Gastric and small intestinal motor function in health and disease – implications for glucose absorption, incretin hormone release, and postprandial blood glucose regulation

A thesis submitted by

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Thesis summary

The human digestive tract is a complex system that, in addition to the digestion and absorption of nutrients, serves an important neuroendocrine role. The focus of this thesis is to examine how changes in the motor function of the gastroduodenal region influence glucose absorption, gut hormone secretion, and postprandial blood glucose regulation, in different human populations, including the healthy young and those with cystic fibrosis. The studies included utilise a mix of established and novel techniques to evaluate gastroduodenal motor function and glucose absorption, and provide insights into the function of the human gut.

Strict overall glycaemic control dramatically reduces the incidence and progression of micro-, and probably macrovascular, complications associated with type 1 and type 2 diabetes. Postprandial glycaemia is now recognised as an important determinant of overall glycaemia, as indicated by the glycated haemoglobin (HbA1c). The rate of glucose absorption after a meal has a major influence on postprandial glycaemia and has, therefore, been a focus of increasing research interest in recent years. Postprandial blood glucose concentrations are a poor indicator of glucose absorption due to peripheral glucose uptake and hepatic glucose release. The glucose analogue 3-O-methylglucose (3-OMG) is absorbed in the small intestine by the same mechanism as glucose, but is not metabolised, and its plasma concentrations are widely used as an index of glucose absorption. However, analysis of plasma 3-OMG concentrations requires chromatographic

methods which are labour-intensive and costly. By labeling 3-OMG with the ^{14}C radioisotope, plasma ^{14}C-3-OMG activity can be measured by the rapid and inexpensive method of liquid scintillation counting. In Chapter 6, plasma ^{14}C-3-OMG activity was shown to correlate closely to plasma concentrations of 3-OMG, after concomitant oral administration. ^{14}C-3-OMG therefore represents a convenient alternative to 3-OMG, for measuring enteral glucose absorption.

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are secreted by the L and K cells in the intestines respectively, in response to nutrient-gut interactions. Their main function is the augmentation of glucose-induced insulin release from the pancreas, the so-called "incretin effect". GLP-1 also possesses a potent inhibitory effect on gastric emptying, arguably the dominant mechanism through which GLP-1 lowers postprandial blood glucose. However, unlike GIP, the release of which is roughly proportional to the amount of glucose entering the small intestine, a caloric threshold of 1.8 kcal/min has been reported to exist for the release of GLP-1, below which the GLP-1 secretory mechanism is not stimulated. In the study described in Chapter 7, by performing a retrospective analysis of data collated from several studies performed previously, a transient, early release of GLP-1, in response to intraduodenal glucose delivery at the rate of 1 kcal/min, was demonstrated. While the functional significance of this observation remains uncertain, this early release of GLP-1 might serve the role of "priming" the gluco-regulatory system, in anticipation for the subsequent arrival of a larger nutrient

load. Furthermore, the mechanism for this early, transient release of GLP-1 remains to be further investigated, as the GLP-1 secreting intestinal L-cells are located most densely in the distal, rather than the proximal, small intestine.

It is established that differences in the rate of gastric emptying contribute to approximately one-third of the variation in the initial rise in postprandial glycaemia, but the contribution made by duodenal motor activity is much less well defined. An increase in duodenal motility, as measured by the number of pressure waves and propagated pressure wave sequences, has been shown to be associated with increased glucose absorption. More recently, using a combined manometry and impedance monitoring technique, it has been demonstrated that duodenal flow events may be a more important determinant of glucose absorption than pressure waves. Impedance monitoring is capable of measuring intraluminal movement of both fluid and air, and can be used in the proximal small intestine to measure the flow of intraluminal chyme. Compared to manometry, impedance monitoring correlates better with fluoroscopy, for measuring movement of small intestinal intraluminal content. In Chapter 8, it was demonstrated that despite stimulating duodenal pressure waves pharmacologically, using the prokinetic agent metoclopramide, there was no concomitant increase in the number of duodenal flow events, as measured by impedance monitoring, and no associated change in glucose absorption. These findings are consistent with those of a previous study using the anti-motility agent, hyoscine butylbromide, and both reinforce the importance of duodenal flow events in determining glucose

absorption, and highlight the value of combining impedance monitoring with manometry in assessing small intestinal motor function and nutrient absorption.

Delayed gastric emptying affects up to 50% of outpatients with long-standing type 1 and type 2 diabetes, often causing persistent upper gut symptoms that are difficult to manage. Acute hyperglycaemia, in a dose-dependent manner, exerts a number of reversible effects on upper gut motor function, including relaxation of the gastric fundus, suppression of antral motility, stimulation of pyloric contractions, and slowing of gastric emptying. In contrast to gastric motor function, data regarding the effects of hyperglycaemia on small intestinal motor function are scarce. Furthermore, there is little information regarding the effects of hyperglycaemia on incretin hormone release and intestinal glucose absorption. The study described in Chapter 9, using the combined manometry and impedance monitoring technique, demonstrated that acute hyperglycaemia in the physiological postprandial range (~9 mmol/L) had minimal impact on duodenal pressure waves and flow events, but reduced fasting plasma GLP-1 concentrations, and increased postprandial GIP secretion and small intestinal glucose absorption. The mechanism for these observations remains to be determined, but may involve changes in the small intestinal mucosa related to hyperglycaemia.

Nitric oxide is a major inhibitory neurotransmitter in the gut, and an increase in its availability has effects on gastropyloric motility and gastric emptying that are

similar to those observed during acute hyperglycaemia. Therefore, nitric oxide may be a mediator of the effects of hyperglycaemia on upper gut motor function. Using the specific nitric oxide synthase inhibitor, NG-nitro-L-arginine-methylester (L-NAME), the study described in Chapter 10 demonstrated that the delay in gastric emptying induced by acute hyperglycaemia (~15 mmol/L) was indeed mediated by nitric oxide, and may involve the modulation of tonic pyloric activity. In addition, nitric oxide may be involved in the release of insulin.

Cystic fibrosis (CF) affects approximately 1 in 2,500 live births in western societies, and the life-expectancy of these patients has risen dramatically as a result of improved medical care. However, this is accompanied by a rapid rise in many long term co-morbidities such as diabetes, which affects ~75 % of all CF patients by the age of 30. Cystic fibrosis-related diabetes (CFRD) is distinct from type 1 and type 2 diabetes, and is characterised by postprandial, rather than fasting, hyperglycaemia. Persistent fat malabsorption occurs in up to 20 % of CF patients, despite pancreatic enzyme supplementation, and fat malabsorption is known to accelerate gastric emptying in both healthy subjects and type 2 diabetes patients. The breakdown of fat is also required to stimulate the release of incretin hormones from the gut. Therefore, fat digestion in CF may be an important factor in determining the rate of gastric emptying and incretin hormone secretion, and consequently, postprandial glycaemia. The study described in Chapter 11 demonstrated that without pancreatic enzyme supplementation, CF patients had more rapid gastric emptying, reduced incretin hormone secretion, and exaggerated

postprandial glycaemic excursions compared to healthy subjects, after a solid high fat, high carbohydrate meal, and that these abnormalities were either substantially improved or normalised by pancreatic enzyme supplementation. Furthermore, the failure of enzyme supplementation to normalise GIP secretion, as opposed to the complete restoration of the GLP-1 response, suggests that mixing of enzymes with food in the proximal small intestine, where GIP-secreting K cells are predominantly located, is suboptimal. Therefore, strategies to optimise mixing between food and enzymes in the proximal small intestine, or incretin-based approaches such as the administration of GIP analogues, represent potential novel approaches in the management of postprandial hyperglycaemia and diabetes in CF.

The recent rapid rise in the prevalence of type 2 diabetes, and the importance of good overall glycaemic control in reducing the long term complications of diabetes, has prompted intense research into new ways to optimise diabetes management. Gastroduodenal motor function has a major influence on glucose absorption, incretin hormone secretion, and postprandial glycaemia, and thus represents an ideal therapeutic target, illustrated by the recent development of GLP-1-based therapies (such as the GLP-1 analogue exenatide, and the DPP-IV inhibitor sitagliptin) for the treatment of type 2 diabetes. However, many areas are still incompletely understood. Further studies are warranted to investigate the relationships between gastroduodenal motor function, glucose absorption, and

incretin hormone secretion, and their impact on postprandial blood glucose regulation.

Declaration

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Publications arising from this thesis

The materials in this thesis formed the basis for the publications listed below:

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Kuo P, Chaikomin R, Pilichiewicz A, O'Donovan D, Wishart JM, Meyer JH, Jones KL, Feinle-Bisset C, Horowitz M, and Rayner CK. Transient, early release of glucagon-like peptide-1 during low rates of intraduodenal glucose delivery. Regulatory Peptides 2008;7;146(1-3):1-3.

Kuo P, Gentilcore D, Nair N, Stevens JE, Hausken T, Gilja OH, Wishart JM, Lange K, Horowitz M, Jones KL, and Rayner CK. The nitric oxide (NO) synthase inhibitor, NG-nitro-L-arginine-methyl-ester (L-NAME), attenuates the delay in gastric emptying induced by hyperglycaemia in healthy humans. Neurogastroenterol Motil 2009;21(11):1175-e103.

Kuo P, Bellon M, Wishart JM, Smout AJ, Holloway RH, Fraser RJ, Horowitz M, Jones KL, Rayner CK. Effects of metoclopramide on duodenal motility and flow events, glucose absorption, and incretin hormone release, in response to intraduodenal glucose infusion. (Submitted for publication)

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Kuo P, Wishart JM, Bellon M, Smout AJ, Holloway RH, Fraser RJ, Horowitz M, Jones KL, Rayner CK. Effects of physiological hyperglycaemia on duodenal motility and flow events, glucose absorption, and incretin hormone secretion in healthy humans. (Submitted for publication)

Kuo P, Chaikomin R, Bellon M, Jones KL, Horowitz M, Rayner CK. Validation of ^{14}C-3-O-methylglucose as a measure of intestinal glucose absorption in humans. (Submitted for publication)

Gastric and small intestinal motor function in health and disease – implications for glucose absorption, incretin hormone release, and postprandial blood glucose regulation

Chapter 1:

Normal physiology of the upper gut

1.1   Introduction

The human gastrointestinal tract is a complex system, beginning at the mouth and ending at the anus, incorporating the oesophagus, the stomach, and the small and large intestines in between. Each of these compartments is separated by a dedicated muscular sphincter that regulates the entry of contents from one compartment into another. The lower oesophageal sphincter separates the stomach and the oesophagus, relaxing to allow passage of ingesta into the stomach, and contracting to prevent reflux of gastric content into the oesophagus; the pylorus separates the stomach and the small intestine, relaxing to allow passage of nutrient between the two compartments, predominantly from the stomach to the duodenum; and the ileocaecal valve separates the small and large intestines. Each part of the gastrointestinal tract serves its own particular set of functions, and together with the liver, gallbladder, and pancreas, form the complex human digestive system.

Embryologically, the human gut is derived from three anatomically distinct origins: the foregut, midgut, and hindgut. The foregut gives rise to all structures

from the mouth to the second part of the duodenum, proximal to the opening of the common bile duct, including the liver, the biliary apparatus, and the pancreas; the hindgut forms all structures from the distal transverse colon to the anus; and all the gut in between arises from the midgut. The term "upper gut" is used in this thesis with particular reference to the stomach, the pylorus, and the proximal small intestine, as these three regions are functionally inseparable, linked by complex mechanical and neuroendocrine mechanisms.

The traditional view that the function of the gastrointestinal tract is merely to process and absorb nutrient understates the importance of this system. The stomach and small intestine, in particular, serve as an important neuroendocrine unit in the body, and together with the central nervous system, play a major role in nutrient processing, hormone secretion, sensory generation, and appetite regulation. These regions interact with one another via a myriad of hormonal and neural feedback loops which are incompletely understood.

The interactions between upper gut motor function, glucose absorption, incretin hormone secretion, and postprandial blood glucose regulation are complex, with specific implications for the management of diabetes mellitus. This chapter aims to provide an overview of the anatomy and physiology of the upper gut in healthy humans. The central role that the upper gut plays in diabetes will be discussed separately in Chapter 2.

