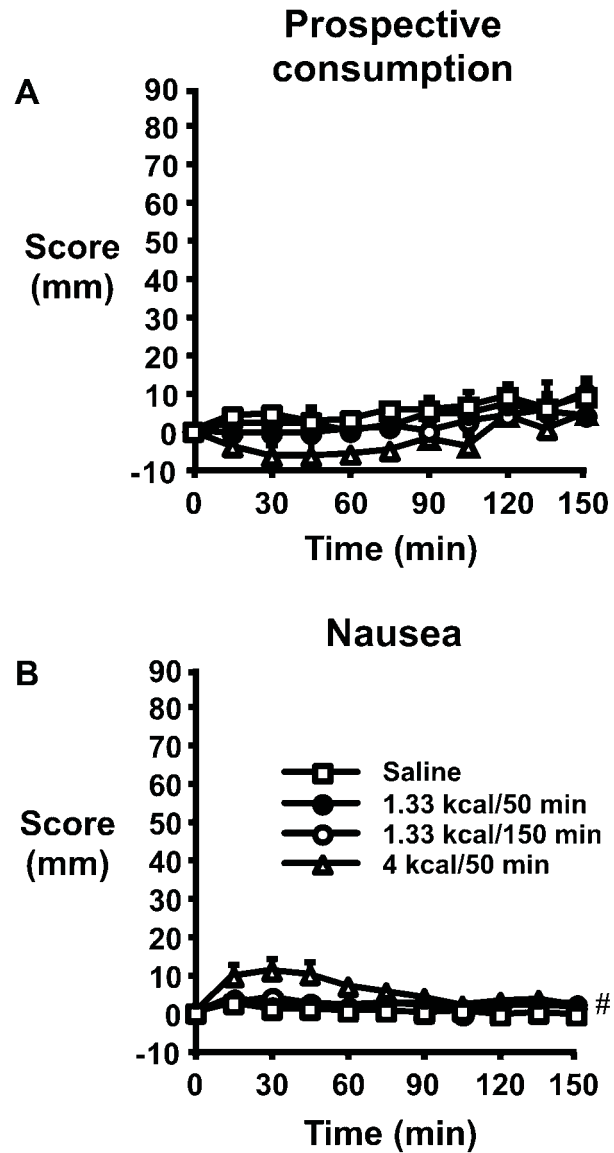


Figure 5.6 Scores for (A) prospective consumption and (B) nausea during intraduodenal infusion of 10 % Intralipid® at 1.33 kcal/min for 50 min (●), 1.33 kcal/min for 150 min (○) and 4 kcal/min for 50 min (▲), or saline for 150 min (□). (A) 4/50 tended to decrease prospective consumption when compared with saline. (B) # vs. saline, 1.33/50 and 1.33/150; $P < 0.05$. Data are means \pm SEM ($n = 11$).

Table 5.1 Motility indices of antral and duodenal pressure waves, basal pyloric pressure, number of isolated pyloric pressure waves, plasma cholecystokinin and peptide YY concentrations and nausea at baseline, before intraduodenal infusions of 10 % Intralipid[®] or saline commenced.

	Control (saline)	1.33 kcal/ 50 min	1.33 kcal/ 150 min	4 kcal/ 50 min
<i>Motility index</i>				
Antral PWs (mmHg)	3.7 ± 0.7	2.3 ± 0.6	3.4 ± 0.8	4.3 ± 0.8
Duodenal PWs (mmHg)	7.0 ± 0.9	5.8 ± 0.6	6.2 ± 0.7	6.3 ± 0.5
<i>Pyloric pressure</i>				
Basal (mmHg)	0.4 ± 4.5	0.0 ± 2.7	-2.7 ± 4.4	-2.1 ± 1.8
IPPWs (number)	1.3 ± 0.6	2.0 ± 0.8	4.1 ± 1.8	1.1 ± 0.6
<i>Plasma hormones</i>				
CCK (pmol/l)	3.6 ± 0.4	3.1 ± 0.5	3.3 ± 0.5	3.1 ± 0.4
PYY (pmol/l)	7.5 ± 1.3	8.7 ± 1.4	9.8 ± 1.3	8.0 ± 1.0
Nausea (mm)	8.1 ± 2.7	6.0 ± 2.5	6.6 ± 2.3	6.7 ± 2.6

Data are means ± SEM (n = 11). PWs, pressure waves; IPPWs, isolated pyloric pressure waves; CCK, cholecystokinin; PYY, peptide YY.

Table 5.2 Energy content (kJ), weight (g) and macronutrient distribution (% energy derived from fat, carbohydrate or protein) of food consumed at a buffet meal after intraduodenal infusions of 10 % Intralipid[®] or saline.

	Control (saline)	1.33 kcal/ 50 min	1.33 kcal/ 150 min	4 kcal/ 50 min	P value (ANOVA)
Energy (kJ)	5712 ± 443	6051 ± 534	5857 ± 488	6102 ± 600	NS
Weight (g)	1433 ± 111	1467 ± 141	1340 ± 87	1489 ± 128	NS
% kJ from fat	29 ± 2	30 ± 2	32 ± 2	29 ± 2	NS
% kJ from CHO	50 ± 3	50 ± 3	47 ± 3	50 ± 3	NS
% kJ from protein	20 ± 1	21 ± 1	21 ± 2	20 ± 1	NS

Data are means ± SEM (n = 11). CHO, carbohydrate; NS, not significant.

5.5 DISCUSSION

As anticipated from previous studies (Little et al., 2005, Andrews et al., 2001, Feinle et al., 2000), APD motor and hormone responses to the intraduodenal infusions between 0 - 50 min were dependent on the rate of lipid infusions, i.e. over the first 50 minutes, responses to 1.33/50 were comparable to those to 1.33/150, but less than those to the 4/50 infusion. Thus, the inhibition of duodenal pressure waves and APD pressure wave sequences, as well as the stimulation of basal pyloric pressures, IPPWs, plasma CCK and PYY and the induction of mild nausea were greater during the 4 kcal/min infusion than either of the 1.33 kcal/min infusions; in turn, the 1.33 kcal/min infusion, over a prolonged period, evoked higher basal pyloric pressures, more IPPWs, greater suppression of antral pressure waves, and greater plasma CCK and PYY responses than saline (control).

Such dose-responsiveness reflects two phenomena: (i) the concentration of the stimulating nutrients (in this case, fatty acids and monoglycerides) exceeds thresholds for detection by sensors located along the gut. For example, as the load (kcal/min) of triglyceride entering the duodenum increases, so do the rates of release, and thus luminal concentrations, of lipolytic products (Meyer et al., 1994), and (ii) with further increases in the rate of duodenal entry of triglycerides, hydrolysis takes longer to complete, and consequently lipolytic products are distributed further downstream. Also, mainly because of the slow rates of aqueous diffusion of poorly soluble, lipolytic products, absorption is also slow, hence, an increasing length of gut is required to complete the absorptive process as duodenal loads increase (Lin et al., 1996, Meyer et al., 1998a, Meyer et al., 1998c). For example, Lin and colleagues reported that a 15 g

fat emulsion emptied initially from the canine stomach at 0.9 kcal/min, but only 7 % of this duodenal load reached the ileum (i.e. was not absorbed proximally); in contrast, a 60 g fat emulsion emptied initially at 2 kcal/min, with 30 % of this load reaching the ileum (Lin et al., 1996). Some responses to luminal nutrients, including the inhibition of gastric emptying of aqueous contents (Lin et al., 1990) and energy intake (Meyer et al., 1998c), increase with the length of gut contacted; other nutrient-driven responses appear to be uniquely mediated by ileal mechanisms (Lin et al., 1997, Spiller et al., 1984, Layer et al., 1990).

In the present study, it was postulated that: (i) intestinal transit would carry the 4/50 bolus into the ileum after the first hour and that this transit would displace lipolytic products from more than 30 % of the infused load from the jejunum to the ileum where digestion and absorption would continue over the 100 min after cessation of the fat infusion, (ii) a correspondingly prolonged stimulation of ileal neural reflexes and/or release of ileal peptides would sustain a feedback inhibition of upper gastrointestinal pressures, but (iii) suppression of energy intake may not be apparent, as this is driven maximally by combined feedback from both proximal and distal gut sensors (Meyer et al., 1998c, Meyer et al., 1998b), and is less when lipolytic products are confined to the ileum (Welch et al., 1988, Meyer et al., 1998b). These observations suggested that these predictions were correct; after the 4/50 load (as opposed to the 1.33/50 infusion) continuing feedback from the ileum is attested to by sustained alterations in upper gastrointestinal pressures and continuing release of PYY, yet CCK had returned to baseline by 90 min and there was no suppression of energy intake at 150 min. While this study did not directly measure the distribution of lipolytic products along the gut, the simplest explanation for the persisting alterations in motility and changing ratios of

CCK:PYY in the plasma beyond 90 min after the 4/50 bolus is the persistence of lipolytic products in the ileum from 90 - 150 min.

Arguably the most striking, and novel, observation was the effect of altering the rate, and duration, of duodenal infusion of 200 kcal of fat on the time-course of plasma CCK and PYY secretion. During the 4/50 infusion, CCK rose rapidly, peaked at 15 min, and then remained elevated for a further 45 min at a concentration which was about twice the level sustained throughout the 1.33/150 infusion. Soon after cessation of the 4/50 infusion at 50 min, CCK fell to basal values. In contrast to the rapid fall of CCK, plasma concentrations of PYY peaked at 60 min and remained elevated between 50 - 150 min after cessation of the 4/50 infusion. Although PYY fell progressively after the 4/50 infusion was stopped at 50 min, plasma PYY levels remained significantly higher between 50 - 120 min when compared with the relatively constant level evident between 30 - 150 min during the 1.33/150 infusion. By contrast, after cessation at 50 min of the lower, 1.33/50, lipid load, both CCK and PYY fell rapidly to levels indistinguishable from control values, presumably because lipolysis of fat was completed, and thus lipolytic products were removed from the gastrointestinal lumen. The elevation of PYY between 50 - 150 min after the 4/50 infusion was stopped is presumably attributable to the presence of lipolytic products from this infusion in the gastrointestinal lumen, as a result of continuing lipolysis in the ileum or ileocolon as the 50 min bolus of fat and lipase moved along the distal small intestine. If so, the rapid decline in plasma CCK after cessation of the 4/50 infusion reflected not the disappearance of lipolytic products from the gastrointestinal lumen, but rather their movement distally, out of the CCK-bearing area of the jejunum (Buffa et al., 1976), into the PYY-rich area of the ileocolon (Adrian et al., 1985). By contrast, the nearly steady

plasma concentrations of both CCK and PYY sustained by the infusion of 1.33/150 must have reflected the continuing presence of lipolytic products in the CCK-bearing area of the proximal small intestine.

Although the explanation put forth in the preceding paragraph adequately explains the time courses for the plasma CCK and PYY responses, it is at odds with a canine study (Lin and Chey, 2003). In dogs, as in humans, CCK is stored in jejunal (Buffa et al., 1976), while PYY is stored in ileocolonic (Taylor, 1985), mucosa. In the study by Lin and colleagues (Lin and Chey, 2003), duodenally infused oleic acid was either confined to the proximal small intestine, infused into the distal small intestine, or simultaneously infused into both the proximal, and distal, small intestine in dogs. When oleic acid infusion was confined to the proximal small intestine, PYY rose slowly (peaking at 90 min), but substantially. On the other hand, PYY concentrations were some 20 % higher when the oleic acid was in direct contact with the distal bowel mucosa (whether infused distally or both proximally and distally), and the release of PYY by fat confined to the proximal small intestine was shown to be mediated, at least in part, by CCK (Lin et al., 2000). The observations described thus far are not in conflict with the scenario suggested in the preceding paragraph. However, in contrast to the present study, in dogs infusion of oleic acid induced similar rises in plasma CCK, whether confined to the proximal or distal small intestine (Lin and Chey, 2003). Thus, it can be speculated that the mechanism(s) for fat-induced CCK release in the dog may differ substantially from that in humans.

The much higher CCK release between 15 - 60 min in response to the 4 kcal/min, compared with 1.33 kcal/min, infusion might reflect a more rapid release, and thus

higher luminal concentrations, of lipolytic products in the proximal small intestine (Meyer et al., 1994); that is, a concentration-dependent release of CCK by fatty acids. Alternatively, it may reflect the much longer length of jejunal exposure to lipolytic products in the CCK-bearing region. If the latter were correct, the modest, but significant, release of PYY by the 1.33 kcal/min loads must have resulted from indirect, probably CCK-mediated, stimulation of PYY release (Lin et al., 2000), rather than release by direct contact, because the maximal CCK-bearing areas of the small intestine are proximal to the maximal PYY bearing areas, with minimal overlap.

A second interesting observation in the present study was the difference in the effect of lipid on antral and duodenal pressure waves during the first 50 min of infusion. The suppression of antral pressure waves was similar for all lipid infusions, however, there was no suppressive effect of both 1.33 kcal/min infusions on duodenal pressure waves, when compared with saline. This demonstrates that the 1.33 kcal/min load may be sufficient to have a maximal inhibitory effect on the antrum, but did not reach the threshold needed for suppression in the duodenum. To date, only one study has demonstrated load-related effects of lipid on intestinal motility, in which the number of duodenal pressure wave sequences was reported to decrease as the duodenal load of lipid increased (Andrews et al., 2001). However, in this study only relatively low loads of lipid were used (0.25 - 1.5 kcal/min), and the effects of lipid on pressures in the antrum and pylorus were not evaluated. In contrast, proximal gastric relaxation in response to duodenal lipid (Intralipid® as used in this study) at 1, 2 and 3 kcal/min, as measured by an electronic barostat, was found not to be load-dependent, as maximal relaxation occurred at the lowest load of 1 kcal/min (Feinle et al., 2000). These observations taken together indicate that there are regional differences in the upper

gastrointestinal tract in relation to the nutrient load required for a maximal inhibitory effect. It is also interesting that the inhibitory effect of the 4/50 infusion on antral pressure waves persisted to some extent after this infusion was stopped, particularly between 50 - 100 min, and were still present, between 100 - 150 min. Similarly, antral pressure waves continued to be strongly inhibited for the entire 150 min during the 1.33/150 infusion. The sustained inhibition of antral pressure waves after the 4/50 or the 1.33/150 infusion might be explained by persisting elevation of PYY, although it should be recognised that Raybould and colleagues (Raybould et al., 1999) have demonstrated in rats that PYY does not play a role in the inhibition of gastric emptying (not exactly the same as inhibition of antral or duodenal motility) after either duodenal or ileal infusion of lipid. By contrast, the time-courses of both basal pyloric pressures and IPPWs correspond closely to the time-courses of plasma CCK observed after the three lipid infusions, if it is assumed that the pylorus responded similarly to plasma CCK concentrations of 6 pmol/l or above.

Appetite scores and energy intake were not affected by any of the lipid infusions, although there was a significant, albeit slight, increase in nausea, and a tendency for reduced scores for prospective consumption, during 4/50 compared with the other infusions. The lack of effect of the 4/50 infusion on energy intake is likely to be accounted for by the experimental paradigm in which the meal was consumed 100 min after cessation of the lipid infusion, at a time when plasma concentrations of CCK and the number of IPPWs had declined to baseline, concentrations of PYY had waned, and unabsorbed lipolytic products had diminished and probably contacted only a portion of distal bowel mucosa. For example, Castiglione and colleagues (Castiglione et al., 1998) assessed the effect of 20 % Intralipid® on energy intake over different durations of

infusion (15 min (30 kcal), 45 min (90 kcal), or 90 min (180 kcal)) in healthy subjects and found a reduction in energy intake only for the 45 min and 90 min infusions. In contrast, in this study the 1.33/50 (67 kcal) and 4/50 (200 kcal) lipid infusions were discontinued 100 min before the evaluation of energy intake, so that only the 1.33/150 (200 kcal) infusion, which ceased immediately before the meal, was comparable to the 90 min infusion in Castiglione's study. Moreover, in this study there was no reduction in energy intake after the 150 min infusion. This may reflect the higher rate of infusion (2 kcal/min) used by Castiglione and colleagues (Castiglione et al., 1998) over a period of time sufficient to permit caudal movement of digesting fat and lipase, thereby exposing lipolytic products to a sufficient length of bowel to induce an effect on energy intake (Meyer et al., 1998c). In contrast, in this study, lipolysis and absorption were probably completed over a shorter length of small intestine during a slower lipid infusion of 1.33/150. Higher loads of intraduodenal lipid (2 - 2.9 kcal/min) have been shown to reduce hunger and suppress subsequent energy intake, even after shorter infusion periods in healthy young (Feinle et al., 2003), old (Cook et al., 1997, MacIntosh et al., 1999) and obese (Chapman et al., 1999) subjects.

Suppression of energy intake by small intestinal fat is likely mediated by combinations of neural responses to luminal nutrients and nutrient-stimulated release of anorexogenic peptides, including CCK, PYY, and GLP-1. Recently, it has been observed that energy intake was not suppressed by increasing loads of duodenally infused lauric acid until the rate of fatty acid entry was high enough to sustain a plasma level of CCK around 7.5 pmol/l (4 pmol/l above basal) (Little et al., 2005), substantially greater than the CCK concentration at 150 min after the sustained 1.33/150 infusion. Thus, it is probable that this study did not observe a reduction in energy intake after any of our infusions

because the potent effects of the short-duration, but high-dose, 4/50, infusion had disappeared by 150 min, while the sustained 1.33/150 infusion was an impotent dose.

5.6 CONCLUSIONS

In summary, this study establishes that there are regional differences in the regulation of APD motility and the release of CCK and PYY in response to small intestinal fat, as demonstrated by altering the load, and duration, of intraduodenal lipid infusion. While sustained exposure of the small intestine to lipid was associated with sustained suppression of antral pressure waves, there was an attenuation in the stimulation of pyloric pressures and plasma CCK, which may account for the absence of a significant effect on energy intake.

Chapter 6

LOAD-DEPENDENT EFFECTS OF DUODENAL LIPID ON ANTROPYLORODUODENAL MOTILITY, PLASMA CCK AND PYY, AND ENERGY INTAKE IN HEALTHY MEN

6.1 SUMMARY

Both the load and duration of small intestinal lipid infusion affect APD motility and CCK and PYY release at loads comparable to, and higher than, the “normal” gastric emptying rate. The effects of intraduodenal lipid loads well below the mean rate of gastric emptying on (i) APD motility, CCK and PYY, and appetite and energy intake, and (ii) the relationships with APD motility, CCK and PYY, appetite and energy intake, were determined. 16 healthy males were studied on four occasions in double-blind, randomised fashion. APD motility, plasma CCK and PYY, and appetite perceptions were measured during 50 min intraduodenal lipid (Intralipid®) infusions at; (i) 0.25 (“IL0.25”), 1.5 (“IL1.5”) and 4 (“IL4”) kcal/min or, (iv) saline (“control”), after which energy intake at a buffet meal was quantified. IL0.25 stimulated IPPWs and CCK release, albeit transiently, and suppressed antral PWs, PWSs and hunger ($P < 0.05$), but had no effect on basal pyloric pressure or PYY, when compared with control. Loads ≥ 1.5 kcal/min were required for the stimulation of basal pyloric pressures and PYY and

suppression of duodenal PWs ($P < 0.05$). All these effects were related to the lipid load ($r > 0.5$ or < -0.5 , $P < 0.05$). Only IL4 reduced energy intake ([kJ], control: 5388 ± 257 , IL0.25: 5360 ± 186 , IL1.5: 5164 ± 296 , IL4: $4753 \pm 272^*$; *vs. control and IL0.25, $P < 0.05$). In conclusion, in healthy males the effects of intraduodenal lipid on APD motility, plasma CCK and PYY, appetite and energy intake are load-dependent, and the threshold-loads required to elicit responses vary for these parameters.

6.2 INTRODUCTION

The presence of fat in the small intestine is associated with a number of changes in gastrointestinal function, including modulation of APD motility (specifically, suppression of antral (Hedde et al., 1989) and duodenal (Andrews et al., 2001) pressures and stimulation of IPPWs and basal pyloric pressure (Cook et al., 1997, Hedde et al., 1989)), resulting in the modulation of gastric emptying (Hedde et al., 1989) and gastrointestinal hormone secretion (MacIntosh et al., 1999), including CCK from the proximal (Buffa et al., 1976), and PYY from the distal (Adrian et al., 1985), small intestine. Small intestinal fat also has the capacity to suppress appetite and energy intake (Chapman et al., 1999).

The effects of fat on gastrointestinal function and energy intake are dependent on fat digestion (Feinle et al., 2001b, Feinle et al., 2003, Borovicka et al., 2000, Schwizer et al., 1997a). On contact with sensors in the small intestine, lipolytic products, especially fatty acids, stimulate gut hormone secretion, modulate gastrointestinal motility, and suppress energy intake (Feinle et al., 2003, Little et al., 2005, Feltrin et al., 2004). These responses depend on the load of lipid delivered to the small intestine (Feltrin et

al., 2007, Chapter 5), as well as the region (Welch et al., 1988, Meyer et al., 1998b, Meyer et al., 1998c), and length (Lin et al., 1990, Meyer et al., 1998b, Meyer et al., 1998c) of small intestine contacted. The rate of postprandial gastric emptying of lipid is highly variable, ranging from ~ 0.4 to 4.1 kcal/min in normal human subjects (Edelbroek et al., 1992b, Kunz et al., 2005, Little et al., 2007, Meyer et al., 1996). Much of this variability is accounted for by how thoroughly water-insoluble fat is dispersed in meal contents along the antropyloric region. There is very little information as to how triglycerides may evoke the above responses during digestion after entering the small intestine over this range of caloric loads.

A recent study has suggested that suppression of energy intake may relate to changes in APD motility, particularly that of the pylorus (Brennan et al., 2005). In healthy subjects intravenous infusion of CCK (1.8 pmol/kg/min) markedly stimulated IPPWs and basal pyloric pressures, which was associated with suppression of energy intake; while GLP-1, in the dose used (0.9 pmol/kg/min), did not stimulate pyloric pressures or suppress energy intake (Brennan et al., 2005). The association between nutrient-induced changes in APD motility and energy intake has not been assessed.

The first aim of this study was, therefore, to evaluate the load-dependent effects of intraduodenal lipid on APD motility, plasma CCK and PYY, and appetite and energy intake. For this purpose, Intralipid® (the most commonly used triglyceride emulsion in physiological studies of this kind) was infused into the duodenum at rates of 0.25, 1.5 and 4 kcal/min and the effects compared with those of a 0.9 % saline control infusion. The doses selected, thus, encompassed the range of rates of gastric emptying of dietary fat hitherto reported in humans (Edelbroek et al., 1992b, Kunz et al., 2005, Little et al.,

2007, Meyer et al., 1996). The second aim was to determine potential relationships between the effects of intraduodenal lipid on appetite and energy intake and those on APD motility and plasma CCK and PYY.

6.3 SUBJECTS AND METHODS

6.3.1 Subjects

16 healthy males (age: 31 ± 3 yrs; BMI: 23.8 ± 0.5 kg/m²) were recruited according to the guidelines described in Chapter 4.2.

6.3.2 Study outline

Each subject was studied on four occasions, each separated by 3 - 7 days, on which they received, in randomised, double-blind fashion, an intraduodenal infusion of a lipid emulsion, at (i) 0.25 kcal/min (“IL0.25”), (ii) 1.5 kcal/min (“IL1.5”), or (iii) 4 kcal/min (“IL4”), or (iv) isotonic saline (“control”), each for 50 min. APD pressures, plasma CCK and PYY concentrations, appetite and energy intake were evaluated.

6.3.3 Intraduodenal infusions

The intraduodenal lipid emulsion (10 % Intralipid®, 300 mOsmol/kg, 1.1 kcal/ml, Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia) was administered as: 0.25 kcal/min, 1.5 kcal/min and 4 kcal/min. These rates were chosen to encompass loads lower than the rate of normal gastric emptying (0.25 kcal/min), as well as loads reflecting the lower to intermediate (1.5 kcal/min) and higher (4 kcal/min) rates

observed during the postprandial time-course of gastric emptying (Meyer et al., 1996, Little et al., 2007).

Lipid solutions were diluted with isotonic saline to achieve the specific loads, and all infusions were administered at a rate of 4 ml/min, so that the total volume infused in all study conditions was 200 ml (see Chapter 4.4.2.1). The infusions were prepared by an investigator who was not otherwise involved in either the performance of the studies or data analysis. During studies the infusion apparatus was covered at all times.

6.3.4 Protocol

Subjects attended the laboratory in the Discipline of Medicine at 0830 h after an overnight fast (14 hours for solids, 12 hours for liquids). A manometric catheter was positioned, as described in Chapter 4.5.1. An intravenous cannula was placed in a forearm vein for blood sampling.

Once the manometric catheter was positioned correctly, fasting motility was monitored until the occurrence of a phase III of the interdigestive MMC (Feltrin et al., 2004). Immediately after cessation of the phase III (at $t = -10$ min), a baseline blood sample was taken, and a VAS questionnaire, assessing appetite-related sensations and gastrointestinal symptoms (Parker et al., 2004), was completed. At $t = 0$ min, during phase I of the MMC, the duodenal infusion of lipid or saline commenced and continued for 50 min. APD pressures were recorded during the 10 min baseline period ($t = -10 - 0$ min) and during the infusion period ($t = 0 - 50$ min). Throughout the infusion, 10 ml blood samples were taken and VAS completed at 10 min intervals. At $t = 50$ min, the

infusion was terminated, the subject immediately extubated, and then offered a cold, buffet-style, meal (Feltrin et al., 2004). Each subject was given 30 min (i.e. $t = 150 - 180$ min) to consume the meal and instructed to eat until comfortably full. After the meal, the intravenous cannula was removed, and the subject allowed to leave the laboratory.

6.3.5 Measurements

6.3.5.1 *Antropyloroduodenal pressures*

APD pressures were assessed, and analysed for (i) number and amplitude of antral and duodenal PWs, (ii) number and amplitude of IPPWs, (iii) basal pyloric pressure and (iv) number and length of PWSs, as described in Chapter 4.5.1.

6.3.5.2 *Plasma cholecystokinin and peptide YY concentrations*

Blood sample collection and analysis of plasma CCK and PYY are described in Chapters 4.5.7 and 4.5.8.

6.3.5.3 *Appetite perceptions*

Appetite perceptions (desire to eat, hunger, fullness, prospective consumption ('how much would you eat if given a meal now?')) were rated on VAS, as described in Chapter 4.5.5.2 (Parker et al., 2004). Nausea and bloating were also assessed (Chapter 4.5.5.2).

6.3.5.4 Energy intake

Assessment of energy intake is described in Chapter 4.5.5.3.

6.3.6 Data and statistical analyses

Baseline values ("0") were calculated as the mean values obtained between $t = -10 - 0$ min for the number and amplitude of IPPWs and antral and duodenal PWs, basal pyloric pressures and PWSs, and at $t = -10$ and 0 min for plasma hormone concentrations and VAS scores. The numbers of antral and duodenal PWs were expressed as total numbers, whereas amplitudes and MI were expressed as mean values, over the 50 min infusion (i.e. $t = 0 - 50$ min). Antral and duodenal MI were derived using the equation, $MI = \text{natural logarithm} [(\text{sum of amplitudes} \times \text{number of phasic pressure waves}) + 1]$ (Camilleri and Malagelada, 1984). Basal pyloric pressures and the number and amplitude of IPPWs were expressed as means of 10 min periods over the 50 min infusion period. APD PWSs were expressed as the total number of PWs spanning over two (1.5 - 3 cm), three (3 - 4.5 cm), ... , fifteen (21 - 22.5 cm) channels during the 50 min infusion period. All motility and VAS data were expressed as changes from baseline, whilst plasma CCK and PYY were expressed as absolute values.

The number, amplitude and MI of antral and duodenal PWs and the parameters measured from the buffet meal (energy intake (kJ), amount eaten (g), macronutrient distribution (%)) were analysed by one-way ANOVA. Basal pyloric pressure, the number and amplitude of IPPWs, plasma concentrations of CCK and PYY and VAS scores were analysed by repeated-measures ANOVA, with time and treatment as factors. The total number of PWSs was analysed by repeated-measures ANOVA, with

number and length (cm) as factors. Post-hoc paired comparisons, corrected for multiple comparisons by Bonferroni's correction, were performed if ANOVAs revealed significant effects.

Correlations, corrected for repeated measures, were determined for (i) the total number of antral and duodenal PWs and APD PWSs, mean amplitude and MI of antral and duodenal PWs, area under the curve (AUCs; calculated using the trapezoidal rule) for number and amplitude of IPPWs, basal pyloric pressures, plasma CCK and PYY and appetite perception scores between $t = 0 - 50$ min, as well as energy intake and amount eaten at the buffet meal, with the \ln (natural logarithm) - transformed loads of lipid administered and (ii) appetite perceptions and energy intake (and amount eaten) with APD motility and plasma CCK and PYY, for both AUCs and values at $t = 50$ min, using the method described by Bland and Altman (Bland and Altman, 1995). Only r values > 0.5 were considered physiologically relevant. When correlations between the above variables were found, multiple regression analysis was performed to establish determinants of energy and food intake. Statistical significance was accepted at $P < 0.05$, and data are presented as means \pm SEM.

6.4 RESULTS

All subjects tolerated the experimental conditions well. Baseline values for motility and VAS data are given in **Table 6.1**. There were no differences between the four experimental conditions.

6.4.1 Antropyloroduodenal pressures

6.4.1.1 Antral pressure waves

IL0.25, IL1.5 and IL4 decreased the number of antral PWs when compared with control ($P < 0.05$ for all), with no significant differences between the lipid treatments (**Table 6.2**). IL1.5 and IL4 decreased the amplitude and MI of antral PWs, when compared with control and IL0.25 ($P < 0.05$ for all); there was no difference between control and IL0.25 or between IL1.5 and IL4 (**Table 6.2**). There was an inverse relationship between the number ($r = -0.69$, $P < 0.05$), amplitude ($r = -0.81$, $P < 0.05$) and MI ($r = -0.74$, $P = 0.05$) of antral PWs with the load of lipid administered.

6.4.1.2 Pyloric pressures

6.4.1.2.1 Basal pressures

There was a treatment by time interaction for basal pyloric pressures ($P < 0.001$) (**Figure 6.1A**). IL1.5 ($P < 0.05$) and IL4 ($P < 0.001$) stimulated basal pyloric pressure between $t = 10 - 50$ min when compared with control. IL1.5 stimulated basal pyloric pressure between $t = 20 - 40$ min ($P < 0.05$), and IL4 between $t = 10 - 50$ min ($P < 0.01$), when compared with IL0.25. IL4 stimulated basal pressure between $t = 20 - 50$ min when compared with IL1.5 ($P < 0.01$). There was no difference between control and IL0.25. By the end of the infusion (i.e. $t = 40 - 50$ min) basal pyloric pressures had returned to levels close to baseline for control, IL0.25 and IL1.5, however remained elevated for IL4 ($P < 0.01$). There was a direct relationship between the AUC for basal pyloric pressure with the load of lipid administered ($r = 0.82$, $P < 0.05$).

6.4.1.2.2 Phasic pressures

There was a treatment by time interaction for the number of IPPWs ($P < 0.001$) (**Figure 6.1B**). IL0.25, IL1.5 and IL4 increased the number of IPPWs when compared with control; IL0.25 between $t = 0 - 20$ min ($P < 0.001$), and IL1.5 ($P < 0.01$) and IL4 ($P < 0.001$) between $t = 0 - 50$ min. IL1.5 and IL4 increased the number of IPPWs when compared with IL0.25; IL1.5 between $t = 20 - 50$ min ($P < 0.01$), and IL4 between $t = 0 - 50$ min ($P < 0.05$). IL4 increased the number of IPPWs when compared with IL1.5 between $t = 20 - 50$ min ($P < 0.05$). After $t = 20$ min, the number of IPPWs began to slightly decline for IL1.5 and IL4, however, during IL0.25 the numbers were not sustained and fell to values similar to control. There was a treatment by time interaction for the amplitude of IPPWs ($P < 0.05$) (**Figure 6.1C**). IL0.25, IL1.5 and IL4 increased the amplitude of IPPWs when compared with control; IL0.25 between $t = 10 - 30$ min ($P < 0.05$), and IL1.5 ($P < 0.05$) and IL4 ($P < 0.01$) between $t = 10 - 50$ min. IL4 increased the amplitude of IPPWs between $t = 20 - 50$ min ($P < 0.05$) when compared with IL0.25, and between $t = 30 - 40$ min when compared with IL1.5. There was no difference between IL0.25 and IL1.5. There was a direct relationship between the AUC for the number ($r = 0.80$, $P < 0.001$) and amplitude ($r = 0.68$, $P < 0.05$) of IPPWs with the load of lipid administered.

6.4.1.3 *Duodenal pressure waves*

IL1.5 and IL4 decreased the number, amplitude and MI of duodenal PWs when compared with control and IL0.25 ($P < 0.05$ for all), with no differences between control and IL0.25, or between IL1.5 and IL4 (**Table 6.2**). There was an inverse relationship between the number ($r = -0.79$, $P < 0.0001$), amplitude ($r = -0.64$, $P <$

0.001) and MI ($r = -0.67$, $P < 0.05$) of duodenal PWS with the load of lipid administered.

6.4.1.4 *Pressure wave sequences*

Only PWSs that spanned 2 - 6 channels (1.5 - 9 cm) were analysed statistically, as PWSs spanning over 7 - 15 channels were infrequent (no/50 min; control, 6 ± 3 ; IL0.25, 3 ± 2 ; IL1.5, 2 ± 1 ; IL4, 1 ± 1).

There was a treatment by length interaction for the number of PWSs spanning two (1.5 - 3 cm), three (3 - 4.5 cm), four (4.5 - 6 cm), five (6 - 7.5 cm) and six (7.5 - 9 cm) channels ($P < 0.001$) (**Figure 6.2**). IL0.25 decreased the number of PWSs that spanned over two channels ($P < 0.01$), IL1.5 over two and three channels ($P < 0.001$) and IL4 over two to six channels ($P < 0.05$), when compared with control. IL1.5 decreased the number of PWSs spanning over two to four channels ($P < 0.05$), and IL4 over two to five channels ($P < 0.05$), when compared with IL0.25. IL4 decreased the number of PWSs spanning two channels compared with IL1.5 ($P < 0.05$). There was an inverse relationship between the total number of PWSs with the load of lipid administered ($r = -0.80$, $P < 0.001$).

6.4.2 **Plasma hormone concentrations**

6.4.2.1 *Cholecystokinin*

There was a treatment by time interaction for plasma CCK concentrations ($P < 0.001$) (**Figure 6.3A**). Plasma CCK rapidly increased during all lipid infusions, after which

levels plateaued for IL1.5 and IL4, and decreased to baseline values by $t = 30$ min for IL0.25. IL0.25, IL1.5 and IL4 increased plasma CCK when compared with control; IL0.25 between $t = 10 - 20$ min ($P < 0.05$) and IL1.5 ($P < 0.01$) and IL4 ($P < 0.001$) between $t = 10 - 50$ min. IL1.5 ($P < 0.05$) and IL4 ($P < 0.01$) increased plasma CCK between $t = 10 - 50$ min when compared with IL0.25, and IL4 between $t = 20 - 50$ min when compared with IL1.5 ($P < 0.01$). There was a direct relationship between the AUC for plasma CCK concentrations with the load of lipid ($r = 0.96$, $P < 0.001$).

6.4.2.2 Peptide YY

There was a treatment by time interaction for plasma PYY concentrations ($P < 0.001$) (**Figure 6.3B**). There was an ongoing rise in PYY in response to IL1.5 and IL4. IL1.5 and IL4 increased plasma PYY when compared with control and IL0.25; IL1.5 between $t = 10 - 50$ min ($P < 0.05$, for both) and IL4 between $t = 20 - 50$ min ($P < 0.001$, for both). IL4 increased plasma PYY when compared with IL1.5 between $t = 30 - 50$ min ($P < 0.01$). There was no difference between control and IL0.25. There was a direct relationship between the AUC for plasma PYY concentrations and the load of lipid ($r = 0.91$, $P < 0.001$).

6.4.3 Appetite perceptions

There was a treatment by time interaction for hunger ($P < 0.001$) (**Figure 6.4A**). Hunger was less during IL0.25 between $t = 30 - 50$ min ($P < 0.05$) and during IL1.5 and IL4 between $t = 20 - 50$ min ($P < 0.05$ for both), when compared with control, during which hunger increased progressively throughout the infusion period. IL4 decreased hunger at $t = 40$ and 50 min when compared with IL0.25, and at $t = 50$ min when

compared with IL1.5 ($P < 0.05$ for both). There was no difference between IL0.25 and IL1.5. There was no effect of treatment on scores of fullness (data not shown). There was a treatment by time interaction for nausea ($P = 0.05$) (**Figure 6.4B**). Although scores were very low, IL4 increased nausea between $t = 30 - 50$ min, when compared with control, IL0.25 and IL1.5 ($P < 0.01$ for all). During IL1.5 nausea was increased at $t = 50$ min when compared with IL0.25 ($P < 0.05$). There were no differences between control and IL0.25 or IL1.5. There was no effect of treatment on scores of bloating (data not shown). There were no significant relationships between hunger or nausea with the load of lipid.

6.4.4 Energy intake

There was an effect of treatment on the energy (kJ) and amount (g) (**Table 6.2**) consumed at the buffet meal ($P < 0.05$). IL4 reduced the energy and amount of food consumed when compared with control ($P < 0.01$) and IL0.25 ($P < 0.05$). There were no differences between control, IL0.25 and IL1.5, or between IL1.5 and IL4. There was no difference in the macronutrient distribution between the four experimental conditions (**Table 6.2**). There was an inverse relationship between the energy ($r = -0.63$, $P < 0.05$) and amount ($r = -0.61$, $P < 0.05$) consumed at the buffet meal with the load of lipid administered.

6.4.5 Relations of antropyloroduodenal motility and plasma cholecystokinin and peptide YY with appetite perceptions and energy intake

6.4.5.1 Relationships between antropyloroduodenal motility with appetite perceptions, energy intake and amount eaten

There were no significant relationships between antral and duodenal PWs, and the AUC for basal pyloric pressure, IPPWs or PWSs with energy intake. There were direct relationships between the amount eaten with the number ($r = 0.42$, $P < 0.05$) and amplitude ($r = 0.54$, $P < 0.05$) of antral PWs, and an inverse relationship with the number of IPPWs ($r = -0.56$, $P < 0.05$). There were direct relationships between the number ($r = 0.57$, $P < 0.05$) and MI ($r = 0.55$, $P < 0.05$) of duodenal PWs and PWSs ($r = 0.58$, $P < 0.05$) with scores for hunger. There were no relationships between antral and duodenal PWs, basal pyloric pressure, IPPWs or PWSs with scores for fullness, nausea or bloating.

6.4.5.2 Relationships between plasma cholecystokinin and peptide YY with appetite perceptions, energy intake and amount eaten

There were inverse relationships between energy intake with AUC ($r = -0.63$, $P < 0.01$) and the values at $t = 50$ min ($r = -0.61$, $P < 0.01$) for plasma CCK and AUC ($r = -0.50$, $P < 0.05$) and the values at $t = 50$ min ($r = -0.62$, $P < 0.05$) for plasma PYY. There were inverse relationships between the amount eaten with AUC ($r = -0.60$, $P < 0.001$) and values at $t = 50$ min ($r = -0.58$, $P < 0.01$) for CCK and AUC ($r = -0.52$, $P < 0.01$) and values at $t = 50$ min ($r = -0.62$, $P < 0.01$) for plasma PYY. There was an inverse relationship between hunger ($r = -0.49$, $P < 0.05$) and a direct relationship between

nausea ($r = 0.55$, $P < 0.05$) scores with plasma CCK. There were no correlations between appetite perceptions with PYY concentrations.

6.4.5.3 Relationships between appetite perceptions with energy intake

There was a direct relationship between hunger scores with energy intake ($r = 0.51$, $P = 0.07$) and the amount eaten ($r = 0.51$, $P < 0.05$). There were no significant relationships between fullness, nausea or bloating scores with either energy intake or the amount eaten at the buffet meal.

6.4.5.4 Relationships between antropyloroduodenal motility with plasma cholecystokinin and peptide YY

There were inverse relationships between the number ($r = -0.64$, $P < 0.05$) and amplitude ($r = -0.64$, $P < 0.05$) of antral PWs, the number ($r = -0.70$, $P < 0.001$) and amplitude ($r = -0.54$, $P < 0.001$) of duodenal PWs and the number of APD PWSs ($r = -0.72$, $P < 0.001$), and direct relationships between the number ($r = 0.82$, $P < 0.001$) and amplitude ($r = 0.65$, $P < 0.01$) of IPPW and basal pyloric tone ($r = 0.75$, $P < 0.05$), with the AUC for plasma CCK. There were inverse relationships between the number ($r = -0.61$, $P < 0.001$) and amplitude ($r = -0.59$, $P < 0.05$) of antral PWs, the number ($r = -0.68$, $P < 0.001$) of duodenal PWs and the number of APD PWSs ($r = -0.57$, $P < 0.001$), and direct relationships between the number ($r = 0.80$, $P < 0.01$) and amplitude ($r = 0.68$, $P < 0.05$) of IPPWs, with the AUC for plasma PYY.

6.4.5.5 Relationships between plasma peptide YY with plasma cholecystinin

There was a direct relationship between plasma PYY with plasma CCK ($r = 0.89$, $P < 0.001$).

6.4.6 Predictors of energy intake

Multiple regression analysis of the combined data for CCK and PYY identified CCK concentrations at $t = 50$ min as an independent predictor of energy intake ($\beta = -128.148$, $P < 0.05$), but not the amount eaten. For the combined data of the number and amplitude of antral PWs and the number of IPPWs, multiple regression analysis identified the AUC for the number of IPPWs as an independent predictor of the amount eaten ($\beta = -0.467$, $P < 0.01$).

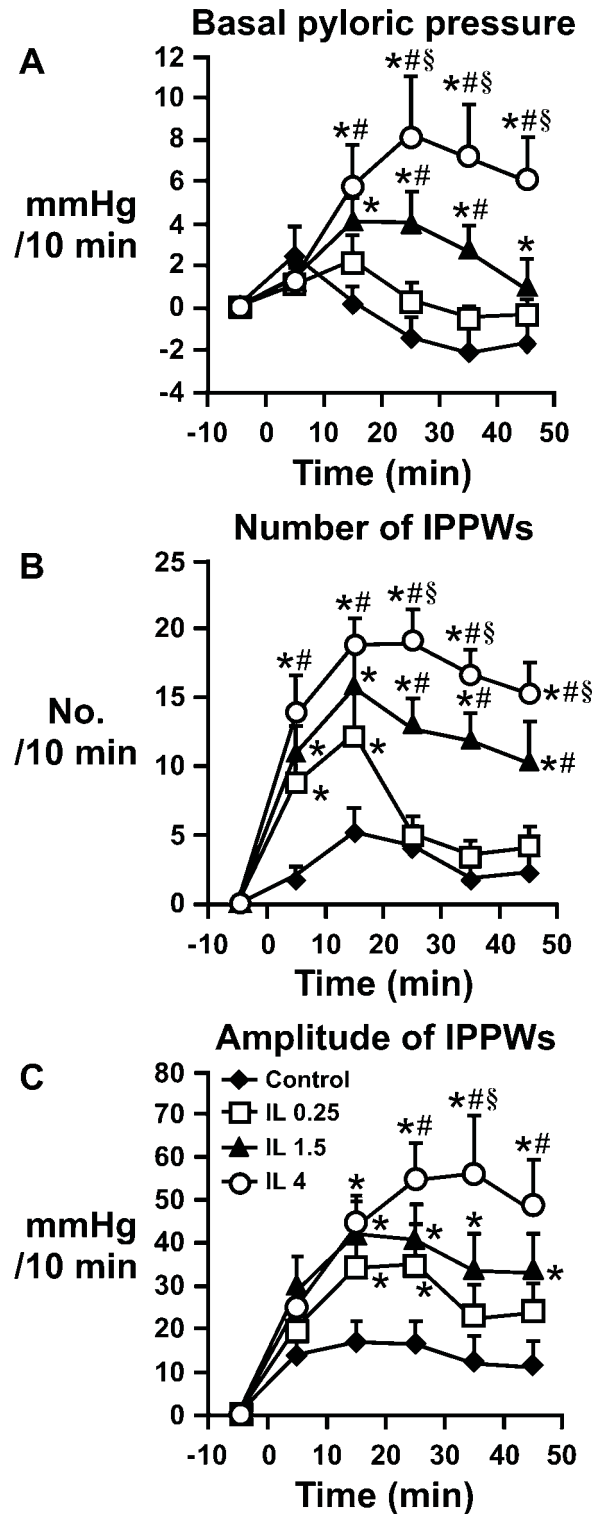


Figure 6.1 Effects of intraduodenal infusion, between $t = 0 - 50$ min, of Intralipid[®] at 0.25 (□) (“IL0.25”), 1.5 (▲) (“IL1.5”) and 4 (○) (“IL4”) kcal/min, or saline (◆) (“control”), on (A) basal pyloric pressure, * vs. control; $P < 0.05$, # vs. IL0.25; $P < 0.05$, § vs. IL1.5; $P < 0.01$, (B) number of isolated pyloric pressure waves (IPPWs), * vs. control; $P < 0.01$, # vs. IL0.25; $P < 0.05$, § IL1.5; $P < 0.05$, and (C) amplitude of IPPWs, * vs. control, # vs. IL0.25, § IL1.5; $P < 0.05$. Data are means \pm SEM ($n = 16$).

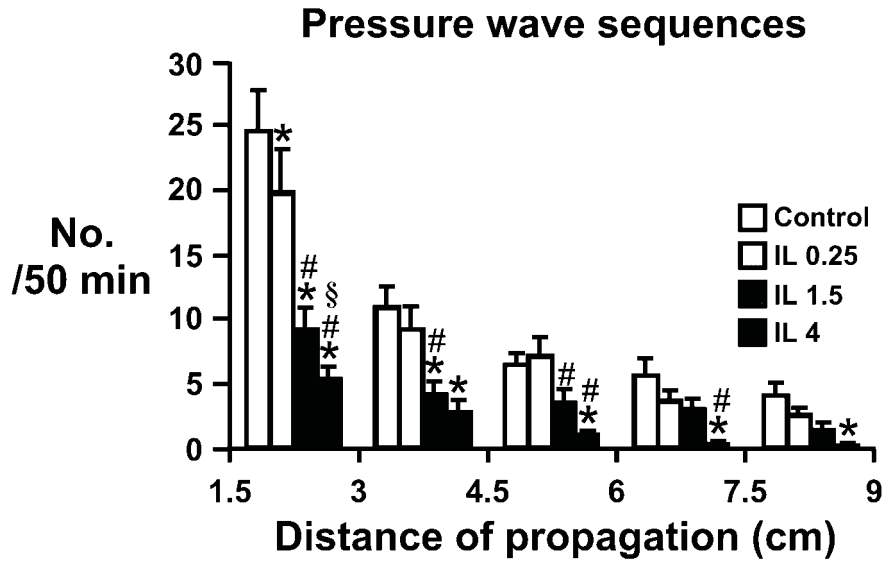


Figure 6.2 Effects of intraduodenal infusion, between t = 0 - 50 min, of Intralipid® at 0.25 (“IL0.25”), 1.5 (“IL1.5”) and 4 (“IL4”) kcal/min, or saline (“control”), on pressure wave sequences, * vs. control, # vs. IL0.25, § vs. IL1.5; P < 0.05. Data are means ± SEM (n = 16).

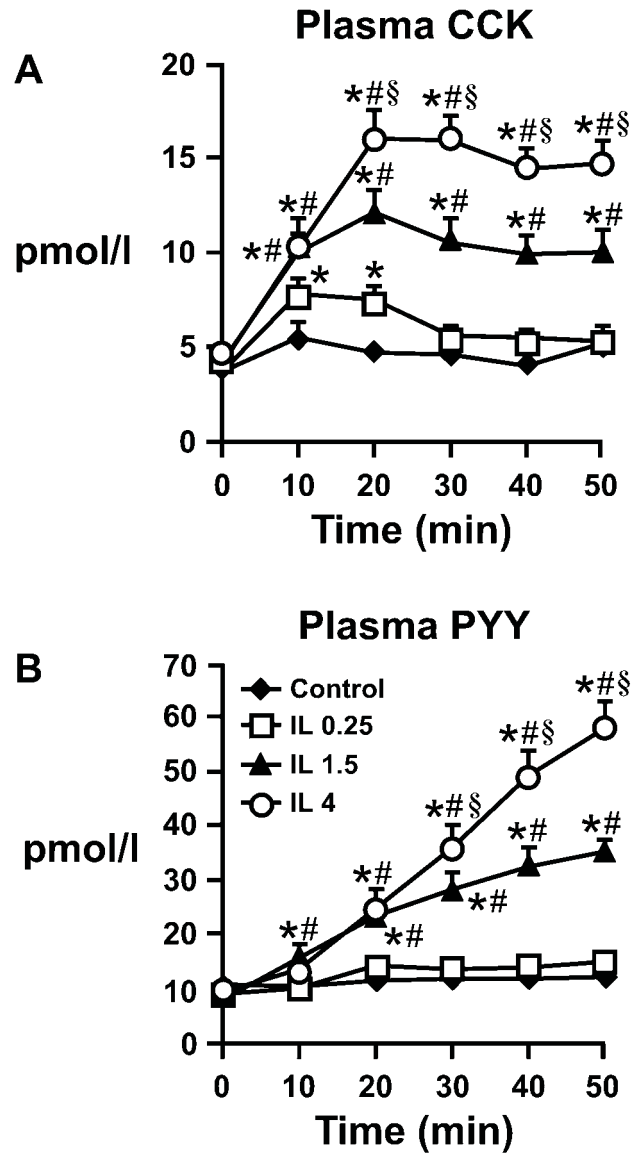


Figure 6.3 Effects of intraduodenal infusion, between $t = 0 - 50$ min, of Intralipid[®] at 0.25 (□) (“IL0.25”), 1.5 (▲) (“IL1.5”) and 4 (○) (“IL4”) kcal/min, or saline (◆) (“control”), on plasma concentrations of (A) cholecystokinin, * vs. control; $P < 0.05$, # vs. IL0.25; $P < 0.05$, § vs. IL1.5; $P < 0.01$, and (B) peptide YY, * vs. control; $P < 0.05$, # vs. IL0.25; $P < 0.05$, § IL4 vs. IL1.5; $P < 0.01$. Data are means \pm SEM ($n = 16$).

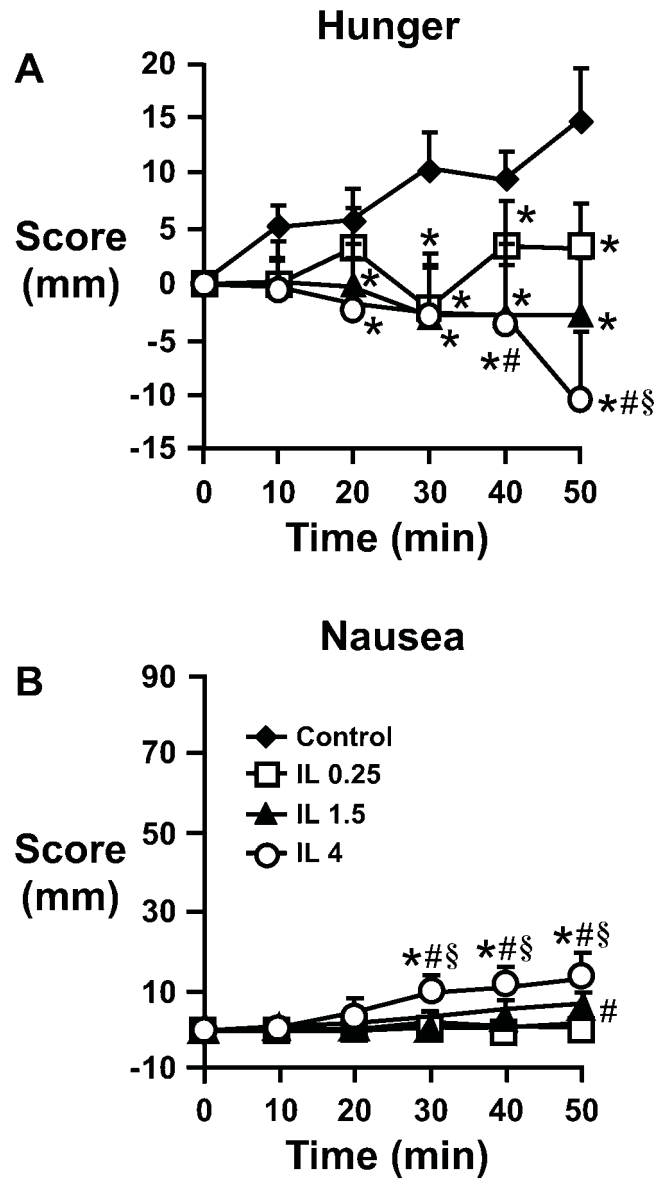


Figure 6.4 Effects of intraduodenal infusion, between t = 0 - 50 min, of Intralipid® at 0.25 (□) (“IL0.25”), 1.5 (▲) (“IL1.5”) and 4 (○) (“IL4”) kcal/min, or saline (◆) (“control”), on perceptions of (A) hunger, * vs. control; P < 0.05, # vs. IL0.25; P < 0.05, § vs. IL1.5; P < 0.001, and (B) nausea, * vs. control, # vs. IL0.25, § vs. IL1.5; P < 0.05. Data are means ± SEM (n = 16).

Table 6.1 Baseline values for antral and duodenal pressure waves, basal pyloric pressure, number and amplitude of isolated pyloric pressure waves, hunger, fullness, nausea and bloating, i.e. before commencement of intraduodenal infusions of 10% Intralipid® or saline.

	Saline (control)	IL0.25	Intralipid IL1.5	IL4
<i>Antral PWs</i>				
Number	2 ± 1	5 ± 2	5 ± 1	3 ± 1
Amplitude (mmHg)	7 ± 2	11 ± 2	9 ± 2	7 ± 2
Motility index (mmHg)	2 ± 1	3 ± 1	3 ± 1	2 ± 1
<i>Duodenal PWs</i>				
Number	29 ± 6	38 ± 9	35 ± 7	29 ± 6
Amplitude (mmHg)	17 ± 2	17 ± 2	18 ± 2	16 ± 1
Motility index (mmHg)	5 ± 0	6 ± 0	6 ± 0	5 ± 0
<i>Pyloric pressures</i>				
Basal (mmHg)	2 ± 4	-3 ± 1	-1 ± 3	-1 ± 2
No of IPPWs	1 ± 0	1 ± 0	1 ± 1	1 ± 0
Amplitude of IPPW (mmHg)	5 ± 3	8 ± 4	8 ± 4	6 ± 3
<i>VAS</i>				
Hunger (mm)	48 ± 7	52 ± 7	54 ± 7	51 ± 7
Fullness (mm)	10 ± 4	7 ± 2	7 ± 2	9 ± 3
Nausea (mm)	7 ± 2	9 ± 3	6 ± 2	8 ± 3
Bloating (mm)	4 ± 1	8 ± 2	8 ± 3	5 ± 2

Data are means ± SEM (n = 16). IPPWs, isolated pyloric pressure waves; PWs, pressure waves; VAS, visual analogue scales; IL0.25, 0.25 kcal/min; IL1.5, 1.5 kcal/min; IL4, 4 kcal/min.

Table 6.2 Mean values for antral and duodenal pressure waves during intraduodenal infusion of 10 % Intralipid and saline, and energy intake, amount eaten and % macronutrient distribution at the buffet meal.

	Saline (control)	IL0.25	Intralipid IL1.5	IL4	P value (ANOVA)
<i>Antral PWs</i>					
Number/50 min	51 ± 12	29 ± 8*	9 ± 3*	10 ± 5*	< 0.001
Amplitude (mmHg)	22 ± 5	26 ± 9	4 ± 2* [#]	8 ± 3* [#]	< 0.05
MI (mmHg)	4 ± 1	3 ± 1	1 ± 1* [#]	1 ± 1* [#]	< 0.01
<i>Duodenal PWs</i>					
Number/50 min	329 ± 32	317 ± 32	207 ± 34* [#]	133 ± 21* [#]	< 0.001
Amplitude (mmHg)	13 ± 2	16 ± 3	5 ± 2* [#]	7 ± 2* [#]	< 0.01
MI (mmHg)	5 ± 0	5 ± 1	3 ± 1* [#]	3 ± 0* [#]	< 0.01
<i>Food intake</i>					
Energy (kJ)	5388 ± 257	5360 ± 186	5164 ± 296	4753 ± 272* [#]	< 0.05
Amount (g)	1425 ± 101	1371 ± 95	1327 ± 111	1231 ± 109* [#]	< 0.05
% kJ from fat	35 ± 1	34 ± 2	35 ± 1	35 ± 1	NS
% kJ from CHO	43 ± 1	42 ± 2	41 ± 2	43 ± 1	NS
% kJ from protein	23 ± 1	24 ± 1	23 ± 1	23 ± 1	NS

Data are means ± SEM (n = 16). CHO, carbohydrate; MI, motility index; NS, not significant; PWs, pressure waves; IL0.25, 0.25 kcal/min; IL1.5, 1.5 kcal/min; IL4, 4 kcal/min. Significant differences: * from control, [#] from IL0.25.

6.5 DISCUSSION

This study has demonstrated for the first time that duodenal lipid loads as low as 0.25 kcal/min are able to stimulate IPPWs and CCK release and suppress antral PWs, APD PWSs and hunger in healthy subjects, while higher loads were required for the stimulation of basal pyloric pressure, PYY release and the suppression of duodenal pressures and energy intake. The effects of duodenal lipid on energy intake/amount of food eaten were related to the release of CCK and stimulation of IPPWs.

In this study the effects of intraduodenal lipid at loads that encompass rates below, similar to, and above, the normal range of gastric emptying were evaluated and demonstrated a load-dependent suppression of antral and duodenal, and stimulation of pyloric, motility. Duodenal lipid loads between 1 - 4 kcal/min are known to stimulate pyloric pressures and suppress antral and duodenal PWs (Hedde et al., 1989, Hedde et al., 1988a, Chapter 5). This study has now demonstrated that lipid loads below the normal range of gastric emptying (0.25 kcal/min) also have the capacity to stimulate IPPWs and suppress antral PWs and APD PWSs. At higher loads (IL1.5 and IL4), hydrolysis takes longer to complete, resulting, as shown in previous studies (Lin et al., 1996, Meyer et al., 1998c, Meyer et al., 1998a), in the digestion process and lipolytic products being distributed further downstream, where more chemo-sensors are contacted, inducing a greater inhibitory response on gastric emptying, as reflected in the observed effects on APD motility with the larger lipid loads. This concept is supported by a study in dogs, which demonstrated that the magnitude of feedback inhibition of gastric emptying is dependent on the length of the gut exposed to lipid (Lin et al., 1990).

The data also confirm the findings in Chapter 5 of regional differences between antrum, pylorus and duodenum in relation to the load of lipid required to stimulate a response. In this study, while the lowest load (0.25 kcal/min) was sufficient to suppress antral PWs and APD PWSs and temporarily stimulate IPPWs, higher loads (≥ 1.5 kcal/min) were required to stimulate basal pyloric pressure and suppress duodenal PWs, suggesting that the mechanisms that regulate duodenal pressures are less sensitive to lipid than those of the antrum and pylorus.

It has been established that there is a load-dependent stimulation of CCK (Feinle et al., 2000, Chapter 5) and PYY (Chapter 5) in response to increasing small intestinal lipid loads. In this study, the lowest load (0.25 kcal/min) stimulated CCK release during the first 20 min of infusion, but did not stimulate PYY. The CCK response to 0.25 kcal/min, however, was not sustained, presumably because the lipolytic products of this small load would have been rapidly digested and absorbed. This may also account for the lack of an increase in PYY during the 0.25 kcal/min infusion, as lipolytic products would not have reached the distal part of the small intestine to directly stimulate PYY secretion. While CCK is known to indirectly stimulate PYY, the data suggest that a certain amount/concentration of CCK is required, confirming previous findings of a lack of PYY release during perfusion of free fatty acids into the first 45 cm of canine small intestine (Aponte et al., 1985). With the higher loads (1.5 kcal/min and 4 kcal/min) plasma PYY increased more slowly, and progressively, than CCK, in that, while plasma CCK peaked within 20 min and then plateaued, plasma PYY continued to rise until the cessation of the infusion. These results are consistent with the release of CCK from I cells located in the proximal small intestine (Buffa et al., 1976) and the release of PYY from L cells located in the distal small intestine (Adrian et al., 1985).

The substantial increase in CCK during IL1.5 and IL4, but not IL0.25, within 10 min of the infusion is, therefore, likely to have contributed to the initial rise in PYY. In contrast, towards the end of infusion, particularly with the highest load (4 kcal/min), lipid would have most likely reached the more distal parts of the small intestine (although it should be recognised that this was not measured in this study), stimulating a greater release of PYY by direct nutrient contact with PYY releasing cells (Adrian et al., 1985).

This is the first study to assess the effect of very low and relatively high loads of lipid on energy intake. A similar study was conducted, in which 20 % Intralipid® was infused at a constant rate (2 kcal/min) over different durations, and a reduction in energy intake was observed after 45 min (90 kcal) and 90 min (180 kcal), but not 15 min (30 kcal), infusions (Castiglione et al., 1998). In this study, only IL4 (200 kcal) reduced energy intake. Taken together these observations indicate that loads greater than 1.5 kcal/min for 50 min (75 kcal), but less than 2 kcal/min for 45 min (90 kcal) (Castiglione et al., 1998), represent the minimum required to reduce energy intake, raising the question as to whether load, rate and/or duration of small intestinal lipid is responsible for the reduction in energy intake. As reported in Chapter 5, duodenal infusion of 10 % Intralipid at 1.33 kcal/min for 150 min, which also yields 200 kcal, is insufficient to reduce energy intake in healthy subjects. During this slower infusion, lipolysis and absorption may well have been completed over a shorter length of small intestine, which may account for the absence of any effect of loads at 0.25 kcal/min and 1.5 kcal/min on energy intake in the present study. As the load of 1.5 kcal/min is considered similar to “average” gastric emptying, the data may also suggest that, if the duration of infusion was longer, plasma PYY may have continued to rise (to a specific

threshold), to have an effect on energy intake. It is important to recognise that, unlike duodenal infusions, gastric emptying of fat is not a steady process, but rather, is usually more rapid early after a meal, slowing later, and presumably predominantly pulsatile, rather than continuous (Malbert and Ruckebusch, 1989), a pattern that is likely to result in a different distribution of free fatty acids along the gut.

It is well documented that gastrointestinal hormones, particularly CCK and PYY, mediate, at least in part, the modulation of gastrointestinal motility by lipid (Fraser et al., 1993, Brennan et al., 2005, Savage et al., 1987, Katschinski et al., 1996), and this study has demonstrated positive correlations between pyloric pressures, and negative correlations between antral and duodenal PWs and APD PWSs, with CCK and PYY. Interestingly, only concentrations of CCK and PYY, but not APD motility, were significantly correlated with energy intake. However, there were correlations between the amount of food eaten at the buffet meal with motility, suggesting that different mechanisms may be responsible for the regulation of food intake vs. energy intake. Previous studies have suggested that the stimulation of the pylorus may have a critical involvement in the suppression of energy intake (Brennan et al., 2005, Xu et al., 2005), however, these studies did not directly assess the effects of nutrient-induced stimulation of pyloric pressures. Intravenous infusions of CCK and PYY have been shown to decrease hunger perceptions and suppress energy intake in humans (Brennan et al., 2005, Batterham et al., 2002), although it is important to recognise that the effects of exogenous PYY relate specifically to PYY(3-36) (Batterham et al., 2002). Nevertheless, in this study the substantial increase in plasma CCK and PYY, as well as pyloric pressures, which were all significantly greater during IL4 infusion compared with the other lipid infusions, may have contributed to the decrease in energy intake, in

that the sum of the various parameters may have reached a critical threshold to result in the decrease in energy intake. There was a slight increase in nausea during IL4 before the buffet meal, however, mean scores were very low, and no subject actually reported nausea. Moreover, there was no correlation between nausea scores with energy intake.

6.6 CONCLUSIONS

This study establishes that intraduodenal lipid at loads that encompass rates below, similar to, and above normal gastric emptying, suppress antral and duodenal PWs, APD PWs, appetite and energy intake and stimulate basal pyloric pressure, IPPWs, plasma CCK and PYY, in a load-dependent manner. While loads as low as 0.25 kcal/min modulate antral and pyloric pressures and transiently increase plasma CCK, higher loads (1.5 and 4 kcal/min) have greater effects and are required for stimulation of PYY, and only the highest load suppresses energy intake. Presumably, at the higher loads, lipid was distributed further along the small intestine, stimulating more chemosensors and, thus, providing greater feedback inhibition/stimulation.

These observations may have implications for our understanding of conditions associated with altered gastrointestinal function in response to consumption of high-caloric diets (Boyd et al., 2003, Castiglione et al., 2002), such as in obesity. The few studies that have compared gastric motility and hormone release between obese and healthy lean subjects have produced inconsistent observations (Tosetti et al., 1996, Maddox et al., 1989). Individuals habitually consuming high-caloric diets may well be less sensitive to nutrient stimuli and, therefore, it is reasonable to assume that higher loads may be required to induce similar responses to those demonstrated in the current

study. It would, accordingly, be of interest to compare the thresholds required for the stimulation, or suppression of gastric motility, hormone release, appetite and energy intake in lean and obese subjects.

Chapter 7

LOAD-DEPENDENT EFFECTS OF DUODENAL GLUCOSE ON GLYCAEMIA, GASTROINTESTINAL HORMONES, ANTROPYLORODUODENAL MOTILITY AND ENERGY INTAKE IN HEALTHY MEN

7.1 SUMMARY

Gastric emptying is a major determinant of glycaemia, gastrointestinal hormone release and appetite. The aim of this study was to determine the effects of different intraduodenal glucose loads on glycaemia, insulinaemia, plasma GLP-1, GIP and CCK, APD motility and energy intake in healthy subjects. Blood glucose, plasma hormone and APD motor responses to 120 min intraduodenal infusions of glucose at (i) 1 (“G1”), (ii) 2 (“G2”), and (iii) 4 (“G4”) kcal/min, or (iv) saline (“control”) were measured in 10 healthy males in double-blind, randomised fashion. Immediately after each infusion energy intake at a buffet meal was quantified. Blood glucose rose in response to all glucose infusions ($P < 0.05$ vs. control), with the effect of G4 and G2 being greater than that of G1 ($P < 0.05$), but with no difference between G2 and G4. The rises in insulin, GLP-1, GIP and CCK were related to the glucose load ($r > 0.82$, $P < 0.05$). All glucose infusions suppressed antral PWs ($P < 0.05$), but only G4 decreased

duodenal PWs ($P < 0.01$), resulted in a sustained stimulation of basal pyloric pressure ($P < 0.01$) and decreased energy intake ($P < 0.05$). In conclusion, variations in duodenal glucose loads have differential effects on blood glucose, plasma insulin, GLP-1, GIP and CCK, APD motility and energy intake in healthy subjects. These observations have implications for strategies to minimise postprandial glycaemic excursions in type-2 diabetes.

7.2 INTRODUCTION

The rate of gastric emptying of carbohydrate, particularly glucose, has an impact on glycaemia (Horowitz et al., 1993), appetite (Cook et al., 1997, Lavin et al., 1996) and energy intake (Lavin et al., 1998). In healthy subjects, glucose solutions are known to empty from the stomach in an overall linear rate of $\sim 2 - 3$ kcal/min (Horowitz et al., 1996); this tight regulation results primarily from a length-dependent feedback arising from the small intestine (Lin et al., 1989), which in turn modulates APD motility and is associated with the release of a number of gastrointestinal hormones, including CCK (Liddle et al., 1985) and the incretin hormones, GLP-1 and GIP (Lavin et al., 1998, O'Donovan et al., 2004, Schirra et al., 1996). The relationships of glycaemia, hormone release and changes in gastric motility with the duodenal glucose load, particularly the time-course of these effects, are poorly defined.

The rate of gastric emptying is a major determinant of the glycaemic response to a meal - even relatively minor changes in small intestinal glucose delivery may have major effects on glycaemic and insulinaemic responses in healthy subjects (Chaikomin et al., 2005) and non-insulin treated type-2 diabetics (O'Donovan et al., 2004). In type-1

diabetes the initial postprandial insulin requirement is less when gastric emptying is slower (Ishii et al., 1994, Ohlsson et al., 2006). These observations have substantial implications for dietary and pharmacological strategies to minimise postprandial glycaemic excursion in diabetes (Rayner et al., 2001) and, thereby, reduce the risk of micro- (Ceriello, 1998) and macrovascular (Saydah et al., 2001) complications. Hence, it is important to define the relationship between glycaemic and insulinaemic responses with enteral glucose delivery. The secretion of GLP-1 and GIP accounts for ~ 50 % of the insulin response to oral glucose in healthy subjects (Nauck et al., 2004). It has been suggested that a threshold of small intestinal glucose delivery of about 1.8 kcal/min needs to be exceeded in order to stimulate GLP-1 release (Schirra et al., 1996). However, this observation is inconsistent with two recent studies, which have demonstrated that duodenal glucose infusion at 1 kcal/min was sufficient for the, albeit transient, stimulation of GLP-1 in healthy subjects and type-2 patients (O'Donovan et al., 2004, Chaikomin et al., 2005). In contrast, there is evidence that the release of GIP is load-dependent; however, the load that elicits the maximum response is not known (O'Donovan et al., 2004, Chaikomin et al., 2005). The presence of glucose in the proximal small intestine also stimulates the release of CCK (Little et al., 2006a), although this response is less marked than that to protein or fat (Liddle et al., 1985). Whether the release of CCK by glucose is load-dependent remains to be determined.