1.2 Motor function of the stomach and proximal small intestine

The stomach is divided anatomically into the fundus, the corpus (or body), and the antrum. Functionally, however, it is more appropriate to divide the stomach into proximal (fundus and upper body) and distal (lower body and antrum) regions (Kelly 1980). When empty, the stomach is only slightly larger in calibre than the large intestine, but can expand up to a capacity of 2 - 3 litres. The stomach's blood supply is derived from branches of the coeliac trunk, and innervation is via the parasympathetic (vagal) and sympathetic autonomic nerves.

1.2.1 Proximal stomach

The proximal stomach has a great capacity to relax and expand in volume, serving as an initial storage compartment for ingesta, regulating the subsequent entry into the distal stomach, where further nutrient processing takes place. Furthermore, it acts as a reservoir for large nutrient loads that would otherwise overwhelm the much smaller distal stomach.

The proximal stomach stores ingested food via both receptive (occurring within seconds of swallowing) and adaptive (induced by mechanical distension of the stomach together with neurohormonal feedback from the duodenum) relaxation (Feinle et al. 2001; Smith 2005). Gastric accommodation is a vagally-mediated

response, with gastric tone determined by the balance between cholinergic excitatory drive and non-adrenergic non-cholinergic (NANC) inhibitory input involving nitric oxide (Desai et al. 1991; Tack et al. 2002). The reduction in gastric tone that occurs with meal ingestion allows gastric distension to occur without a significant increase in intragastric pressure (De Schepper et al. 2004).

1.2.2 Distal stomach

The distal stomach has a thick, muscular wall with much less capacity to expand than the proximal stomach. Its main function is, therefore, as a mechanical grinder that mixes ingesta with gastric secretions and grinds solids into small particles of the order of 1 - 2 mm, prior to their emptying into the duodenum, through the intermittently relaxing pylorus. Nutrient is delivered into the small intestine at an optimal caloric rate for digestion and absorption, averaging 2 - 3 kcal/min (Brener et al. 1983), after an initially more rapid phase of emptying (5 - 15 min) of about 6 kcal/min (Kaplan et al. 1992; Horowitz et al. 2001).

1.2.3 Pylorus

The pylorus is a muscular sphincter that separates the stomach from the small intestine. It undergoes both tonic and phasic contractions to regulate the entry of nutrient into the small intestine. Its actions are regulated by feedback mechanisms arising from exposure of the small intestine to nutrients, via both hormonal

(Schirra et al. 2000) and neural (Treacy et al. 1992), pathways. This feedback increases pyloric contractile activity and reduces the rate of subsequent nutrient delivery into the duodenum (Heddle et al. 1988; Heddle et al. 1989; Rayner et al. 2000; Feltrin et al. 2004).

The pylorus demonstrates two major patterns of pressure waves during the fasting period, namely pressure waves confined to a narrow pyloric zone (isolated pyloric pressure waves [IPPWs]) and those that are less well localised and also involve the antrum and/or duodenum (Heddle et al. 1988). Postprandially, pyloric motor activity is dominated by IPPWs and localised tonic contractions (Heddle et al. 1988). Most phasic contractions occur within a zone <9 mm in length, while tonic contractions are confined to an even narrower zone (<3 mm) (Heddle et al. 1988; Heddle et al. 1988).

1.2.4 Duodenum

The duodenum was named from the latin word "duodeni" ("twelve"), because it is usually about twelve fingerbreadths long (~25 cm). It is divided anatomically into 4 parts (parts 1 - 4, or superior, descending, horizontal, and ascending respectively). The common bile duct and the main pancreatic duct open into the posteromedial wall of the second part of the duodenum. The ducts join to form the hepatopancreatic ampulla (or ampulla of Vater), which opens via the major duodenal papilla, incorporating a sphincter (sphincter of Oddi) which controls the

entrance of bile and pancreatic secretions into the duodenum and prevents reflux of duodenal content into the pancreaticobiliary system. The duodenum's blood supply is derived from the coeliac and superior mesenteric arteries. It is densely populated by various hormone-producing and nutrient-absorbing cells (e.g. L and K cells for the secretion of glucagon-like peptide-1 [GLP-1] and glucose-dependent insulinotropic polypeptide [GIP] respectively, and the glucose receptors SGLT-1 and GLUT-2), as well as being richly innervated by nerve fibres derived from the vagus and sympathetic nerves via plexi accompanying the arteries.

Functionally, the duodenum mixes small food particles with digestive enzymes, regulates gastric emptying via both mechanical means and neuroendocrine feedback loops, absorbs digested nutrients, and controls postprandial glycaemic excursions via the secretion of incretin hormones (the neuroendocrine feedback loops and incretin hormones will be discussed further in Chapter 1.3). Such responses are triggered by the presence of digestive products in the duodenal lumen, or "nutrient-gut interaction". Contractile activity in the duodenum serves to mix nutrient with digestive enzymes, and can lead to either antegrade or retrograde flow, the latter partially slowing gastric emptying, or causing reflux of chyme back into the stomach.

1.2.5 Gastric emptying and its regulation

Gastric emptying results from the coordinated activities of the proximal and distal stomach, together with the upper small intestine (Rayner and Horowitz 2005). An impairment of function of one region is usually compensated for by the others, so that disordered emptying usually implies dysfunction of multiple gastroduodenal regions (Rayner and Horowitz 2005).

The overall pattern of gastric emptying is dependent on the physical and chemical composition of a meal (Gentilcore et al. 2003). Solids empty relatively slowly in an overall linear fashion, after an initial lag phase (20 - 60 min); non-nutrient liquids empty rapidly in an overall mono-exponential pattern, but as the caloric content of liquids increases, emptying slows and approximates a linear pattern (Collins et al. 1983; Gentilcore et al. 2003). When liquids and solids are consumed together, liquids empty preferentially, so that up to 80 % of liquids may empty prior to solid emptying (Horowitz et al. 1989). Increasing the viscosity of a meal slows gastric emptying, independent of any change to antropyloric motility (Russell and Bass 1985). These different patterns of emptying reflect the requirement of solids to be ground into small particles before they empty (Horowitz et al. 2001), and varying degrees of inhibitory feedback from the small intestine (Rayner et al. 2001), determined primarily by the caloric load, and by the length of small intestine exposed to nutrient (Little et al. 2006). Previously, based on animal models, it was thought that larger solid particles (>3 mm) relied on

antral phase III motor activity (of the migrating motor complex [MMC]) to empty from the stomach; however, more recently in humans, emptying of solid particles up to 7 mm has been observed independent of phase III activity (Stotzer and Abrahamsson 2000).

Gastric emptying of fat may be considered separately from that of liquids and solids, as in the upright position, fat tends to redistribute to the proximal stomach due to its low density (Edelbroek et al. 1992; Horowitz et al. 1993). Both its redistribution and its high caloric content means that fat is likely to empty more slowly than liquids or solids containing little or no fat. In a study looking at the effect of posture on the gastric emptying of a combined oil and low nutrient aqueous meal, oil was observed to empty faster than the aqueous meal in the decubitus posture and slower than the aqueous meal in the upright posture, but the amount of oil emptied at 180 minutes was not different between the two postures (Horowitz et al. 1993).

As discussed, the stomach empties at a relatively constant rate of 2 - 3 kcal/min, regulated by neural and hormonal feedback mechanisms generated by nutrient-gut interactions in the small intestine (Horowitz et al. 2002). Direct infusion of nutrient into the small intestine leads to relaxation of the gastric fundus, suppression of antral contractions, and stimulation of tonic and phasic pyloric motor activity (Heddle et al. 1988; Heddle et al. 1989; Rayner et al. 2000; Feltrin et al. 2004). Tonic and phasic contractions of the pylorus, as well as contractions

of the duodenum, play an important role in the regulation of gastric emptying by acting as a "brake" (Horowitz et al. 1994; Gentilcore et al. 2003). Flow in the duodenum helps regulate gastric emptying in a number of ways (Rao et al. 1996; Camilleri 1997; Castedal et al. 1998; Andrews et al. 2001): rapid clearance is thought to facilitate emptying, delayed clearance impedes emptying (Rao et al. 1996; Nguyen et al. 1997), and duodenogastric reflux returns content to the stomach, also effectively slowing emptying (Castedal et al. 1998). Various hormones have been implicated as mediators of small intestinal feedback on gastric motility, including cholecystokinin (CCK), GLP-1, peptide-YY (PYY), amylin, and ghrelin (Gentilcore et al. 2003; Little et al. 2005). The neural feedback mechanisms are less well understood, but for fundic relaxation at least, there is strong evidence for the involvement of nitric oxide, the major inhibitory neurotransmitter in the gut (Desai et al. 1991; Tack et al. 2002; De Schepper et al. 2004).

1.2.6 Transpyloric flow

Contractile activity in the antropyloroduodenal region can lead to either forward, interrupted, or reversed flow across the pylorus (Hausken et al. 1992; Malbert and Mathis 1994), with the overall rate of gastric emptying being determined by the sum of the various flow patterns and their spatiotemporal relationships (Sun et al. 1997; Castedal et al. 1998). Transpyloric flow of chyme is predominantly pulsatile, as a result of peristaltic antral contractions (the "peristaltic pump"), but

tonic contractile activity also results in a common cavity antroduodenal pressure difference (the "pressure pump"), which drives emptying during relative antral quiescence (Anvari et al. 1995; Hausken et al. 1998; Indireshkumar et al. 2000). The relative contribution of each is likely to differ over time after a meal, and possibly influenced by meal composition. For example, the pressure pump, rather than the peristaltic pump, has been suggested to be the dominant mechanism for liquid emptying (Anvari et al. 1995; Indireshkumar et al. 2000). The volume of transpyloric flow appears to be enhanced by increased proximal gastric tone, and inhibited by increased pyloric tone, while antral contractions correlate with the presence but not the volume of flow (Paterson et al. 2000).

Duodenogastric reflux is a normal physiological phenomenon that occurs frequently during gastric emptying, and can occur either in the middle of the peristaltic cycle (mid-cycle reflux), or following immediately after antegrade flow at the end of the cycle (end-cycle reflux); its function and significance, however, are unknown (Hausken et al. 1992).

1.2.7 Small intestinal transit

Little is known about the characteristics of small intestinal transit of chyme. Limited evidence suggests that, in contrast to the stomach, there is little, if any, difference in the way solids and liquids progress through the small intestine (Malagelada et al. 1984). It has been suggested that during the initial postprandial

period, transit through the small intestine may be relatively more rapid, triggering an early rise in GLP-1 secretion (Chaikomin et al. 2008).

1.2.8 Gastroduodenal motility

Contractions of the distal stomach (antrum and pylorus), as well as the small intestine, are controlled by electrical slow waves generated by specialised pacemaker cells, the interstitial cells of Cajal, in the greater curvature of the stomach, located in the myenteric plexus between the longitudinal and circular muscle layers, which discharge at a rate of about 3 per minute (Sanders 1996; Horowitz et al. 2002). This activity can be recorded as "slow waves" from electrodes applied to the skin overlying the stomach, a technique termed "electrogastrography" (EGG) (Koch 2001). The slow waves generated by the interstitial cells of Cajal initiate contractile activity via the generation of action potentials, the frequency and amplitude of which are subject to modulation by neural and hormonal inputs, thus generating different patterns of motility.

1.2.8.1 Fasting motility

During fasting, the stomach and small intestine display cyclical activity termed the "migrating motor complex" (MMC) (Sjovall et al. 1990; Medhus et al. 2000). The MMC is divided into three phases, with a total duration of about 100 min (Horowitz et al. 2001). Phase I (about 40 min) is characterised by motor

quiescence, phase II (about 50 min) by irregular contractions, and phase III (about 5 - 10 min) by regular, high amplitude contractions occurring at the maximum rate of 3 per minute in the stomach and 10 - 12 per minute in the small intestine (Horowitz et al. 2001). The MMC functions as the "intestinal housekeeper" which sweeps indigestible solids and bacteria aborally from the gastric antrum to the distal small intestine (Husebye 1999). Ingestion of a meal interrupts the MMC, and produces a "postprandial" pattern that aids the trituration and propulsion of chyme (Gentilcore et al. 2003).

1.2.8.2 Postprandial motility

The postprandial pattern is characterised by an initial relaxation of the proximal stomach to accommodate ingesta, followed by an increase in tonic contractions to move gastric contents more distally. This is accompanied by an increase in the contractile activity of the antrum that breaks down solids into small 1 - 2 mm-sized particles, prior to their emptying from the stomach. Furthermore, an increase in both tonic and phasic contractions of the pylorus helps regulate the rate of gastric emptying. In the small intestine, an increase in duodenal contractile activity aids the mixing of nutrient with digestive enzymes and facilitates the distal propulsion of chyme.

The arrival of unabsorbed nutrient into the distal small intestine triggers the "ileal brake" (Spiller et al. 1984; Spiller et al. 1988; Siegle et al. 1990; Cuche et al.

2000), whereby gastric emptying, as well as gastric and pancreatic secretions, are inhibited via neurohormonal mechanisms. In rats, the ileal infusion of peptone is associated with a reduction in antroduodenal motor activity, and the duodenal inhibition is abolished by administration of the GLP-1 antagonist, exendin (9-39), suggesting the "ileal brake" mechanism is mediated, at least in part, by GLP-1 (Giralt and Vergara 1999). In a separate study involving pigs, the direct infusion of short-chain fatty acids into the terminal ileum causes a reduction in the amplitude of distal and terminal antral contractions, and an increase in the release of peptide YY, but not GLP-1, suggesting peptide YY may also be important in the mediation of the "ileal brake" (Cuche et al. 2000).

1.3   Neurohormonal regulation of upper gut function

Complex neurohormonal mechanisms allow interactions between various parts of the upper gut and with the central nervous system. These mechanisms maintain a delicate balance to allow the optimal environment for nutrient intake, delivery, digestion, and absorption (Fig. 1.1).

1.3.1 The brain-gut axis

The gastrointestinal system is closely controlled by both an intrinsic and an extrinsic nervous system (De Giorgio et al. 2000; Hansen 2003). The so-called "brain-gut axis" consists of three parts: the enteric nervous system, the autonomic

nervous system, and the central nervous system (Van Oudenhove et al. 2004). The autonomic nervous system transfers information from the gut to the brain via vagal and spinal afferent pathways; at brain level, the information is processed (affective and cognitive dimensions added); finally, efferent signals from the brain are sent back to the gut via the autonomic nerves (vagal and sympathetic efferents) (Van Oudenhove et al. 2004).

It has been suggested that both volume and chemical characteristics of meals in the gut can trigger vagal afferent signals. In rats, intraduodenal infusions of glucose and peptone stimulate antral and duodenal vagal afferent activity, beyond those attributable to osmolarity alone, and that the stimulation is greater with peptone than equicaloric glucose (Schwartz and Moran 1998).

In humans, upper gut function appears to be modified by inputs from the central nervous system. In healthy males, sham feeding is associated with stimulation of gastropancreatic secretion, antroduodenal motility, and pancreatic polypeptide release (Katschinski et al. 1992). Accelerated small bowel transit has been associated with stress (Cann et al. 1983) and anxiety (Gorard et al. 1996). Sleep is associated with a marked increase in phase I of the MMC at the expense of phase II, modifying the MMC towards a biphasic pattern with phases I and III, which reflects enterically dominated MMC activity (Kumar et al. 1990). In patients with peptic ulcer disease, vagotomy causes a reduction in GIP release after intra-

jejunal glucose infusion (Lauritsen et al. 1982), indicating GIP release partially relies on afferent vagal input.

1.3.1.1 The enteric nervous system

The enteric nervous system is subdivided into 3 major ganglionated plexi: the myenteric (situated between the circular and longitudinal muscle layers), the submucous (in the submucosa), and the mucous (in the mucosa) plexus, each containing a different mix of neurons that serve different functions (Hansen 2003). A special population of cells within the gastrointestinal mucosa, the entero-chromaffin cells, release serotonin upon stimulation by intraluminal contents, to activate afferent nerve endings within the lamina propria, which in turn sets off a chain of events within the rest of the enteric nervous system, influencing motility, secretions, blood-flow, immune function, gut sensation, gallbladder contraction, and exocrine pancreatic secretion (Hansen 2003; Hansen 2003). There are regional and topographic differences in the distribution of entero-chromaffin cells, with the highest density in the duodenum (Hansen 2003), potentially playing an important role in providing neuronal feedback control to the rest of the gastrointestinal tract. The extrinsically denervated guinea pig small intestine has preserved enteric motor reflexes, demonstrating the independence of the enteric nervous system (Furness et al. 1995). In pigs, surgical transection and re-anastomosis of the duodenum led to disruption of the enteric nerve fibers, and subsequent attenuation of the slowing of gastric emptying and stimulation of

IPPWs, in response to intraduodenal nutrient infusion (Treacy et al. 1992). Furthermore, in humans, intraduodenal infusion of the local anaesthetic, benzocaine, attenuates the effect of intraduodenal lipid on gastric volume, plasma CCK and nausea (Feinle et al. 2001). Gastrointestinal dysmotility is often a result of disturbed neurological function in the gut; examples of disorders where an enteric neuropathy has been implicated include Hirschsprung's disease, achalasia, diabetes, chronic intestinal pseudo-obstruction, and slow-transit constipation (De Giorgio et al. 2000).

Multiple neurotransmitters are released in the gut, with varying effects on gastrointestinal motility. Vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide, noradrenaline (NAd), opioids, and nitric oxide (NO) are inhibitory, whilst the tachykinins (eg. substance P), acetylcholine (Ach), and serotonin (5-HT) are excitatory (Hansen 2003).

The enteric nervous system also controls gastrointestinal secretion. In response to stimulation by intraluminal contents, the entero-chromaffin cells release serotonin, which subsequently activates the submucous neural network and stimulates gut secretions via a variety of neurotransmitters including Ach and VIP (Cooke 2000).

1.3.1.2 Nitric oxide

In the gastrointestinal tract, nitric oxide (NO) is the major inhibitory non-adrenergic, non-cholinergic (NANC) neurotransmitter (Takahashi 2003), and appears to act as the final common pathway to mediate enteric smooth muscle relaxation. NO is produced from L-arginine by the enzyme nitric oxide synthase (NOS). There are three distinct isoforms of NOS, namely neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS); the latter does not normally exist in significant quantity, but is inducible by tissue injury or inflammation (Takahashi 2003). NO relaxes the smooth muscles of the lower oesophageal sphincter, gastric fundus, pylorus, gallbladder, sphincter of Oddi, anal sphincter, and small and large intestine (Takahashi 2003). There is increasing evidence that dysfunction of the nitrergic pathway occurs in various diseases of the gastrointestinal tract, including oesophageal stenosis, achalasia, functional dyspepsia, infantile hypertrophic pyloric stenosis, diabetic gastroparesis, chronic idiopathic pseudo-obstruction, idiopathic slow transit constipation, Hirschsprung's disease, and Chaga's disease (Takahashi 2003).

Studies using either NO donors, such as intravenous nitroglycerin (Sun et al. 1998) or sublingual glyceryl trinitrate (Gilja et al. 1997), or specific inhibitors of NO production such as NG-monomethyl-L-arginine (L-NMMA) (Konturek et al. 1999), indicate that NO mediates relaxation of the gastric fundus (Gilja et al. 1997), slows gastric emptying (Konturek et al. 1995; Sun et al. 1998; Konturek et

al. 1999; Shah et al. 2000; Calatayud et al. 2002; Wang et al. 2002; Abraham et al. 2004; Patil et al. 2005), decreases antral (Konturek et al. 1995; Konturek et al. 1999) and pyloric (Sun et al. 1998) contractions, and inhibits both fasting (Russo et al. 1999) and postprandial (Kuiken et al. 2002) onset of phase III contractile activity. These observations are, however, not consistent across all human studies. For example, a recent study in healthy older humans showed no effect of intravenous L-NAME on the gastric emptying of an oral glucose load (Gentilcore et al. 2005). Animal studies have also shown inconsistent effects of nitric oxide on upper gut motility. Studies involving pigs (Lefebvre et al. 2005) and rats (Calatayud et al. 2002) have found L-NAME to be associated with a delay in gastric emptying. In contrast, the relaxation of the rat gastric antrum appears to be dependent on nNOS expression (Gangula et al. 2007). Furthermore, delayed gastric emptying and impaired pyloric NANC relaxation in diabetic mice were found to be similar to that observed in neuronal nitric oxide synthase (nNOS) knock-out mice, and administration of the phosphodiesterase-5 inhibitor, sildenafil, reversed the delay in gastric emptying, indicating impaired NO signaling as the mechanism through which gastric emptying is delayed in this model (Watkins et al. 2000). There also appears to be sex-dependent differences in the contribution of nitrergic mechanisms to gastric motor function, with female rats having greater levels of nitrergic activity during health, and a greater propensity for disordered gastric motor function during diabetes, than male rats (Gangula et al. 2007).

Nitric oxide has also been implicated in the regulation of intestinal blood flow, and appears to be a major determinant of vascular tone in mesenteric resistance vessels and submucous arterioles (Hansen 2003).

1.3.2 Incretin hormones

The "incretin effect" describes the phenomenon where a glucose load delivered into the gut leads to a much greater insulin response than an isoglycaemic intravenous glucose infusion (Nauck et al. 1986; Efendic and Portwood 2004; Nauck and Meier 2005). The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released from the small intestine as a result of nutrient-gut interaction, and play an important role in stimulating postprandial insulin secretion (predominantly GIP, to a lesser extent GLP-1) (Elliott et al. 1993; Holst and Gromada 2004) and slowing of gastric emptying (GLP-1). In incretin receptor knockout mice, significantly reduced insulin secretion is observed following oral, but not intraperitoneal, glucose administration (Hansotia and Drucker 2005). Limited evidence suggests incretin hormone release can also be modulated via neural and hormonal mechanisms (Deacon 2005).

The incretin hormones contribute about 60 % of the insulin response to an oral glucose load in healthy humans (Meier and Nauck 2004). However fat, rather than carbohydrate, has been suggested to be a more potent stimulus for incretin

hormone release, particularly that of GLP-1, although studies that have suggested this did not deliver fat and carbohydrate at equicaloric rates, and this may explain the differences observed (Feinle et al. 2003; Pilichiewicz et al. 2007). For example, when fat was infused intraduodenally at 2.8 kcal/min, plasma GLP-1 rose to approximately 25 pmol/L at 120 minutes (Feinle et al. 2003), whilst in another study glucose was infused at 2 and 4 kcal/min, and plasma GLP-1 rose to approximately 12 and 43 kcal/min at 120 minutes (Pilichiewicz et al. 2007). The stimulation of incretin hormones by fat is attenuated by the lipase inhibitor orlistat, in both healthy subjects (Feinle et al. 2003) and patients with type 2 diabetes (Pilichiewicz et al. 2003), indicating that triglycerides need to be digested to free fatty acids in order to stimulate incretin release..

The relative contribution of GLP-1 and GIP to the incretin effect is controversial. Earlier studies found GLP-1 to be several times more potent than GIP in stimulating insulin release after an oral glucose load (Kreymann et al. 1987; Elahi et al. 1994). However, more recent studies indicate that GIP is the dominant mediator of the incretin effect, with GLP-1 making only a small (20 - 25 %) contribution (Nauck et al. 1993; Gault et al. 2003), in healthy humans. The overall weight of evidence supports the latter.

Incretin hormones are metabolised rapidly to inactive forms after release by the enzyme dipeptidyl peptidase IV (DPP-IV) This is particularly the case for GLP-1, where 90 % is broken down prior to reaching the systemic circulation (Deacon

2004; Holst 2004). DPP-IV exists predominantly in the endothelium of the local capillary bed of the intestines, and inactivates GLP-1 and GIP by removing the N-terminal, rendering them incapable of binding to receptors (Deacon 2004; Deacon 2005). Prior to its degradation, GLP-1 activates local afferent nerve fibres in the enteric nervous system, relaying signals to the rest of the gut and the central nervous system, thereby exerting its effects on gastrointestinal motility, appetite, and food intake (Holst 2004; Deacon 2005). Once in the portal circulation, the remaining intact GLP-1 and GIP are further inactivated during passage across the liver by DPP-IV associated with hepatocytes, followed by ongoing degradation in peripheral tissues, with the final metabolites being cleared renally (Deacon 2004; Meier et al. 2004).

1.3.2.1 GLP-1

GLP-1 is secreted by specialised endocrine cells embedded in the intestinal mucosa, the L cells. L cells are found most densely in the distal small intestine (distal jejunum and ileum), but are also present throughout the remaining small and large intestine (Mortensen et al. 2003; Deacon 2005). It has been suggested that a minimum caloric rate of 1.8 kcal/min of glucose delivery is required to stimulate GLP-1 secretion (Schirra et al. 1996). Despite the predominantly distal location of L cells, GLP-1 is released rapidly (within 5 - 15 min) in response to the presence of nutrient in the small intestine, prompting suggestions of the early rise in GLP-1 being either due to release from the proximal small intestinal L cells

(Holst 2004), or the possible existence of a "duodeno-jejunoileal loop" (Rocca and Brubaker 1999; Hansen and Holst 2002; Deacon 2005), or more rapid initial transit of nutrient through the small intestine (Little et al. 2006; Chaikomin et al. 2008). In rats, exogenous GIP was found to stimulate the release of GLP-1 (Roberge and Brubaker 1993), but this has not been demonstrated in humans.