The slowing of gastric emptying by glucose in the small intestine is associated with suppression of antral and duodenal PWs and stimulation of phasic and tonic pressures localised to the pylorus (Hedde et al., 1988c, Edelbroek et al., 1992a). Studies in animals indicate that small intestinal feedback on gastric emptying is load-, but not concentration-, dependent (Lin et al., 1989). In humans, intraduodenal infusions of

glucose at 2.4 kcal/min and 4 kcal/min for 10 min stimulate phasic pressure waves localised to the pylorus and increase basal pyloric pressure, with a greater response to 4 kcal/min, indicative of load-dependence (Hedde et al., 1988c). However, in another study, duodenal infusion of glucose at 2.4 kcal/min for 120 min increased the number of IPPWs and basal pyloric pressure during the first 20 min, but after this time there was a decrease, with a subsequent return to baseline (Edelbroek et al., 1992a), suggesting that there may be “adaptation” in the pyloric motor response during more sustained small intestinal glucose exposure. In contrast, antral PWs remained suppressed throughout the glucose infusion (Edelbroek et al., 1992a). The effects of enteral glucose on gastric motility may also be secondary to the consequent rise in blood glucose - acute hyperglycaemia (blood glucose 12 - 16 mmol/l) is associated with suppression of antral waves (Hasler et al., 1995) and stimulation of pyloric contractions (Fraser et al., 1991b). Changes in blood glucose that are within the normal postprandial range also affect gastrointestinal motility and gastric emptying (Schvarcz et al., 1997, Hasler et al., 1995).

Perceptions of appetite and energy intake are suppressed by small intestinal infusion of glucose in young, obese, and healthy older subjects (Cook et al., 1997, Lavin et al., 1998, Chapman et al., 1999). These effects may relate to the release of CCK and GLP-1 (Kissileff et al., 1981, Verdich et al., 2001a). It has recently been suggested that the suppression of energy intake may relate to changes in APD motility, particularly that of the pylorus (Brennan et al., 2005, Xu et al., 2005). In a recent study, intravenous infusion of CCK was shown to markedly stimulate IPPWs and basal pyloric pressures, and this was associated with suppression of energy intake; while GLP-1, at least in the dose used, failed to suppress energy intake, or stimulate pyloric pressures (Brennan et

al., 2005). A further study in animals also supports the concept that the stimulation of pyloric motility may, per se, reduce energy intake (Xu et al., 2005). - It is not known whether the effects of enteral glucose on energy intake and APD motility are related, nor whether the suppression of energy intake by glucose is load-dependent in humans.

The aims of this study were to evaluate, in healthy subjects, the effects of different intraduodenal glucose loads on glycaemia, insulin, incretin and CCK release, APD motility and energy intake, as well as the relationships between these parameters. It was hypothesised that: (i) the effects of intraduodenal infusion of glucose at loads lower than (1 kcal/min), comparable to (2 kcal/min) and higher than (4 kcal/min) the rate of normal gastric emptying on these parameters would be load-dependent (and in the case of GLP-1 be evident early) and (ii) the effects of small intestinal glucose on energy intake would be related to those on APD motility.

7.3 SUBJECTS AND METHODS

7.3.1 Subjects

10 healthy males (age: 32 ± 4 yrs; BMI: 25.1 ± 0.4 kg/m²) were recruited according to guidelines described in Chapter 4.2.

7.3.2 Study outline

Each subject was studied on four occasions, each separated by 3 - 7 days, on which they received, in randomised, double-blind fashion, an intraduodenal infusion of a 25 % glucose (1390 mosmol/L) solution, at (i) 1 kcal/min (“G1”), (ii) 2 kcal/min (“G2”), or

(iii) 4 kcal/min (“G4”), or (iv) intraduodenal hypertonic (4.2 %, 1390 mosmol/L) saline (“control”), for 120 min. Blood glucose, plasma insulin, GLP-1, GIP and CCK, APD pressures and energy intake were evaluated.

7.3.3 Intraduodenal infusions

The intraduodenal glucose infusion was administered as: (i) 1 kcal/min, (ii) 2 kcal/min and (iii) 4 kcal/min, for 120 min. The intraduodenal glucose solutions were prepared by dissolving glucose powder (Glucodin, Boots Healthcare, North Ryde, NSW, Australia) in distilled water and diluting with hypertonic saline to achieve the specific loads. Thus, all infusions had a concentration of 1390 mosmol/l and were administered at a rate of 4 ml/min, so that the total volume infused was 480 ml in all study conditions (Chapter 4.4.2.2). The infusions were prepared by an investigator who was not otherwise involved in either the performance of the studies or data analysis. During studies the infusion apparatus was covered at all times.

7.3.4 Protocol

Each subject attended the laboratory in the Discipline of Medicine at 0830 h after an overnight fast (14 hours for solids, 12 hours for liquids). A manometric catheter was positioned, as described in Chapter 4.5.1. An intravenous cannula was placed in a forearm vein for blood sampling.

Once the manometric catheter was positioned correctly, fasting motility was monitored until the occurrence of a phase III of the interdigestive MMC (Feltrin et al., 2004). Immediately after the cessation of phase III activity (at $t = -15$ min), a baseline blood

sample was taken. At $t = 0$ min, during phase I of the MMC (Feltrin et al., 2004), the intraduodenal infusion commenced and was continued for 120 min. APD pressures were recorded during the 15 min baseline period (i.e. $t = -15 - 0$ min) and the infusion period (i.e. $t = 0 - 120$ min). Blood samples were taken at 15 min intervals between $t = 0 - 60$ min, and then at 30 min intervals between $t = 60 - 120$ min. At $t = 120$ min, the infusion was terminated and the manometric catheter removed. Each subject was then presented with a standard, cold, buffet-style meal and allowed 30 min (i.e. $t = 120 - 150$ min) to eat freely until they were comfortably full (Feltrin et al., 2004). Further blood samples were taken at $t = 150$ min and 180 min. At $t = 180$ min the intravenous cannula was removed and the subject allowed to leave the laboratory.

7.3.5 Measurements

7.3.5.1 *Blood glucose, plasma insulin, glucagon-like peptide-1, glucose-dependent insulintropic polypeptide and cholecystokinin concentrations*

Blood sample collection and analysis of blood glucose, plasma insulin, GLP-1, GIP and CCK are described in Chapters 4.5.7 and 4.5.8.

7.3.5.2 *Antropyloroduodenal pressures*

APD pressures were assessed, and analysed for (i) number and amplitude of antral and duodenal PWs, (ii) number and amplitude of isolated IPPWs, (iii) basal pyloric pressure and (iv) number and length of pressure wave sequences PWSs, as described in Chapter 4.5.1.

7.3.5.3 Energy intake

Assessment of energy intake is described in Chapter 4.5.5.3.

7.3.6 Statistical analysis

Baseline values (“0”) were calculated as the total values obtained for the number of IPPWs, antral and duodenal PWs and PWSs, and the mean values obtained for amplitude of IPPWs, antral and duodenal PWs and basal pyloric pressure, between $t = -15 - 0$ min, and the mean values for plasma hormone concentrations at $t = -15$ and 0 min. Antral and duodenal PWs were expressed as total numbers in all six antral, and all seven duodenal, side-holes, respectively. Basal pyloric pressures and number and amplitude of IPPWs were expressed as the mean of 15 min periods over the 120 min infusion period. APD PWSs were expressed as the total number of PWs spanning over two (1.5 - 3 cm), three (3 - 4.5 cm), ... , fifteen (21 - 22.5 cm) channels during the 120 min infusion period. All motility data were expressed as changes from baseline, whilst blood glucose and plasma insulin, GLP-1, GIP, and CCK were expressed as absolute values. Blood glucose and plasma hormone data were evaluated, a priori, for two time periods; $t = 0 - 120$ min (“entire infusion” period) and $t = 120 - 180$ min (“post-meal” period). For GLP-1 an additional period from $t = 0 - 30$ min was evaluated, to define the “early response” (O'Donovan et al., 2004, Chaikomin et al., 2005). Peak hormone concentrations and the times at which they occurred were calculated by determining the highest concentration and its timing in each subject. The AUC for hormone concentrations, basal pyloric pressure, and number and amplitude of IPPWs during the infusion period were determined using the trapezoidal rule.

Numbers and amplitudes of antral and duodenal PWs and energy intake were analysed by one-way ANOVA. Blood glucose and plasma hormone concentrations, basal pyloric pressure and number and amplitude of IPPWs were analysed by repeated-measures ANOVA, with time and treatment as factors. The total number of PWSs was analysed by repeated-measures ANOVA, with number and length (cm) as factors. Peak glucose and hormone concentrations (and the times at which these occurred) and the AUCs were analysed using Student's t-test.

Correlations, corrected for repeated measures, were determined for (i) the total number and mean amplitude of antral and duodenal PWs, AUC for basal pyloric pressures, number and amplitude of IPPWs, APD PWSs, blood glucose and plasma insulin, GLP-1, GIP, and CCK concentrations between $t = 0 - 120$ min, and energy intake and amount eaten at the buffet meal with the \ln (natural logarithm) - transformed glucose loads, and (ii) energy intake and amount eaten with APD motility, blood glucose and plasma insulin, GLP-1, GIP and CCK concentrations, as their total or AUC values and values at $t = 120$ min, using the method described by Bland and Altman (Bland and Altman, 1995). Only r values > 0.5 were considered physiologically relevant. When correlations between the above variables were found, multiple regression analysis was performed to establish determinants of insulin release and energy intake at the buffet meal. Statistical significance was accepted at $P < 0.05$, and data are presented as means \pm SEM.

7.4 RESULTS

The studies were well tolerated by all, but one, subject who experienced quite marked nausea during the control infusion. That study was completed and all data were included in the analysis. Fasting concentrations of blood glucose and plasma insulin, GIP, GLP-1 and CCK did not differ between the four study days (**Table 7.1**). For the analysis of GLP-1, data in 9 of the 10 subjects were available for analysis.

7.4.1 Blood glucose and plasma hormone concentrations

7.4.1.1 *Blood glucose (Figure 7.1A)*

7.4.1.1.1 Infusion period

Blood glucose concentrations were higher during all glucose infusions, from $t = 15 - 120$ min, when compared with control ($P < 0.05$). The increase in blood glucose between $t = 15 - 60$ min was greater during G2 and G4, when compared with G1 ($P < 0.05$ for both). There was no difference between G2 and G4. Peak glucose concentrations were higher during G2 and G4, when compared with G1 ($P < 0.01$), with no difference between G2 and G4 (**Table 7.2**). After $\sim t = 60$ min blood glucose progressively fell ($P < 0.01$) during G2 and G4, to concentrations close to baseline by $t = 120$ min ($P = 0.07$ for both). The AUC between $t = 0 - 120$ min, was greater for G1, G2 and G4 compared with control (**Table 7.2**). There was no relationship between the AUC for blood glucose with the load of glucose administered.

7.4.1.1.2 Post-meal period

Blood glucose concentrations decreased after all glucose infusions when compared with both control and levels immediately before the meal (i.e. $t = 120$ min) ($P < 0.001$) - the magnitude of the fall was greater for G4 than for G1 and G2 ($P < 0.05$). In contrast, there was an increase in blood glucose ($P < 0.001$) after the control infusion.

7.4.1.2 *Insulin (Figure 7.1B)*

7.4.1.2.1 Infusion period

There was a rise in insulin with all glucose infusions when compared with control ($P < 0.05$); between $t = 60 - 120$ min for G1, $t = 45 - 120$ min for G2, and $t = 15 - 120$ min for G4, with no difference between G1 and G2. Insulin concentrations were substantially greater during G4 when compared with G1 and G2 between $t = 30 - 120$ min ($P < 0.05$). The AUC for plasma insulin was greater for G2 and G4, when compared with control, and for G4 when compared with G1 and G2 ($P < 0.001$), with no difference between G1 and G2 (**Table 7.2**). The AUC for plasma insulin and the load of glucose administered were related ($r = 0.89$, $P < 0.01$).

7.4.1.2.2 Post-meal period

Plasma insulin concentrations fell after G4 ($P < 0.001$) to levels comparable to G1 and G2. In contrast, there was an increase in plasma insulin after control ($P < 0.001$) - at $t = 180$ min, insulin concentrations after control were greater than those after the glucose infusions ($P < 0.05$).

7.4.1.3 *Glucagon-like peptide-1 (Figure 7.1C)*

7.4.1.3.1 Infusion period

Between $t = 0 - 30$ min, there was a prompt rise (i.e. within 15 min) in plasma GLP-1 with all glucose infusions, when compared with control ($P < 0.05$), with no difference between G1 and G2. GLP-1 concentrations were higher during G4 when compared with G1 and G2 ($P < 0.01$). Between $t = 15 - 30$ min, plasma GLP-1 fell during G1 and G2, and there were no longer any differences between G1, G2 and control. At $t = 30$ min plasma GLP-1 remained higher during G4 when compared with control, G1 and G2 ($P < 0.001$).

Between $t = 0 - 120$ min, G2 and G4, but not G1, increased plasma GLP-1 when compared with control; G2 at $t = 15$ min and G4 between $t = 15 - 120$ min ($P < 0.05$). During G4 there was a progressive rise in plasma GLP-1 ($P < 0.001$) and levels were much higher compared with G1 between $t = 15 - 120$ min and with G2 between $t = 30 - 120$ min ($P < 0.05$ for both). There was no difference between control, G1 and G2, except at $t = 15$ min. The AUC for plasma GLP-1 was greater for G4 when compared with control, G1 and G2 (**Table 7.2**). The AUC for plasma GLP-1 and the load of glucose administered were related ($r = 0.89$, $P < 0.01$).

7.4.1.3.2 Post-meal period

Plasma GLP-1 decreased after G4 and increased with control, and both these concentrations were higher than those following G1 and G2 ($P < 0.01$). At $t = 180$ min, there was no difference between control and any glucose infusion.

7.4.1.4 *Glucose-dependent insulintropic polypeptide (Figure 7.1D)*

7.4.1.4.1 Infusion period

All treatments increased plasma GIP between $t = 15 - 120$ min when compared with control ($P < 0.001$ for all), with rapid rises followed by relatively stable levels. G2 and G4 increased plasma GIP when compared with G1 between $t = 15 - 120$ min, and $t = 30 - 120$ min, respectively ($P < 0.05$ for all). Plasma GIP was higher during G4 when compared with G2 between $t = 30 - 90$ min ($P < 0.05$). Peak GIP concentrations were greater in response to G2 and G4 when compared with G1 ($P < 0.001$), and G4 when compared with G2 ($P < 0.05$) (**Table 7.2**). The AUC for plasma GIP was greater for all glucose treatments, when compared with control (**Table 7.2**). The AUC for plasma GIP and the load of glucose administered were related ($r = 0.91$, $P < 0.01$).

7.4.1.4.2 Post-meal period

There were no changes in GIP for G2 and G4, with levels remaining elevated compared with control and G1 ($P < 0.01$), but there were increases after control and G1 (i.e. $t = 150$ min). At $t = 180$ min, there was no difference between control and any glucose infusion.

7.4.1.5 *Cholecystinin (Figure 7.1E)*

7.4.1.5.1 Infusion period

There was a rapid increase in CCK during all glucose treatments, after which concentrations remained relatively stable. G1 and G2 increased plasma CCK when compared with control at $t = 15$ min ($P < 0.01$). CCK remained elevated compared

with baseline during G1 and G2 between $t = 15 - 120$ min ($P < 0.01$). G4 increased plasma CCK when compared with control, G1 and G2 between $t = 15 - 120$ min ($P < 0.001$ for all). Both peak plasma CCK and the AUC for plasma CCK were greater for G4 when compared with control, G1 and G2 ($P < 0.01$) (**Table 7.2**). The AUC for plasma CCK and the load of glucose administered were related ($r = 0.82$, $P < 0.01$).

7.4.1.5.2 Post-meal period

CCK concentrations increased after control, G1 and G2 ($P < 0.001$ for all), but not after G4, immediately after consumption of the buffet meal (i.e. $t = 150$ min). There were no differences between the treatments.

7.4.2 **Antropyloroduodenal pressures**

7.4.2.1 *Antral pressure waves*

There was a treatment effect for the number, but not the amplitude, of antral PWs ($P < 0.01$) (**Table 7.3**). G1 ($P < 0.05$), G2 ($P < 0.05$) and G4 ($P < 0.001$) decreased the number of antral PWs when compared with control, with no significant difference between them, although the mean value was least for G4. There was no significant relationship between the number or amplitude of antral PWs and the load of glucose administered.

7.4.2.2 *Pyloric pressures*

7.4.2.2.1 Basal pressures

There was a treatment by time interaction for basal pyloric pressures ($P < 0.001$) (**Figure 7.2A**). G1, G2 and G4 stimulated basal pyloric pressure when compared with control; G1 and G2 between $t = 90 - 105$ min ($P < 0.05$) and G4 between $t = 15 - 120$ min ($P < 0.01$). During G4 basal pyloric pressure progressively rose until $t = 60$ min ($P < 0.001$), after which it fell slightly. G2 stimulated basal pyloric pressure between $t = 15 - 45$ min ($P < 0.05$) and G4 between $t = 15 - 120$ min ($P < 0.01$), when compared with G1. G4 stimulated basal pyloric pressure between $t = 30 - 120$ min when compared with G2 ($P < 0.01$). The AUC for basal pyloric pressure and the load of glucose administered were related ($r = 0.83$, $P < 0.01$).

7.4.2.2.2 Phasic pressures

There was no effect of treatment on the number (**Figure 7.2B**) or amplitude of IPPWs (data not shown). All infusions initially stimulated IPPWs ($P < 0.05$), and this increase was greater during G4 when compared with control (between $t = 15 - 45$ min), G1 (between $t = 0 - 45$ min) and G2 (between $t = 15 - 30$ min) ($P < 0.05$ for all). With all infusions there was a subsequent decline in the number of IPPWs. There were no significant relationships between the number or amplitude of IPPWs and the load of glucose administered.

7.4.2.3 *Duodenal pressures waves*

There was a treatment effect for the number, but not the amplitude, of duodenal PWs ($P < 0.001$) (**Table 7.3**). G4 decreased the number of duodenal PWs compared with control ($P < 0.001$), G1 ($P < 0.001$) and G2 ($P < 0.01$), with no difference between G1, G2 and control. There was an inverse relationship between the number, but not the amplitude, of duodenal PWs with the load of glucose administered ($r = - 0.75$, $P < 0.001$).

7.4.2.4 *Pressure wave sequences*

Only PWSs that spanned 2 - 9 channels (1.5 - 13.5 cm) were analysed statistically, as PWSs spanning over 10 - 15 channels were infrequent (control: 3.4 ± 0.3 ; G1: 1.6 ± 0.2 ; G2: 2.3 ± 0.2 ; G4: 1.2 ± 0.1). There was a treatment by length interaction for the number of PWSs spanning two (1.5 - 3 cm), three (3 - 4.5 cm), four (4.5 - 6 cm), five (6 - 7.5 cm), six (7.5 - 9 cm), seven (9 - 10.5 cm), eight (10.5 - 12 cm) and nine (12 - 13.5 cm) channels ($P < 0.05$) (data not shown). G2 decreased the number of PWSs that spanned over two channels ($P < 0.001$) and G4 over two to four channels ($P < 0.001$), when compared with control. G2 ($P < 0.05$) and G4 ($P < 0.01$) decreased the number of PWSs that spanned over two and three channels compared with G1. There were no differences between control and G1 or G2 and G4. There was an inverse relationship between the number of PWSs with the load of glucose administered ($r = - 0.70$, $P < 0.05$).

7.4.3 Energy intake

There was an effect of treatment on the amount eaten (g) at the buffet meal ($P < 0.001$) (**Table 7.4**). G2 ($P < 0.05$) and G4 ($P < 0.001$) reduced the amount eaten when compared with control, and G4 when compared with G1 ($P < 0.001$) and G2 ($P < 0.01$). There was also an effect of treatment on the energy consumed at the meal ($P < 0.01$), so that G4 reduced energy intake when compared with G1 ($P < 0.001$) and G2 ($P < 0.01$), with a trend for a decrease compared with control ($P = 0.09$) (**Table 7.4**). There was no effect of treatment on % energy from fat, carbohydrate or protein (**Table 7.4**). There was an inverse relationship between the amount of food ($r = -0.79$, $P < 0.001$) and energy ($r = -0.73$, $P < 0.01$) consumed at the buffet meal with the load of glucose administered.

7.4.4 Relations between antropyloroduodenal motility, blood glucose, hormones and energy intake

7.4.4.1 *Relations of antropyloroduodenal motility with blood glucose and plasma hormone concentrations*

There was an inverse relationship between the number ($r = -0.66$, $P < 0.05$) of antral PWs and the number ($r = -0.52$, $P < 0.01$) and amplitude ($r = -0.61$, $P < 0.01$) of duodenal PWs, and a direct relationship between basal pyloric pressure ($r = 0.68$, $P < 0.05$), with the AUC for blood glucose. There was an inverse relationship between the number of antral PWs ($r = -0.74$, $P < 0.05$), the number ($r = -0.71$, $P < 0.001$) and amplitude ($r = -0.70$, $P < 0.05$) of duodenal PWs and number of PWSs ($r = -0.53$, $P < 0.05$), and a direct relationship between basal pyloric pressure ($r = 0.89$, $P < 0.01$), with the AUC for plasma insulin. There was an inverse relationship between the number (r

= -0.64, $P < 0.01$) and amplitude ($r = -0.66$, $P < 0.05$) of duodenal PWs, and a direct relationship between basal pyloric pressure ($r = 0.84$, $P < 0.05$), with the AUC for plasma GLP-1. There was an inverse relationship between the number ($r = -0.63$, $P < 0.05$) of antral PWs, the number ($r = -0.57$, $P < 0.01$) and amplitude ($r = -0.67$, $P < 0.01$) of duodenal PWs and the number of PWSs ($r = -0.57$, $P < 0.05$), and a direct relationship between basal pyloric pressure ($r = 0.68$, $P < 0.001$), with the AUC for plasma GIP. There was an inverse relationship between the number of antral PWs ($r = -0.59$, $P < 0.05$) and the number ($r = -0.76$, $P < 0.01$) and amplitude ($r = -0.62$, $P < 0.05$) of duodenal PWs, and a direct relationship between basal pyloric pressure ($r = -0.81$, $P < 0.05$), with the AUC for plasma CCK.

7.4.4.2 *Relation of blood glucose and plasma hormone concentrations with energy intake and amount eaten*

There was an inverse relationship between the AUC for blood glucose ($r = -0.50$, $P < 0.05$), insulin ($r = -0.77$, $P < 0.01$), GLP-1 ($r = -0.71$, $P = 0.06$) and GIP ($r = -0.61$, $P = 0.01$) and the values at $t = 120$ min for insulin ($r = -0.76$, $P < 0.05$) and GLP-1 ($r = -0.71$, $P < 0.71$) with the amount eaten. There was an inverse relationship between the AUC for insulin ($r = -0.62$, $P < 0.01$) and GLP-1 ($r = -0.80$, $P = 0.07$) and the values at $t = 120$ min for insulin ($r = -0.62$, $P < 0.01$) and CCK ($r = -0.52$, $P < 0.05$) with energy intake.

7.4.4.3 *Relation of antropyloroduodenal motility with energy intake and amount eaten*

There was an inverse relationship between the amount eaten at the meal and the AUC for basal pyloric pressure ($r = 0.70$, $P < 0.05$), but no relationships between any other motility parameter with energy intake.

7.4.4.4 *Relation of plasma insulin with blood glucose and plasma glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide*

There was a direct relationship between the AUC for blood glucose ($r = 0.76$, $P < 0.001$), plasma GLP-1 ($r = 0.85$, $P < 0.001$) and GIP ($r = 0.77$, $P < 0.01$) with plasma insulin.

7.4.5 **Predictors of insulin concentrations and energy intake**

Multiple regression analysis of the combined data for blood glucose and incretin hormones demonstrated that blood glucose ($\beta = 9.18$, $P < 0.05$), plasma GLP-1 ($\beta = 2.08$, $P < 0.001$) and GIP ($\beta = 0.65$, $P < 0.05$) were independent predictors of plasma insulin concentrations.

Multiple regression analysis of the combined data for blood glucose, all hormones and motility parameters showed that only basal pyloric pressure was associated with the amount eaten at the buffet meal ($\beta = -0.35$, $P < 0.05$). The AUC for plasma insulin ($\beta = 0.54$, $P < 0.01$) and values at $t = 120$ min for plasma insulin ($\beta = -29.33$, $P < 0.01$) and CCK ($\beta = -437.79$, $P < 0.01$) were predictors of energy intake.

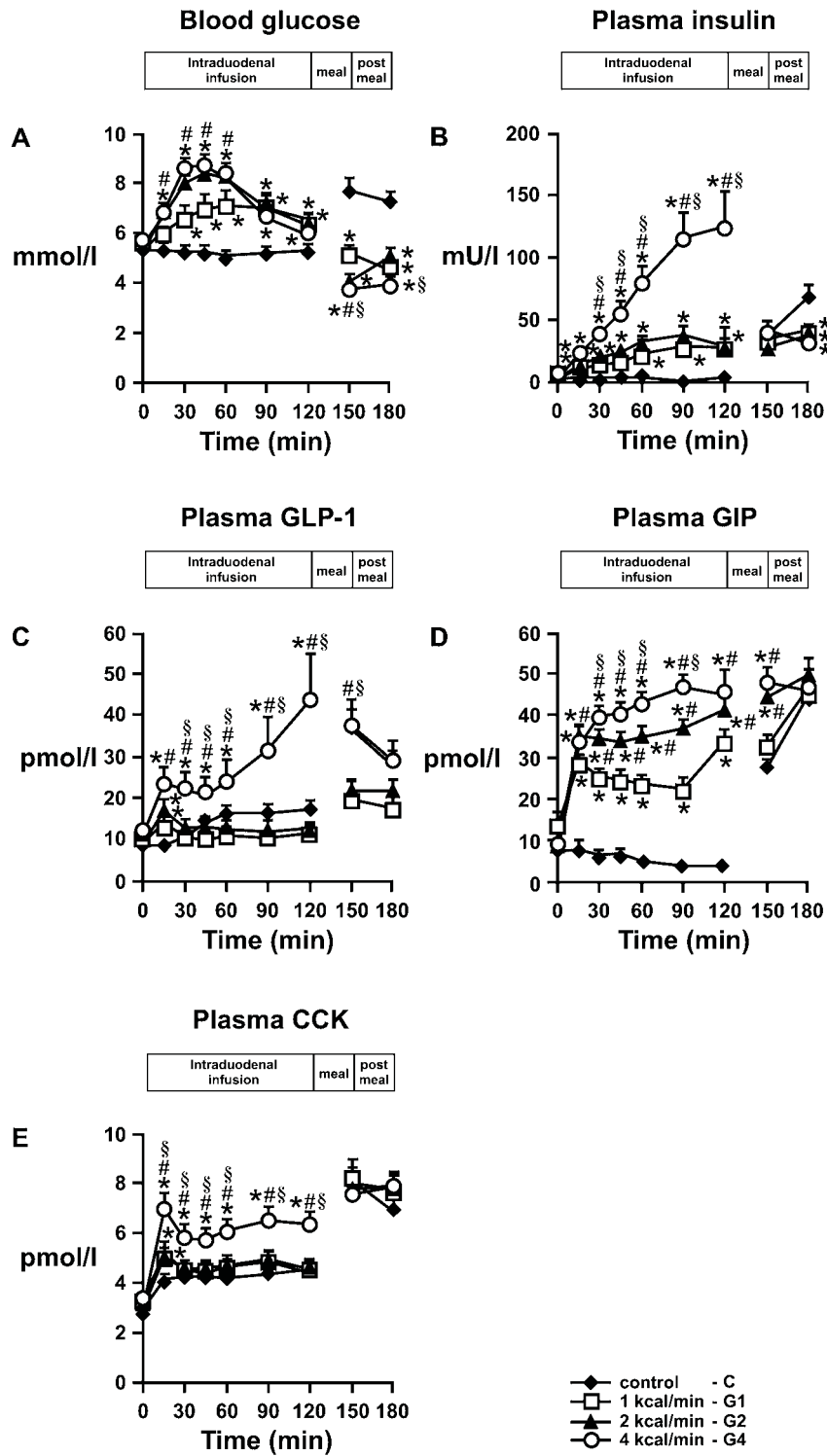


Figure 7.1 (A) Blood glucose, and plasma (B) insulin, (C) glucagon-like peptide-1 (GLP-1), (D) glucose-dependent insulinotropic polypeptide (GIP), and (E) cholecystikinin (CCK) concentrations in response to 120 min intraduodenal glucose (25 %, 1390 osmol/l) infusions at 1 (□) (“G1”), 2 (▲) (“G2”), or 4 (○) (“G4”) kcal/min, or saline (4.2 %, 1390 osmol/l) control (◆) (“C”), in 10 healthy males. (A) * vs. control; $P < 0.05$, # vs. G1; $P < 0.05$, § vs. G2; $P < 0.05$. (B) * vs. control; $P < 0.05$, # vs. G1; $P < 0.05$, § vs. G2; $P < 0.05$. (C) * vs. control; $P < 0.05$, # vs. G1; $P < 0.05$, § vs. G2; $P < 0.05$. (D) * vs. control; $P < 0.01$, # vs. G1; $P < 0.05$, § vs. G2; $P < 0.05$. (E) * vs. control; $P < 0.05$, # vs. G1; $P < 0.01$, § vs. G2; $P < 0.01$. Data are means \pm SEM ($n = 10$).

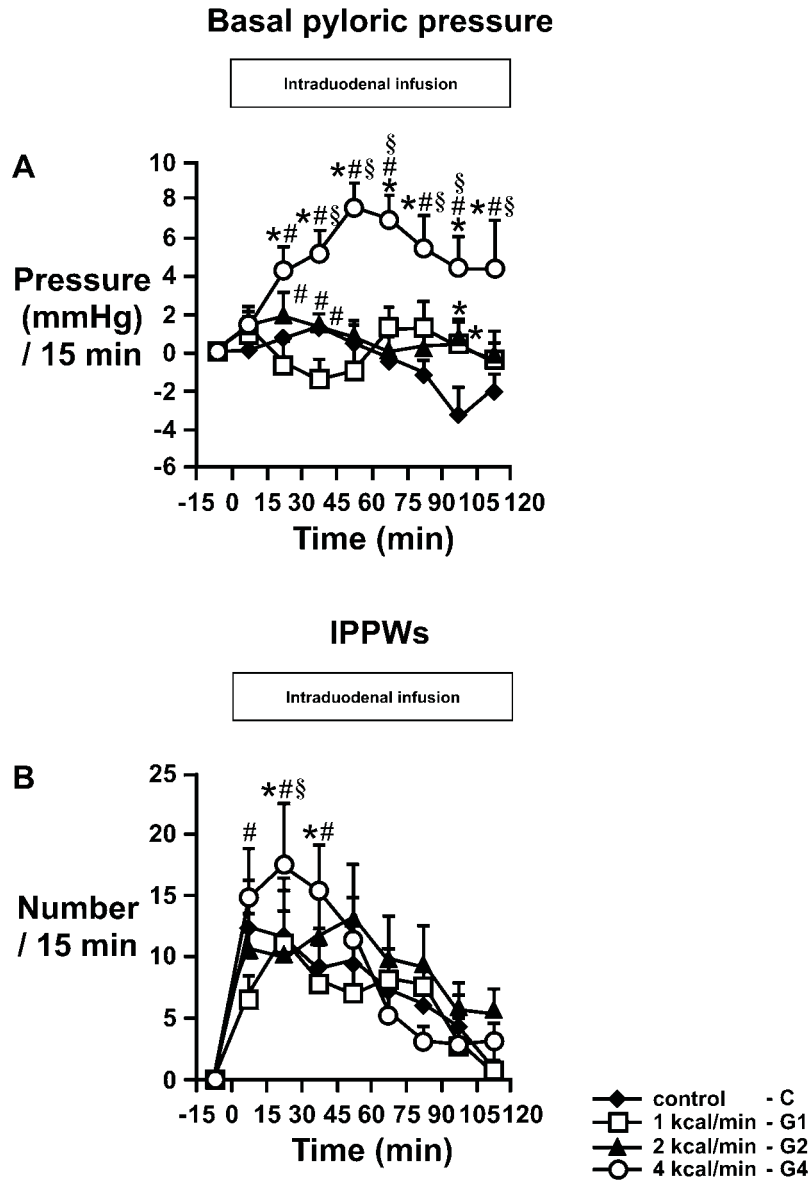


Figure 7.2 (A) Basal pyloric pressures, and (B) isolated pyloric pressure waves (IPPWs) in response to 120 min intraduodenal glucose (25 %, 1390 osmol/l) infusions at 1 (□) (“G1”), 2 (▲) (“G2”), or 4 (○) (“G4”) kcal/min, or saline (4.2 %, 1390 osmol/l) control (◆) (“C”), in 10 healthy males. (A) * vs. control; $P < 0.05$, # vs. G1; $P < 0.05$, § vs. G2; $P < 0.01$. (B) * vs. control; $P < 0.05$, # vs. G1; $P < 0.05$, § vs. G2; $P < 0.05$. Data are means \pm SEM ($n = 10$).

Table 7.1 Baseline values for antral and duodenal pressure waves, basal pyloric pressure, number and amplitude of isolated pyloric pressure waves, blood glucose and plasma insulin, glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide and cholecystokinin concentrations (i.e. prior to commencement of intraduodenal infusions of 25 % (1390 mosmol/l) glucose (at 1 (“G1”), 2 (“G2”) or 4 (“G4”) kcal/min) or 4.2 % (1390 mosmol/l) saline).

	Saline (Control)	G1	Glucose G2	G4	P value (ANOVA)
<i>Antral PWs</i>					
Number	1.7 ± 0.7	4.2 ± 2.0	4.0 ± 2.1	3.3 ± 2.2	NS
Amplitude (mmHg)	7.7 ± 2.1	7.5 ± 2.6	12.0 ± 2.0	10.4 ± 5.6	NS
<i>Pyloric pressures</i>					
Basal (mmHg)	-0.2 ± 1.4	2.1 ± 2.3	-3.0 ± 5.6	5.6 ± 6.1	NS
IPPW (number)	0.1 ± 0.1	1.6 ± 1.6	0 ± 0	0 ± 0	NS
IPPW (mmHg)	3.0 ± 3.0	4.2 ± 4.2	0 ± 0	0 ± 0	NS
<i>Duodenal PWs</i>					
Number	32.2 ± 10.1	41.1 ± 11.2	41.4 ± 9.0	46.8 ± 18.1	NS
Amplitude (mmHg)	14.1 ± 2.8	19.9 ± 2.5	17.4 ± 3.1	16.4 ± 2.3	NS
Glucose (mmol/l)	5.2 ± 0.3	5.0 ± 0.2	5.4 ± 0.2	5.1 ± 0.2	NS
Insulin (mU/l)	2.3 ± 0.3	2.9 ± 0.3	2.6 ± 0.3	2.6 ± 0.2	NS
GLP-1 (pmol/l)	8.6 ± 1.4	9.1 ± 1.9	8.8 ± 0.6	10.9 ± 1.7	NS
GIP (pmol/l)	7.4 ± 1.3	13.4 ± 3.3	8.9 ± 1.9	7.3 ± 1.5	NS
CCK (pmol/l)	2.9 ± 0.3	3.3 ± 90.3	3.0 ± 0.2	3.0 ± 0.2	NS

Data are means ± SEM (n = 10). CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IPPWs, isolated pyloric pressure waves; NS, not significant; PWs, pressure waves.

Table 7.2 Peak concentrations, their timing and areas under the curve (AUC) for blood glucose and plasma insulin, glucagon-like peptide-1, glucose-dependent polypeptide and cholecystokinin concentrations during intraduodenal infusions of 25 % (1390 mosmol/l) glucose (at 1 (“G1”), 2 (“G2”) or 4 (“G4”) kcal/min) or 4.2 % (1390 mosmol/l) saline) between t = 0 - 120 min.

	Saline (Control)	G1	Glucose G2	G4	P value (ANOVA)
<i>Peak concentration</i>					
Glucose (mmol/l)	5.5 ± 0.3	7.5 ± 0.6*	8.7 ± 0.4* [#]	9.1 ± 0.4* [#]	< 0.001
Insulin (mU/l)	4.3 ± 0.5	29.8 ± 16.2	40.9 ± 20.7	137.0 ± 24.9	NS
GLP-1 (pmol/l)	69.0 ± 9.8	75.3 ± 10.8	78.4 ± 4.9	96.0 ± 8.7	NS
GIP (pmol/l)	9.0 ± 2.3	31.9 ± 2.3*	41.8 ± 3.5* [#]	52.2 ± 3.2* ^{#§}	< 0.001
CCK (pmol/l)	5.2 ± 0.3	5.6 ± 0.5	5.4 ± 0.3	7.4 ± 0.6* ^{#§}	< 0.01
<i>Timing of peak (min)</i>					
Glucose	74 ± 14	68 ± 9	48 ± 7	45 ± 4	NS
Insulin	69 ± 10	75 ± 11	78 ± 5	96 ± 9	NS
GLP-1	98 ± 10	51 ± 16	26 ± 7	75 ± 17	NS
GIP	20 ± 5	20 ± 4	80 ± 17	99 ± 8	NS
CCK	62 ± 1	53 ± 1	63 ± 13	30 ± 8	NS
<i>AUCs</i>					
Glucose (mmol/l.min)	-6 ± 9	147 ± 38*	198 ± 36*	226 ± 26*	< 0.001
Insulin (mU/l.min)	72 ± 26	1885 ± 1037	2625 ± 390*	7980 ± 1209* ^{#§}	< 0.05
GLP-1 (pmol/l.min)	591 ± 119	212 ± 301	447 ± 182	1805 ± 445* ^{#§}	< 0.001
GIP (pmol/l.min)	-225 ± 106	1146 ± 486*	2846 ± 306*	3648 ± 329*	< 0.001
CCK (pmol/l.min)	142 ± 28	146 ± 40	156 ± 23	344 ± 4* ^{#§}	< 0.001

Data are means ± SEM (n = 10). AUC, area under the curve; CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IPPWs, isolated pyloric pressure waves; NS, not significant; PWs, pressure waves. Significant differences: * from control, [#] from G1, [§] from G2.

Table 7.3 Antral and duodenal pressure wavers during intraduodenal infusions of 25 % (1390 mosmol/l) glucose (at 1 (“G1”), 2 (“G2”) or 4 (“G4”) kcal/min) or 4.2 % (1390 mosmol/l) saline) between t = 0 - 120 min.

	Saline (Control)	G1	Glucose G2	G4	P value (ANOVA)
<i>Antral PWs</i>					
Number	70 ± 16	40 ± 10*	40 ± 12*	24 ± 7*	< 0.01
Amplitude (mmHg)	26 ± 6	27 ± 7	8 ± 3	22 ± 11	NS
<i>Duodenal PWs</i>					
Number	928 ± 78	1132 ± 25	865 ± 118	390 ± 93*#§	< 0.001
Amplitude (mmHg)	15 ± 3	11 ± 4	9 ± 4	7 ± 2	NS

Data are means ± SEM (n = 10). NS, not significant; PWs, pressure waves. Significant differences: * from control, # from G1, § from G2.

Table 7.4 Amount eaten, energy intake and % macronutrient distribution at the buffet meal (i.e. between t = 120 - 150 min) following intraduodenal infusions of 25 % (1390 mosmol/l) glucose (at 1 (“G1”), 2 (“G2”) or 4 (“G4”) kcal/min) or 4.2 % (1390 mosmol/l) saline).

	Saline (Control)	G1	Glucose G2	G4	P value (ANOVA)
Amount (g)	1568 ± 142	1461 ± 126	1395 ± 132*	1157 ± 166* [#] [§]	< 0.01
Energy (kJ)	4444 ± 492	4999 ± 255	5020 ± 364	3935 ± 480 [#] [§]	< 0.001
% kJ from fat	28 ± 2	29 ± 2	30 ± 4	27 ± 2	NS
% kJ from CHO	53 ± 3	50 ± 3	50 ± 2	57 ± 4	NS
% kJ from protein	20 ± 2	20 ± 1	20 ± 1	19 ± 1	NS

Data are means ± SEM (n = 10). CHO, carbohydrate; NS, not significant. Significant differences: * from control, # from G1, § from G2.

7.5 DISCUSSION

This study provides novel insights into the effects of administration of glucose directly into the small intestine, at loads lower than (1 kcal/min), comparable to (2 kcal/min), and higher (4 kcal/min) than the rate at which gastric emptying normally occurs, on blood glucose, plasma insulin, GLP-1, GIP and CCK concentrations, APD motility and energy intake in healthy males. Of particular note are that: (i) while there was a rise in blood glucose in response to all glucose infusions, there was no difference in the overall response to 2 and 4 kcal/min, (ii) while a progressive rise in plasma insulin occurred in response to all glucose infusions, there was no difference between 1 and 2 kcal/min, and a substantially greater response to 4 kcal/min, (iii) there was a transient, modest, rise in plasma GLP-1 in response to intraduodenal glucose, but a sustained elevation was only evident with 4 kcal/min, which was progressive from $\sim t = 45$ min, and a “meal-related” rise in GLP-1 occurred following the 1 and 2 kcal/min, but not the 4 kcal/min, infusion, (iv) there was a load-dependent stimulation of GIP and CCK with a subsequent plateau and a “meal-related” increase only after control and 1 kcal/min for GIP, and after control and the 1 and 2 kcal/min infusions for CCK, (v) antral pressures were suppressed by all glucose infusions, while the stimulation of basal pyloric pressure and suppression of duodenal PWs only occurred during the 4 kcal/min infusion, (vi) a reduction in food intake was evident after the 2 and 4 kcal/min, but not the 1 kcal/min, infusion, and (vii) there were significant relationships between blood glucose, plasma insulin, GLP-1, GIP and CCK with APD motility, energy intake and the amount eaten, and between pyloric pressures with the amount eaten.

This study establishes that there are substantial variations in the effect of different duodenal glucose loads on glycaemia, insulinaemia and incretin hormones. While, predictably, all loads increased blood glucose, the 2 and 4 kcal/min infusions resulted in comparable blood glucose profiles, suggesting that the 2 kcal/min load was sufficient to cause a maximal response. The maximal capacity of glucose absorption from the small intestine into the systemic circulation is probably ~ 0.5 g per minute or 2 kcal/min per 30 cm (Duchman et al., 1997, Holdsworth and Dawson, 1964), accordingly, the 2 and 4 kcal/min loads used are equal to, or exceed, the maximal absorption capacity of a segment of the small intestine the length of the duodenum. The comparable glucose responses are likely to reflect the substantially greater insulin response to the 4 kcal/min load. Similarly, presumably as a result of the insulin response, blood glucose concentrations fell during the 2 and 4 kcal/min infusions after $\sim t = 60$ min despite the ongoing entry of glucose into the small intestine. The differential insulin response to the 2 and 4 kcal/min infusions, accordingly, cannot be accounted for by glycaemia and is likely to reflect the differential secretion of GLP-1 and GIP. As GIP is released from duodenal K cells (Fehmann et al., 1995), which are located near the site of infusion, the observed load-dependent increase and subsequent plateau are not unexpected (Schirra et al., 1996). However, the GIP response to the 4 kcal/min was only marginally greater than that to the 2 kcal/min glucose infusion, and GIP did not increase postprandially after the 4 kcal/min infusion, suggesting that the latter results in a maximal, or close to maximal, response. GLP-1 is released from L cells whose density is greatest in the distal small intestine (Eissele et al., 1992). Hence, a gradual, progressive rise in GLP-1 may have been anticipated. Schirra and colleagues have suggested that GLP-1 secretion requires a threshold of glucose delivery of ~ 1.8 kcal/min to be exceeded (Schirra et al., 1996). However, the present, as well as two

other (O'Donovan et al., 2004, Chaikomin et al., 2005), studies have established the capacity of loads as low as 1 kcal/min to cause an early, transient, stimulation of plasma GLP-1, albeit modest in magnitude. While the latter may reflect the presence of L cells in the duodenum (Theodorakis et al., 2006), a recent study suggests that GLP-1 is only released in response to glucose when more than 60 cm of small intestine is exposed (Little et al., 2006a). Hence, the “early”, transient GLP-1 response may be accounted for by, initially, relatively more rapid small intestinal transit and subsequent slowing. Studies in rodents also indicate that GLP-1 may be released through a neuroendocrine loop to the distal small intestine, whereby the release of GIP from duodenal K cells in response to glucose acts through vagal afferent pathways to stimulate the L cell indirectly (Rocca and Brubaker, 1999, Roberge and Brubaker, 1993). Accordingly, the mechanism(s) underlying the initial increase in plasma GLP-1 warrant further investigation. The GLP-1 response to the 4 kcal/min infusion was substantially greater than to the other glucose loads and progressive; this is likely to reflect the spread of glucose over a longer length of small intestine; the enteral glucose load which results in a maximum GLP-1 response remains to be determined. Given the comparable GIP, but vastly different GLP-1, responses to the 2 and 4 kcal/min infusions, it seems reasonable to speculate that the stimulation of GLP-1 was primarily responsible for the increased insulin response. In contrast, GIP may be responsible for the insulin response to the lower duodenal glucose loads, although there are clear limitations in attempting to estimate the relative contributions of GLP-1 and GIP to postprandial insulin release on a molar plasma level basis (Schirra et al., 2006). Clearly, the observations in the present study, in healthy subjects, should not be extrapolated directly to type-2 diabetes, which is characterised by a delay in insulin release (O'Donovan et al., 2004), impaired secretion of GLP-1 (Toft-Nielsen et al.,

2001), an impaired insulinotropic effect of GIP, but not GLP-1 (Holst and Gromada, 2004), and a high prevalence of disordered gastric emptying and gastroduodenal motility (Horowitz et al., 1989). However, it will be important to determine the effects of different small intestinal glucose loads in this group. CCK is released from I cells, which are confined to the proximal small intestine (Polak et al., 1975), therefore, as expected with GIP, all glucose infusions stimulated plasma CCK concentrations in a load-dependent fashion.

The tight regulation of glucose entry into the small intestine in health reflects the integration of motor activity in the proximal stomach, antrum, pylorus and duodenum (Hedde et al., 1988c, Edelbroek et al., 1992a) - the stimulation of pressure waves localised to the pylorus may be the most important mechanism (Tougas et al., 1992). All glucose treatments, as well as the control, stimulated IPPWs during the first 30 min of infusion, followed by a decline. The response to control is not surprising, given that it would have provided an osmotic stimulus known to slow gastric emptying (Barker et al., 1974) and the highest glucose load (4 kcal/min) stimulated IPPWs more, for some 45 min. Consistent with our observations, Edelbroek and colleagues, reported in healthy men that intraduodenal glucose infusion at 2.4 kcal/min for 120 min initially stimulated IPPWs and basal pyloric pressure, but these responses were not sustained (Edelbroek et al., 1992a), suggesting that there may be adaptation of the pylorus to the presence of glucose in the small intestine. The current study also confirms previous data, showing sustained suppression of antral PWs during intraduodenal glucose infusion (Rayner et al., 2000b, Hedde et al., 1988c, Edelbroek et al., 1992a). In a recent study, suppression of antral motility was evident when glucose was infused at 3.5 kcal/min for 60 min into both the duodenum and distal small intestine (i.e. allowed

access to the entire small intestine), and not when it was confined to the proximal 60 cm (Little et al., 2006a). Hence, the observed suppression of antral PWs by the 1 kcal/min load is perhaps surprising, as one would not expect this load to reach beyond the proximal 60 cm of the small intestine before being absorbed, but this may reflect the longer duration of glucose infusion in this study. The effects of intraduodenal glucose on motility may also be secondary to the consequent elevation in blood glucose (Barnett and Owyang, 1988, Fraser et al., 1991b, Hebbard et al., 1996), although this cannot explain the discordant duodenal and pyloric responses to the 2 and 4 kcal/min infusions given that the glycaemic responses were comparable. That the magnitude of the rise in blood glucose was related to the suppression of antral PWs is not surprising given that blood glucose concentrations as low as ~ 8 mmol/l can inhibit antral pressures (Barnett and Owyang, 1988) and slow gastric emptying when compared with euglycaemia ($\sim 4 - 6$ mmol/l) (Barnett and Owyang, 1988). The effects of hyperglycaemia on motility do not appear to be mediated by hyperinsulinaemia (Kong et al., 1999b).

There is a close relationship between the initial rise in blood glucose after oral carbohydrate and gastric emptying (Horowitz et al., 1993). Accordingly, interventions which result in a slowing of gastric emptying, and are associated with a pattern of APD motility which favours this, may reduce postprandial glycaemic excursions (Gentilcore et al., 2006a). In this study there were significant relationships between rises in blood glucose, plasma insulin, GLP-1, GIP and CCK with the stimulation of basal pyloric tone and the suppression of duodenal PWs, which may account for slowing of gastric emptying. The observed relationship between plasma GIP with the suppression of antral PWs is surprising given that GLP-1, not GIP, appears to be important in the

regulation of gastric emptying (Meier et al., 2004, Schirra et al., 2000). Although there was a significant relationship between both plasma GLP-1 and CCK concentrations with the glucose load, this was not reflected in the AUCs and should, accordingly, be viewed circumspectly.

Energy intake was only suppressed after the highest glucose load (4 kcal/min; total 480 kcal), although the amount (in grams) of food consumed was also reduced by the 2 kcal/min infusion. That a decrease in energy intake was only evident after 4 kcal/min is not surprising, as previous studies employing glucose loads of 2 kcal/min for 90 min (180 kcal) (Rayner et al., 2000b) and 2.86 kcal/min for 120 min (343 kcal) (Chapman et al., 1999, MacIntosh et al., 2001a) failed to show any reduction in energy intake in healthy young males. The only study, to date, which has determined a reduction in energy intake during intraduodenal glucose infusion, compared with saline, used a 3.2 kcal/min infusion of glucose for 90 min, which approximates to 288 kcal (Lavin et al., 1998). Interestingly, this load (288 kcal) is less than that in studies providing 2.86 kcal for 120 min (Chapman et al., 1999, MacIntosh et al., 2001a), in which no reduction in energy intake was observed. This may indicate that the rate of glucose infusion or, similarly, the length of small intestinal exposure to glucose, rather than the duration of infusion *per se*, are more important in the regulation of energy intake, since digestion and absorption are likely to be completed over a shorter length of intestine during slower infusions. The effect of exposing greater lengths of the small intestine (e.g. to the mid-jejunum) warrants further investigation. There is evidence that interplays exist between gastrointestinal hormones, motility, insulin release and energy intake. The present study suggests basal pyloric tone, and CCK and insulin were independent predictors of food and energy intake, respectively. Both exogenous and endogenous

CCK slow gastric emptying/motility and decrease energy intake, but have no effect on insulin secretion (Brennan et al., 2005, Kissileff et al., 1981, Fried et al., 1991, Schwizer et al., 1997b, Beglinger et al., 2001). Exogenous and endogenous GLP-1 modulate gastric motility, stimulate insulin secretion and decrease energy intake (Little et al., 2006b, Schirra et al., 2000, Schirra et al., 2006). The substantial increase in GLP-1 concentrations during the 4 kcal/min may have contributed to the observed decrease in energy intake, by stimulating insulin release (Verdich et al., 2001b). It has recently been suggested that the stimulation of pyloric motility may, per se, diminish energy intake (Brennan et al., 2005, Xu et al., 2005). In a recent study, intravenous CCK stimulated pyloric pressures and decreased energy intake, whereas GLP-1, at least in the dose used, failed to stimulate pyloric pressures and did not decrease energy intake (Brennan et al., 2005).

7.6 CONCLUSIONS

In summary, this study has established that variations in the delivery of glucose into the small intestine have differential effects on blood glucose, insulin, incretin and CCK responses, gastrointestinal motility and energy intake in healthy subjects. These observations have implications for an understanding of the regulation of postprandial glycaemia and energy intake in type-2 diabetes.

Chapter 8

IN FUNCTIONAL DYSPEPSIA ORAL CARBOHYDRATE AND FAT DIFFERENTIALLY MODULATE SYMPTOMS, GUT HORMONES AND ANTRAL AREA

8.1 SUMMARY

In patients with FD symptoms are frequently triggered, or exacerbated, by fatty foods. It was hypothesised that in FD a high-fat (“high-FAT”) meal would induce more symptoms than a high-carbohydrate (“high-CHO”) preload, associated with altered secretion of CCK, PYY and ghrelin and increased antral size, when compared with healthy subjects. FD symptoms, appetite perceptions, plasma hormones and antral area were measured in 8 FD patients and 8 healthy subjects on three separate days, after ingestion of high-CHO or high-FAT (500 kcal/400 g) preloads, or a low-nutrient control (180 kcal/400 g); energy intake was quantified 60 min later. Nausea ($P < 0.01$) and pain ($P = 0.05$) were greater in FD after the high-FAT, when compared with high-CHO and control, meals and with healthy subjects. Discomfort was greater after all preloads in FD when compared with healthy subjects ($P < 0.05$). Fasting CCK and stimulation of CCK by the high-FAT preload were greater in FD ($P < 0.01$), while fasting and postprandial PYY were lower in FD than in healthy subjects ($P < 0.001$), with no

differences in fasting, or postprandial, plasma ghrelin between FD and healthy subjects. Fasting antral area was greater in FD ($P < 0.05$), with no differences postprandially between FD and healthy subjects. There were no differences in energy intake between the two groups. In conclusion, in FD (i) a high-FAT preload induces more symptoms than an isocaloric high-CHO preload and (ii) fasting and postprandial plasma CCK are greater and PYY concentrations less. These findings have important implications for the development of diet-based therapies for the treatment of FD.

8.2 INTRODUCTION

FD is a common chronic condition, for which the aetiology and pathophysiology remain poorly defined (Talley et al., 1999a). Many patients report that ingestion of food, particularly high-fat meals, induces, or exacerbates, their symptoms (Kaess et al., 1988, Kearney et al., 1989, Mullan et al., 1994). While such observations suggest that FD patients may be hypersensitive to foods rich in fat, the role of dietary fat in symptom induction has hitherto received relatively little attention. The addition of 30 g margarine to a soup has been reported to result in greater symptoms (including epigastric pain, bloating, fullness and nausea) in patients, when compared with a soup without fat (Houghton et al., 1993). Furthermore, ingestion of a palatable yoghurt containing 24 g fat increased bloating, fullness and nausea in patients with FD, when compared with a control yoghurt containing 1 g fat, by 30 - 40 % (Feinle-Bisset et al., 2003). In these studies symptoms were unrelated to either the rate of gastric emptying (Houghton et al., 1993) or intragastric volume (Feinle-Bisset et al., 2003), suggesting that the symptomatic responses may be mediated from the small intestine. Intraduodenal infusion of lipid has been reported to induce symptoms in FD patients,

but not in healthy subjects (Barbera et al., 1995b, Feinle et al., 2001a). Moreover, FD patients may be particularly sensitive to the presence of fat in the small intestine in that while intraduodenal lipid induced symptoms (Barbera et al., 1995b, Barbera et al., 1995a, Feinle et al., 2001a), an isocaloric glucose infusion did not (Barbera et al., 1995b). Whether the apparent “specificity” in the symptomatic response to nutrients, i.e. to lipid, but not glucose, also applies to orally ingested meals, is not known. This information is of fundamental relevance to the design of diet-based therapies for the treatment of FD.

The gastrointestinal hormones, CCK, PYY and ghrelin, play an important role in the regulation of appetite and gastrointestinal motor function, and their release is modulated by food ingestion (Adrian et al., 1985, Erdmann et al., 2003, Liddle et al., 1985). With the exception of CCK, there is little information about their possible role in symptom generation in FD. The stimulation of plasma CCK by intraduodenal lipid has been reported to be comparable in healthy subjects and FD patients (Feinle et al., 2001a), while intravenous CCK induced greater bloating, nausea and fullness in FD patients when compared with healthy subjects (Chua et al., 1994), suggesting that CCK hypersecretion may not be an underlying mechanism, but that FD patients may be more sensitive to CCK. The specific CCK1 receptor antagonist, loxiglumide, attenuated the symptomatic responses to duodenal lipid in FD (Feinle et al., 2001a), thus, the effects of dietary fat on symptoms may be mediated by CCK. While both fat and carbohydrate stimulate CCK (Liddle et al., 1985) and suppress ghrelin (Erdmann et al., 2003) in healthy subjects, fat is a more effective CCK-secretagogue and carbohydrate suppresses ghrelin more effectively, while fatty acids are the most potent stimulants of PYY secretion (Onaga et al., 2002). It is, therefore, conceivable that gut hormone responses

may be exaggerated in FD, particularly to high-fat meals, leading to greater symptoms. Fasting plasma ghrelin concentrations have been reported to be greater (Lanzini et al., 2006), or not different (Shinomiya et al., 2005), in FD. There is no information about plasma PYY responses, or the relationships between symptoms with hormone responses, in FD.

Changes in gut motor function have been described in FD, including accelerated (initial) and delayed (overall) gastric emptying (Delgado-Aros et al., 2004, Stanghellini et al., 1996), impaired proximal gastric relaxation (Tack et al., 1998), increased perception of gastric distension (Mearin et al., 1991), and antral and duodenal dysmotility (Malagelada and Stanghellini, 1985, Stanghellini et al., 1992). A wide fasting antrum and increased postprandial antral filling have been described in subgroups of FD patients in some (Hausken and Berstad, 1992b, Troncon et al., 1994), but not all (Ahluwalia et al., 1996), studies. In healthy subjects, the only function that has been related to “meal-related fullness” and a decrease in energy intake is that of the antrum (Jones et al., 1997, Sturm et al., 2004). In contrast, the relationship between dyspeptic symptoms and antral area is unclear (Hausken and Berstad, 1992b, Hausken and Berstad, 1994).

Therefore, it was hypothesised that in FD patients, a high-fat preload would induce more symptoms than a high-carbohydrate preload, and this will correspond to changes in plasma CCK, PYY and ghrelin and antral area.

8.3 SUBJECTS AND METHODS

8.3.1 Subjects

8 female FD patients (age range: 23 - 56 years; BMI range: 20.0 - 26.7 kg/m²) were recruited according to guidelines described in Chapter 4.2. Details of the symptoms and their severity reported by FD patients at screening are described in **Table 8.1**. 8 female healthy subjects (age range: 20 - 50 years; BMI range: 19.9 - 24.2 kg/m²) were recruited according to guidelines described in Chapter 4.2, in addition these subjects were age, BMI and gender “matched” to those of a patient.

8.3.2 Study outline

Each subject attended the laboratory at 0900 h, after an overnight fast (14 hours for solids, 12 hours for liquids) on three occasions, separated by 3 - 10 days, for assessments of meal-related gastrointestinal symptoms, appetite, hormone secretion, antral area and energy intake in response to (i) a high-carbohydrate, (ii) a high-fat or (iii) a control preloads. The subjects were informed that the study aim was to evaluate the effects of the meals on “stomach function and hormone secretion”. They were unaware that the main endpoint was the symptomatic response.

8.3.3 Preloads

Three yoghurts, one high in carbohydrate (‘high-CHO’), one high in fat (‘high-FAT’) and a low-nutrient volume ‘control’, were used as preloads. The composition of these preloads are described in Chapter 4.4.1 and Table 4.1.

8.3.4 Protocol

Each subject was seated in a 75° recumbent position, and an intravenous line established in a forearm vein for blood sampling. At baseline ($t = -10$ min), a VAS, assessing symptoms and appetite, was completed, a blood sample taken, and antral area measured. Then subjects consumed 400 g of one of the preloads, in double-blind, randomised fashion, over 10 min. Subsequently, VAS were completed, blood samples taken and antral areas measured at 10 min intervals for 60 min ($t = 0 - 60$ min). Subjects were then presented with a standardised, cold, buffet style meal and allowed 30 min ($t = 60 - 90$ min) to eat freely until they were comfortably full (Feltrin et al., 2004). A further VAS was completed, blood sample taken and antral area measured at $t = 90$ min, after which the intravenous line was removed and the subject allowed to leave.

8.3.5 Measurements

8.3.5.1 *Gastrointestinal symptoms and appetite*

Symptoms of nausea, bloating, abdominal discomfort and pain, as well as fullness and hunger, were rated on 100 mm VAS, as described in Chapter 4.5.5.2 (Parker et al., 2004).

8.3.5.2 *Energy intake*

Assessment of energy intake is described in Chapter 4.5.5.3. Habitual diets were also assessed prior to study commencement. For this purpose, each subject was asked to record everything they ate and drank, weighing foods where possible, and otherwise

recording portion sizes, over a complete 7-day period in diet diaries designed specifically for this purpose, as described in Chapter 4.5.5.1 and Appendix 4.1.

8.3.5.3 *Blood glucose and plasma cholecystinin, peptide YY and ghrelin*

Blood sample collection and analysis of blood glucose and plasma CCK, PYY and ghrelin were performed as described in Chapters 4.5.7 and 4.5.8.

8.3.5.4 *Antral area*

Measurement of antral area is described in Chapter 4.5.4.

8.3.6 *Data and statistical analyses*

Mean values for VAS scores, blood glucose, plasma hormone concentrations and antral area, obtained at $t = -10$ and 0 min, were taken as baseline values (“0”). Subsequently, these parameters were evaluated over two time periods; between $t = 0 - 60$ min (in response to the test-meal), and between $t = 60 - 90$ min (in response to the buffet meal).

VAS scores, plasma hormone concentrations and antral area in response to the preloads were analysed by repeated-measures ANOVA, with time, treatment and subject group as factors. Energy intake and the amount eaten were analysed by one-way ANOVA. Post-hoc paired comparisons, corrected for multiple comparisons by Bonferroni’s correction, were performed if ANOVAs revealed significant effects.

Correlations, corrected for repeated measures, were determined between AUCs (calculated using the trapezoidal rule) for dyspeptic symptoms, appetite-related sensations, blood glucose, plasma hormones, antral area and energy intake and amount eaten, using values at $t = 60$ min, as described (Bland and Altman, 1995). Only r values > 0.5 were considered physiologically relevant. Statistical significance was accepted at $P < 0.05$, and data are presented as means \pm SEM.

8.4 RESULTS

The preloads were all well tolerated. Habitual dietary intakes did not differ between FD patients and healthy subjects (energy intake/week, FD: 47967 ± 6908 kJ, health: 50585 ± 4038 kJ). The eating restraint score in healthy subjects was 4.5 ± 0.9 (range: 2 - 7) and in FD patients 8.9 ± 1.7 (range: 4 - 18), thus, only one FD patient (score: 18) was a restrained eater.

8.4.1 Gastrointestinal symptoms (Figure 8.1) and appetite perceptions (Figure 8.2)

8.4.1.1 Baseline

There were no differences in symptoms or appetite perceptions between study days (data not shown). Scores for bloating ($P < 0.001$) and pain ($P = 0.07$) were higher, and hunger was less ($P < 0.05$), in FD patients than healthy subjects, with no differences in scores for nausea, discomfort or fullness, between the two groups (**Table 8.2**).

8.4.1.2 After preload (t = 0 - 60 min)

In the patients, there were treatment and time effects for both nausea and pain ($P < 0.05$). Scores for nausea ($P < 0.01$) and pain ($P = 0.05$) rose immediately after the high-FAT meal, were greater when compared with control and high-CHO ($P < 0.05$) and remained elevated until $t = 60$ min and $t = 40$ min, respectively. There was no difference between control and high-CHO. There was an effect of time, but not treatment, on scores for bloating and discomfort ($P < 0.001$). Bloating increased from baseline after control between $t = 0 - 40$ min ($P < 0.05$) and after high-FAT between $t = 0-60$ min ($P < 0.01$), but not significantly after high-CHO. Discomfort increased from baseline after control between $t = 0-50$ min ($P < 0.05$), after high-CHO between $t = 0 - 40$ min ($P = 0.05$) and after high-FAT between $t = 0-60$ min ($P < 0.001$). There was a treatment by time interaction for fullness ($P < 0.05$). Scores for fullness rose immediately after ingestion of all meals ($P < 0.001$), plateaued subsequently with high-FAT, and decreased after control and high-CHO. Scores were less after high-CHO at $t = 10$ and 20 min ($p = 0.05$), and greater after high-FAT at $t = 50$ min ($P < 0.01$), when compared with control, and greater at $t = 20$ and 30 min after high-FAT, when compared with high-CHO ($P < 0.05$).

After high-FAT, scores for nausea ($P < 0.05$), pain ($P < 0.05$) and bloating ($P = 0.08$) were greater in FD patients than in healthy subjects. There were no significant differences in these scores between FD patients and healthy subjects following high-CHO or control, although mean scores were higher in the patients. Scores for discomfort were greater in FD patients than in healthy subjects after all preloads ($P < 0.05$), with no differences in scores for fullness or hunger.

8.4.1.3 After buffet meal (t = 60 - 90 min)

In FD patients, scores for nausea increased following control ($P < 0.05$), but not high-CHO or high-FAT. Scores for pain increased following high-FAT ($P < 0.05$), but not high-CHO or control. Scores for bloating, discomfort and fullness increased, and hunger decreased, following all treatments ($P < 0.05$), with no differences between treatments for any symptom or appetite score.

Scores for nausea and pain were greater after high-FAT, and for bloating after both high-CHO and high-FAT, in FD patients than healthy subjects ($P < 0.05$), with no differences following control. Discomfort was greater after all preloads in FD patients than healthy subjects ($P < 0.05$). There were no differences in scores for fullness and hunger after any preloads, between the two groups.

8.4.2 **Blood glucose and plasma hormone concentrations (Figure 8.3)**

8.4.2.1 *Blood glucose*

8.4.2.1.1 Baseline

There was no difference in blood glucose concentrations between study days (**Figure 8.3A**) or between the two subject groups (**Table 8.2**).

8.4.2.1.2 After preload (t = 0 - 60 min)

In the patients there was a treatment by time interaction for blood glucose ($P < 0.001$). Blood glucose rose immediately after ingestion of high-CHO ($P < 0.05$), and 10 min after ingestion of control ($P < 0.001$), with no significant rise after high-FAT. Blood

glucose was higher after high-CHO between $t = 0-60$ min when compared with control ($P < 0.05$), and lower after high-FAT when compared with control ($P < 0.01$) between $t = 10 - 30$ min and high-CHO ($P < 0.05$) between $t = 0 - 60$ min.

There was no difference in the blood glucose response to any preload between FD patients and healthy subjects.

8.4.2.1.3 After buffet meal (t = 60 - 90 min)

There was a significant rise in blood glucose following control ($P < 0.001$) and high-FAT ($P < 0.05$), but not high-CHO, with no differences between treatments in the patients.

There was no difference in the blood glucose response following the buffet meal between FD patients and healthy subjects.

8.4.2.2 *Cholecystokinin*

8.4.2.2.1 Baseline

There was no difference in plasma CCK between study days (**Figure 8.3B**). Plasma CCK was slightly, but significantly, higher in FD patients than in healthy subjects ($P < 0.05$) (**Table 8.2**).

8.4.2.2.2 After preload (t = 0 - 60 min)

In the patients there was a treatment by time interaction for plasma CCK ($P < 0.01$). Plasma CCK rose immediately after all preloads ($P < 0.001$) and was greater after high-CHO ($P < 0.01$) and high-FAT ($P < 0.01$) between t = 0 - 10 min and 30 - 60 min, respectively, when compared with control, with no difference between high-CHO and high-FAT.

After high-FAT, plasma CCK was greater in FD patients between t = 0 - 60 min, when compared with healthy subjects ($P < 0.05$). In contrast, there were no significant differences in plasma CCK between FD patients and healthy subjects following high-CHO or control, although mean CCK concentrations were higher ($P = 0.1$) following high-CHO in FD patients.

8.4.2.2.3 After buffet meal (t = 60 - 90 min)

There was a significant rise in plasma CCK following control ($P < 0.01$), and a trend for a rise following high-CHO ($P = 0.07$) and high-FAT ($P = 0.06$), with no differences between treatments.

Plasma CCK was greater in patients than healthy subjects following high-CHO ($P < 0.01$), but not following high-FAT and control, although mean values were greater in the patients following both preloads.

8.4.2.3 *Peptide YY*

8.4.2.3.1 Baseline

There was no difference in plasma PYY concentrations between study days (**Figure 8.3C**). Plasma PYY was lower in FD patients than in healthy subjects ($P < 0.001$) (**Table 8.2**).

8.4.2.3.2 After preload (t = 0 - 60 min)

In the patients there was a treatment by time interaction for plasma PYY ($P < 0.05$). Plasma PYY rose immediately after ingestion of high-CHO and high-FAT ($P < 0.01$), but not after control, reached a maximum at $t = 10$ min, and then plateaued. Plasma PYY was greater after high-CHO and high-FAT, between $t = 10 - 40$ min and $10 - 60$ min, respectively, when compared with control ($P < 0.05$), and greater after high-FAT at $t = 20$ min and $t = 50$ min, when compared with high-CHO ($P < 0.05$).