GLP-1 serves the dual function of being both an incretin hormone and an enterogastrone (i.e. a hormone that influences gut motility) (Schirra and Goke 2005). Its incretin effect derives from its ability to augment the release of insulin from the pancreatic beta cells in response to glucose stimulation; in addition, it inhibits the release of glucagon from the pancreatic alpha cells. It acts as an enterogastrone in its effects on the motor activity of the upper gut; in healthy humans, intravenous administration of exogenous GLP-1 (7-36) causes relaxation of the gastric fundus (Schirra et al. 2002), inhibition of antral and duodenal motor activity (Schirra et al. 1997; Schirra et al. 2000; Brennan et al. 2005), and stimulation of pyloric contractions (Schirra et al. 2000), all of which contribute to a slowing of gastric emptying (Nauck et al. 1997; Little et al. 2006). These effects have been observed at both "physiological" (~0.3 pmol/kg/min) and pharmacological (0.8 - 1.2 pmol/kg/min) doses of GLP-1, in a dose-dependent fashion (Nauck et al. 1997; Schirra et al. 2000; Schirra et al. 2002; Little et al. 2006). Recent evidence suggests that the slowing of gastric emptying may in fact be the dominant action of GLP-1 in reducing postprandial glycaemic excursions, rather than its insulinotropic effect (Nauck et al. 1997).

Exogenous GLP-1 (7-36), both at physiological and pharmacological levels, dose dependently slows gastric emptying of solid and liquid meals in healthy volunteers (Nauck et al. 1997; Little et al. 2006), and truncated GLP-1 (proglucagon 78-107-amide) causes a delay in gastric half-emptying time (Wettergren et al. 1993). The synthetic GLP-1 analogue exenatide (Exendin-4) has also been shown to delay gastric emptying and reduce postprandial glycaemia in healthy subjects (Edwards et al. 2001) and patients with type 2 diabetes (Kolterman et al. 2003); however, the effect of exenatide on antropyloroduodenal motility has not been directly studied. In rats, exogenous GLP-1 inhibits small intestinal motility and transit (Tolessa et al. 1998; Tolessa et al. 1998), which could represent an additional mechanism in the lowering of postprandial glycaemia; so far, this has not been evaluated in humans. Inhibition of endogenous GLP-1, by administering the GLP-1 receptor antagonist exendin (9-39) amide, stimulated antroduodenal motility and reduced pyloric contractions (Schirra et al. 2006), and inhibited gastric fundal relaxation, in response to a duodenal nutrient load (Schirra and Goke 2005). In contrast, others have reported that the subcutaneous administration of exogenous GLP-1 at pharmacological doses, dose-dependently prolonged the lag phase of gastric emptying, but maximal emptying velocity and total emptying time of a liquid meal in healthy subjects (Schirra et al. 1997), and half emptying time of a solid meal in type 2 diabetic patients (Schirra et al. 1998), were unchanged, indicating that the

subcutaneous, compared to the intravenous, route of administration, may result in differing pharmacokinetics and subsequent pharmacological effect.

The mechanism via which GLP-1 exerts its effects on gastrointestinal motility remains unclear. In animal models, GLP-1 failed to change basal muscular tone in smooth muscle strips from the gastric fundus and corpus (Tolessa et al. 1998), or the length of antral smooth muscle cells (Rodier et al. 1997), making a direct effect of GLP-1 on gastric smooth muscle unlikely. Vagal mediation seems a possible mechanism as GLP-1 inhibits vagally-stimulated antral motility in pigs (Wettergren et al. 1998), and in rats, GLP-1-induced delay in gastric emptying appears to require an intact vagal afferent pathway (Imeryuz et al. 1997). Furthermore, the secretion of pancreatic polypeptide (PP) in response to an orally ingested meal, which is under vagal cholinergic control, is inhibited by GLP-1 in both healthy subjects (Schirra et al. 1997) and patients with type 2 diabetes (Schirra et al. 1998). GLP-1 also inhibits the PP response to duodenal lipid infusion in healthy subjects (Schirra et al. 2000), thereby discounting the possibility of reduced duodenal nutrient delivery as a cause for decreased PP secretion. A direct action of GLP-1 on pancreatic polypeptide cells appears unlikely, as GLP-1 stimulates, rather than inhibits, PP release from isolated human pancreatic islets (Fehmann et al. 1995).

The term "ileal brake" describes the inhibition of gastric emptying and small intestinal pressure waves and transit (Read et al. 1984; Spiller et al. 1984; Spiller

et al. 1988), as well as exocrine pancreatic secretion (Layer et al. 1990), triggered by nutrient-gut interaction in the distal small intestine. The exact pathways that mediate this reflex are unclear, with both humoral (GLP-1 (Brennan et al. 2005; Little et al. 2006), CCK (Brennan et al. 2005), and PYY (Cuche et al. 2000)) and neurologic (opioid (Zhao et al. 2000), serotonergic (Lin and Chen 2003), and alpha- and beta-adrenergic (Brown et al. 1992; Lin et al. 2003)) mechanisms being implicated. Endogenous GLP-1 is likely to be involved, given the elevation in plasma levels of GLP-1 during ileal nutrient stimulation parallels the inhibitory effects of the "ileal brake" on antral motility, and gastric acid and exocrine pancreatic secretions (Layer et al. 1990; Layer et al. 1995).

GLP-1 augments the insulin response of pancreatic beta-cells to glucose i.e. conveying "glucose competence" to beta cells (Holst and Gromada 2004), and directly stimulates beta cell insulin gene transcription and translation (Fehmann and Habener 1992). It has been reported that GLP-1 synergistically potentiates the stimulatory effect of glucose on insulin release from the beta cells in rats, by increasing intracellular cyclic adenosine monophosphate (cAMP) levels via activation of adenylyl cyclase (Delmeire et al. 2003). The effects of GLP-1 on pancreatic beta cells are therefore "glucose-dependent", so that this peptide does not cause hypoglycaemia (Gallwitz 2005; Nauck and Meier 2005). In healthy humans, intravenous infusion of GLP-1 has also been shown to suppress hepatic glucose production (Prigeon et al. 2003) and increase glucose disposal (D'Alessio

et al. 1994; D'Alessio et al. 1995), independent of its actions on pancreatic islet hormone secretion.

In addition to its glucoregulatory effects, limited evidence suggests that GLP-1 plays a role in mediating visceral illness and anxiety responses (Seeley et al. 2000; Kinzig et al. 2002; Kinzig et al. 2003), suppression of food intake and appetite (Kinzig et al. 2002), and reduction in intestinal lymph flow and lipid absorption (Qin et al. 2005).

In rats, the postprandial GLP-1 level in intestinal lymph has been found to be 5 - 6 times greater than in the portal plasma, with the difference being even greater compared to peripheral plasma, raising the possibility that intestinal lymph may act as a special signaling conduit for regulatory peptides secreted by the gastrointestinal tract (D'Alessio et al. 2007).

1.3.2.2 GIP

GIP is secreted by K cells embedded in the small intestinal mucosa and situated most densely in the duodenum (Mortensen et al. 2003; Deacon 2005). GIP is responsible for the majority of the insulinotropic effect exerted by the incretin hormones (Nauck et al. 1993; Gault et al. 2003), and has a trophic effect on pancreatic beta cells, but does not appear to have the other functions of GLP-1 (Yamada and Seino 2004; Hansotia and Drucker 2005). GIP potentiates the

stimulatory effect of glucose on pancreatic insulin release, like GLP-1, by increasing intracellular cAMP levels via activation of adenylyl cyclase (Delmeire et al. 2003).

In contrast to GLP-1, the administration of exogenous GIP does not delay gastric emptying (Meier et al. 2004). One additional property of GIP is its link to lipid metabolism and promotion of lipid storage (Holst 2004). Studies in GIP receptor-knockout mice indicate that GIP may play a role in linking over-nutrition to obesity (Yamada and Seino 2004). The effect of GIP on glucagon secretion is less clear, with limited evidence suggesting that GIP has little or no effect (Meier et al. 2007; Cassidy et al. 2008).

1.4   Sensory function

Sensations arising from the upper gut are a result of both mechanical (distension) and chemical (nutrient, pH, osmotic) stimulation. Combinations of stimuli may generate different, and potentially synergistic, sensations when compared to each stimulus alone.

Distension of the proximal stomach increases the sensation of fullness, but has no effect on hunger, and appears to be mediated by mechanisms other than tension receptors in the gastric wall (Carmagnola et al. 2005). Distension of the distal stomach also increases fullness (Mundt et al. 2005), and causes greater degrees of

nausea, pain and bloating than proximal gastric distension (Ladabaum et al. 1998), suggesting a greater role of distal than proximal stomach in appetite regulation.

Chemical stimuli, including nutrients, act predominantly in the small intestine, stimulating perceptions of fullness and nausea, involving the release of peptides such as CCK and GLP-1 (Feinle 1998; Feltrin et al. 2004).

1.5   Conclusion

The gastropyloroduodenal region acts as a functional unit that serves complex motor, sensory, and endocrine roles, closely regulated by various neuroendocrine feedback loops. In addition to the grinding, mixing, and forward propulsion of nutrient, it is also involved in sensory detection and appetite regulation, as well as postprandial blood glucose homeostasis. The duodenum is densely populated with nerve fibres, endocrine cells, and various nutrient receptors, and serves as a crucial control point that regulates the delivery and absorption of nutrient. Despite a substantial compensatory capacity, the delicate balance between the stomach, the pylorus, and the duodenum can be disturbed by certain diseases, a classic example of which is diabetes mellitus, which will be discussed in Chapter 2.

The human small intestine, due to its length and the difficulty in assessing much of its lumen, remains a poorly understood region in the gastrointestinal tract.

Greater efforts are required to help define the function of the small intestine, how it interacts with other regions of the gut, and possibly, with other organ systems in the body.
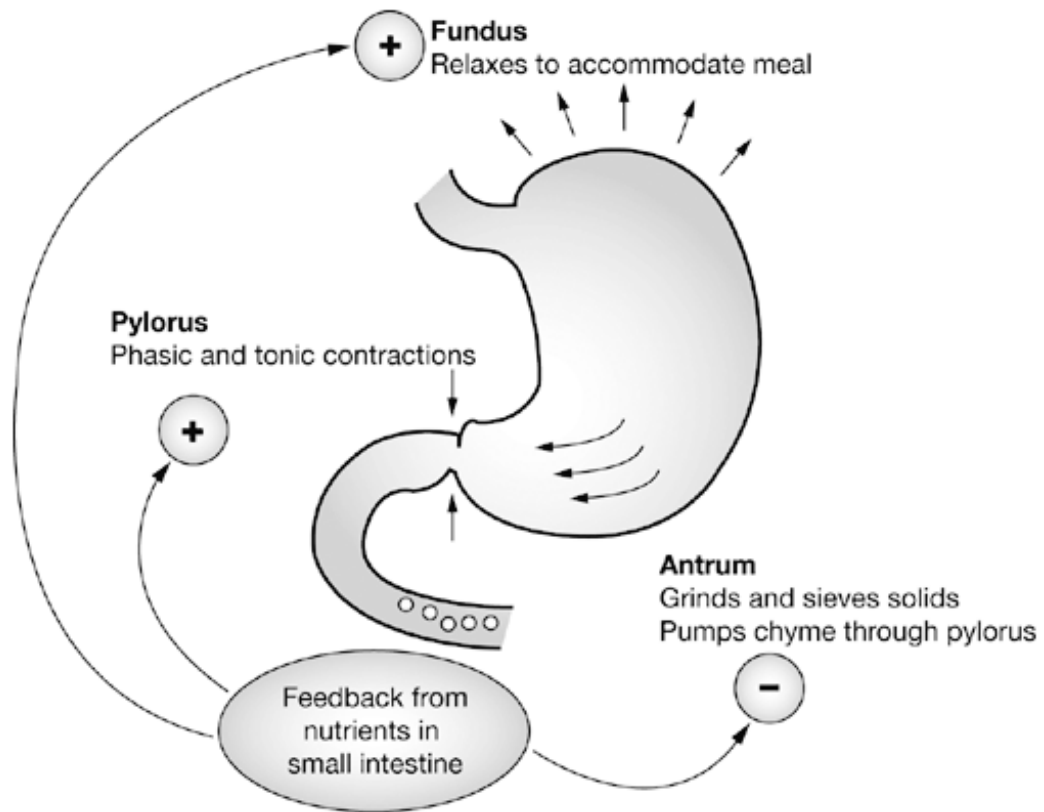
Figure 1.1. Motor control of normal gastric emptying (Reproduced from Rayner et. al. 2005)

Chapter 2:

Gastroduodenal function in diabetes

(Adapted from Kuo et al. Pathophysiology and management of diabetic gastropathy: a guide for endocrinologists. Drugs 2007;67:1671-87)

2.1 Introduction

The gastropyloroduodenal region of the gut forms a functional entity of interdependent components. As discussed in Chapter 1, gastric emptying (Jones et al. 1996) and proximal small intestinal flow events (Chaikomin et al. 2007) are important determinants of glucose absorption and postprandial glycaemia. Conversely, variations in blood glucose levels also induce reversible changes in upper gut motor activity, hence the use of the phrase "chicken and egg" to describe their interdependence (Rayner and Horowitz 2006). In addition, diabetes is itself associated with various abnormalities in upper gut motility, independent of the blood glucose concentration (Jones et al. 2001). Furthermore, the incretin hormones secreted from the small bowel, as a result of nutrient-gut interaction, play an important role in postprandial insulin secretion and blood glucose regulation and, in the case of GLP-1, regulation of gastric emptying.