Plasma PYY was less following control ($P < 0.05$) and high-CHO ($P < 0.01$) between $t = 0 - 60$ min in patients when compared with healthy subjects, with no difference between the two groups following high-FAT, although mean PYY concentrations were lower in the patients.

8.4.2.3.3 After buffet meal (t = 60 - 90 min)

There was a rise in plasma PYY following control ($P = 0.05$) and high-CHO ($P < 0.05$), and a trend for a rise following high-FAT ($P = 0.07$), with no differences between treatments in the patients.

Plasma PYY was lower following control ($P = 0.05$) and high-CHO ($P < 0.05$), and tended to be lower following high-FAT ($P = 0.07$), in FD patients compared with healthy subjects.

8.4.2.4 *Ghrelin*

8.4.2.4.1 Baseline

There was no difference in plasma ghrelin concentrations between study days (**Figure 8.3D**) or between the two subject groups (**Table 9.2**).

8.4.2.4.2 After preload (t = 0 - 60 min)

In the patients there was a time effect for plasma ghrelin ($P < 0.05$). Both high-CHO and high-FAT, but not control, suppressed plasma ghrelin, compared with baseline, high-CHO between t = 0 - 60 min ($P < 0.01$) and high-FAT at t = 20 min and between t = 40 - 60 min ($P = 0.05$).

There was no difference in plasma ghrelin between preloads or between FD patients and healthy subjects.

8.4.2.4.3 After buffet meal (t = 60 - 90 min)

Plasma ghrelin was less following high-CHO and high-FAT when compared with control ($P < 0.05$), with no difference between the two nutrients in the patients.

There was no difference in plasma ghrelin following any preload between the two subject groups.

8.4.3 Antral area (Figure 8.4)

8.4.3.1 Baseline

There was no difference in antral area between study days (data not shown), but antral area was slightly, but significantly, greater in FD than in healthy subjects ($P < 0.05$) (**Table 8.2**).

8.4.3.2 After preload (t = 0 - 60 min)

In the patients there was a treatment by time interaction for antral area ($P < 0.001$). Antral area rose immediately after ingestion of all preloads ($P < 0.001$), and was greater after control and high-FAT, between $t = 0 - 10$ min, when compared with high-CHO ($P < 0.01$). Antral area decreased ($P = 0.05$) after both control and high-FAT, from $t = 10$ min and $t = 40$ min, respectively, but not for high-CHO, and remained higher for high-CHO between $t = 40 - 60$ min ($P = 0.05$), and for high-FAT between $t = 30 - 60$ min ($P < 0.05$), when compared with control, with no difference between high-CHO and high-FAT between $t = 20 - 60$ min.

There was no difference in antral area after any preload between the two subject groups, except after high-CHO between $t = 0-10$ min when antral area was lower in the patients ($P < 0.05$).

8.4.3.3 After buffet meal (t = 60 - 90 min)

There were no differences in antral area between preloads or between the two subject groups.

8.4.4 Energy intake

There were no differences in energy intake or the amount eaten at the buffet meal between preloads, or between FD patients and healthy subjects (**Table 8.3**).

8.4.5 Relations between symptoms, appetite-related sensations, blood glucose, plasma hormones, antral area and energy intake

There were direct relationships between scores for nausea ($r = 0.67$, $P = 0.08$) and pain ($r = 0.72$, $P < 0.01$) with the AUC for CCK and inverse relationships between AUCs of scores for discomfort ($r = -0.86$, $P = 0.08$), bloating ($r = -0.63$, $P < 0.05$) and fullness ($r = -0.77$, $P = 0.09$) with the amount eaten.

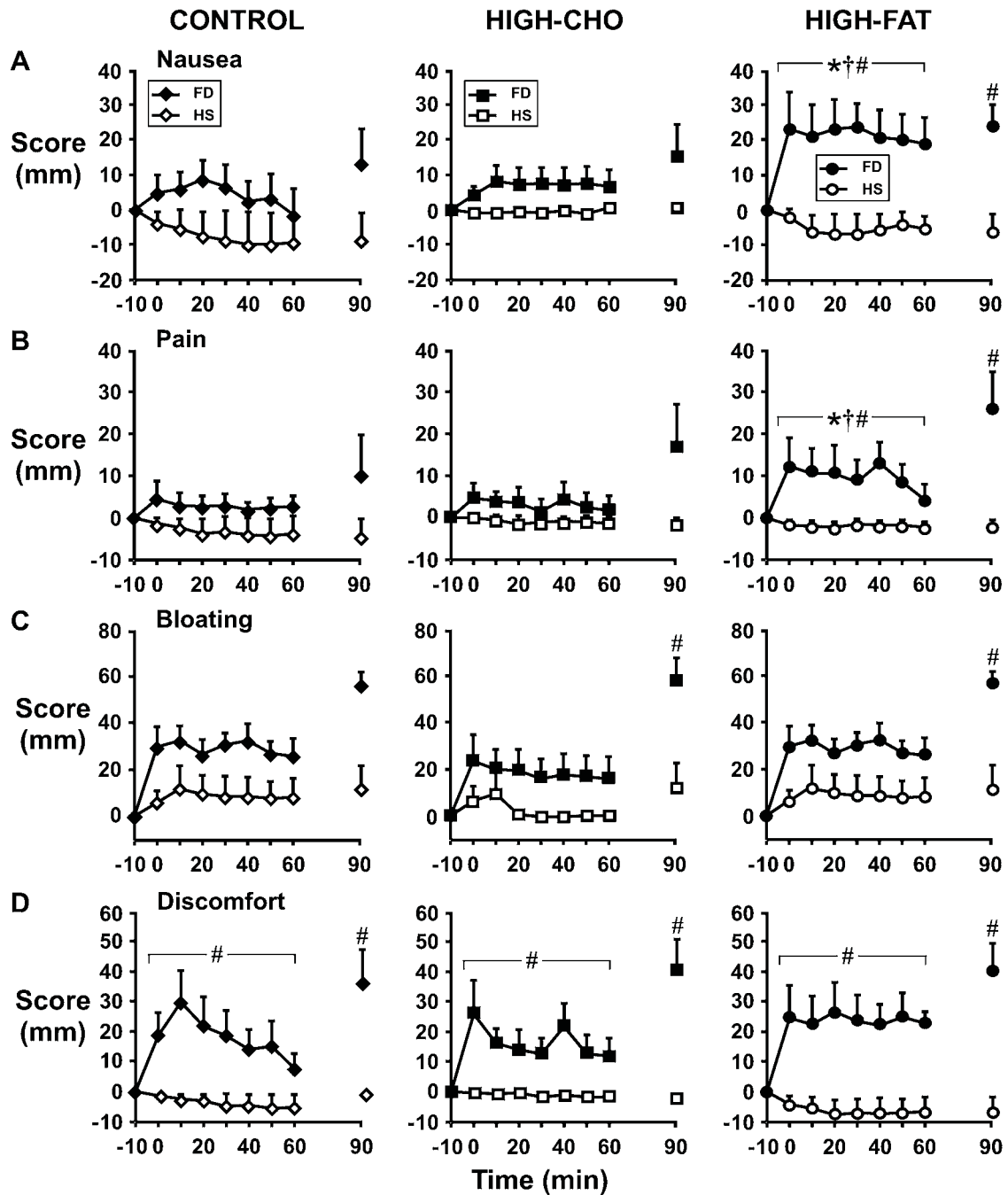


Figure 8.1 Effects of orally ingested control (◆), high-carbohydrate (high-CHO) (■) and high-fat (high-FAT) (●) yoghurt preloads on (A) nausea, (B) pain, (C) bloating, and (D) discomfort in patients with functional dyspepsia (FD) (closed symbols) and healthy subjects (HS) (opened symbols). * vs. control, † vs. high-CHO, # vs. healthy subjects; $P < 0.05$. Data are means \pm SEM ($n = 8$ FD and HS).

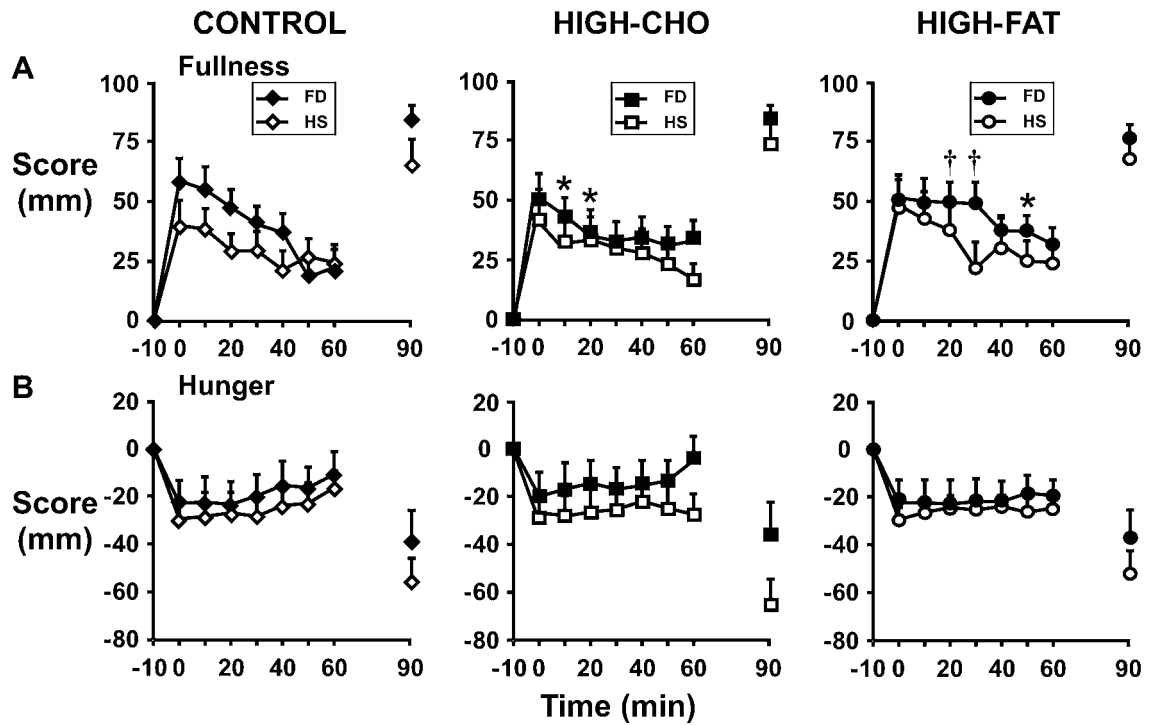


Figure 8.2 Effects of orally ingested control (◆), high-carbohydrate (high-CHO) (■) and high-fat (high-FAT) (●) yoghurt preloads on (A) fullness, and (B) hunger in patients with functional dyspepsia (FD) and healthy subjects (HS). * vs. control, † vs. high-CHO; $P < 0.05$. Data are means \pm SEM ($n = 8$ FD and HS).

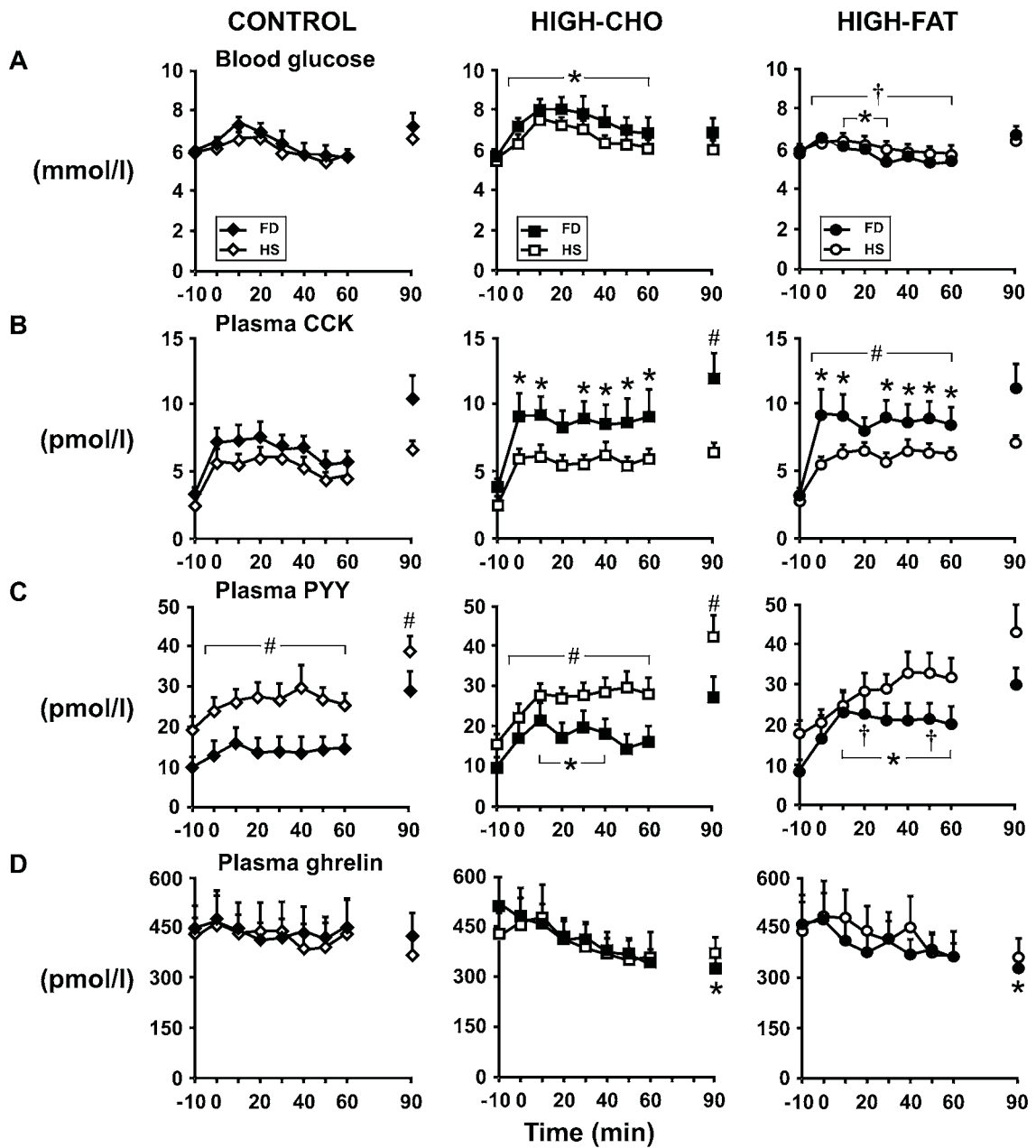


Figure 8.3 Effects of orally ingested control (◆), high-carbohydrate (high-CHO) (■) and high-fat (high-FAT) (●) yoghurt preloads on (A) blood glucose, and plasma (B) cholecystikinin (CCK), (C) peptide YY (PYY), and (D) ghrelin concentrations in patients with functional dyspepsia (FD) and healthy subjects (HS). * vs. control, † vs. high-CHO, # vs. healthy subjects; $P < 0.05$. Data are means \pm SEM ($n = 8$ FD and HS).

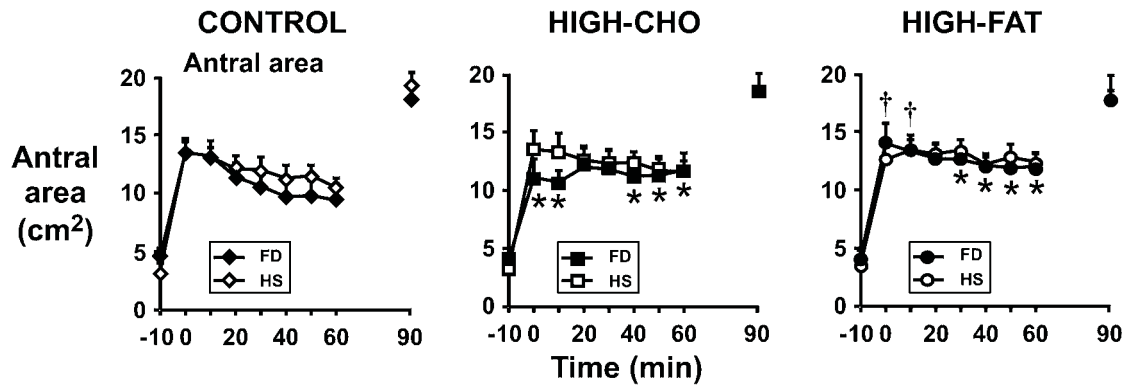


Figure 8.4 Effects of orally ingested control (◆), high-carbohydrate (high-CHO) (■) and high-fat (high-FAT) (●) yoghurt preloads on antral area in patients with functional dyspepsia (FD) and healthy subjects (HS). * vs. control, † vs. high-CHO; $P < 0.05$. Data are means \pm SEM ($n = 8$ FD and HS).

Table 8.1 Symptoms reported by the functional dyspepsia patients at screening interview.

Patient no	Symptom	Severity (out of 3)
1	Upper abdominal/epigastric pain	1
	Bloating	2
	Early fullness after meals	2
	Nausea/vomiting	1
2	Upper abdominal/epigastric pain	3
	Bloating	3
	Early fullness after meals	2
	Nausea/vomiting	2
3	Upper abdominal/epigastric pain	2
	Bloating	2
	Early fullness after meals	2
	Nausea/vomiting	2
4	Upper abdominal/epigastric pain	1
	Bloating	2
	Early fullness after meals	2
	Nausea/vomiting	2
5	Upper abdominal/epigastric pain	3
	Bloating	3
	Nausea/vomiting	1
6	Upper abdominal/epigastric pain	2
	Bloating	3
	Early fullness after meals	3
	Nausea/vomiting	1
7	Upper abdominal/epigastric pain	2
	Bloating	3
	Early fullness after meals	2
	Nausea/vomiting	1
8	Upper abdominal/epigastric pain	1
	Bloating	3
	Nausea/vomiting	2

Table 8.2 Baseline values (i.e. prior to ingestion of preloads) for gastrointestinal symptoms, appetite-related sensations, blood glucose, plasma cholecystokinin, peptide YY and ghrelin and antral area, in patients with functional dyspepsia and healthy subjects.

	FD patients	Healthy subjects	P value
<i>Gastrointestinal symptoms</i>			
Nausea (mm)	17 ± 5	9 ± 4	NS
Pain (mm)	8 ± 2	4 ± 2	0.07
Bloating (mm)	19 ± 4	2 ± 1	< 0.001
Discomfort (mm)	12 ± 2	9 ± 3	NS
<i>Appetite-related sensations</i>			
Fullness (mm)	12 ± 3	17 ± 5	NS
Hunger (mm)	40 ± 7	61 ± 6	< 0.05
<i>Hormone concentrations</i>			
Blood glucose (mmol/l)	5.8 ± 0.1	5.8 ± 0.2	NS
Plasma CCK (pmol/l)	3.4 ± 0.3	2.7 ± 0.2	< 0.05
Plasma PYY (pmol/l)	9.4 ± 1.4	17.7 ± 1.7	< 0.001
Plasma ghrelin (pmol/l)	475 ± 131	428 ± 144	NS
<i>Antral area (cm²)</i>	4.2 ± 0.3	3.4 ± 0.2	< 0.05

Data are means of three study days ± SEM (n = 8). CCK, cholecystokinin; FD, functional dyspepsia; NS, not significant; PYY, peptide YY.

Table 8.3 Energy intake and amount consumed at the buffet meal (i.e. between t = 60 - 90 min) following consumption of control, high-carbohydrate and high-fat preloads, in functional dyspepsia patients and healthy subjects.

	Control	High-CHO	High-FAT	P value
<i>Functional dyspepsia</i>				
Energy (kJ)	3387 ± 707	3382 ± 564	3653 ± 758	NS
Amount consumed (g)	876 ± 160	863 ± 144	833 ± 152	NS
<i>Healthy subjects</i>				
Energy (kJ)	2786 ± 347	2542 ± 340	2587 ± 235	NS
Amount consumed (g)	861 ± 94	719 ± 87	777 ± 84	NS

Data are means ± SEM (n = 8). CHO, carbohydrate; NS, not significant.

8.5 DISCUSSION

This is the first study to evaluate in FD the comparative effects of orally ingested meals varying in their macronutrient composition (i.e. either high in fat or carbohydrate) on symptoms, plasma CCK, PYY and ghrelin concentrations, antral area and energy intake. The major findings are that in FD patients, (i) a high-FAT meal induced substantially greater symptoms, particularly nausea and pain, than an equicaloric high-CHO meal, (ii) fasting plasma CCK concentrations were greater, as was the stimulation of plasma CCK by the high-FAT meal, than in healthy subjects, (iii) fasting and postprandial PYY concentrations were less than in healthy subjects, (iv) fasting ghrelin and the magnitude of postprandial ghrelin suppression did not differ from that in healthy subjects, (v) fasting antral area was slightly greater than in healthy subjects, and (vi) scores for nausea and pain were related directly to plasma CCK concentrations.

One of the major findings was that the induction of gastrointestinal symptoms after the oral preloads was “nutrient-specific”. Consumption of a high-FAT preload was associated with a substantially greater increase in nausea and pain when compared with a high-CHO meal in FD, with scores increasing immediately after completion of ingestion. These data extend findings from studies which found that both small intestinal infusion, and oral ingestion, of fat induce symptoms in FD (Barbera et al., 1995b, Feinle-Bisset et al., 2003, Houghton et al., 1993) and that equicaloric small intestinal fat, but not glucose, loads exacerbate dyspeptic symptoms during concomitant gastric distension (Barbera et al., 1995b). This study has now demonstrated, in a standardised fashion, that dietary factors, specifically the macronutrient composition of a meal, affect symptoms in FD, particularly nausea and pain. In contrast, scores for

discomfort rose after all preloads (including control) in the patients, and a further rise was apparent following ingestion of the buffet meal, suggesting that simple distension of the stomach, irrespective of the caloric or macronutrient content of a meal, underlies certain symptoms in FD (Mearin et al., 1991).

A second novel observation relates to the effects of meals on gut hormones involved in appetite regulation i.e. CCK, PYY and ghrelin, in FD. While plasma CCK concentrations increased promptly in response to all preloads in both subject groups, the responses to both the high-FAT and high-CHO meals were greater in FD patients than in controls (although in case of the high-CHO preloads this difference was not statistically significant), with no difference between the two preloads. The results are unexpected for two reasons. Firstly, the only previous study comparing plasma CCK concentrations in FD and health reported no differences in fasting concentrations or the stimulation of CCK by intraduodenal lipid (Feinle et al., 2001a), which led to the suggestion that FD patients may be hypersensitive to the actions of CCK (Feinle et al., 2001a). The present study results of greater, albeit modestly, fasting levels in the FD patients were clear-cut and consistent across all subjects. The cause of this is uncertain, but may be attributable to different patterns of prior nutrient intake (French et al., 1995). It is also unclear why the responses to oral and intraduodenal lipid should be different. FD patients would, as a group, be expected to have slower gastric emptying (Delgado-Aros et al., 2004, Stanghellini et al., 1996), which, in turn, would be expected to be associated with a slower rise in plasma CCK. Secondly, given that in the patients the high-FAT preload resulted in more symptoms, particularly nausea and pain, compared with the high-CHO preload, and the data from previous studies implicating a role for CCK in symptom induction in FD (Chua et al., 1994, Feinle et al., 2001a), it would be

expected that plasma CCK to be greater following the high-FAT, compared with the high-CHO, preload, although it is now well established that both carbohydrate and fat release CCK (Liddle et al., 1985, Parker et al., 2005). Nonetheless, this study found significant correlations between scores for nausea and pain and plasma CCK concentrations.

Both fasting and postprandial PYY concentrations were lower in the patients, which was unexpected, particularly also in the light of elevated CCK levels; it is well established that CCK stimulates PYY (Lin et al., 2000). This suggests a disturbance in CCK-induced PYY stimulation, however, whether, and, if so, how, this could relate to symptom generation is unclear. Interestingly, in the patients plasma PYY concentrations following the high-FAT preload were greater than following the high-CHO preload, a difference that may, at least in part, have contributed to greater symptoms following the high-fat preload.

Ghrelin is an orexigenic hormone and implicated in preprandial hunger and meal initiation, in part because circulating levels increase before, and decrease after, meals (Cummings et al., 2001). Fasting plasma ghrelin has been reported to be greater (Lanzini et al., 2006), or not different (Shinomiya et al., 2005), in FD, and this study found no differences between patients and healthy subjects. The effects of nutrients on postprandial ghrelin concentrations in patients with FD have hitherto not been evaluated. Glucose and amino acids suppress ghrelin more rapidly and potently than lipids (Erdmann et al., 2003). Both the high-FAT and high-CHO meals suppressed ghrelin secretion compared with the control preload, and to a comparable extent in both

subject groups, suggesting that nutrient-induced suppression of ghrelin secretion is intact in FD and, thus, unlikely to contribute to symptoms.

Antral area increased immediately after ingestion of all preloads and remained higher following both the high-CHO and high-FAT preloads, and slightly higher following the high-FAT, than the high-CHO, preloads. Except in the fasting state, there were no other differences between the two subject groups. Antral dysfunction has been reported to occur in about 35 % of patients with FD (Hausken and Berstad, 1992b), therefore, other motor functions, not measured in the current study, including impaired proximal gastric accommodation (Tack et al., 1998), delayed or accelerated gastric emptying (Delgado-Aros et al., 2004, Stanghellini et al., 1996), or accelerated emptying in the “early phase”, particularly of fat (Lin et al., 1999), may have contributed to symptom induction after the high-FAT preload.

Energy intake, as reported in the one-week diet diaries or from the buffet meal, did not differ between the two subject groups or when comparing the nutrient-meals with control, although the lack of effect of the nutrient-containing meals to suppress energy intake in the healthy subjects could be a type 2 error, given that mean energy intake following the high-CHO and high-FAT preloads was ~ 200 kJ less when compared with the control preload. It is surprising that the patients consumed amounts that were comparable with those of the healthy subjects and continued to eat despite experiencing significant symptoms, although this indicates that the patients appear to ignore signals from the gastrointestinal tract that indicate pathophysiological changes.

This study design warrants some comment, particularly given that it is the first to address in FD patients the comparative effects of the macronutrients, fat and carbohydrate, on symptom induction. For this purpose the investigator designed semi-solid yoghurts rich in either carbohydrate or fat. While most meals ingested on a daily basis would have different textures and consistencies and contain protein, and the fat and carbohydrate contents of the preloads used in the study were at the extreme ends of what would be ingested in a normal meal, the investigator believed that the preloads were appropriate to evaluate the hypotheses as to the potential differences in symptoms induced by carbohydrate- and fat-containing meals. The fat component of the high-fat yoghurt was cream and the carbohydrate was predominantly maltodextrin, hence, the possibility that the response may be specific to these nutrients cannot be excluded. The time interval between the preloads and the buffet meal (i.e. 60 min) was selected on the basis of a previous study, in which energy intake from a buffet meal 60 min after a nutrient preload was shown to be inversely related to antral area in healthy young and older subjects (Sturm et al., 2004). This study evaluated antral area only, hence, any conclusions as to the relevance of any disturbances in proximal gastric function cannot be drawn, and the study period of 60 min was insufficient to quantify overall gastric emptying. While the subject numbers were relatively small, they were based on a previous study (Feinle-Bisset et al., 2003), and the data, particularly those relating to symptoms, are very clear-cut. Nevertheless, this study cannot exclude with entire certainty the occurrence of type 2 errors with some parameters, such as the lack of a statistically significant difference in plasma CCK between FD patients and healthy subjects following the high-CHO meal. Also this study cannot draw any firm conclusions as to the applicability of the data to men, given that our cohort included females only.

8.6 CONCLUSIONS

In summary, this study has shown that in FD (i) a high-FAT meal potently induces meal-related symptoms, particularly nausea and pain, and (ii) both fasting and postprandial CCK concentrations are elevated. This data suggest that a reduction in both the amount eaten and the fat content of the diet may help to alleviate meal-related symptoms in FD. This issue should now be evaluated in a large cohort of patients.

Chapter 9

RELATIONSHIP BETWEEN DYSPEPTIC SYMPTOMS AND DIETARY PATTERNS IN FUNCTIONAL DYSPEPSIA

9.1 SUMMARY

Patients with FD often report that their symptoms are related to the ingestion of food, particularly fatty foods. There is limited evidence that eating patterns and nutrient intake may differ between FD patients and healthy individuals. The relationship between symptoms and dietary factors in FD has not been evaluated. 20 FD patients (17 female; age: 45 ± 3 (range: 23 - 73) yr; BMI: 24.0 ± 0.9 (range: 19.3 - 25.7) kg/m^2) and 21 healthy subjects (18 female; age: 40 ± 4 (range: 20 - 74) yr; BMI: 22.9 ± 0.5 (range: 18.6 - 26.5) kg/m^2) completed detailed diet and symptom diaries in which all foods and drinks and the times when these were consumed, as well as the occurrence, timing, and severity of dyspeptic symptoms (including nausea, discomfort, fullness, bloating, upper abdominal/epigastric pain), were recorded over a 7-day period. Data from the diet diaries were analysed for the number of meals, light meals, snacks and drinks, as well as energy intake and macronutrient distribution. The number of “meals” was less (healthy subjects: 7 (2 - 16), FD: 5 (1 - 10); $P < 0.01$), and total energy (healthy subjects: 56570 (34780 - 98997) kJ, FD: 48131 (17844 - 97548) kJ; $P = 0.1$) and fat (healthy subjects: 514 (301 - 703) g, FD: 479 (89 - 798) g; $P = 0.1$) intake tended to be

less, in FD patients compared with HS. In FD patients, symptoms were usually modest in severity (score out of 10; 5 (3 - 8)) and occurred within 31 (8 - 64) min of eating. There were a number of relationships between symptoms and dietary factors; fullness was related directly to both the amount of fat ingested ($z = 1.91$, $P < 0.05$) and overall energy intake ($z = 2.12$, $p < 0.05$), and inversely to the amount of carbohydrate ingested ($z = -1.9$, $P = 0.05$). There was a tendency for bloating to be related directly to the amount of fat ingested ($z = 1.68$, $P = 0.09$). There were no significant relationships between symptom severity and any of the dietary variables. These observations suggest that FD patients may consume a smaller number of meals and that symptoms are associated with energy and fat intake; the consumption of smaller meals with a low fat content may, accordingly, prove beneficial in the management of FD.

9.2 INTRODUCTION

The pathophysiology and aetiology of FD remain poorly defined. Many patients report that their symptoms are related to the ingestion of food (Kearney et al., 1989, Mullan et al., 1994), however, only few studies have investigated this putative association. Two studies reported that dyspeptic symptoms were associated with ingestion of onions, peppers, fried and fatty foods, alcohol, citrus fruits, and spicy foods (Kearney et al., 1989, Mullan et al., 1994), or “rich” cakes and carbonated beverages (Mullan et al., 1994). Another study, using a questionnaire of about 39 different foods, found that subjects with FD identified 22 of these which they believed aggravated their symptoms, and, therefore, avoided them (Kaess et al., 1988). Of these foods, the highest rates of intolerance were for mayonnaise (80 %), nuts (70 %), fish (66 %) and chocolate (62 %) - three out of these four foods have a high fat content (Kaess et al., 1988).

It is uncertain whether patterns of eating and nutrient intake are different in FD. The prevalence of snacking has been reported to be greater (by about 9 %), and the number of larger meals to be lower (by about 24 %), in FD patients compared with healthy subjects, but these differences were not statistically significant (Mullan et al., 1994). Furthermore, in this study only 55 % of FD patients consumed three meals per day compared with 80 % of healthy subjects (Mullan et al., 1994), although interpretation is difficult as definitions of “meals” and “snacks” were not provided (Mullan et al., 1994). In contrast, a study using food frequency diaries reported no differences in eating patterns, including the number of regular meals and total number of eating episodes between patients with FD and healthy subjects (Cuperus et al., 1996), however, food intake was only evaluated in food categories, and not as individual food items. A further study, which employed a 7-day diet history to assess nutrient intake, found no significant differences in nutrient intake, except that the subjects with FD had a significantly lower intake of dietary fibre (Kearney et al., 1989). Accordingly, previous studies have yielded inconsistent observations with regard to eating patterns in FD. Furthermore, none of these studies evaluated the relationship between symptoms with eating patterns by concurrent measurement.

Many FD patients report that they can only eat small meals and do not tolerate fat, consistent with the outcome of laboratory-based studies demonstrating diminished tolerance of volume/pressure, as indicated by increased sensitivity to distension of the proximal (Mertz et al., 1998b, Barbera et al., 1995b, Barbera et al., 1995a) and distal (Caldarella et al., 2003) stomach in ~ 35 % of patients, reduced ingestion capacity during an oral nutrient drink test (Tack et al., 1998) in ~ 40 % of patients (Tack et al., 2001) and increased sensitivity to oral and duodenal fat administration in ~ 60 - 70 % of

patients (Houghton et al., 1993, Barbera et al., 1995a, Feinle et al., 2001a) . While the potential contribution of specific dietary macronutrients to symptom generation in FD has not been evaluated, a recent study has demonstrated that meals high in fat increase symptoms of nausea and pain to a greater extent than high carbohydrate preloads in FD (see Chapter 8).

Patients with FD as a group report to have more life stress and psychological distress, as well as a reduced quality of life compared with healthy individuals (Talley et al., 1990, Talley and Piper, 1986, Talley et al., 1986a, Hui et al., 1991, Bennett et al., 1991). While it would not be surprising if eating attitudes in FD were abnormal and impact on eating behaviour and/or symptoms, these factors have not been formally analysed in FD and related to food intake.

The aims of this study were to assess eating habits, and the occurrence and severity of symptoms, as well as relationships between them, in FD. It was hypothesised that patients with FD (i) would consume smaller meals and experience more “meal-associated” symptoms, but eat more frequently, when compared with healthy subjects and (ii) the occurrence and severity of symptoms would be related directly to the amount eaten, as well as the amount of fat in the diet.

9.3 SUBJECTS AND METHODS

9.3.1 Subjects

20 FD patients (17 female, 3 male; age: 45 ± 3 (range: 23 - 73) years; BMI: 24.0 ± 0.9 (range: 19.3 - 35.7) kg/m^2) were recruited, as described in Chapter 4.2. 21 healthy

subjects (18 female, 3 male; age: 40 ± 4 (range: 20 - 74) years; BMI: 22.9 ± 0.5 (range: 18.6 - 26.5) kg/m^2) were recruited, as described in Chapter 4.2. Subjects were also age, BMI and gender “matched” to those of a patient.

9.3.2 Protocol

Each patient and healthy subject completed a diet diary, in which they recorded all foods and drinks consumed, and the time of each eating or drinking episode, over an entire 7-day period (i.e. five week days and two weekend days) (Karvetti and Knuts, 1992), while maintaining their usual eating habits, as described in Chapter 4.5.5.1. All symptoms experienced (including abdominal pain, cramps, bloating, nausea, uncomfortable fullness after meals), were also recorded in a symptom diary, described in Chapter 4.5.5.1. The subjects were informed that the study aim was to determine whether there were differences in food intake in patients who suffer digestive symptoms when compared with healthy subjects. They were unaware that the main endpoint was the association between symptom occurrence and food intake.

9.3.3 Measurements

9.3.3.1 Assessment of eating attitudes, upper and lower gastrointestinal symptoms, quality of life, personality and psychological distress

To obtain demographic information in each patient and healthy subject, regarding eating attitudes, upper and lower gastrointestinal symptoms, quality of life, personality and psychological distress, a number of questionnaires were completed. These included; (i) Three Factor Eating questionnaire (Stunkard and Messick, 1985) (Chapter 4.5.5.4), (ii)

Eating Attitudes Test (Garner and Garfinkel, 1979) (Chapter 4.5.5.5), (iii) Northwest Lipid Research Clinical Fat Intake Scale (Retzlaff et al., 1997) (Chapter 4.5.5.6), (iv) Nepean Dyspeptic Index (Talley et al., 1999b) (Chapter 4.5.6.1), (v) Eysenck Personality Questionnaire (Eysenck and Eysenck, 1964) (Chapter 4.5.6.2), (vi) Hospital anxiety and depression (“HAD”) scale (Zigmond and Snaith, 1983) (Chapter 4.5.6.3), and (vii) Zung self-rating depression scale (Zung, 1965) (Chapter 4.5.6.4).

9.3.3.2 *Diet diaries*

The total number of consecutive eating/drinking episodes for the week was determined. These episodes were classified in five categories; (i) meals, (ii) light meals, (iii) snacks, (iv) caloric drinks and (v) non-caloric drinks. Breakfast, lunch and dinner were generally classified as either “meals” or “light meals”. “Meals” were defined as the main eating occasion(s) of the day and comprised foods traditionally eaten as a main meal (e.g. continental breakfast, pasta, meat and vegetables, 4 slices of pizza etc). “Light meals” were defined as episodes where the amount ingested would be less than expected at a main meal (by ~ 30 %) (e.g. cereals, sandwiches, 2 slices of pizza, salads containing meat). “Snacks” were defined as eating episodes in which consumption was less and easier to prepare than “meals” or “light meals”, usually consists of one food item (e.g. biscuits, chocolate, crisps, fruit, yoghurt, slice of pizza) and consumed at times between meals. A “caloric drink” was defined as any beverage that contained nutrients (e.g. coca cola, coffee with milk and/or sugar, juice, milkshakes, alcohol). A “non-caloric” drink was defined as any beverage that contained no calories (e.g. water, diet drinks, black coffee/tea).

Foods consumed within a time interval of less than 15 min were considered to be part of one eating episode. The intervals between eating episodes during the day and night were also calculated.

9.3.3.3 *Symptom diaries*

Symptoms were divided into three categories: “meal-associated” symptoms (bloating, nausea, upper abdominal pain, belching, epigastric pain, fullness, vomiting, discomfort), which occurred within a 2-hour period after completion of eating (Caldarella et al., 2003), “other” gastrointestinal symptoms - any other symptom(s) (heartburn, diarrhoea, constipation, lower abdominal pain) that occurred within a 2-hour period after eating, or “meal-unrelated” - any symptom which occurred more than 2 hours after, or before, an eating episode (e.g. when the subject awoke). Symptom severity was classified as; “mild” (score between 1 - 3 out of 10) not influencing usual activities, “modest” (score between 4 - 7) diverting from, but not requiring modification of, daily activities, or “strong” (score between 8 - 10) impairing daily activities. The times at which these symptoms occurred were evaluated.

9.3.4 *Data and statistical analysis*

Questionnaire data, numbers of total eating and drinking episodes, meals, light meals, snacks, and drinks, energy intake, weight, macronutrient distribution, and inter-meal intervals were compared between patients with FD and healthy subjects using the Mann Whitney U-test (Wilcoxon rank sum test). To evaluate the relationship between symptoms and food intake, due to repeated measures over time and day, food intake and occurrence/severity were modelled with a generalised estimating question, with

multinomial distribution and cumulative log link. Relationships between NDI subscales and food intake were analysed, using Spearman's rho correlations. Statistical significance was accepted at $P < 0.05$, and data are presented as median (ranges).

9.4 RESULTS

9.4.1 Eating attitudes, upper and lower gastrointestinal symptoms, quality of life, personality and psychological distress (Table 9.1)

There was a significant difference in eating attitudes between FD patients and healthy subjects ($P < 0.01$), indicating that the FD patients had greater concerns about eating. There were no differences in scores for any factor of the Three Factor Eating questionnaire or the fat intake scale between the two groups. Upper abdominal symptoms, as assessed by the Nepean Dyspeptic Index, were greater in FD patients when compared with healthy subjects ($P < 0.0001$). There was a significant difference in the quality of life between FD patients and healthy subjects. Scores for (i) interference, or difficulty with activities of daily living, or work because of dyspepsia, combined with impaired enjoyment of life and emotional well being ($P < 0.0001$), (ii) lack of knowledge of, and control over, the illness ($P < 0.0001$), (iii) disturbances in eating and drinking ($P < 0.0001$) and (iv) sleep disturbances ($P < 0.001$), were greater in FD patients when compared with healthy subjects, indicating that the patients with FD had a poorer quality of life. There was no difference in personality, i.e. neuroticism and extroversion, between the two groups. Both FD patients and healthy subjects presented with the same degree of anxiety, as measured by the HAD scale, however, depression tended to be higher in FD patients ($P = 0.08$). Psychological distress, as measured by the Zung depression scale, was greater in FD patients when compared with healthy

subjects ($P < 0.01$). However, only 2 FD patients (and no healthy subjects) were “depressed” (i.e. score > 50).

9.4.2 Eating behaviour

The number of meals consumed ($P < 0.01$) was less in FD patients when compared with healthy subjects. There were no other significant differences in total eating or drinking episodes, light meals, snacks and drinks consumed (**Table 9.2**). There was no difference in the total weight, carbohydrate, protein or alcohol content of foods consumed, or the % distribution of macronutrients, between FD patients and healthy subjects, although fat and energy intake tended to be less in the FD patients ($P = 0.1$) (**Table 9.3**). There was also no difference in the time interval between meals (FD: median = 162 min (range: 100 - 320 min) vs. healthy subjects: median = 172 min (120 - 347 min)) or the duration of the overnight fast (FD: median = 733 min (450 - 905 min) vs. healthy subjects: median = 669 min (527 - 910 min)) between FD patients and healthy subjects.

9.4.3 Symptom assessment

No healthy subject experienced any symptoms during the 7-day assessment. FD patients reported a total of 612 symptoms, or 26 (1 - 92) per patient; 64 % (0 - 100 %) of these were “meal-associated” with a severity of 5 (3 - 8) and occurring 31 min (8 - 64 min) after eating, 9 % (0 - 43 %) were “other” gastrointestinal symptoms with a severity of 5 (3 - 8) and occurring 25 min (5 - 120 min) after eating, while 14 % (0 - 36 %) were “meal-unrelated” with a severity of 5 (2 - 7) and occurring 135 min (-180 - 0 min and 120 - 200 min) before, or after, eating. The percentage of total, meal-associated, other

gastrointestinal, and meal-unrelated, symptoms experienced and their severity are summarised in **Table 9.4**. The occurrence, severity and timing of individual “meal-associated” symptoms are summarised in **Table 9.5**.

9.4.4 Relationship between food intake with symptom occurrence and severity

Only the relationship between “meal-associated” symptoms with dietary factors was analysed as the number of “other” and “meal unrelated” symptoms was low.

The occurrence of overall meal-associated symptoms was positively related to energy intake ($z = 2.02$, $P < 0.05$), and inversely related to the ingestion of carbohydrate (%: $z = -2.08$, $P < 0.05$). There was no relation between symptoms with fat, protein, alcohol (g and %) or weight consumed. When individual symptoms were analysed, fullness was related directly to fat (absolute and %: $z = 1.91$, $P < 0.05$), protein (absolute: $z = 2.64$, $P < 0.001$; %: $z = 1.82$, $P = 0.06$) and energy intake ($z = 2.12$, $P < 0.05$), and inversely related to carbohydrate (%: $z = -1.9$, $P = 0.05$), consumption. Bloating was related to the ingestion of fat ($z = 1.68$, $P = 0.09$). There was no significant relationship between the severity of symptoms and energy intake, or the amount and macronutrient content of foods consumed

9.4.5 Relationship between food intake and quality of life

In FD patients there was a direct relationship between fat ($r = 0.50$, $P < 0.05$) and protein ($r = 0.4$, $P = 0.06$), and an inverse relationship between carbohydrate ($r = -0.60$,

P < 0.01) intake with scores for disturbance in eating and drinking, as measured by the Nepean Dyspeptic Index, in FD patients. There was no other significant relationship between food intake and quality of life scores in FD patients or healthy subjects.

Table 9.1 Scores for eating attitudes, quality of life, and personality/psychological distress, in functional dyspepsia patients and healthy subjects.

	FD patients	Healthy subjects	P value
<i>Eating attitudes</i>			
TFEQ (Factor 1) (cut off = 12; max =21)	7 (range: 1 - 18)	6 (range: 1 - 14)	NS
TFEQ (Factor 2) (max =16)	4 (range: 0 - 13)	5 (range: 1 - 10)	NS
TFEQ (Factor 3) (max =14)	4 (range: 0 - 11)	4 (range: 1 - 10)	NS
Eating attitudes test (cut off = 30)	15 (range: 5 - 47)	9 (range: 1 - 31)	< 0.01
Fat intake scale (max = 44)	26 (range: 15 - 35)	27 (range: 21 - 40)	NS
<i>Upper abdominal symptoms and quality of life</i>			
Symptoms (max = 195)	56 (range: 6 - 132)	4 (range: 0 - 31)	< 0.0001
Interference/difficulty with activities (max = 65)	28 (range: 17 - 46)	13 (range: 13 - 13)	< 0.0001
Lack of knowledge or control (max = 35)	16 (range: 10 - 28)	7 (range: 7 - 10)	< 0.0001
Eating/drinking disturbances (max =15)	8 (range: 4 - 14)	3 (range: 3 - 5)	< 0.0001
Sleep disturbances (max = 10)	4 (range: 0 - 10)	2 (range: 2 - 2)	< 0.001
<i>Personality/psychological distress</i>			
EPQ (neuroticism) (max score = 12)	4 (range: 0 - 12)	4 (range: 0 - 9)	NS
EPQ (extroversion) (max score =12)	8 (range: 0 - 11)	7 (range: 2 - 12)	NS
HAD (anxiety) (cut off = 11)	14 (range: 7 - 23)	12 (range: 8 - 17)	NS
HAD (depression) (cut off = 11)	11 (range: 7 - 20)	8 (range: 7 - 16)	= 0.08
Zung depression scale (cut off = 50)	37 (range: 27 - 55)	32 (range: 21 - 46)	< 0.05

Data are median (ranges) (n = 20 FD patients and 21 healthy subjects). EPQ, Eysenck personality questionnaire; FD, functional dyspepsia; HAD, Hospital Anxiety and Depression scale; NS, not significant; NDI, Nepean Dyspeptic Index; TFEQ, Three Factor Eating questionnaire. Factor 1: cognitive restraint of eating, Factor 2: disinhibition of eating and Factor 3: hunger.

Table 9.2 Number of eating and drinking episodes, meals, light meals, snacks, caloric and non-caloric drinks and total weight and energy consumed during a 7-day period in functional dyspepsia patients and healthy subjects.

	FD patients	Healthy subjects	P value
Total number episodes	46 (range: 25 - 96)	44 (range: 21 - 84)	NS
Eating episodes	32 (range: 18 - 40)	29 (range: 21 - 46)	NS
Drinking episodes	14 (range: 1 - 57)	10 (range: 0 - 39)	NS
Meals	5 (range: 1 - 10)	7 (range: 2 - 16)	< 0.01
Light meals	13 (range: 4 - 19)	10 (range: 4 - 17)	NS
Snacks	15 (range: 3 - 25)	11 (range: 0 - 29)	NS
Caloric drinks	4 (range: 1 - 36)	3 (range: 0 - 30)	NS
Non-caloric drinks	9 (range: 0 - 21)	5 (range: 0 - 29)	NS
% meals	122 (range: 2- 26)	15 (range: 6 - 52)	= 0.06
% light meals	28 (range: 6 - 57)	21 (range: 10 - 57)	NS
% snacks	30 (range: 8 - 44)	27 (range: 0 - 54)	NS
% caloric drinks	10 (range: 2 - 46)	7 (range: 0 - 46)	NS
% non-caloric drinks	17 (range: 0 - 36)	10 (range: 0 - 48)	NS

Data are median (ranges) (n = 20 FD patients and 21 healthy subjects). FD, functional dyspepsia; NS, not significant.

Table 9.3 Macronutrient distribution (g and % kJ) of fat, carbohydrate, protein and alcohol during a 7-day period, in functional dyspepsia patients and healthy subjects.

	FD patients	Healthy subjects	P value
Weight (g)	16404 (range: 7827 - 31454)	18825 (range: 6683 - 22491)	NS
Energy (kJ)	48131 (range: 17844 - 97548)	56570 (range: 34780 - 78997)	= 0.1
Fat (g)	479 (range: 89 - 798)	564 (range: 301 - 703)	= 0.1
Carbohydrate (g)	1337 (range: 629 - 2459)	1578 (range: 856 - 2496)	NS
Protein (g)	511 (range: 237 - 1051)	531 (range: 335 - 846)	NS
Alcohol (g)	14 (range: 0 - 490)	19 (range: 0 - 279)	NS
% fat	29.5 (range: 15.3 - 45.0)	28.3 (range: 16.0 - 38.3)	NS
% carbohydrate	49.6 (range: 25.8 - 60.4)	51.2 (range: 37.0 - 62.7)	NS
% protein	17.8 (range: 10.3 - 25.1)	16.0 (range: 12.5 - 22.7)	NS
% alcohol	0.8 (range: 0 - 259)	1.3 (range: 0 - 19.2)	NS

Data are median (ranges) (n = 20 FD patients and 21 healthy subjects). FD, functional dyspepsia; NS, not significant.

Table 9.4 The % and severity of total, meal-associated, other gastrointestinal, and meal-unrelated symptoms, experienced.

	Mild (0 - 3)	Modest (4 - 7)	Strong (8 - 10)
Total (%)	25 (0 - 100)	54 (0 - 100)	18 (0 - 30)
Meal-related (%)	36 (0 - 100)	52 (0 - 100)	3 (0 - 42)
Other (%)	0 (0 - 100)	73 (0 - 100)	0 (0 - 71)
Meal-unrelated (%)	0 (0 - 100)	75 (0 - 100)	0 (0 - 27)

Data are median (ranges) (n = 20 functional dyspepsia patients).

Table 9.5 The occurrence and severity of individual meal-associated symptoms and the timing of their occurrence after meals.

	Occurrence (%)	Severity (out of 10)	Timing (min)
Bloating	28 (7 - 57)	5 (2 - 9)	30 (1 - 94)
Nausea	11 (4 - 51)	5 (3 - 8)	37 (10 - 95)
Upper abdominal pain	11 (2 - 72)	6 (3 - 8)	25 (6 - 60)
Belching	37 (3 - 41)	4 (3 - 8)	40 (12 - 59)
Epigastric pain	28 (5 - 97)	4 (3 - 6)	31 (2 - 105)
Fullness	20 (3 - 86)	6 (2 - 7)	14 (1 - 68)
Vomiting	3 (3 - 3)	8 (8 - 8)	1 (1 - 1)
Discomfort	22 (14 - 50)	5 (4 - 7)	45 (16 - 60)

Data are median (ranges) (n = 20 functional dyspepsia patients).

9.5 DISCUSSION

This is the first study to formally evaluate eating behaviour in FD patients and healthy subjects and the relationship between symptoms with food intake. The major findings are that in FD patients: (i) symptoms are related to food intake, specifically there were relationships between the occurrence of fullness with fat and energy intake and bloating with fat intake, (ii) the number “meals” consumed is less, but there is no difference in the number of eating episodes (i.e. light meals, snacking, drinking), or the weight or macronutrient content consumed, except for a tendency for a reduction in fat and energy intake, (iii) symptoms occurred in all patients, within 30 min of eating and usually at a modest severity, and (v) the quality of life is a determinant of fat and protein intake.

This study demonstrated a relationship between symptom occurrence and food intake. The occurrence of symptoms was related to energy intake and inversely related to the ingestion of carbohydrate; in particular, fullness and bloating were related to the intake of fat, and fullness to energy intake. These observations are consistent with those of a laboratory-study by Barbera and colleagues, which demonstrated that duodenal infusions of fat, but not glucose, exacerbated symptoms in FD (Barbera et al., 1995b). In the latter study, duodenal infusion of fat induced the perception of discomfort at lower gastric volumes (as measured by a barostat), in FD patients, while in healthy subjects the fat infusion increased gastric volume (Barbera et al., 1995b). Conversely, in both FD patients and healthy subjects, intraduodenal infusion of glucose increased gastric volume comparably (Barbera et al., 1995b). The increased feeling of discomfort during the fat infusion may be transposed to the present study to account for the greater

perception of “fullness” during fat, and why symptoms were inversely related to intake of glucose/carbohydrate.

The second observation was that while there was no overall difference in the total number of eating episodes, FD patients consumed fewer full meals. Taken together, with the outcome of a previous study (Mullan et al., 1994), these findings suggest that patients with FD may eat smaller meals in an attempt to reduce their symptoms. In the present study there was no difference in the weight, carbohydrate, protein or alcohol consumed, but a trend for decreased fat and energy intake in FD patients. The absence of significant differences may represent a type 2 error. Given that the occurrence of symptoms was related to fat and energy intake, this may suggest an important first “point-of-call” for dietary therapy, i.e., reducing fat and energy intake (coupled with diminished meal size) may alleviate symptoms, particularly fullness and bloating. It has been reported previously that there are differences in energy, carbohydrate and vitamin C intakes in both male and female FD patients; which are gender specific, i.e. energy, carbohydrate, fat, protein and vitamin C intakes were reported to be less in female FD patients, compared with healthy subjects, with no differences in male subjects (Mullan et al., 1994). As the cohort that comprises the current study included three males, by chance, it was not possible evaluate the possible effect of gender.

All FD patients reported some symptoms throughout the week, being “meal-associated”, “other” or “meal-unrelated” - this observation is interesting as, despite small differences in fat and energy intake, total food intake was not different between FD and healthy subjects. The majority of these symptoms, as expected, were “meal-

associated”, reflecting the cohort recruited and rigorous inclusion criteria. These symptoms were perceived ~ 30 min after meal ingestion and were of moderate severity. The effect of food intake on these symptoms, within this time frame, may reflect other gastrointestinal factors, such as the secretion of the hormones, CCK and PYY. Both hormones have been demonstrated to mediate, at least in part, the effect of meals to reduce of hunger and increase fullness (Gutzwiller et al., 2000, Degen et al., 2005). The study reported in Chapter 8 has demonstrated that fasting and postprandial CCK concentrations after a high fat preload are higher in FD patients when compared with healthy subjects - this increase in CCK was also associated with higher scores for nausea and pain.

As expected, FD patients had a poorer quality of life, as well as greater symptom scores, when compared with healthy subjects – given the inclusion criteria, it was not surprising that no healthy subject experienced gastrointestinal symptoms. A previous study indicated that patients had the capacity to identify foods that exacerbate their symptoms and, therefore, avoided them (Kearney et al., 1989), however, this association was anecdotal and not formally assessed. In the present study, however, there was a significant relationship between fat intake and the subscale of the Nepean Dyspeptic Index relating to disturbances in eating or drinking. These items include statements of “the ability to eat/drink is disturbed by stomach problems”, “diet changed due to stomach problems” and “enjoyment of eating/drinking reduced due to stomach problems”. This observation is consistent with the concept that patients are aware that eating is the cause of their symptoms, as suggested by their baseline questionnaire answers, and may indicate why fat ingestion was less when compared with healthy subjects, given that this may be associated with the occurrence of symptoms. It has

been reported that eating attitudes, as assessed by the eating attitudes test, did not differ between patients with IBS and healthy subjects (Sullivan et al., 1997). However, in the current study differences were apparent which were indicative of higher prevalence of anorectic behaviours in the FD group. Depression scores were also significantly greater in patients when compared with healthy subjects consistent with previous observations (Van Oudenhove et al., 2007, Soo et al., 2003, Koloski et al., 2002). It may be possible that depression, anxiety and poor quality of life explain a particular dietary behaviour or at least the increased aversion against certain nutrients psychologically.

Our study design warrants some comment, particularly given that it is the first to address the influence of dietary factors, and its relationship on symptom induction in FD patients. While our subject numbers were relatively small, the resulting data, particularly those relating symptoms to energy and fat intake, appear very clear-cut. Nevertheless, as mentioned, type 2 errors cannot be excluded and to the applicability of these observations to men, remain uncertain.

9.6 CONCLUSIONS

In summary, this study has shown in a cohort of FD patients that (i) symptoms are related to fat and energy intake, (ii) FD patients eat fewer “meals” compared with healthy subjects, with a tendency to consume less fat and energy intake, (iii) symptoms usually occur within 30 min of eating and are of a modest severity, and (v) the quality of life was a determinant of fat and protein intake. These observations suggest that FD patients may consume a smaller number of meals and that symptoms are associated

with energy and fat intake; the consumption of smaller meals with a low fat content may, accordingly, prove beneficial in the management of FD.

Chapter 10

EFFECTS OF THE HERBAL MEDICATION, IBEROGAST® ON PROXIMAL GASTRIC VOLUME, ANTROPYLORODUODENAL MOTILITY AND GASTRIC EMPTYING IN HEALTHY MEN

10.1 SUMMARY

The herbal preparation, Iberogast®, has been reported to improve upper abdominal symptoms in FD and to decrease fundic tone, increase antral contractility and decrease afferent nerve sensitivity in experimental animals. The effects of Iberogast® on the human gastrointestinal tract have not been evaluated. The aim of this study was to investigate the effects of Iberogast® and control, each administered orally as a single dose (1.1 ml), in double-blind, randomised fashion, on proximal gastric volume (Part A), APD motility (Part B) and gastric emptying and intragastric distribution of a solid/liquid meal (Part C) for 120 min, in 9 (Part A), 12 (Part B) and 8 (Part C) healthy males. Iberogast® increased proximal gastric volume (max volume; control: 104 ± 12 ml, Iberogast®: 174 ± 23 ml; $P < 0.05$) (Part A), increased the MI of antral PWs in the first 60 min ($P < 0.05$) without affecting pyloric or duodenal pressures (Part B), and slightly increased the retention of liquid in the total stomach between 10 - 50 min ($P <$

0.01), but had no effect on gastric emptying of solids or intragastric distribution (Part C). Iberogast® affects gastric motility in humans, probably in a region-dependent manner. The stimulation of gastric relaxation and antral motility may contribute to the reported therapeutic efficacy of Iberogast® in FD.

10.2 INTRODUCTION

Abnormalities in gastric motor and sensory function have been investigated widely as a potential cause of symptoms in FD (Stanghellini et al., 1996, Gilja et al., 1996, Tack et al., 1998, Kim et al., 2001, Hausken and Berstad, 1992b, Schwartz et al., 2001, Bradette et al., 1991, Lemann et al., 1991, Mearin et al., 1991, Barbera et al., 1995b, Feinle et al., 2001a). The documented disturbances include delayed gastric emptying (Stanghellini et al., 1996), impaired proximal gastric relaxation (Gilja et al., 1996, Kim et al., 2001, Tack et al., 1998), decreased contractile activity of the antrum (Hausken and Berstad, 1992b) and abnormal duodenal motility (Schwartz et al., 2001), as well as increased sensitivity to mechanical (Bradette et al., 1991, Mearin et al., 1991, Lemann et al., 1991) and chemical (Barbera et al., 1995b, Feinle et al., 2001a) stimuli. Treatment for symptom relief is, accordingly, frequently directed at the normalisation of gastroduodenal motility using prokinetic drugs (Talley et al., 2000, Moayyedi et al., 2003, Corinaldesi et al., 1987). However, the beneficial effect of these drugs is relatively small and variable (Talley et al., 2000, Corinaldesi et al., 1987, Moayyedi et al., 2003), and their adverse effects can be substantial (Vitola et al., 1998).

Herbal drug preparations have recently received considerable interest as an alternative treatment option in FD (Arora and Sharma, 1990, Holtmann et al., 2003, May et al.,

2000). Clinical trials of herbal medicines, administered either alone (Arora and Sharma, 1990, Holtmann et al., 2003), or as combination preparations (May et al., 2000), have established their capacity to improve symptoms. A commercially available herbal preparation, Iberogast®, which contains nine plant extracts, has been evaluated in a number of trials and demonstrated to be superior to placebo (Buchert, 1994, Madisch et al., 2004), and of comparable efficacy to pharmacological agents, including metoclopramide (Nicolay, 1984) and cisapride (Rösch et al., 2002), in improving symptoms in FD and irritable bowel syndrome. In these studies, Iberogast® was administered at a dose of 20 drops (1.1 ml) three times per day over periods of 2 (Nicolay, 1984) or 4 (Buchert, 1994, Madisch et al., 2004, Rösch et al., 2002) weeks. Iberogast® has not been associated with any adverse effects.

To date, the limited number of studies which have investigated the potential mechanisms of action underlying the beneficial effects of Iberogast® have been performed primarily in animal models (Liu et al., 2004, Storr et al., 2004, Hohenester et al., 2004), with some preliminary evidence from human gastric tissue (Schemann et al., 2006). These studies suggest that Iberogast® has a dual action on the gastrointestinal tract. For example, Iberogast® decreases fundic tone, while increasing antral motility, in muscle strips from guinea pig stomach in a concentration-dependent manner (Hohenester et al., 2004). Preliminary experiments on human gastric muscle preparations demonstrated that Iberogast® relaxes the proximal stomach, comparable in magnitude to the effect in the guinea pig stomach (Schemann et al., 2006). Additional actions of Iberogast® in rats include decreased sensitivity of vagal and spinal afferents to low and high pressure distension in response to chemical (5-HT and bradykinin) and

mechanical (distension) stimuli (Liu et al., 2004), indicating that Iberogast® also affects sensory gut function.

The aims of this study were to determine the effects of Iberogast® on proximal gastric volume, pressures in the antrum, pylorus and duodenum, and gastric emptying (including intragastric meal distribution) in healthy men.

10.3 MATERIALS AND METHODS

10.3.1 Subjects

Healthy male subjects were recruited according to guidelines described in Chapter 4.2.

10.3.2 Study outline

The study consisted of three parts which evaluated the effects of Iberogast® on (i) proximal gastric volume (“relaxation”) (Part A), (ii) APD motility (Part B) and (iii) gastric emptying and intragastric distribution (Part C). Subjects attended at 0900 h after an overnight fast (14 hours for solids and 12 hours for liquids), either the Discipline of Medicine (Parts A and B) or the Department of Nuclear Medicine, PET and Bone Densitometry (Part C). In each part, subjects were studied on two occasions, separated by 3 - 10 days, on which they received, in double-blind, randomised fashion, an oral dose (1.1 ml) of either Iberogast® or control, each with 50 ml water. Part A was performed first, followed by Part B, then Part C. Ten of the fourteen subjects recruited participated in more than one part.

10.3.3 Composition of Iberogast® and preparation of control solution

Iberogast® (Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany; donated by Mr N. Pollard, Flordis Herbal Medicines Pty Ltd, Epping, NSW, Australia) is a complex herbal preparation, containing nine constituents, including fresh plant extract of *Iberis amara* (bitter candy tuft) and the extracts of eight dried herbs (*Angelicae radix* (angelica roots), *Matricariae flos* (camomile flowers), *Carvi fructus* (caraway fruit), *Cardui mariae fructus* (St. Mary's thistle fruit), *Melissae folium* (balm leaves), *Menthae peperitae folium* (peppermint leaves), *Chelidonii herba* (greater celandine), *Liquiritiae radix* (liquorice roots)) in 30.9 % ethanol (see Chapter 4.4.3 and **Table 4.3**).

The control solution was prepared by diluting 100 % ethanol with water to achieve an alcohol content of 30.9 %, thus 1.1 ml of solution contained 0.34 ml ethanol. Iberogast® was administered in the recommended dose of 1.1 ml (20 drops). The solutions were drawn into a syringe that had been covered with aluminum foil, by one of the investigators who was not directly involved in the performance of the study or data analysis, and injected into the subject's mouth.

10.3.4 Protocol

10.3.4.1 Part A: Effect of Iberogast® on proximal gastric volumes

9 healthy males (age: 29 ± 4 yrs; BMI: 23 ± 1 kg/m²) were included. Subjects swallowed a single-lumen polyvinyl orogastric catheter as described in Chapter 4.5.2. Subjects were then seated in a 75° recumbent position. Intrabag pressure was set at MDP, as described in Chapter 4.5.2, and following a 10 min "baseline" period, each subject was given either Iberogast® or control with 50 ml water. Volume changes in

the bag were recorded for 120 min. The catheter was then removed, and the subject allowed to leave the laboratory.

Intrabag pressures and volumes were digitised and recorded on a computer-based system running commercially available software (Protocol Plus™, G & J Electronics, Toronto, Ontario, Canada). Absolute volumes were expressed as means of 10 min segments for baseline (i.e. $t = -10 - 0$ min) and during the following 120 min. The average maximum volume was calculated by determining the highest volume, and time point at which this occurred, in each subject. The AUC for gastric volume between $t = 0 - 120$ min was determined using the trapezoidal rule.

10.3.4.2 Part B: Effect of Iberogast® on antropyloroduodenal pressures

12 healthy males (age: 28 ± 4 yrs; BMI: 24 ± 2 kg/m²) were included. A manometric catheter was positioned, as described in Chapter 4.5.1. Once the manometric catheter was positioned correctly, fasting motility was monitored until the occurrence of a phase III of the interdigestive MMC (Feltrin et al., 2004). At $t = 0$ min, i.e. during phase I of the MMC, the subject was given either Iberogast® or control with 50 ml water, and APD pressures were monitored for 120 min. The manometric assembly was then removed and the subject allowed to leave the laboratory.

APD pressures were assessed as described in Chapter 4.5.1. APD pressures were analysed for (i) number and amplitude of antral and duodenal PWs, (ii) number and amplitude of IPPWs, (iii) basal pyloric pressure and (iv) number and length of PWSs (Chapter 4.5.1).

Baseline values were calculated as the means of values obtained between $t = -10 - 0$ min for the number and amplitude of antral and duodenal PWs, IPPWs and basal pyloric pressure. Basal pyloric pressures and the number and amplitude of IPPWs and antral and duodenal PWs were expressed as means of 10 min segments. Antral and duodenal MI were also calculated (Camilleri and Malagelada, 1984). The AUC for the number, amplitude and MI of antral and duodenal PWs, basal pyloric pressure and number and amplitude of IPPWs from $t = 0 - 120$ min was determined using the trapezoidal rule. Episodes of phase III of the MMC were excluded if they occurred more than 60 min after the treatment was given (i.e. $t = 60 - 120$ min), i.e. when they were more likely to be the result of fasting rather than of the treatment.

10.3.4.3 Part C: Effect of Iberogast® on gastric emptying and intragastric distribution

8 healthy males (age: 31 ± 4 yrs; BMI: 25 ± 1 kg/m²) were included. Subjects were seated with their back upright against a gamma camera. Each subject was given either Iberogast® or control with 50 ml of water, immediately before a mixed solid/liquid meal, described in Chapter 4.5.3, which was consumed within 5 min. The time of completion of the meal was defined as $t = 0$ min. Gastric emptying was measured for 120 min.

Radioisotopic data were acquired as described in Chapter 4.5.3 For both the solid and the liquid meal components, the amounts remaining in the total, proximal and distal stomach between $t = 0 - 120$ min were derived at 10 min intervals, as well as the AUC. The lag phase for solid and liquid was determined and the amount of solid remaining in

the stomach at $t = 100$ min and the time for 50 % of the liquid to empty (T50) were calculated as described in Chapter 4.5.3.

10.3.5 Statistical analysis

Gastric volume (Part A), number, amplitude and MI of antral and duodenal PWs, basal pyloric pressure, number and amplitude of IPPWs (Part B) and amount of solid and liquid remaining in the total, proximal and distal stomach (Part C), between 0 - 60 min and 0 - 120 min, were analysed by repeated-measures ANOVA, with time and treatment as factors. AUCs for gastric volume, APD motility and the amount of solid and liquid remaining in the total, proximal and distal stomach, the maximal effect of treatment (and the time at which this occurred) on gastric volume, the lag phase of solid and liquid, retention of solid at $t = 100$ min and the T50 of liquids were analysed by Student's t - test. Statistical significance was accepted at $P < 0.05$, and data are presented as means \pm SEM.

10.4 RESULTS

The studies were tolerated well. Only one subject was able to distinguish between control and Iberogast®; four subjects reported a mildly unpleasant taste after both treatments which lasted for a few seconds. None of the subjects experienced any adverse effects (including nausea).

10.4.1 Part A: Effect of Iberogast® on intrabag volume changes (“gastric relaxation”)

Mean MDP was 8 ± 0 mmHg for control and 8 ± 1 mmHg for the study with Iberogast®. The balloon volumes at MDP, before administration of the treatments, did not differ on the two days (control: 49 ± 4 ml, Iberogast®: 53 ± 5 ml). There were effects of treatment and time on intra-bag volume between 0 - 120 min (**Figure 10.1**) - while intra-bag volume increased gradually on both days, the magnitude of this increase was greater with Iberogast® than control ($P < 0.05$). The increase in intra-bag volume from baseline was marginal for control, and evident only between 100 - 120 min ($P < 0.05$), whereas the rise after Iberogast® was substantial and occurred between 40 - 120 min ($P < 0.05$). Maximum intra-bag volume (control: 104 ± 12 ml at 64 ± 15 min, Iberogast®: 174 ± 23 ml at 69 ± 12 min; $P < 0.05$ for volume) and the AUC (control: 7130 ± 765 ml.min, Iberogast®: 12400 ± 1850 ml.min; $P < 0.05$) were also greater with Iberogast®.

10.4.2 Part B: Effect of Iberogast® on antropyloroduodenal motility

Phase III episodes were observed in five subjects (in two subjects they occurred during both control and Iberogast®, in two subjects during Iberogast® only and in one subject during control only), and on three occasions, this was in the first 60 min (twice during control and once during Iberogast® treatment).