2.2   Glycaemic control and diabetic complications

The association between diabetic microvascular and, probably, macrovascular complications, with overall glycaemic control (as indicated by glycated haemoglobin [HbA1c]), is now well established in both type 1 and type 2 diabetes (The Diabetes Control and Complications Trial [DCCT] Research Group 1993; UK Prospective Diabetes Study [UKPDS] Group 1998; Nathan et al.). The major contribution of postprandial, as opposed to fasting, glycaemia, to glycated haemoglobin has, however, only recently been recognised (Rayner and Horowitz 2006). In type 2 patients, retinopathy and neuropathy have been more closely linked with postprandial hyperglycaemia, than either fasting blood glucose or HbA1c levels (Shiraiwa et al. 2005). Postprandial hyperglycaemia appears to be an independent risk factor for cardiovascular disease and mortality, even in non-diabetic populations (Gerich 2003; Beisswenger et al. 2004). The 2-hour blood glucose concentration after an oral glucose load, predicts cardiovascular and all-cause mortality better than fasting glucose or HbA1c (Balkau et al. 1998; de Vegt et al. 1999; The DECODE study group 1999), and this is evident even when the latter two indices are normal (Barrett-Connor and Ferrara 1998; Shaw et al. 1999; Gerich 2003). Lowering postprandial blood glucose concentrations, even at the expense of higher fasting glucose levels, can improve overall glycaemic control, as indicated by HbA1c (Bastyr et al. 2000). The contribution of postprandial blood glucose to glycated haemoglobin appears greatest when diabetic control is satisfactory or good (as indicated by HbA1c < 7.3%), whilst the importance of

fasting glucose increases as glycaemic control worsens (Monnier and Colette 2006).

Existing pharmacological therapies for type 2 diabetes, including the sulphonylureas, metformin, and the thiazolidinediones, are unsatisfactory in treating postprandial hyperglycaemia because they do not target the deficiency in "early-phase" insulin release, a major contributor to postprandial hyperglycaemia (Mitrakou et al. 1992; Gerich 2003). Meglitinides (repaglinide, nateglinide), other short-acting insulin secretagogues that specifically target early insulin release, and the new shorter-acting insulins (insulin aspart, insulin lispro), may be better alternatives for managing postprandial hyperglycaemia (Home et al. 1999; Gerich 2003).

2.3   Upper gut function in diabetes mellitus

In patients with diabetes, regardless of the blood glucose level, various abnormal patterns of upper gut motility have been observed, including impairment of meal-induced relaxation of the gastric fundus (Samsom et al. 1998), excessive pyloric contractile activity (Mearin et al. 1986), reduced antral contraction (Samsom et al. 1996), impaired coordination of contractile activity between the antrum and the duodenum (Rayner and Horowitz 2006), altered gastric emptying (Jones et al. 1996), and prolonged fasting phase II and reduced or absent phase III activity of the migrating motor complex (Samsom et al. 1996). Gastric emptying is delayed

in about 30 - 50 % of outpatients with long-standing type 1 and type 2 diabetes (Rayner et al. 2001; Horowitz et al. 2002). Abnormally rapid gastric emptying occurs infrequently in long-standing type 1 and type 2 patients (Keshavarzian et al. 1987; Nowak et al. 1995; Lipp et al. 1997), but has been reported more often in patients with "early" type 2 diabetes (Phillips et al. 1992; Schwartz et al. 1996), although this has not been a consistent observation (Jones et al. 1996). Diabetes is also associated with gastric dysrythmias on electrogastrography (EGG), either slow (bradygastria), fast (tachygastria), or mixed (Koch 2001). However, the presence of gastric dysrythmia, although common in patients with diabetes, is not necessarily predictive of disordered (delayed or more rapid) gastric emptying (Nohara et al. 2006). There is scarce information regarding small intestinal motor activity in diabetes. In a small study, abnormal duodenal motility, such as early recurrence of phase III MMC activity and bursts of activity after a meal, have been reported in 50 % of type 1 diabetic patients (Samsom et al. 1996). In patients with insulin-dependent diabetes and gastroparesis, more retrograde and complex duodenal flow patterns have been observed postprandially, compared to healthy subjects (Nguyen et al. 1997).

Apart from the above-mentioned motor dysfunctions, patients with diabetes also more frequently report gastrointestinal symptoms such as nausea, bloating, and abdominal pain, compared to healthy subjects (Jebbink et al. 1993; Samsom et al. 1995). However, the correlation between gastrointestinal symptoms and disordered motility is poor in diabetes.

A distinct clinical entity exists, termed "diabetic gastropathy", that describes the delay in gastric emptying and the presence of upper abdominal symptoms, that frequently affect both type 1 and type 2 patients. Diabetic gastropathy will be discussed in detail in the latter part of this chapter.

2.3.1 Influence of gastric emptying on postprandial glycaemia

The rate of gastric emptying is a major determinant of the postprandial glycaemic response, and contributes to about one-third of the variation in blood glucose after a meal in both healthy subjects (Horowitz et al. 1993) and patients with type 2 diabetes (Jones et al. 1996) (Fig. 2.1). Manipulation of gastric emptying, through either pharmalogical or non-pharmalogical means, has therefore been the subject of intense investigation over recent years. Dietary modifications which slow gastric emptying are beneficial in improving glycaemic control in patients with non-insulin requiring type 2 diabetes. A 6-week diet high in fibre, particularly of the soluble type, decreased the area under the curve for 24-hour plasma glucose and insulin concentrations in type 2 patients (Chandalia et al. 2000). Similarly, when guar gum is added to a glucose drink in type 2 patients, the postprandial glycaemic excursions are reduced, attributable to both slower gastric emptying and inhibition of small intestinal glucose absorption (Russo et al. 2003).

Slowing of gastric emptying induced by consuming oil before a meal (Gentilcore et al. 2006), or by administering morphine (Gonlachanvit et al. 2003), the amylin analogue pramlintide (Kolterman et al. 1995; Thompson et al. 1997; Thompson et al. 1997), cholecystokinin-8 (Phillips et al. 1993), the oral proteinase inhibitor POT-II (Schwartz et al. 1994), or GLP-1 and its analogues (e.g. exenatide, liraglutide) (Nauck 1996; Egan et al. 2002), have been shown consistently to attenuate the postprandial rise in blood glucose and reduce the total area under the blood glucose curve. In fact, the dominant mechanism of GLP-1 analogues in lowering postprandial glucose is by slowing gastric emptying, rather than stimulating insulin secretion, as evidenced by reduced or unchanged, rather than increased, postprandial plasma insulin levels (Nauck et al. 1997; Dupre et al. 2004).

The goal of modulating gastric emptying may differ substantially depending on whether a patient is treated with insulin. In patients with type 1 or insulin-requiring type 2 diabetes, gastric emptying may need to be either slowed or accelerated, in order to improve the coordination between nutrient absorption and insulin action. Conversely, in type 2 patients not requiring exogenous insulin, it is often advantageous to delay gastric emptying (provided this does not provoke gastrointestinal symptoms), due to the deficiency of the early phase of insulin secretion that is characteristic of this disorder (Bruce et al. 1988).

2.3.2 Influence of glycaemia on upper gut function

Acute changes in the blood glucose concentration have reversible effects on motility in every region of the gastrointestinal tract (Rayner et al. 2001). Elevation of blood glucose from fasting (~4 mmol/L) to physiological postprandial concentrations (~8 mmol/L) slows gastric emptying in both healthy subjects and patients with uncomplicated type 1 diabetes (Schvarcz et al. 1997; Jones et al. 1999), while marked hyperglycaemia (blood glucose 16 - 20 mmol/L) substantially retards gastric emptying of solids and liquids in type 1 patients, including those with established autonomic neuropathy (Fraser et al. 1990; Samsom et al. 1997). Changes in blood glucose concentration appear to act synergistically with stimuli arising from the small intestine to slow gastric emptying (Rayner et al. 2001). On the other hand, insulin-induced hypoglycaemia (blood glucose ~2.6 mmol/L) accelerates gastric emptying in type 1 patients (Schvarcz et al. 1997; Russo et al. 2005). The slowing of gastric emptying induced by acute hyperglycaemia has been shown to be associated with reduced tone of the proximal stomach (Hebbard et al. 1996; Rayner et al. 2000), inhibition of antral pressure waves (Barnett and Owyang 1988; Hasler et al. 1995), stimulation of pyloric contractions (Fraser et al. 1991), and induction of abnormal gastric electrical rhythms (Jebbink et al. 1994; Hasler et al. 1995). There is limited evidence that hyperglycaemia-induced gastric arrhythmias are reversed by increasing available nitric oxide, using nitroglycerin or sildenafil (Coleski et al. 2005), and by inhibiting prostaglandin synthesis, using the non-steroidal anti-

inflammatory drug indomethacin (Hasler et al. 1995). However, the role of NO mechanisms in mediating the effects of hyperglycaemia on gastric motility has hitherto not been evaluated. It also remains to be established whether the response to hyperglycaemia is dependent on the rate of gastric emptying during euglycaemia, long-term glycaemic control, or autonomic nerve function (Horowitz et al. 2002).

Currently, there is limited understanding of the mechanism(s) mediating the effect of hyperglycaemia on gastric emptying. Autonomic nerve dysfunction has been suggested as one potential mechanism. In animal models, glucose-responsive neurons have been demonstrated in the central nervous system (Mizuno and Oomura 1984) and the small intestine (Liu et al. 1999), and acute hyperglycaemia is known to cause reversible inhibition of vagal efferent activity (Takahashi et al. 2003). Furthermore, autonomic nerve function is impaired by acute hyperglycaemia in healthy humans (Yeap et al. 1996). Insulin does not appear to play a major role, as euglycaemic hyperinsulinaemia does not affect gastric emptying (Kong et al. 1999), and the effects of hyperglycaemia are also evident in type 1 (insulin-deficient) patients (Fraser et al. 1990).

Compared to the stomach, information on how hyperglycaemia affects small intestinal motility is very limited. In healthy subjects, marked hyperglycaemia (blood glucose 12 - 15 mmol/L) slows small intestinal transit (de Boer et al. 1993; Russo et al. 1996), decreases the motility index and the occurrence of propagated

contractions (Bjornsson et al. 1994), and reduces the cycle length of the migrating motor complex (Oster-Jorgensen et al. 1992). Furthermore, blood glucose levels at the upper end of the physiological range (10 mmol/L) decrease duodenal compliance, as measured by balloon distension (Lingenfelser et al. 1999). Hyperglycaemia also inhibits gallbladder contraction (de Boer et al. 1993), and affects upper gastrointestinal exocrine secretory function, with suppression of gastrin, gastric acid, and pancreatic exocrine secretion in healthy subjects (MacGregor et al. 1976; Lam et al. 1993; Lam et al. 1997; Andrews et al. 2001).

Acute changes in the blood glucose level also reversibly influence upper gut sensations. Marked hyperglycaemia (blood glucose 15 mmol/L) increases sensory perceptions such as nausea and fullness, during proximal gastric distension, both in the fasted state and during intraduodenal lipid infusion in healthy subjects (Hebbard et al. 1996; Hebbard et al. 1996; Hebbard et al. 1997; Andrews et al. 2001). Although acute hyperglycaemia did not increase the perception of proximal gastric distention in type 1 patients in one study, when compared to euglycaemia, these patients already had much greater symptom scores than healthy controls during euglycaemia, potentially making any additional increase difficult to demonstrate (Rayner et al. 2000). Both type 1 and type 2 diabetic patients report more upper gut symptoms if they have worse glycaemic control, as assessed by HbA1c (Schvarcz et al. 1996) or self-report (Bytzer et al. 2001; Bytzer et al. 2002), while the perception of fullness in type 1 patients is related directly to postprandial blood glucose levels (Jones et al. 1997).

The evidence regarding how chronic glycaemia affects gastrointestinal symptoms is unclear. In a longitudinal study involving patients with both types 1 and 2 diabetes, gastric emptying and gastrointestinal symptoms did not change over a 12 year period, despite a modest improvement in glycaemic control, as indicated by lower mean blood glucose and HbA1c levels (Jones et al. 2002). In a population study using questionnaires, the prevalence of gastrointestinal symptoms was found to be associated with poorer levels of glycaemic control, but not with the duration of diabetes (Bytzer et al. 2001).

2.4   Incretin hormones in diabetes

The insulin response to infusions of exogenous GLP-1 is relatively intact in patients with type 1 (Dupre 2005) and type 2 (Elahi et al. 1994; Hansotia and Drucker 2005; Nauck and Meier 2005) diabetes, but exogenous GIP appears essentially ineffective, for unknown reasons. In patients with type 2 diabetes, the incretin effect in response to a meal is decreased, perhaps mainly due to loss of the GIP-regulated second phase (20 - 120 min) insulin secretion, but also, to a lesser extent, to decreased secretion of GLP-1 (Vilsboll et al. 2001; Efendic and Portwood 2004; Holst and Gromada 2004; Nauck et al. 2004). In about 50 % of first degree relatives of patients with type 2 diabetes, there is a reduced insulinotropic response to GIP, without a significant change in the postprandial levels of GLP-1 and GIP, suggesting reduced responsiveness to GIP as a possible

contributor to the pathogenesis of, or even indicating a genetic predisposition to, type 2 diabetes (Meier et al. 2001; Nauck et al. 2004).

GLP-1, when infused at pharmacological doses in patients with type 2 diabetes, normalises fasting (Nauck et al. 1998) and postprandial (Willms et al. 1996) blood glucose concentrations. However, GLP-1 is inactivated rapidly in vivo, making longer-acting analogues of GLP-1 a more feasible option for clinical use. Examples of GLP-1 analogues include exenatide and liraglutide, which have been shown to reduce HbA1c by about 1 %, with associated moderate weight loss (2 - 3 kg), but have adverse effects of nausea and, less commonly, vomiting (Nauck and Meier 2005). Another class of agents, the dipeptidyl peptidase-IV (DPP-IV) inhibitors, such as sitagliptin, prolongs the action of endogenous incretin hormones by limiting their metabolism, has also been shown to reduce HbA1c by 0.7 % after 1 year of treatment, and appears to be well tolerated (Scott et al. 2007; Chan et al. 2008; Nonaka et al. 2008; Scott et al. 2008). Both exenatide and sitagliptin have now been approved for clinical use in the treatment of type 2 diabetes in Australia.

2.5   Diabetic gastropathy

2.5.1 Definition

"Gastroparesis" refers to severely delayed gastric emptying (i.e. more than two standard deviations from the healthy mean), in the absence of mechanical obstruction (Horowitz et al. 2002; Jones and Maganti 2003; Rayner and Horowitz 2005). In recent years, the term "diabetic gastropathy" has emerged, to encompass not only delayed gastric emptying, but also the presence of upper gastrointestinal symptoms such as bloating, nausea, and discomfort, and is used occasionally to describe symptoms only. It is recommended, however, that the term not to be used in isolation, but rather be accompanied by a more detailed description of the symptoms and the magnitude of delay in gastric emptying, as the focus of management may differ accordingly.

Glycaemic control has received inadequate attention in the management of diabetic gastropathy. As discussed, acute hyperglycaemia not only contributes to a delay in gastric emptying, but can exacerbate upper gastrointestinal symptoms (Horowitz et al. 2002). Furthermore, hyperglycaemia may attenuate the effect of prokinetic drugs such as erythromycin (Jones et al. 1999). Optimisation of glycaemic control is, therefore, likely to be important in the management of diabetes-related upper gastrointestinal motor and sensory dysfunction.

2.5.2 Pathophysiology

Studies of the pathogenesis of diabetic gastroparesis to date have been limited by small numbers and heterogeneous patient profiles. In animal models, diabetes has been associated with a wide range of reversible, and irreversible, pathologies, including axonopathy of extrinsic autonomic nerves, loss of interstitial cells of Cajal, apoptosis of enteric neurons, loss of inhibitory neurotransmission, and gastric myopathy (Wrzos et al. 1997; Watkins et al. 2000; Horvath et al. 2006; Rayner and Horowitz 2006; Yamamoto et al. 2008). Nitrergic mechanisms appear to play a crucial role in the pathogenesis of diabetes-related gastric motor dysfunction. A reduction in pyloric neuronal nitric oxide synthase (nNOS) levels has been reported in diabetic mice, and is reversed by insulin (Watkins et al. 2000). In a separate study, nitric oxide synthase expression in the myenteric plexus was found to be reduced in the antrum of streptozotocin-induced diabetic rats (Wrzos et al. 1997). Furthermore, nitrergic mechanisms appear to play a greater role in gastric motor function in female rats, compared to male rats, and become more impaired during streptozotocin-induced diabetes (Gangula et al. 2007). Data in humans are scarce due to limited patient numbers and the requirement for invasive biopsy. A loss of interstitial cells of Cajal has been identified in patients with medically-refractory gastroparesis (Forster et al. 2005), while gastric myopathy was reported in a small group of patients with type 1 diabetes and intractable vomiting (Ejskjaer et al. 1999). Among a group of gastric cancer patients, reduced densities of interstitial cells of Cajal, neuronal nitric

oxide synthase, and substance P, were associated with the presence of diabetes (Iwasaki et al. 2006). The role of vagal axonopathy in the pathogenesis of diabetic gastroparesis remains controversial in humans (Guy et al. 1984; Yoshida et al. 1988; Britland et al. 1990). Recently, it has been suggested that diabetic gastroparesis may be due to a combination of multiple pathologies, and the severity of these changes may correlate with a longer duration of diabetes and poorer glycaemic control (Pasricha et al. 2008).

There is limited information regarding the mechanism of diabetes-related abdominal symptoms. In type 1 diabetes patients, the administration of sublingual glyceryl trinitrate failed to improve postprandial symptoms such as nausea, fullness, and abdominal pain, despite a reduction in antral area, arguing against a role for nitric oxide in symptom generation (Undeland et al. 1998).

Diabetic gastroparesis is often attributed to irreversible autonomic neuropathy, based on the observation of similar symptoms in vagotomised patients (Rundles 1950). This concept was reinforced by studies which demonstrated an association between delayed gastric emptying and abnormal cardiovascular autonomic function (Buysschaert et al. 1987; Ziegler et al. 1996; Merio et al. 1997; Nohara et al. 2006). However, this association is weak and inconsistent (Jones et al. 2001; Horowitz et al. 2002; Asakawa et al. 2005). In part, this may reflect the lack of specific tests for the assessment of gastrointestinal autonomic function, but it also

suggests that other mechanisms are involved (Clarke et al. 1979; Clarke and Ewing 1982).

2.5.3 Clinical manifestations

Abnormal gastric emptying is clinically important because it is associated with poor glycaemic control, altered drug absorption, malnutrition, decreased quality of life and, in severe cases, recurrent hospitalisations with significant associated costs (Gallar et al. 1993; Horowitz et al. 2001; Bell et al. 2002; Horowitz et al. 2002). Gastroparesis is also now recognised as a risk factor for hypoglycaemia in insulin-treated patients (Horowitz et al. 2006). Information regarding the natural history of diabetic gastroparesis is scarce, but available data suggest a relatively stable long term course, and no substantial association with increased mortality (Kong et al. 1999; Gentilcore et al. 2003). Gastric bezoar is a well recognised, but rare complication, which may result from a reduction in gastric phase III activity (Brady and Richardson 1977; Horowitz et al. 2002), since the latter is responsible for the emptying of indigestible solids.

The prevalence of upper gastrointestinal symptoms among patients with both type 1 and type 2 diabetes has been reported to be higher than in the general community (Schvarcz et al. 1996; Ricci et al. 2000), affecting particularly women (Schvarcz et al. 1996; Ricci et al. 2000), and is associated with decreased quality of life (Farup et al. 1998; Talley et al. 2001) and psychological dysfunction

(Lustman et al. 1991; Talley et al. 2001), independent of other diabetic complications (Horowitz et al. 2002). Common symptoms include early satiation, postprandial fullness, epigastric pain, nausea, vomiting, and weight loss (O'Donovan et al. 2003); however, only postprandial fullness and bloating have been shown to correlate with delayed gastric emptying (Jones et al. 2001), suggesting that the aetiology of symptoms is multifactorial. Symptoms are usually most severe postprandially, but may persist long after food ingestion (O'Donovan et al. 2003). Conversely, some patients with markedly delayed gastric emptying experience no upper gut symptoms (Rayner and Horowitz 2005).

Other than disordered motility, gastrointestinal symptoms in diabetes could potentially be influenced by glycaemic control, psychological and demographic variables, autonomic neuropathy, visceral hypersensitivity, gastric myoelectrical activity, and the use of medications (Horowitz et al. 2002).

Little attention has been paid to the potential for the absorption of orally administered drugs, which occurs predominantly in the small intestine, to be affected by disordered gastric motility. Delayed gastric emptying is of particular clinical significance where a rapid onset of drug effect is desired, including some oral hypoglycaemic drugs (Groop et al. 1989). Reduced or absent phase III activity, which may be secondary to hyperglycaemia, could also be important, particularly for drugs that are not degraded easily in the stomach (Horowitz et al.

2002). Nevertheless, steady-state concentrations for a majority of drugs are usually not substantially affected (Horowitz et al. 2002).

2.5.4 Investigations

Diabetic gastroparesis is usually chronic, and indeed has been defined arbitrarily as persisting for more than three months (Horowitz et al. 2001). A slow onset of typical symptoms (early satiation, bloating, fullness, nausea, vomiting, and weight loss) in a patient with long-standing diabetes, without features on history or physical examination to indicate another cause, is suggestive of diabetic gastroparesis. However, in all patients, it is mandatory that mechanical obstruction be excluded by way of upper gastrointestinal endoscopy, with or without other imaging such as a small bowel series, prior to embarking on treatment.

Acute onset of upper abdominal symptoms in patients with diabetes should not automatically be attributed to gastroparesis, and efforts should be made to exclude other potential causes such as drugs, viral infection, electrolyte abnormalities, and endocrine disorders (Rayner and Horowitz 2005). Gastroparesis can also be a complication of malignancy, particularly associated with carcinomas of the pancreas, lung, and breast (Horowitz et al. 2001).

2.5.4.1 Measurements of gastric emptying

Scintigraphy is still regarded as the gold-standard test for evaluating gastric emptying (Horowitz and Dent 1991; Horowitz et al. 2002; O'Donovan et al. 2003; Talley 2003; Rayner and Horowitz 2005). Breath tests and gastric ultrasound are two promising, non-invasive, techniques to measure gastric emptying. They are simple (in the case of breath tests), are cheap to perform, and do not require exposure to ionising radiation.

Scintigraphy

Recent efforts have been made to improve a lack of standardisation between centres (Tougas et al. 2000; Ziessman et al. 2004). It has been suggested that a solid test meal alone is adequate, since solid emptying is more often delayed than that of liquids (Wright et al. 1985; Rayner and Horowitz 2005); however, nutrient liquid emptying is often impaired, and ideally, emptying of both solids and liquids should be evaluated concurrently using a dual isotope technique (Camilleri et al. 1998; Horowitz et al. 2002; Rayner and Horowitz 2005). Measurements should be carried out with blood glucose monitoring (Horowitz et al. 2002), and should be continued for at least 2, and possibly up to 4, hours, in order to optimise specificity and accuracy (Park and Camilleri 2006). Some have also advocated a simplified approach involving hourly scans for 4 hours, with gastric retention of over 10 % at 4 hours indicating gastroparesis (Camilleri et al. 1991; Tougas et al.