10.4.2.1 Antral pressure waves

There was no effect of treatment on the number (**Figure 10.2A**) or amplitude (**Figure 10.2B**) of antral PWs, although mean values were slightly greater following Iberogast®

when compared with control. However, there was an effect of treatment on the MI (**Figure 10.2C**) of antral PWs. The MI was greater following Iberogast® compared with control in the first 60 min ($P < 0.01$). There was no effect of treatment on the AUCs for the number, amplitude or MI (**Table 10.1**), although mean values were greater following Iberogast® when compared with control.

10.4.2.2 Pyloric pressures

There was no effect of treatment on basal pyloric pressure (**Figure 10.3A**), or the number (**Figure 10.3B**) or amplitude (**Figure 10.3C**) of IPPWs. There was an effect of time on both the number and amplitude of IPPWs; after an initial rise during the first 20 min following administration of both control (non-significant) and Iberogast® ($P < 0.05$), values returned to near baseline. There was no effect of treatment on the AUCs for basal pyloric pressure, or the number or amplitude of IPPWs (**Table 10.1**).

10.4.2.3 Duodenal pressure waves

There was an effect of time, but not of treatment, on the number (**Figure 10.4A**), amplitude (**Figure 10.4B**) and MI (**Figure 10.4C**) of duodenal PWs. During both treatments there was an initial rise ($P < 0.05$) in all three parameters, and while the effect of Iberogast® appeared to be slightly greater than that of control, this was not significant. There was no effect of treatment on the AUCs for the number, amplitude or MI (**Table 10.1**).

10.4.3 Part C: Effect of Iberogast® on gastric emptying and intragastric distribution

10.4.3.1 Solid meal

There was no difference in the lag phase (control: 16 ± 4 min, Iberogast®: 19 ± 5 min), the amount of solid remaining in the total (**Figure 10.5A**), proximal (**Figure 10.5B**) or distal (**Figure 10.5C**) stomach, or the AUCs (**Table 10.2**) between treatments. There was no difference in the % retention of solid at $t = 100$ min between the two treatments (control: 40 ± 4 %, Iberogast®: 43 ± 5 %). Gastric emptying was delayed in one subject during Iberogast® treatment (retention at 100 min = 63 %).

10.4.3.2 Liquid meal

There was no difference in the lag phase between treatments (control: 1 ± 0 min, Iberogast®: 1 ± 0 min), however, there was a treatment by time interaction for the amount of liquid remaining in the total stomach ($P < 0.01$) (**Figure 10.5D**). Intragastric retention of liquid was slightly greater during Iberogast® when compared with control between 10 - 50 min ($P < 0.05$). There was no difference between treatments in the amount of liquid remaining in the proximal (**Figure 10.5E**) or distal (**Figure 10.5F**) stomach. There was no effect of treatment on AUCs for liquid emptying in total, proximal or distal stomach (**Table 10.2**). There was no difference in the T50 of liquid between the two treatments (control: 20 ± 3 min, Iberogast®: 23 ± 2 min). Gastric emptying was delayed in one subject during Iberogast® treatment (T50 = 32 min).

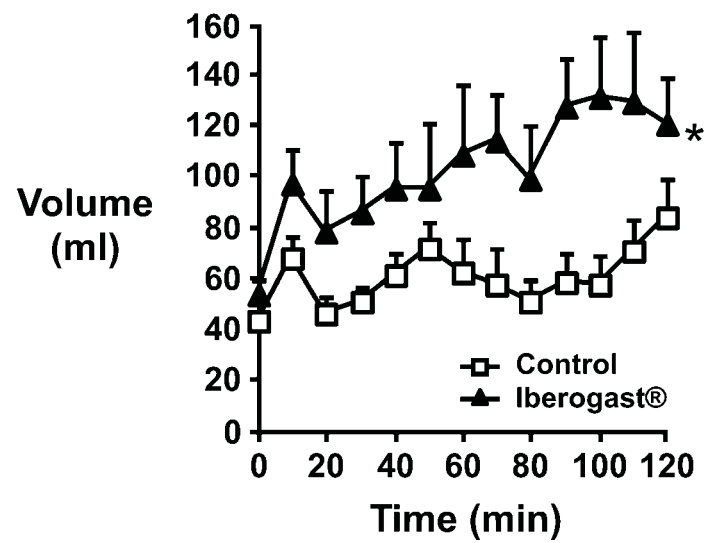


Figure 10.1 Intra-bag volume, as means of 10 min segments, after oral administration of 1.1 ml control solution (□), or Iberogast® (▲), with 50 ml water. * vs. control; $P < 0.05$. Data are means \pm SEM ($n = 9$).

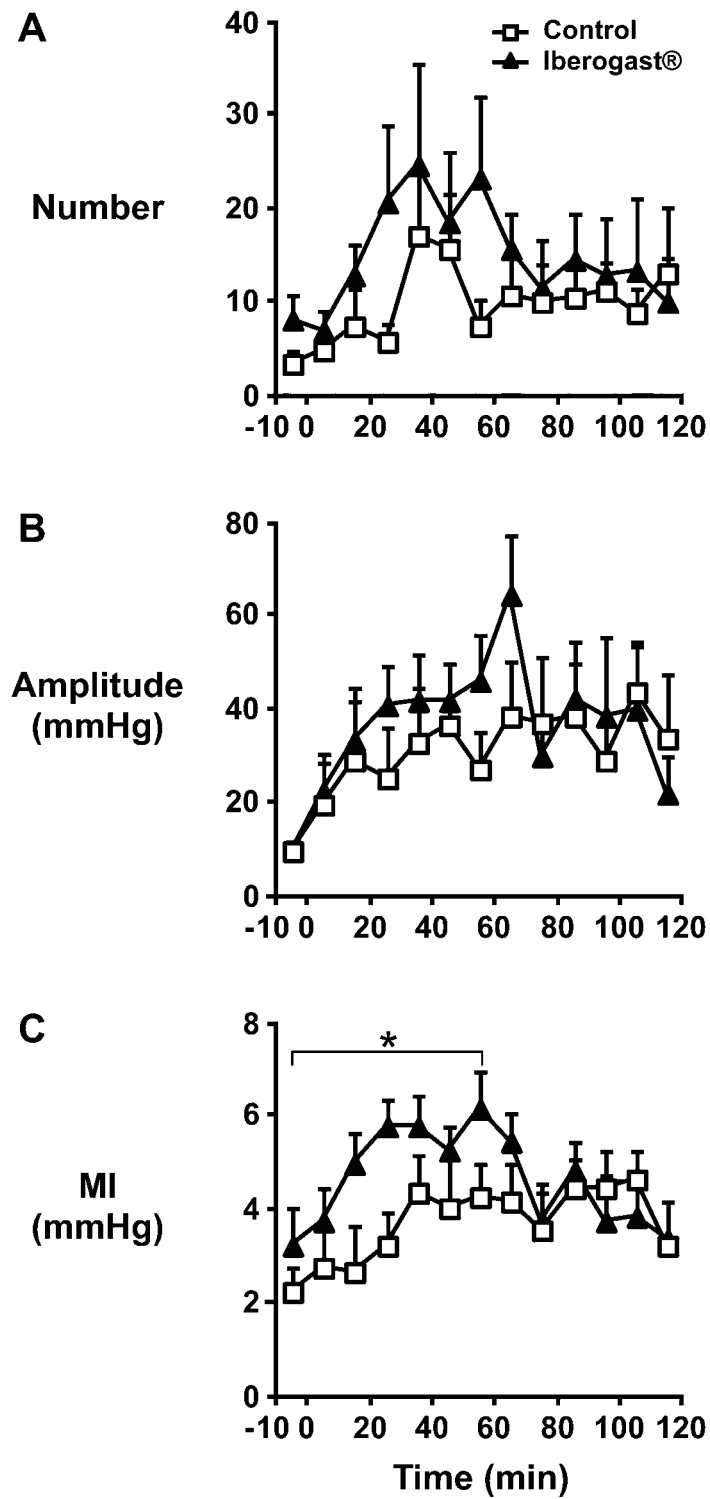


Figure 10.2 (A) Number, (B) amplitude and (C) motility index (MI) of antral pressure waves, as means of 10 min segments, after oral administration of 1.1 ml control solution (□), or Iberogast® (▲), with 50 ml water. * vs. control; $P < 0.01$. Data are means \pm SEM ($n = 12$).

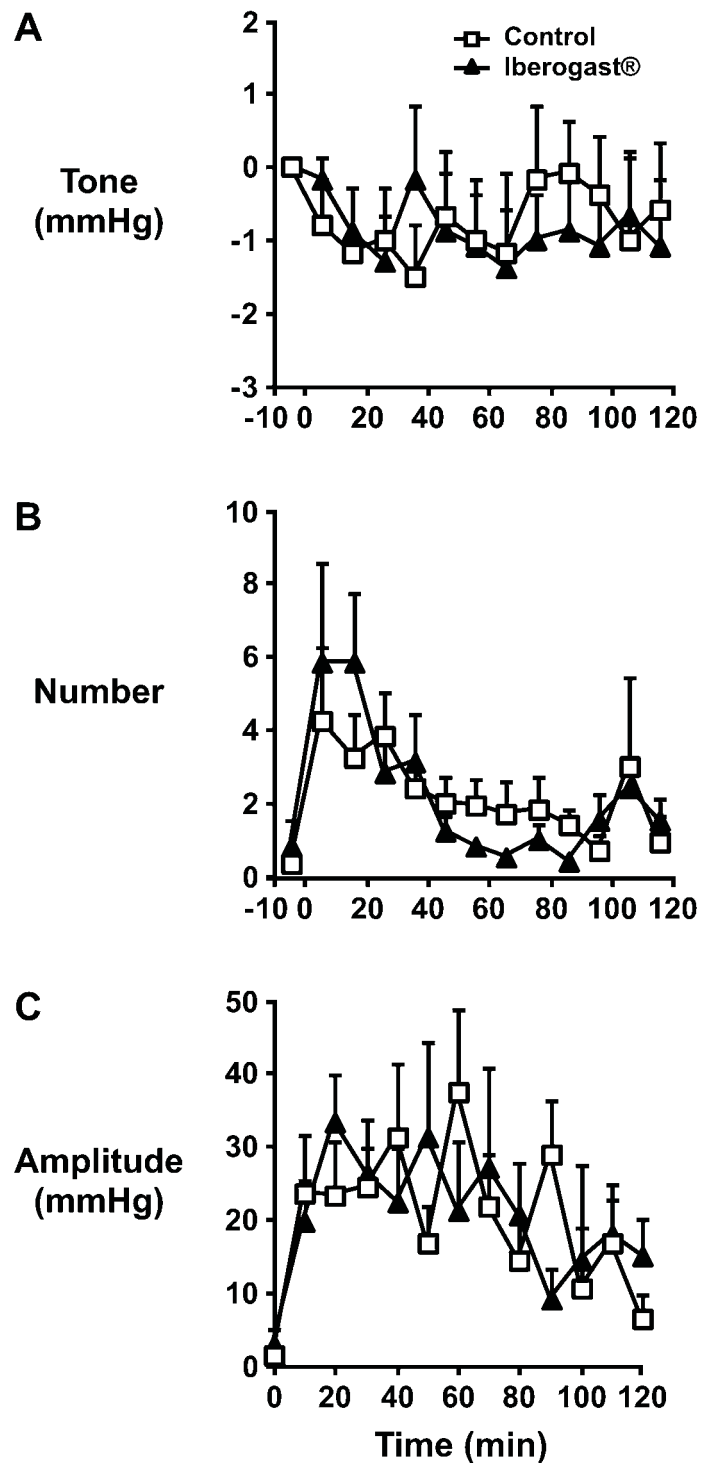


Figure 10.3 (A) Basal pyloric pressure, (B) number and (C) amplitude of isolated pyloric pressure waves, as means of 10 min segments, after oral administration of 1.1 ml control solution (□), or Iberogast® (▲), with 50 ml water. Data are means ± SEM (n = 12).

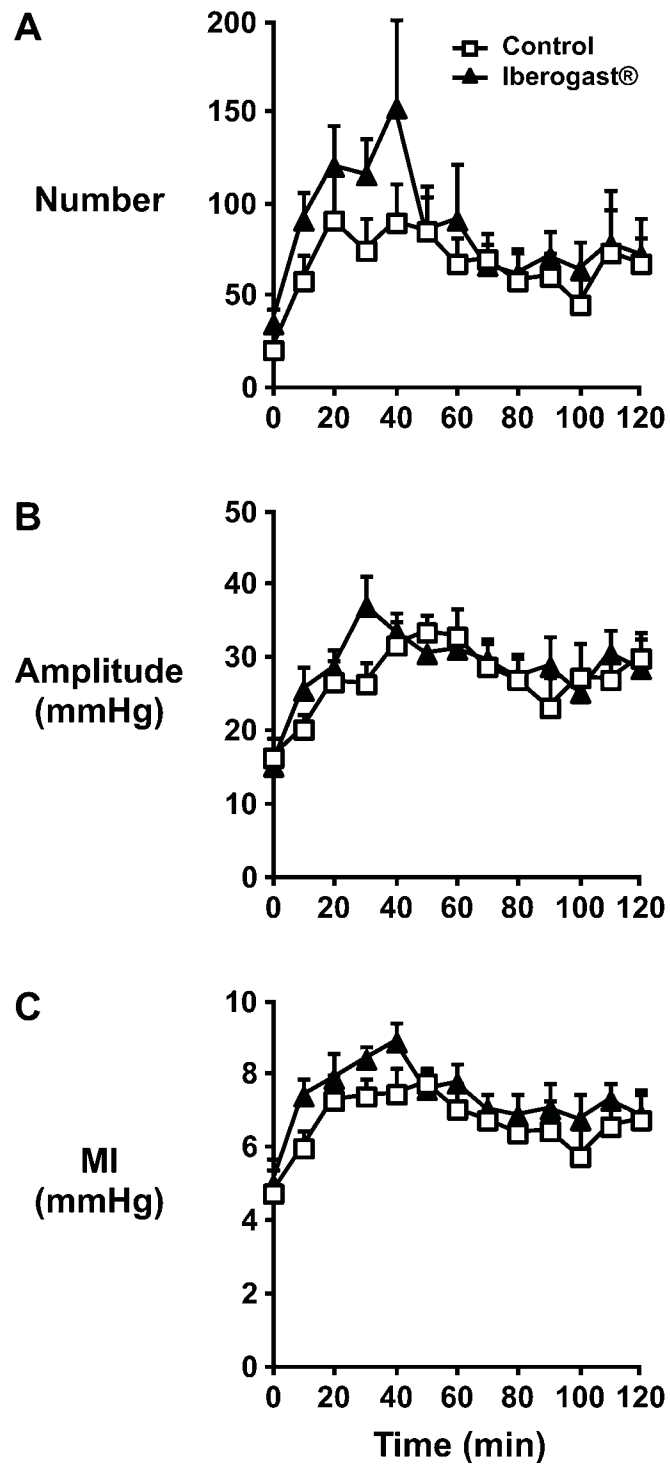


Figure 10.4 (A) Number, (B) amplitude and (C) motility index (MI) of duodenal pressure waves, as means of 10 min segments, after oral administration of 1.1 ml control solution (□), or Iberogast® (▲), with 50 ml water. Data are means ± SEM (n = 12).

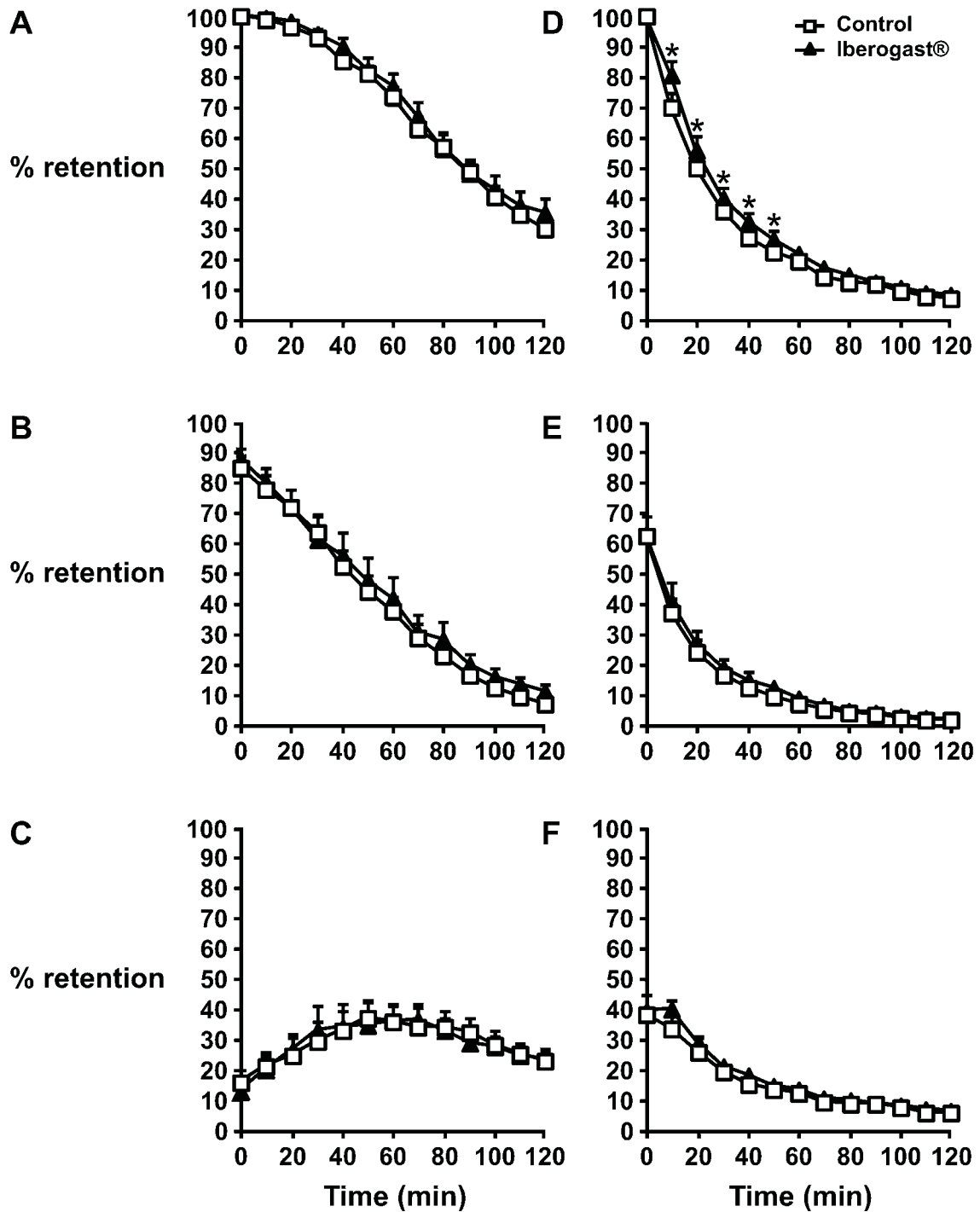


Figure 10.5 Gastric emptying and intragastric distribution of solid (A, B, C) and liquid (D, E, F) meal components, after oral administration of 1.1 ml control solution (□), or Iberogast® (▲), with 50 ml water. * vs. control; P < 0.05. Data are means ± SEM (n = 8).

Table 10.1 Area under the curves for the number, amplitude and motility index of antral and duodenal pressure waves, basal pyloric pressure and number and amplitude of isolated pyloric pressure waves after control or Iberogast® between t = 0 - 120 min.

	Control	Iberogast®
<i>Antral pressure waves</i>		
Number (min)	1150 ± 279	1810 ± 598
Amplitude (mmHg.min)	3730 ± 480	4490 ± 612
MI (mmHg.min)	447 ± 49	559 ± 48
<i>Pyloric pressures</i>		
Basal pyloric pressure (mmHg.min)*	-94 ± 89	-102 ± 76
Number IPPWs (min)	267 ± 79	264 ± 57
Amplitude IPPWs (mmHg.min)	2510 ± 484	2500 ± 493
<i>Duodenal pressure waves</i>		
Number (min)	8040 ± 1150	10390 ± 1940
Amplitude (mmHg.min)	3230 ± 192	3450 ± 226
MI (mmHg.min)	799 ± 38	880 ± 42

Data are means ± SEM (n = 12). MI, motility index; IPPWs, isolated pyloric pressure waves. * The negative values indicate that both treatments decreased tone compared with baseline.

Table 10.2. Area under the curves for the gastric emptying profiles of solid and liquid meal components from the total, proximal and distal stomach after control or Iberogast® from t = 0 - 120 min.

	Control	Iberogast®
<i>Solid gastric emptying</i>		
Total (%.min)	8330 ± 286	8580 ± 348
Proximal (%.min)	4810 ± 439	5120 ± 600
Distal (%.min)	3530 ± 593	3490 ± 539
<i>Liquid gastric emptying</i>		
Total (%.min)	3310 ± 247	3720 ± 237
Proximal (%.min)	1520 ± 200	1720 ± 242
Distal (%.min)	1780 ± 243	2000 ± 175

Data are means ± SEM (n = 8).

10.5 DISCUSSION

This study establishes for the first time that Iberogast® affects gastric motility in humans and suggests that these effects may be region-dependent. Specifically, Iberogast® increased proximal gastric relaxation substantially, increased the MI of antral PWs and slowed gastric emptying of liquid slightly, but had no significant effect on fasting duodenal or pyloric motility, solid gastric emptying or intragastric meal distribution, in healthy males.

The pathophysiology of FD remains poorly defined, and heterogenous. Treatment options, including prokinetics (Talley et al., 2000, Corinaldesi et al., 1987, Moayyedi et al., 2003) and proton pump inhibitors (Talley et al., 1998), have resulted in variable and less than optimal responses. The therapeutic benefits of relatively inexpensive herbal drugs in patients with functional gastrointestinal disorders appear to be significant and their use is devoid of adverse effects. For example, Iberogast® is superior to placebo (Buchert, 1994, Madisch et al., 2004) and comparable to metoclopramide (Nicolay, 1984) and cisapride (Rösch et al., 2002) in the management of FD. The current study demonstrated that Iberogast® increased proximal gastric relaxation, as reflected by an increase in intra-bag volume at constant pressure, which was sustained for the duration of the study (120 min), and also increased the MI of antral PWs during the first 60 min after administration. These results are consistent with observations in animals (Hohenester et al., 2004) and suggest that the effects of Iberogast® on gut motility are also region-dependent in healthy humans. Moreover, these effects may potentially account for improvement in dyspeptic symptoms, as impaired proximal gastric relaxation and antral hypomotility affect some 40 - 70 % of FD patients (Gilja et al.,

1996, Tack et al., 1998, Kim et al., 2001, Malagelada and Stanghellini, 1985). In contrast, Iberogast® had no apparent effect on either duodenal pressures, phasic or tonic pyloric motility, or solid gastric emptying and had a minimal, albeit significant, effect to slow liquid emptying. The latter is not surprising, given that Iberogast® relaxes the fundus and increases contractile activity in the antrum; one would, therefore, not expect any major change in gastric emptying. The absence of any effect of Iberogast® on intragastric meal distribution is perhaps surprising, given the enhancement of proximal gastric relaxation, but this may be because the test meal was relatively small and low in energy (330 kcal). It would be of interest, to evaluate the effects of Iberogast® on the intragastric distribution of meals of higher caloric content and volume. In a rat model Iberogast® decreased afferent nerve sensitivity in response to mechanical ramp distension of an intestinal loop at increasing pressures (0 - 60 cmH₂O) (Liu et al., 2004), accordingly, evaluation of the effects of Iberogast® on gastric sensitivity in humans is also warranted. Studies to evaluate the effects of Iberogast® on proximal gastric accommodation, antral, pyloric and small intestinal motility and gastric emptying, as well as gastric sensitivity, in FD patients are indicated, particularly given that our study has now demonstrated that Iberogast® modulates gastric motility in humans.

While the data do not provide any insight regarding the site of action of Iberogast®, a recent study on isolated guinea pig stomach preparations clearly indicates a direct muscle effect of the drug (Hohenester et al., 2004). In contrast, the site of action of Iberogast® in regards to its effect on gastric sensitivity has not been established, and the concept that the effect of Iberogast® may be mediated from the small intestine, as is the case with nutrient-regulation of gastric emptying (Hedde et al., 1989), warrants exploration.

It is important to recognise potential limitations of the study. Iberogast® contains a number of components, and it is, therefore, unknown, which extract(s) is (are) responsible for the demonstrated effects on proximal gastric relaxation, antral motility and liquid gastric emptying in humans. However, the mode of action of Iberogast® has recently been investigated in guinea pig stomach preparations, and the data provide evidence that the region-dependent effects of Iberogast® are not due to the differential actions of the individual components of Iberogast® on the fundus or antrum, but rather the specific properties of fundus vs. antrum muscle (Kong et al., 1986). The effects of Iberogast® on gastric motility are not attributable to the ethanol in this preparation, as the control solution contained an identical amount, and concentration, of alcohol. The effects of a single-dose of Iberogast® was evaluated, and the effects of different doses, as well as chronic administration, are unknown. In addition, our study was an investigation in a relatively small number of healthy subjects, therefore, future studies in male and female patients with FD are required. Furthermore, while this study evaluated the effects of Iberogast® in the fasting gastrointestinal tract, the evaluation of the effects of Iberogast® on postprandial gastrointestinal motor function is of high clinical relevance. Iberogast® and control solutions were consumed immediately before 50 ml of water, and only one subject was able to distinguish between control and Iberogast® and only four reported a transient (i.e. immediately after the treatment was taken) unpleasant taste following both treatments. Accordingly, the observed effects on motility are most unlikely to be attributable to an aversive effect.

10.6 CONCLUSIONS

In summary, this study demonstrated that Iberogast® has region-dependent effects on gastric motility in humans - Iberogast® increased proximal gastric relaxation, increased antral motility and slowed gastric emptying of liquid slightly, but had no effect on pyloric and duodenal motility or solid gastric emptying in healthy male subjects. The effects on gastric motility may contribute to the beneficial effect of Iberogast® in FD.

Chapter 11

CONCLUSIONS

Gastrointestinal function is influenced by a number of factors, including exposure of the small intestine to nutrients and gastrointestinal hormone release/suppression, all of which play a role in the regulation of appetite and energy intake, i.e. suppression of appetite and subsequent energy intake by meal ingestion is mediated, at least in part, by gastric emptying, gastrointestinal motility and the release of CCK, PYY, GLP-1, GIP and suppression of ghrelin. In patients with FD, gastrointestinal function is frequently disturbed and symptoms are anecdotally often induced, or exacerbated by food ingestion, however there is limited information about the associations between symptoms, food ingestion and gut function. Furthermore, the therapeutic benefit of currently available pharmacotherapies targeted at symptom improvement by normalising gastrointestinal function is marginal at best, so that there is an urgent need for more effective treatments.

The studies included in this thesis have focused on the effects of different macronutrients, predominantly fat and carbohydrate, on gastrointestinal motility, hormone release/suppression, appetite and energy intake in healthy subjects and on symptom generation in FD by addressing three broad issues: (i) the effect of load, and

duration, of small intestinal nutrient exposure on APD motility, hormone release/suppression, appetite and energy intake in healthy subjects (Chapters 5 - 7), (ii) potential dietary factors that contribute to symptom generation in patients with FD (Chapters 8 and 9), and (iii) the effects of the herbal medication, Iberogast®, on gastric motility in healthy subjects (Chapter 10).

A numbers of factors may influence the effects of small intestinal nutrients on gastrointestinal motility, hormone release/suppression, appetite and energy intake. Studies in animals and, one recent study in humans, have demonstrated that the length of the small intestine exposed to nutrients is important. While the effects of the load of small intestinal nutrients and duration of nutrient exposure have been evaluated in humans, the effects of energy loads lower than, comparable to, and greater than, the normal rate of gastric emptying on gastrointestinal motility, hormone release/suppression, appetite and energy intake, in healthy humans, or the relationships between these parameters, have hitherto not been evaluated.

The study described in Chapter 5 compared the effects of a low (1.33 kcal/min) and high (4 kcal/min) intraduodenal load of lipid during a “short infusion” (50 min), with a low load (1.33 kcal/min) during a “long infusion” (150 min). Antral PWs and PWSs were suppressed, and basal pyloric pressure, IPPWs, plasma CCK and PYY stimulated, by both the low, and high, loads. The effect of the high lipid load was sustained, so that the suppression of antral PWs and PWSs and increase in PYY were evident after the cessation of the infusion. The prolonged intraduodenal lipid infusion suppressed antral PWs, stimulated CCK and PYY and basal pyloric pressure, and tended to stimulate IPPWs, when compared with saline throughout the infusion period. These observations

indicate that both the load, and duration, of small intestinal lipid have an influence on APD motility and patterns of CCK and PYY release.

The study described in Chapter 6 evaluated the effects of intraduodenal lipid, at loads lower than (0.25 kcal/min for 50 min: 12.5 kcal), comparable to (1.5 kcal/min for 50 min: 75 kcal) and higher (4 kcal/min for 50 min: 200 kcal) than normal gastric emptying in healthy subjects. The lowest lipid load transiently stimulated IPPWs and CCK release and suppressed PWSs and hunger scores. Loads comparable to, and higher than, the normal rate of gastric emptying, were required to stimulate basal pyloric pressure and PYY release, and suppress antral and duodenal PWs. Only the highest load suppressed energy intake. The effects of intraduodenal lipid on all parameters, with the exception of hunger, were load-dependent, but the threshold-loads required to elicit responses varied between parameters. There were also significant relationships between energy intake with APD motility and CCK and PYY concentrations. In obese subjects, there is evidence that gastrointestinal motility and hormone secretion are abnormal. Many of these individuals habitually consuming high-caloric diets and may well be less sensitive to nutrient stimuli, therefore, it is possible that higher small intestinal lipid loads may be required to induce similar responses to those observed in this study. It would, accordingly, be of interest to compare the thresholds required, and “dose-responses” for the stimulation, or suppression of gastric motility, hormone release/suppression, appetite and energy intake in lean and obese subjects.

The study described in Chapter 7 evaluated the effect of intraduodenal glucose loads lower than (1 kcal/min for 120 min: 120 kcal), comparable to (2 kcal/min for 120 min:

240 kcal), and higher than (4 kcal/min for 120 min: 480 kcal) the normal rate of gastric emptying, in healthy subjects. All loads stimulated blood glucose, plasma insulin, GLP-1, GIP and CCK concentrations and suppressed antral PWs, however, loads comparable, and higher than, normal gastric emptying were required for the suppression of duodenal PWs and PWSs and the stimulation of basal pyloric pressure, and suppression of energy intake was only evident after the highest load. There were also significant relationships between food/energy intake with insulin, GLP-1 and GIP and pyloric pressures. Interestingly, the glycaemic responses to the 2 kcal/min and 4 kcal/min infusions were comparable, which is attributable to the substantially greater insulin and GLP-1 stimulation in response to the latter. Type-2 diabetes is associated with abnormalities in gastrointestinal function and hormone release, therefore the observations derived from this study have implications for an understanding of the regulation of postprandial glycaemia and energy intake in type-2 diabetes and studies in this group are warranted.

Patients with FD, report that their symptoms are related to the ingestion of food, and while there is evidence that there are differences in eating behaviour between FD patients and healthy subjects, information is limited and inconsistent. In addition, no study has hitherto correlated the occurrence, and severity, of symptoms with food intake. A limited number of laboratory-based studies have reported that fat, not glucose, exacerbates dyspeptic symptoms, and fasting CCK concentrations and fasting and postprandial antral size, are increased in FD compared, however the relationship between symptoms with fat intake, antral size and gastrointestinal hormone release has not been assessed. In the study described in Chapter 8, symptoms, plasma CCK, PYY and ghrelin, and antral area and energy intake were compared in FD patients and

healthy subjects after ingestion of high-carbohydrate, high-fat and low energy control yoghurt preloads. Nausea and pain were greater in FD after the high-fat, when compared with high-carbohydrate, and control, preloads and with healthy subjects. Discomfort was greater after all preloads in FD when compared with healthy subjects. Fasting CCK and the stimulation of CCK by the high-fat preload were greater in FD, while fasting and postprandial PYY were less in FD than in healthy subjects, with no differences in fasting, or postprandial, plasma ghrelin between FD and healthy subjects. Fasting antral area was greater in FD, with no differences postprandially between FD and healthy subjects. There were no differences in energy intake between the two groups. In the study reported in Chapter 9, dietary factors, and the occurrence and severity of symptoms, were compared in cohorts of FD patients and healthy subjects. The number of “meals” was slightly less, and total energy and fat intake tended to be less, in FD patients. Symptoms experienced by the patients were modest and occurred within ~ 30 min of eating. There were a number of relationships between symptoms with dietary factors: perhaps of most interest was that fullness was related directly to the amount of fat ingested and to overall energy intake, and inversely to the amount of carbohydrate and that there was a tendency for bloating to be related directly to the amount of fat ingested. Taken together, the observations from these two studies suggest that FD patients may consume a smaller number of meals and that symptoms are associated with energy and fat intake. Hence, the consumption of smaller meals with a low fat content may prove beneficial in the management of FD. The cause(s) of the elevated fasting and postprandial plasma CCK and reduced postprandial PYY concentrations remain to be determined. It is, however, clear that the observations reported in Chapters 8 and 9 have important implications for the development of diet-based therapies for FD.

There has been considerable interest in the use of herbal preparations in patients with FD, in particular the use of the combination preparation, Iberogast®. While a number of clinical studies have reported that Iberogast® was superior to placebo, with an efficacy comparable to other pharmacotherapies, to improve symptoms in FD patients, the mechanisms underlying the action of Iberogast® have only been evaluated in animal models. In these studies, Iberogast® decreased fundic tone, while increasing antral motility in muscle strips from guinea pig stomach in a concentration-dependent manner, and decreased sensitivity of vagal and spinal afferent to low and high pressure distension. These studies therefore indicate that Iberogast® affects gastrointestinal motility and sensory function. In the study described in Chapter 10, the effects of Iberogast® on proximal gastric volume, pressures in the antrum, pylorus and duodenum, and gastric emptying were determined in healthy subjects. Iberogast® increased proximal gastric volume and the motility index of antral PWs, without affecting pyloric or duodenal pressures, and increased the retention of liquid in the total stomach, without affecting gastric emptying of solids or intragastric distribution, in healthy men. This study, accordingly establishes that Iberogast® has region-dependent effects on gastric motility in humans, by increasing proximal gastric relaxation and decreasing antral motility. As FD is a heterogenous disorder, in which subgroups of patients have impaired proximal gastric relaxation and increased antral filling, these effects may contribute to the beneficial effect of Iberogast® in FD. An immediate priority is, therefore, to investigate the effects of Iberogast® on gastric motility and sensitivity in patients with FD.