2000; Camilleri 2007). The scintigraphic technique will be discussed in greater detail in Chapter 4.

Breath tests

Stable isotope breath tests usually employ ^{13}C-octanoic acid for labelling of solids (Bromer et al. 2002; Zahn et al. 2003; Nohara et al. 2006; Punkkinen et al. 2006), and ^{13}C-acetate for liquids (Chew et al. 2003). Solid and liquid emptying can be measured concurrently using a ^{14}C/^{13}C dual label technique (Chew et al. 2003). Breath tests are based on the principle that gastric emptying is the rate-limiting step in the absorption of a ^{13}C-labelled meal, its metabolism in the liver, and appearance in exhaled air as ^{13}CO$_2$. Breath samples are usually collected for 4-6 hours (Bromer et al. 2002; Chew et al. 2003; Zahn et al. 2003; Braden et al. 2004; Punkkinen et al. 2006). Validation studies have generally indicated a high sensitivity and specificity in detecting delayed gastric emptying in both adults and children (Viramontes et al. 2001; Bromer et al. 2002; Chew et al. 2003; Zahn et al. 2003; Braden et al. 2004), but further evaluation is needed in certain patient groups, such as those with previous gastrointestinal surgery, pancreatic insufficiency, and liver or lung diseases, in whom gastric emptying may not be the only rate-limiting step in conversion of the substrate to ^{13}CO$_2$. Furthermore, the test is not well validated in patients with markedly delayed gastric emptying. Nevertheless, it shows substantial promise as a screening tool.

Ultrasonography

Conventional 2D-ultrasonography measures the cross-sectional antral area as an indicator of gastric emptying, and correlates well with scintigraphy (Irvine et al. 1993; Hveem et al. 1996; Benini et al. 1999; Darwiche et al. 2003; Gomes et al. 2003; Pedersen 2003). Its limitations are that it is operator dependent, and is not suited to evaluating gastric emptying of solid meals or obese subjects. 3D-ultrasonography, with the aid of a magnetometer-based position and orientation measurement (POM) device, offers more comprehensive imaging of the whole stomach (Gilja et al. 1997), and has been shown to correlate closely with scintigraphy in measuring gastric emptying (Gentilcore et al. 2006). The use of ultrasonography in gastrointestinal research will be discussed in greater detail in Chapter 4.

Despite the development of alternative modalities, scintigraphy remains the "gold-standard", but is not recommended for screening all long-standing diabetic patients, due to significant radiation exposure and the uncertain impact on subsequent management (e.g. whether to treat someone who has markedly delayed gastric emptying but is otherwise well). However, it is reasonable to perform such a test if either the diagnosis is suspected on clinical grounds, for example, a patient with symptoms suggestive of delayed gastric emptying, or if the test is performed to exclude abnormal gastric emptying as a cause of poor

glycaemic control, for example, an insulin-treated patient with postprandial hypoglycaemia.

2.5.5 Management

The goals of treatment are to relieve symptoms, improve oral intake and nutritional status, and optimise glycaemic control (Horowitz et al. 2002; Rayner and Horowitz 2005). In symptomatic patients, dietary modifications are usually inadequate, and most will require medical therapy (O'Donovan et al. 2003).

Once mechanical obstruction and other reversible causes are excluded, it is reasonable to commence an empirical trial of prokinetic therapy for perhaps 4 weeks, while recognising there is a substantial placebo response (Horowitz et al. 2001; Horowitz et al. 2002; O'Donovan et al. 2003; Rayner and Horowitz 2005). Those who fail to improve, or relapse after cessation of therapy, should have a scintigraphic gastric emptying study to confirm the presence of delayed gastric emptying, prior to embarking on further therapy (Horowitz et al. 2002).

2.5.5.1 Dietary modification

Fat slows gastric emptying more than protein or carbohydrate, while non-digestible solids may predispose to gastric bezoar formation. Therefore, small volume, frequent meals, low in insoluble fibre and fat, are generally

recommended, despite a lack of evidence to support this approach (Rayner and Horowitz 2005; Abell et al. 2006). Thorough chewing, remaining upright for 1-2 hours postprandially, and supplementation with multivitamins have also been advocated (Abell et al. 2006). Increasing the proportion of energy provided as liquids, rather than solids, may be beneficial, as liquid emptying is often less affected (Abell et al. 2006). An elemental diet is limited by unpalatability, but may be a short term option, despite no evidence to support its superiority over polymeric feeding. Total parenteral nutrition is expensive and impractical, and is associated with potentially serious complications, including sepsis (Dissanaike et al. 2007).

Indications for nutritional supplementation include weight loss of 10 % or more during a period of 3 to 6 months, inability to maintain recommended body weight, and severe symptoms requiring hospitalisation or non-pharmacological interventions e.g. nasogastric tube, to relieve nausea and vomiting (Abell et al. 2006; Camilleri 2007). Glycaemic control should be optimised and, if necessary, the use of an insulin pump be considered, as hyperglycaemia potentially further slows gastric emptying.

2.5.5.2 Medical therapy

The mainstay of pharmacological therapy involves the use of prokinetic agents. Efficacy appears to be greater when gastric emptying is more delayed (Horowitz

et al. 2002). The most commonly used prokinetic agents include metoclopramide, domperidone, erythromycin, and cisapride (Horowitz et al. 2001; Horowitz et al. 2002; O'Donovan et al. 2003; Talley 2003; Rayner and Horowitz 2005). The aim of therapy is to improve symptoms by accelerating gastric emptying, despite a poor correlation between the two (O'Donovan et al. 2003; Talley 2003). Some prokinetic drugs have additional properties which may be beneficial, including centrally mediated antiemesis, proximal gastric relaxation, suppression of visceral sensations, and improvement in gastric dysrhythmias (Tack et al. 1998; Rayner and Horowitz 2005). Agents that provide the greatest symptom relief are, therefore, not necessarily the most potent in accelerating gastric emptying (Rayner and Horowitz 2005). The main targets of pharmacotherapy include dopamine (D_2), serotonin (5-HT$_4$ and 5-HT$_3$), and motilin receptors (O'Donovan et al. 2003; Rayner and Horowitz 2005). D_2 and 5-HT$_4$ receptor activation have been shown to accelerate gastric emptying by stimulating acetylcholine release in the gastrointestinal tract (Tonini et al. 1999).

Most drugs are well tolerated, although side-effects can occur, especially at high doses. Tachyphylaxis is a problem in the long term use of erythromycin (Richards et al. 1993; Dhir and Richter 2004), and may potentially occur with metoclopramide and domperidone (O'Donovan et al. 2003), and is probably secondary to down-regulation of receptors.

Studies of prokinetic medications in gastroparesis are limited by small sample sizes, relatively few "prolonged" studies, and heterogeneity among patient groups. In addition, there are few data directly comparing different prokinetic agents, but there is limited evidence that combination therapy may be more effective than a single agent (Tatsuta et al. 1992). It has been suggested that serotonergic drugs may be more potent than antidopaminergic agents in accelerating gastric emptying (Tonini et al. 1999). All of the four most commonly used prokinetic agents have been shown to improve symptoms and quality of life (Horowitz et al. 2001; O'Donovan et al. 2003; Camilleri 2007). In general, compared to placebo, these agents increase gastric emptying by about 25 - 72 % and reduce symptom severity by 25 - 68 % (Camilleri 2007).

Erythromycin

Erythromycin is a motilin receptor agonist and the most potent drug for accelerating gastric emptying, when given acutely by the intravenous route (at doses <3 mg/kg) (Janssens et al. 1990). Therefore, it is often used as the initial treatment for patients hospitalised with severe gastroparesis (Rayner and Horowitz 2005). Of the oral formulations, a suspension may have greater efficacy than tablets (Ehrenpreis et al. 1998). Benefit is reported with long term use, albeit with some limitations due to probable down-regulation of motilin receptors (Richards et al. 1993; Dhir and Richter 2004). Hyperglycaemia should be corrected, as it attenuates the effect of erythromycin on gastric emptying (Jones et

al. 1999; Jones et al. 1999), and may have contributed to a loss of efficacy in some studies (Maganti et al. 2003). Long term exposure to an antibiotic represents an additional concern with prolonged therapy (Edelbroek et al. 1993). Other adverse effects include abdominal pain, vomiting, diarrhoea, headache, rash, and prolongation of the cardiac QT interval with a consequent risk of cardiac arrhythmias. Motilin agonists devoid of antibacterial properties appeared promising initially, but have been disappointing in clinical trials. For example, the motilin agonist ABT-229 failed to result in symptomatic improvement in patients with either type 1 diabetes (Talley et al. 2001) or functional dyspepsia (Talley et al. 2000), with or without delayed gastric emptying. However, blood glucose was not measured in these studies, and glycaemic influences on gastric emptying can therefore not be excluded. Another motilin agonist (KC 11458), showed no benefit on either gastric emptying or symptoms (Russo et al. 2004), but the efficacy may have been limited by postprandial hyperglycaemia. Newer motilin agonists include atilmotin, which has been shown to accelerate solid and liquid emptying in healthy subjects (Park et al. 2006), and mitemcinal (GM-611), which improves gastric emptying in those with diabetic and idiopathic gastroparesis (McCallum and Cynshi 2007) and, possibly, gastroparetic symptoms (McCallum and Cynshi 2007).

Cisapride

Cisapride is the most intensively studied prokinetic drug (Havelund et al. 1987; Horowitz et al. 1987; Camilleri et al. 1989; Abell et al. 1991; Horowitz et al. 2001; Veysey et al. 2001; Horowitz et al. 2002), and stimulates oesophageal (Horowitz et al. 1987), and small and large intestinal motility (Veysey et al. 2001), in addition to its effects on gastric emptying. Its use in gastroparesis from a variety of causes has been associated with prolonged symptomatic relief of more than one year (Abell et al. 1991).

Prior to recognition of its cardiac arrhythmogenic effect, cisapride was the oral prokinetic of first choice. Its arrhythmogenic potential stems from its class III antiarrhythmic property, rather than its action on the 5-HT$_4$ receptors, leading to prolongation of the cardiac QT-interval and concomitant risk of potentially lethal ventricular arrhythmias, such as torsade de pointes (Tonini et al. 1999). Most reports of arrhythmia were associated with high doses (80 mg per day) (Horowitz et al. 2001; O'Donovan et al. 2003). Cisapride has since been withdrawn from widespread use, but remains available in some countries through special access schemes. At greatest risk of arrhythmia are those with pre-existing QT prolongation, the young, and those receiving another drug that either prolongs the QT-interval or slows the metabolism of cisapride via CYP450 3A4 inhibition (e.g. class III antiarrhythmic agents, certain antihistamines, -azole antifungals, and macrolide antibiotics) (Tonini et al. 1999); combination with erythromycin is,

therefore, contraindicated. A careful medical history, measurement of serum electrolytes, and an ECG to exclude a prolonged QT-interval (>450 ms) are essential, prior to commencing treatment. A significant abnormality on any of these tests represents a contraindication to the use of cisapride; however, borderline abnormality in the presence of debilitating symptoms refractory to other therapies may warrant a trial of low dose therapy (up to 40 mg daily in divided doses) with close monitoring for adverse effects, provided the patient is fully informed of the potential risks.

Metoclopramide

Metoclopramide is less effective than cisapride in accelerating gastric emptying (McHugh et al. 1992), but has additional anti-emetic properties. Its D_2 antagonist and 5-HT_4 agonist effects result in prokinesis, while central D_2 and 5-HT_3 antagonist effects result in anti-emesis. Its low cost, wide availability, and the ability to be given parenterally are further advantages. Subcutaneous administration achieves 80 % of the plasma level achieved by the intravenous route, and three times the level after oral dosing (Rabine and Barnett 2001), and provides an option for outpatient therapy. Central nervous system adverse reactions affect up to 20 % of patients, particularly women, children, and the elderly (Tonini et al. 1999). Tardive dyskinesia is an uncommon, but potentially irreversible side effect, occurring in about 1 %. Endocrine side-effects, including hyperprolactinaemia, are not uncommon.