The studies presented in this thesis provide novel insights into the effects of nutrients and the herbal medication, Iberogast®, on gastrointestinal function. Specifically, the effects of fat and carbohydrate on gastroduodenal motility, hormone release/suppression and appetite and energy intake in healthy subjects, and symptom generation in patients with FD, and the effects of Iberogast® on gastroduodenal motility in healthy subjects. Gastric motor function, hormone release and energy intake may be disturbed in obesity, type-2 diabetes and FD, and it would be appropriate to extend these observation to these groups.

APPENDIX 1

DIET DIARY

FOOD INTAKE			SYMPTOM OCCURRENCE		
Time	Description of food and drink consumed	Amount	Time	Symptom	Severity (1 – 10)
	EXAMPLE				
0700	'Weetbix'	3 biscuit			
	milk (full cream)	1/2 cup			
	bread (white)(toasted)	1 slice			
	margarine (low-salt, polyunsat)	2 tsp			
	orange juice (unsweetened)	1 glass	0705 - 0725	Upper abdominal pain	4
1000	coffee (instant)	1 cup			
	sugar (white)	2 tsp			
	biscuits (milk arrowroot) Arnott's	2 biscuit			
1230	bread (white)	2 slices			
	margarine (low-salt, polyunsat) Flora	2 tsp			
	ham (shaved, shoulder)	1 slice	1235 – 1240	Upper abdominal pain	3
	cheese (low-fat cheddar)	1 slice	1235 - 1255	Bloating	2
1730	steak (beef)(grilled)	200 g			
	potato (with skin)(baked)	200 g			
	beans (french)(boiled)	60 g			
	bread (white)	1 roll	1735 - 1745	Nausea	1
2200	milk (full cream)	250 ml			
	'Milo'	2 tsp			
	biscuits (Arnott's milk coffee)	2 biscuits			

APPENDIX 2

VISUAL ANALOGUE SCALE

Name: **Code:** **Date:**

Visit number: **Treatment:** **TIME**

Please indicate how you are feeling at this moment by placing a **vertical mark** at the appropriate point on each scale below. Please **do not** make a cross or sloping mark. Please mark all scales.

Nauseated ?
 Not at all | | Very

Alert ?
 Not at all | | Very

Bloated ?
 Not at all | | Very

Anxious ?
 Not at all | | Very

Energetic ?
 Not at all | | Very

Full in your stomach ?
 Not at all | | Very

Happy ?
 Not at all | | Very

How much food do you think you would eat ?
 None | | A large amount

Hungry ?
 Not at all | | Very

How strong is your desire to eat ?
 Weak | | Strong

Are you in discomfort ?
 Not at all | | Very

How much pain are you in ?
 None | | A lot

APPENDIX 3

VISUAL ANALOGUE SCALE

Name: **Study code:**

Date: **Visit number:**

Please rate the characteristics of the yoghurt by placing a **vertical mark** at the appropriate point on each scale below. Please **do not** make a cross or sloping mark. Please mark **all** scales.

How tasty was the yoghurt ?

Very tasty |-----| Not tasty at all

How sweet was the yoghurt ?

Very sweet |-----| Not sweet at all

Rate the colour of the yoghurt:

Intense |-----| Not intense

How bitter was the yoghurt ?

Bitter |-----| Not bitter at all

How fatty was the yoghurt ?

Fatty |-----| Not fatty at all

How sour was the yoghurt ?

Sour |-----| Not sour at all

APPENDIX 4

THREE FACTOR EATING QUESTIONNAIRE

Read each of the following 36 statements carefully. If you agree with the statement or feel that it is true as applied to you, answer true by circling the (T). If you disagree with the statement, or feel that it is false as applied to you, answer false by circling the (F). Be certain to answer all of the questions.

*F: Factor

1. When I smell a freshly baked pizza, I find it very difficult to keep from eating, even if I have just finished a meal. (F2)
 (T) (F)
2. I usually eat too much at social occasions, like parties and picnics. (F2)
 (T) (F)
3. I am usually so hungry that I eat more than three times a day. (F3)
 (T) (F)
4. When I have eaten my quota of calories/fat, I am usually good about not eating any more. (F1)
 (T) (F)
5. Dieting is so hard for me because I just get too hungry. (F3)
 (T) (F)
6. I deliberately take small helpings as a means of controlling my weight. (F1)
 (T) (F)
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry. (F2)
 (T) (F)
8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat. (F3)
 (T) (F)
9. When I feel anxious, I find myself eating. (F2)
 (T) (F)
10. Life is too short to worry about dieting. (F1)
 (T) (F)

11. Since my weight goes up and down, I have gone on reducing diets more than once.
(F2)
 (T) (F)
12. I often feel so hungry that I just have to eat something. (F3)
 (T) (F)
13. When I am with someone who is overeating, I usually overeat too. (F2)
 (T) (F)
14. I have a pretty good idea of the number of calories/grams of fat in common foods. (F1)
 (T) (F)
15. Sometimes when I start eating, I just can't seem to stop. (F2)
 (T) (F)
16. It is not difficult for me to leave something on my plate. (F2)
 (T) (F)
17. At certain times of the day, I get hungry because I have got used to eating then. (F3)
 (T) (F)
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it. (F1)
 (T) (F)
19. Being with someone who is eating often makes me hungry enough to eat also. (F3)
 (T) (F)
20. When I feel blue, I often overeat. (F2)
 (T) (F)
21. I enjoy eating too much to spoil it by counting calories, counting grams of fat or watching my weight. (F1)
 (T) (F)
22. When I see a real delicacy, I often get so hungry that I have to eat right away. (F3)
 (T) (F)
23. I often stop eating when I am not really full as a conscious means of limiting the amount I eat. (F1)
 (T) (F)
24. I get so hungry that my stomach often seems like a bottomless pit. (F3)
 (T) (F)

25. My weight has hardly changed at all in the last ten years. (F2)
 (T) (F)
26. I am always hungry, so it is hard for me to stop eating before I finish the food on my plate. (F3)
 (T) (F)
27. When I feel lonely, I console myself by eating. (F2)
 (T) (F)
28. I consciously hold back at meals in order not to gain weight. (F1)
 (T) (F)
29. I sometimes get very hungry late in the evening or at night. (F3)
 (T) (F)
30. I eat anything I want any time I want. (F1)
 (T) (F)
31. Without even thinking about it, I take a long time to eat. (F2)
 (T) (F)
32. I count calories/grams of fat as a conscious means of controlling my weight. (F1)
 (T) (F)
33. I do not eat some foods because they make me fat. (F1)
 (T) (F)
34. I am always hungry enough to eat at any time. (F3)
 (T) (F)
35. I pay a great deal of attention to changes in my figure. (F1)
 (T) (F)
36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods. (F2)
 (T) (F)

Each question in this section is followed by a number of options. After reading each question carefully, choose one option which most applies to you, and circle the appropriate answer.

37. How often are you dieting in a conscious effort to control your weight? (F1)

- | | | | |
|--------|-----------|---------|--------|
| 1 | 2 | ③ | ④ |
| rarely | sometimes | usually | always |

38. Would a weight fluctuation of 3 kg affect the way you live your life? (F1)

- | | | | |
|------------|----------|------------|-----------|
| 1 | 2 | ③ | ④ |
| not at all | slightly | moderately | very much |

39. How often do you feel hungry? (F3)

- | | | | |
|-----------------------|-------------------------------|---------------------------|------------------|
| 1 | 2 | ③ | ④ |
| only at
meal times | sometimes
between
meals | often
between
meals | almost
always |

40. Do your feelings of guilt about overeating help you to control your food intake? (F1)

- | | | | |
|-------|--------|-------|--------|
| 1 | 2 | ③ | ④ |
| never | rarely | often | always |

41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours? (F3)

- | | | | |
|------|-----------------------|-------------------------|-------------------|
| 1 | 2 | ③ | ④ |
| easy | slightly
difficult | moderately
difficult | very
difficult |

42. How conscious are you of what you are eating? (F1)

- | | | | |
|------------|----------|------------|-----------|
| 1 | 2 | ③ | ④ |
| not at all | slightly | moderately | extremely |

43. How frequently do you *avoid* “buying large” on tempting foods? (F1)

- | | | | |
|-----------------|--------|---------|------------------|
| 1 | 2 | ③ | ④ |
| almost
never | seldom | usually | almost
always |

44. How likely are you to shop for low calorie or low fat foods? (F1)

1	2	③	④
unlikely	slightly likely	moderately likely	very likely

45. Do you eat sensibly in front of others and splurge alone? (F2)

1	2	③	④
never	rarely	often	always

46. How likely are you to consciously eat slowly in order to cut down on how much you eat? (F1)

1	2	③	④
unlikely	slightly likely	moderately likely	very likely

47. How frequently do you skip dessert because you are no longer hungry (F3)

①	②	3	4
almost never	seldom	at least once a week	almost every day

48. How likely are you to consciously eat less than you want? (F1)

1	2	③	④
unlikely	slightly likely	moderately likely	very likely

49. Do you go on eating binges even though you are not hungry? (F2)

1	2	③	④
never	rarely	sometimes	at least once a week

50. To what extent does this statement describe your eating behaviour? (F1)

“I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.”

1	2	③	④
not like me	little like me	pretty good description of me	describes me perfectly

51. On a scale of 1 to 6, where 1 means no restraint in eating (eat whatever you want, whenever you want it) and 6 means total restraint (constantly limiting food intake and never “giving in”), what number would you give yourself? (F2)

- ① eat whatever you want, whenever you want it
- ② usually eat whatever you want, whenever you want it
- ③ often eat whatever you want, whenever you want it
- 4 often limit food intake, but often “give in”
- 5 usually limit food intake, rarely “give in”
- 6 constantly limit food intake, never “give in”

APPENDIX 5

EATING ATTITUDES TEST

Please place an (X) under the column which applies best to each of the statements. Please answer each question carefully.

	always	very often	often	some- times	rarely	never
1. I like eating with other people	()	()	()	(1)	(2)	(x)
2. I prepare food for others, but do not eat what I prepare	(x)	(2)	(1)	()	()	()
3. I become anxious prior to eating	(x)	(2)	(1)	()	()	()
4. I am terrified about being overweight	(x)	(2)	(1)	()	()	()
5. I avoid eating when I am hungry	(x)	(2)	(1)	()	()	()
6. I find myself preoccupied with food	(x)	(2)	(1)	()	()	()
7. I have gone on eating binges where I feel that I may not be able to stop	(x)	(2)	(1)	()	()	()
8. I cut my food into small pieces	(x)	(2)	(1)	()	()	()
9. I am aware of the caloric content of foods that I eat	(x)	(2)	(1)	()	()	()
10. I particularly avoid foods with a high carbohydrate content (eg bread, potatoes, rice, etc)	(x)	(2)	(1)	()	()	()
11. I feel bloated after meals	(x)	(2)	(1)	()	()	()
12. I feel that others would prefer if I ate more	(x)	(2)	(1)	()	()	()
13. I vomit after I have eaten	(x)	(2)	(1)	()	()	()
14. I feel extremely guilty after eating	(x)	(2)	(1)	()	()	()
15. I am preoccupied with a desire to be thinner	(x)	(2)	(1)	()	()	()
16. I exercise strenuously to bum off calories	(x)	(2)	(1)	()	()	()
17. I weigh myself several times a day	(x)	(2)	(1)	()	()	()
18. I like my clothes to fit tightly	()	()	()	(1)	(2)	(x)
19. I enjoy eating meat	()	()	()	(1)	(2)	(x)
20. I wake up early in the morning	(x)	(2)	(1)	()	()	()
21. I eat the same foods day after day	(x)	(2)	(1)	()	()	()
22. I think about burning up calories when I exercise	(x)	(2)	(1)	()	()	()
23. I have regular menstrual periods	()	()	()	(1)	(2)	(x)
24. Other people think that I am too thin	(x)	(2)	(1)	()	()	()
25. I am preoccupied with the thought of having fat on my body	(x)	(2)	(1)	()	()	()
26. I take longer than others to eat my meals	(x)	(2)	(1)	()	()	()
27. I enjoy eating at restaurants	()	()	()	(1)	(2)	(x)
28. I take laxatives	(x)	(2)	(1)	()	()	()
29. I avoid foods with sugar in them	(x)	(2)	(1)	()	()	()
30. I eat diet foods	(x)	(2)	(1)	()	()	()
31. I feel that food controls my life	(x)	(2)	(1)	()	()	()
32. I display self control around food	(x)	(2)	(1)	()	()	()
33. I feel that others pressure me to eat	(x)	(2)	(1)	()	()	()
34. I give too much time and thought to food	(x)	(2)	(1)	()	()	()
35. I suffer from constipation	(x)	(2)	(1)	()	()	()
36. I feel uncomfortable after eating sweets	(x)	(2)	(1)	()	()	()
37. I engage in dieting behaviour	(x)	(2)	(1)	()	()	()
38. I like my stomach to be empty	(x)	(2)	(1)	()	()	()
39. I enjoy trying rich new foods	()	()	()	(1)	(2)	(x)
40. I have the impulse to vomit after meals	(x)	(2)	(1)	()	()	()

Score: the selected column receives the corresponding score; 0, 1, 2, 3 (“x”)

APPENDIX 6

NWRLC FAT INTAKE SCALE

Check the answer which best describes the way you have been eating over the past month.

1. How many ounces of meat, fish or poultry do you usually eat?

- 1. I do not eat meat, fish or poultry.
- 2. I eat 3 ounces or less per day.
- 3. I eat 4-6 ounces per day.
- 4. I eat 7 or more ounces per day.

*3 ounces of meat, fish or chicken in any **ONE** of the following: 1 regular hamburger, 1 chicken breast, 1 chicken leg (thigh and drumstick), 1 pork chop or 3 slices of pre-sliced lunch meat.

2. How much cheese do you eat per week?

- 1. I do not eat cheese.
- 2. I eat whole milk cheese less than once a week and/or use only low fat cheese such as diet cheese, low fat cottage cheese, or ricotta.
- 3. I eat whole milk cheese once or twice per week (such as cheddar, swiss, monterey jack).
- 4. I eat whole milk cheese three or more times per week.

3. What type of milk do you use?

- 1. I use only skim or 1% milk or don't use milk.
- 2. I usually use skim milk or 1% milk but use others occasionally.
- 3. I usually use 2% or whole milk.

4. How many visible egg yolks do you use per week?

- 1. I avoid all egg yolks or use less than one per week and/or use on egg substitute.
- 2. I eat 1-2 egg yolks per week.
- 3. I eat 3 or more egg yolks per week.

5. How often do you eat these meats: regular hamburger, bologna, salami, hot dogs, corned beef, spareribs, sausage, bacon, braunsweiger, or liver? Do not count others.

- 1. I do not eat any of these meats.
- 2. I eat them about once per week or less.
- 3. I eat them about 2 to 4 times per week.
- 4. I eat more than 4 servings per week.

6. **How many commercial baked goods and how much regular ice cream do you usually eat? (Example: cake, cookies, coffee cake, sweet rolls, donuts, etc. Do not count low fat versions.)**
- 1. I do not eat commercial baked goods and ice cream.
 - 2. I eat commercial baked goods or ice cream once per week or less.
 - 3. I eat commercial baked goods or ice cream 2 to 4 times per week.
 - 4. I eat commercial baked goods or ice cream more than 4 times per week.
7. **Which is the main type of fat you cook with?**
- 1. I use nonstick spray or I do not use fat in cooking.
 - 2. I use liquid oil (Examples: safflower, sunflower, corn, soybean, and olive oil.)
 - 3. I use margarine.
 - 4. I use butter, shortening, bacon drippings, or lard.
8. **How often do you eat snack foods such as chips, fries or party cracker?**
- 1. I do not eat these snack foods.
 - 2. I eat one serving of these snacks per week.
 - 3. I eat these snacks 2 to 4 times per week.
9. **What spread do you usually use on bread, vegetables, etc?**
- 1. I do not use any spread.
 - 2. I use diet or light margarine.
 - 3. I use margarine.
 - 4. I use butter.
10. **How often do you eat as a snack candy bars, chocolate or nuts?**
- 1. Less than once per week.
 - 2. One to 3 times per week.
 - 3. More than 3 times per week.
11. **When you use recipes or convenience foods, how often are they low fat?**
- 1. Almost always.
 - 2. Usually.
 - 3. Sometimes.
 - 4. Seldom or never.
12. **When you eat away from home, how often do you choose low fat foods?**
- 1. Almost always.
 - 2. Usually.
 - 3. Sometimes.
 - 4. Seldom or never.

To score: Add the points for each answer. If you have 24 or less, your diet is moderate to low in fat and cholesterol. If your score is greater than 24, look for high fat choices you could change.

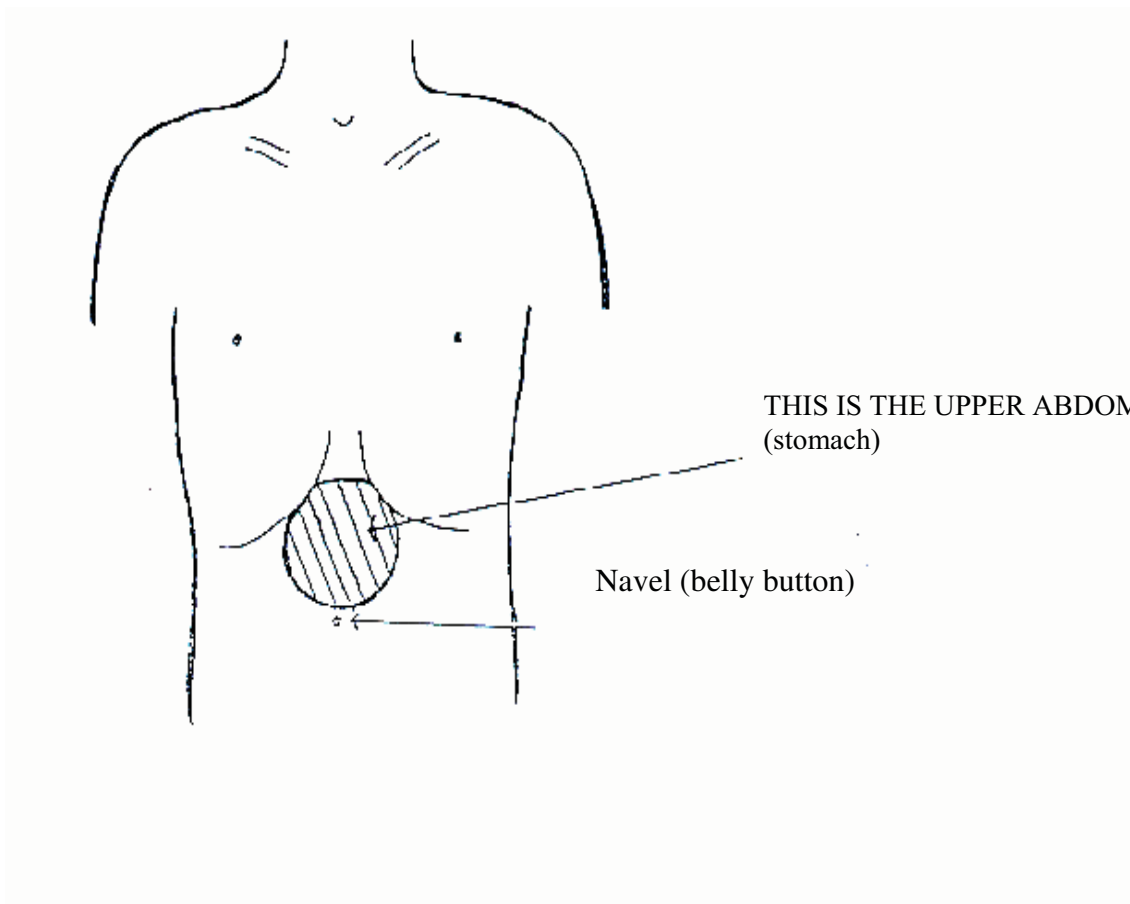
APPENDIX 7

NEPEAN DYSPEPSIA INDEX

NDI[®]

Thank you for helping us with this research. This questionnaire contains detailed questions about your stomach problems, and how they are affecting you and your life.

Some of the questions are quite personal, but the information you provide will be treated with confidentiality and sensitivity.



1. In the table below, please **WRITE NUMBERS** to indicate the frequency, intensity, and bothersomeness of any stomach problems you have had in the last **TWO WEEKS**. The diagram on the front page shows your "UPPER ABDOMEN" - please look at this diagram when answering items about problems with your "UPPER ABDOMEN".

<p>DURING THE LAST TWO WEEKS,</p> <p>DID YOU HAVE ANY OF THE FOLLOWING STOMACH PROBLEMS?</p>	<p>How OFTEN did you have it?</p> <p>0 = Not at all 1 = One to four days 2 = Five to eight days 3 = Nine to twelve days 4 = Every day / almost every day</p>	<p>If you had this problem, how INTENSE was it usually?</p> <p>0 = Not at all 1 = Very mild 2 = Mild 3 = Moderate 4 = Severe 5 = Very severe</p>	<p>If you had this problem, how BOTHERSOME was it?</p> <p>0 = Not at all 1 = A little bit 2 = Moderately 3 = Quite a bit 4 = Extremely</p>
PAIN OR ACHE IN UPPER ABDOMEN			
DISCOMFORT IN UPPER ABDOMEN			
BURNING SENSATION IN UPPER ABDOMEN			
BURNING SENSATION IN CHEST (HEARTBURN)			
CRAMPS IN UPPER ABDOMEN			
PAIN OR ACHE IN CHEST			
INABILITY TO FINISH A REGULAR MEAL			
BITTER / SOUR TASTING FLUID THAT COMES UP INTO YOUR MOUTH OR THROAT			
FULLNESS AFTER EATING OR SLOW DIGESTION			
PRESSURE IN UPPER ABDOMEN			
BLOATING IN UPPER ABDOMEN			
NAUSEA			
BURPING / BELCHING			
VOMITING			
BAD BREATH			

THE FIGURE ON THE FRONT PAGE SHOWS YOUR **UPPER ABDOMEN**. THE REMAINING QUESTIONS WHICH ASK ABOUT "STOMACH PROBLEMS" ARE REFERRING TO PAIN, DISCOMFORT, OR OTHER PROBLEMS WITH YOUR **UPPER ABDOMEN**.

1. Have your stomach problems (remember, this means pain, discomfort or other problems in your **upper abdomen**) been interfering with your daily activities during the **last 2 weeks**?

- 1 STOMACH PROBLEMS DID NOT INTERFERE WITH USUAL ACTIVITIES
- 2 STOMACH PROBLEMS DISTRACTED ME FROM MY USUAL ACTIVITIES
- 3 STOMACH PROBLEMS PREVENTED MY USUAL ACTIVITIES
- 4 STOMACH PROBLEMS HAVE REQUIRED BEDREST

2. Have you felt UPSET or ANNOYED in the **last 2 weeks** by not being able to adequately CONTROL or CURE your stomach problems?

- 1 NOT AT ALL OR NOT APPLICABLE (my symptoms are adequately controlled or cured)
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

3. Have you felt UPSET or ANNOYED in the **last 2 weeks** by NOT KNOWING what CAUSES your stomach problems?

- 1 NOT AT ALL OR NOT APPLICABLE (I know what causes my stomach problems)
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

IN THE NEXT QUESTIONS, "DRINKING" MEAN BOTH NON-ALCOHOLIC DRINKS (SOFT DRINKS, JUICE, MILK, WATER, TEA, COFFEE, ETC) AND ALCOHOLIC DRINKS (WINE, BEER, SPIRITS, ETC). PLEASE THINK ABOUT ALL THESE TYPE OF DRINKS WHEN ANSWERING THE NEXT THREE QUESTIONS.

4. Has your ABILITY to EAT or DRINK (including when, what, and how much) been disturbed by your stomach problems in the **last 2 weeks**?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

QUESTIONS ASKING ABOUT "STOMACH PROBLEMS" REFER TO PAIN, DISCOMFORT, OR OTHER PROBLEMS WITH YOUR **UPPER ABDOMEN**.

5. Did you **CHANGE YOUR DIET** because of your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY
6. In the **last 2 weeks**, have your stomach problems disturbed your **ENJOYMENT** of **EATING** and/or **DRINKING** (including your appetite, and how you feel afterwards)?
- 1 NOT AT ALL
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY
7. Has your **ABILITY** to **SLEEP** been disturbed by your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY
8. Has the **QUALITY** of your **SLEEP** been disturbed by your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY
9. Has your **ABILITY** to **WORK** or **STUDY** been disturbed by your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL OR NOT APPLICABLE (I do not work or study)
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY

10. Has your ENJOYMENT of WORK or STUDY been disturbed by your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL OR NOT APPLICABLE (I have not worked or studied in the last 2 weeks)
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY
11. Excluding work or study, has your ABILITY to perform your usual daily tasks (like housework, yard work, or other necessary activities of everyday life) been disturbed by your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY
12. Excluding work or study, has your ENJOYMENT of your usual daily tasks (like housework, yard work, or other necessary activities of everyday life) been disturbed by your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY
13. Has your ENJOYMENT of time spent with FRIENDS or other SOCIAL ACTIVITIES been disturbed by your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL OR NOT APPLICABLE
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY
14. Has your ABILITY to engage in things you usually do for LEISURE (like recreation, going out, hobbies, sports) been disturbed by your stomach problems in the **last 2 weeks**?
- 1 NOT AT ALL
 - 2 A LITTLE
 - 3 MODERATELY
 - 4 QUITE A LOT
 - 5 EXTREMELY

15. Has your ENJOYMENT of things you usually do for LEISURE (like recreation, going out, hobbies, sports) been disturbed by your stomach problems in the **last 2 weeks**?

- 1 NOT AT ALL OR NOT APPLICABLE (I have not been able to do any of these things in the past 2 weeks)
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

QUESTIONS ASKING ABOUT "STOMACH PROBLEMS" REFER TO PAIN,
DISCOMFORT, OR
OTHER PROBLEMS WITH YOUR **UPPER ABDOMEN**.

16. Has your GENERAL EMOTIONAL WELL-BEING been disturbed by your stomach problems in the **last 2 weeks**?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

17. Have you been ANXIOUS, NERVOUS, or WORRIED in the **last 2 weeks** because of your stomach problems?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

18. Have you been DEPRESSED, SAD, or MISERABLE in the **last 2 weeks** because of your stomach problems?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

19. Have you been IRRITABLE, TENSE or FRUSTRATED in the **last 2 weeks** because of your stomach problems?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

20. Have you felt HELPLESS or LACKING EMOTION or MOTIVATION in the **last 2 weeks** because of your stomach problems?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

21. Have you had DIFFICULTY THINKING or CONCENTRATING in the **last 2 weeks** because of your stomach problems?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

QUESTIONS ASKING ABOUT "STOMACH PROBLEMS" REFER TO PAIN, DISCOMFORT, OR OTHER PROBLEMS WITH YOUR **UPPER ABDOMEN**.

22. Have you WONDERED whether your stomach problems might be due to a very SERIOUS illness (such as cancer or a heart problem), in the **last 2 weeks**?

- 1 ALMOST NEVER
- 2 SOMETIMES
- 3 FAIRLY OFTEN
- 4 VERY OFTEN
- 5 ALWAYS

23. Have you WONDERED whether you will ALWAYS have these stomach problems, in the **last 2 weeks**?

- 1 ALMOST NEVER
- 2 SOMETIMES
- 3 FAIRLY OFTEN
- 4 VERY OFTEN
- 5 ALWAYS

24. Have you felt TIRED, WEAK OR LOW IN ENERGY in the **last 2 weeks** because of your stomach problems?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

25. Has your OVERALL HEALTH been affected by your stomach problems in the **last 2 weeks**?

- 1 NOT AT ALL
- 2 A LITTLE
- 3 MODERATELY
- 4 QUITE A LOT
- 5 EXTREMELY

26. Please rate the following items according to their importance in determining the QUALITY OF YOUR LIFE.

Take your time, and think carefully about each item. Use the following scale:

- 1 = Not at all important
- 2 = Somewhat important
- 3 = Moderately important
- 4 = Very important
- 5 = Extremely important

GENERALLY, HOW IMPORTANT IN DETERMINING THE QUALITY OF YOUR LIFE IS:
(Rate 1 - 5 as above)

- A) UNDERSTANDING THE CAUSE and ability to CONTROL the stomach problems _____
- B) EATING OR DRINKING (when, what, how much, and being able to enjoy it)..... _____
- C) SLEEP..... _____
- D) WORK (or study, if a student) _____
- E) USUAL DAILY TASKS, like housework, yardwork, and other necessary activities of everyday life (excluding work or study) _____
- F) Spending time with FRIENDS, and other SOCIAL activities..... _____
- G) Things you usually do for LEISURE (like recreation, going out, hobbies, sports)..... _____
- H) Your EMOTIONAL state _____
- I) Your ability to THINK and CONCENTRATE _____
- J) CONSIDERING your illness (for example, "it could be cancer" or "it may never get better") _____
- K) Your ENERGY levels and how generally WELL you feel _____

APPENDIX 8

EYSENCK

The following questions refer to how you have been feeling generally, not just how you feel today. Please answer each question by putting a circle around the 'YES' or 'NO' that follows each question. There are no right or wrong answers, and no trick questions. Please work quickly and do not think too long about the exact meaning of the question.

		YES	NO
1.	Does your mood often go up and down?	1	2
2.	Are you a talkative person?	1	2
3.	Do you ever feel 'just miserable' for no reason?	1	2
4.	Are you rather lively?	1	2
5.	Are you an irritable person?	1	2
6.	Do you enjoy meeting new people?	1	2
7.	Are your feelings easily hurt?	1	2
8.	Can you usually let yourself go and enjoy yourself at a lively party?	1	2
9.	Do you often feel 'fed up'?	1	2
10.	Do you usually take the initiative in making new friends?	1	2
11.	Would you call yourself a nervous person?	1	2
12.	Can you easily get some life into a rather dull party?	1	2
13.	Are you a worrier?	1	2
14.	Do you tend to keep in the background on social occasions?	1	2
15.	Would you call yourself highly strung?	1	2
16.	Do you like mixing with people?	1	2
17.	Do you worry too long after an embarrassing experience?	1	2
18.	Do you like plenty of bustle and excitement around you?	1	2
19.	Do you suffer from nerves?	1	2
20.	Are you mostly quiet when you are with other people?	1	2
21.	Do you often feel lonely?	1	2
22.	Do other people think of you as being lonely?	1	2
23.	Are you often troubled about feelings of guilt?	1	2
24.	Can you get a party going?	1	2

Score: Yes = 1, No = 0 (questions 1 - 13 and 15 - 19 and 21 - 24)

Yes = 0, No = 1 (Question 14 and 20)

APPENDIX 9

HAD (VI)

Instructions: Read each item and place a firm cross in each box opposite the reply which comes closest to how you have been feeling in the past week

1. I feel tense or 'wound up':

- | | |
|-----------------------------|--------------------------|
| Most of the time | <input type="checkbox"/> |
| A lot of the time | <input type="checkbox"/> |
| Time to time / occasionally | <input type="checkbox"/> |
| Not at all | <input type="checkbox"/> |

2. I still enjoy the things I used to enjoy:

- | | |
|--------------------|--------------------------|
| Definitely as much | <input type="checkbox"/> |
| Not quite as much | <input type="checkbox"/> |
| Only a little | <input type="checkbox"/> |
| Hardly at all | <input type="checkbox"/> |

3. I get sort of frightened feeling as if something awful was about to happen:

- | | |
|----------------------------------|--------------------------|
| Very definitely and quite badly | <input type="checkbox"/> |
| A lot of the time | <input type="checkbox"/> |
| A little but it doesn't worry me | <input type="checkbox"/> |
| Not at all | <input type="checkbox"/> |

4. I can laugh and see the funny side of things:

- | | |
|----------------------------|--------------------------|
| As much as I always could | <input type="checkbox"/> |
| Not quite as much now | <input type="checkbox"/> |
| Definitely not so much now | <input type="checkbox"/> |
| Not at all | <input type="checkbox"/> |

5. I feel as if I am slowed down:

- | | |
|---------------------|--------------------------|
| Nearly all the time | <input type="checkbox"/> |
| Very often | <input type="checkbox"/> |
| Sometimes | <input type="checkbox"/> |
| Not at all | <input type="checkbox"/> |

6. I get a sort of frightened feeling like 'butterflies' in the stomach:

- | | |
|--------------|--------------------------|
| Not at all | <input type="checkbox"/> |
| Occasionally | <input type="checkbox"/> |
| Quite often | <input type="checkbox"/> |
| Very often | <input type="checkbox"/> |

7. I have lost interest in my appearance:

- | | |
|---------------------------------------|--------------------------|
| Definitely | <input type="checkbox"/> |
| I don't take so much care as I should | <input type="checkbox"/> |
| I may not take quite so much care | <input type="checkbox"/> |
| I take just as much care as ever | <input type="checkbox"/> |

8. I feel restless as if I have been on the move:

- Very much indeed 4
 Quite a lot 3
 Not very much 2
 Not at all 1

9. Worrying thoughts go through my mind:

- A great deal of the time 4
 A lot of the time 3
 From time to time but not that often 2
 Not at all 1

10. I feel cheerful:

- Not at all 4
 Not often 3
 Sometimes 2
 Most of the time 1

11. I can sit at ease and relaxed:

- Definitely 1
 Usually 2
 Not often 3
 Not at all 4

12. I look forward with enjoyment to things:

- As much as I ever did 1
 Rather less than I used to 2
 Definitely 3
 Hardly at all 4

13. I get sudden feelings of panic:

- Very often indeed 4
 Quite often 3
 Not very often 2
 Not at all 1

14. I can enjoy a good book or radio or TV program:

- Often 1
 Sometimes 2
 Not often 3
 Very seldom 4

APPENDIX 10

ZUNG SELF-RATING SCALE

Please answer the questions by marking the box that best describes your response. If a question does not apply, mark the box that is closest to answering your question.

	None or a little of the time	Some of the time	Good part of the time	Most or all of the time
1. I feel downhearted, blue and sad	1	2	3	4
2. Morning is when I feel the best	4	3	2	1
3. I have crying spells or feel like it	1	2	3	4
4. I have trouble sleeping through the night	1	2	3	4
5. I eat as much as I used to do	4	3	2	1
6. I enjoy looking at, talking to, an being with attractive women/men	4	3	2	1
7. I notice that I am losing weight	1	2	3	4
8. I have trouble with constipation	1	2	3	4
9. My heart beats faster than usual	1	2	3	4
10. I get tired for no reason	1	2	3	4
11. My mind is as clear as it used to be	4	3	2	1
12. I find it easy to do the things I used to	4	3	2	1
13. I am restless and can't sleep	1	2	3	4
14. I feel hopeful about the future	4	3	2	1
15. I am more irritable than usual	1	2	3	4
16. I find it easy to make decisions	4	3	2	1
17. I feel that I am useful and needed	4	3	2	1
18. My life is pretty full	4	3	2	1
19. I feel that others would be better off if I were dead	1	2	3	4
20. I still enjoy the things I used to do	4	3	2	1

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