Domperidone

Domperidone crosses the blood-brain-barrier poorly, so has fewer central nervous system adverse effects than metoclopramide, and is emerging as the oral drug of choice, especially in the elderly. Non-central nervous system side-effects are similar to those of metoclopramide. It acts on gastrointestinal D_2 receptors to accelerate gastric emptying, but also influences the vomiting centre, which lies outside the blood brain barrier. Its prokinetic effect appears to be comparable to that of metoclopramide (Rayner and Horowitz 2005). Use of domperidone over a period of 4 weeks in the treatment of diabetic gastroparesis has been shown to provide significant symptomatic and quality of life improvements, and is well tolerated (Farup et al. 1998; Silvers et al. 1998). Combination with cisapride has been shown to be more effective than cisapride alone in patients with functional dyspepsia, in terms of accelerating gastric emptying and relieving symptoms (Tatsuta et al. 1992). However, a lack of parenteral formulation limits its use in patients with vomiting.

Antiemetics

Antiemetic agents, including the phenothiazines (promethazine, prochlorperazine) can be useful adjuncts to prokinetic therapy (Rayner and Horowitz 2005). Anti-serotonergic antiemetics (ondansetron, tropisetron, dolasetron, granisetron), and butyrophenones (haloperidol, droperidol) are alternatives, but their role in

gastroparesis has not been established, and the cost is substantial. The option of parenteral administration is an advantage of all of these medications. Aprepitant, a new class of potent antiemetic agent targeting the neurokinin NK-1 receptor, is available for chemotherapy-related nausea and vomiting, but efficacy in diabetic gastropathy is unknown.

Other therapies

Ghrelin is a peptide produced in the gastric mucosa which stimulates appetite and growth hormone release. Early trials using parenteral ghrelin have demonstrated improvements in gastric emptying in patients with diabetic and idiopathic gastroparesis (Murray et al. 2005; Tack et al. 2005; Binn et al. 2006), and in upper gut symptoms in idiopathic gastroparesis (Tack et al. 2005), but only small numbers of subjects have been studied and there are no long-term data.

Other $5-HT_4$ agonists, in particular tegaserod and mosapride, have been reported to accelerate gastric emptying, without significant potential for cardiac arrhythmias (Crowell et al. 2005; Abell et al. 2006). Tegaserod has since been withdrawn from the market due to potential cardiovascular adverse events. Renzapride is a combined $5-HT_4$ receptor agonist and $5-HT_3$ antagonist, whose effect on gastroparesis is currently under investigation (Abell et al. 2006). Levosulpiride, a selective dopamine (D_2) antagonist, is apparently effective in the acute and chronic (up to six months) treatment of diabetic gastroparesis, leading

to improvements in gastric emptying and upper gut symptoms (Mansi et al. 1995; Melga et al. 1997), as well as overall glycaemic control (HbA1c) (Melga et al. 1997), but is not available outside Europe. Itopride, a prokinetic agent that possesses antagonistic effects on both dopaminergic (D_2) receptors and acetylcholinesterase, has been reported to reduce upper gut symptoms in patients with functional dyspepsia (Sawant et al. 2004; Holtmann et al. 2006), with efficacy comparable to that of domperidone (Sawant et al. 2004). A study of itopride in diabetic gastroparesis found modest acceleration of gastric emptying of liquids, but not solids (Stevens 2006).

The phosphodiesterase-5 inhibitor, sildenafil, reverses delayed gastric emptying in rodent models of diabetes (Watkins et al. 2000); however, evidence is limited and inconsistent in humans with diabetic gastroparesis (Bianco et al. 2002; Dishy et al. 2004). The CCK-1 receptor antagonist, dexloxiglumide, attenuates the inhibition of gastric emptying by small intestinal feedback, in patients with functional dyspepsia (Feinle et al. 2001); its use in gastroparesis is yet to be reported. Activation of the endocannabinoid receptor (CB-1) slows gastric emptying (Massa et al. 2005), and its antagonist is, therefore, a potential prokinetic agent. The aldose reductase inhibitor, epalrestat, has been reported to improve diabetic autonomic neuropathy (heart rate variation at rest and during deep breathing) (Goto et al. 1995), and normalise the gastric slow wave (Okamoto et al. 2003), in patients with type 2 diabetes. Antioxidants have been found to reverse the retardation of gastric emptying caused by the generation of free

radicals in rats (Sharma et al. 2000), and celecoxib has been shown to reverse the delay in gastric emptying caused by glucagon in dogs (Xu and Chen 2007), but data are lacking in humans. The kappa-opioid agonist, fedotozine, failed to improve either symptoms or gastric emptying in patients with diabetic gastroparesis (Jones et al. 2000). There is evidence that C-peptide can improve autonomic function, but its administration failed to accelerate gastric emptying in a small cohort of patients with long-standing type 1 diabetes (Stevens et al. 2006). Acetylcholinesterase inhibitors, neostigmine and physostigmine, stimulate motor activity in the gut (Abell et al. 2006), but are not associated with significant clinical benefit, probably because the stimulation of motility is uncoordinated (Bortolotti et al. 1995). Herbal medicines such as rikkunshi-to and Zhishi-Xiaopiwan, have been reported to accelerate gastric emptying and improve upper abdominal symptoms in small studies (Lin et al. 1998; Kido et al. 2005). The effects of acupuncture on gastric emptying and gastric motility are inconsistent (Tatewaki et al. 2003; Tabosa et al. 2004; Wang 2004).

Injection of botulinum toxin into the pyloric sphincter was reported to improve gastric emptying and gastrointestinal symptoms in small uncontrolled studies in patients with diabetic and idiopathic gastroparesis, without significant complications (Ezzeddine et al. 2002; Miller et al. 2002; Lacy et al. 2004; Bromer et al. 2005; Abell et al. 2006; Arts et al. 2006; Ben-Youssef et al. 2006). The largest of these studies involved 63 patients with refractory gastroparesis (26 diabetic, 35 idiopathic, 2 post-operative), 43 % experienced symptomatic

improvements that lasted a mean duration of 5 months; vomiting was associated with poor response (Bromer et al. 2005). A separate study involving 20 gastroparetic patients (3 diabetic, 17 idiopathic) also demonstrated an improvement in solid, but not liquid, gastric emptying, after intrapyloric botulinum toxin injection, accompanied by significant reductions in meal-related symptoms (Arts et al. 2006). However, in the only placebo-controlled study, involving 23 gastroparesis patients (19 idiopathic), intrapyloric botulinum toxin injection failed to improve either symptom or gastric emptying (Arts et al. 2007). Therefore, more trials are required before this therapy can be recommended, especially as there are no good data on the prevalence of excessive pyloric contraction in patients with diabetic gastroparesis.

Many patients will have suffered from prolonged symptoms and are subject to substantial psychological stresses as that associated with other chronic illnesses. In functional dyspepsia, psychological stress is well known to exacerbate gastrointestinal symptoms (Creed et al. 1988; Olden and Drossman 2000). Therefore, it is important to recognise the impact of psychological influences on symptoms, although there is no evidence to support the use of any particular psychotherapeutic intervention.

2.5.5.3 Gastric electrical stimulation

Gastric electrical stimulation has been the subject of recent attention in the treatment of refractory gastroparesis. Two types of stimulation have been described; one uses low frequency, long duration pulses at or just above the frequency of gastric slow waves (3/min), and the other uses high frequency, short duration, pulses at four times the slow wave frequency (12/min) (Rayner and Horowitz 2005). The latter protocol, delivered by the Enterra gastric electrical stimulator (Medtronic Inc., USA), was approved in 2000 by the FDA in the USA for patients with refractory diabetic or idiopathic gastroparesis (Rayner and Horowitz 2005; Abell et al. 2006). The device consists of a pair of electrodes sutured to the anterior gastric wall, connected to a subcutaneous generator implanted in the anterior abdominal wall (Abell et al. 2006). Temporary, endoscopically placed electrodes can be used initially, to assess efficacy (Liu et al. 2006).

In diabetic and idiopathic gastroparesis, gastric electrical stimulation using the high frequency protocol has been reported to improve symptoms and quality of life, despite minimal acceleration of gastric emptying (Abell et al. 2003; Forster et al. 2003). Several studies have also reported improvements in glycaemic control in patients with diabetes, as indicated by a reduction in HbA1c of about 1 - 2 % (Lin et al. 2004; van der Voort et al. 2005; Lin et al. 2006); benefits were sustained at 6 and 12 months (Abell et al. 2003; Lin et al. 2004; van der Voort et

al. 2005), and may extend beyond 3 years (Lin et al. 2006). In the only placebo-controlled study involving 33 patients with chronic gastroparesis (17 diabetic, 16 idiopathic), all had gastric electrical stimulation device implanted, but were randomised to have the device either turned on or off, in a double-blinded design, for 1 month; those who had the device activated experienced a reduction in self-reported frequency of vomiting (Abell et al. 2003). Another study suggested that stimulation parameters need to be individualised for different patient groups, in order to achieve greatest efficacy, and that an algorithm can be used to identify optimal stimulation parameters for each patient (Abidi et al. 2006). The mechanism of clinical benefit is unclear, given the lack of entrainment of slow waves or substantial acceleration of gastric emptying (Abell et al. 2006). Possible effects include modulation of sensory function, or proximal gastric accommodation (Abell et al. 2006). Infection is the major risk, requiring removal of the device in up to 10 % (Forster et al. 2003; Lin et al. 2004). The absence of interstitial cells of Cajal on gastric biopsy may be predictive of more severe symptoms and a poor response to treatment (Forster et al. 2005). Anecdotal evidence suggests that patients with diabetic gastroparesis respond better to gastric electrical stimulation compared to the idiopathic group. Overall, the available evidence argues favourably for the benefit of electrical stimulation, but the device is expensive and invasive and should, at the present time, be used for refractory patients in referral centres, preferably in the context of a clinical trial. A new device utilising sequential neural electrical stimulation with multiple

electrodes implanted in the distal two-thirds of the stomach is currently being evaluated (Bortolotti 2002).

2.5.5.4 Gastrostomy/jejunostomy

Insertion of a venting gastrostomy has been advocated to improve symptoms and nutritional status in gastroparesis, although supporting data are limited (Jones and Maganti 2003). Feeding jejunostomy may improve symptoms, nutritional status, and overall health, and lead to fewer hospitalisations, but is associated with a high incidence of complications, often requiring further hospitalisation or surgery (Fontana and Barnett 1996; Jones and Maganti 2003).

2.5.5.5 Surgical therapy

Surgical treatments are usually reserved as last resort, since the benefit of these procedures is uncertain (Jones and Maganti 2003; Rayner and Horowitz 2005). There is a paucity of data regarding gastrectomy in diabetic gastroparesis. Most studies are small and uncontrolled, and involve patients with previous surgical procedures. Most authors favour either a complete, or extensive subtotal gastrectomy (Jones and Maganti 2003). One study involved seven women with type 1 diabetes and severe vomiting secondary to refractory gastroparesis, reported good symptomatic relief in six of these patients after extensive subtotal gastrectomy with Roux-en-Y gastrojejunostomy (Watkins et al. 2003).

Limited studies have reported an improvement in glycaemic control, gastric emptying, and gastrointestinal symptoms, following kidney-pancreas transplantation (Gaber et al. 1991; Cashion et al. 2004), while isolated pancreatic transplant may improve autonomic function (Kennedy et al. 1990; Navarro et al. 1997). It should be stressed, however, that not all patients experience an improvement in symptoms after either isolated pancreatic or combined kidney-pancreas transplant, and that frequent assessments are still required post-operatively (Cashion et al. 2004; Ben-Youssef et al. 2006). There is currently no information on the effect of pancreatic islet cell transplantation on diabeteic gastropathy. In a rodent model of gastroparesis, deficiency of nitric oxide synthase was overcome by intrapyloric injection of neural stem cells, with subsequent improvement in gastric emptying (Micci et al. 2005); it is unclear whether this approach could be translated to humans.

2.6   Conclusion

Both gastric motor and sensory functions are often disturbed in patients with diabetes, leading to great demands on the health care system. Optimisation of glycaemic control is the first step in the management of these patients, as hyperglycaemia can contribute to many of these abnormalities.

Diabetic gastropathy represents perhaps the most challenging complication of long-term diabetes. The pathogenesis is incompletely understood, and the limited relationship between symptoms and impaired gastric emptying represents a challenge to its management, and suggests that both motor and sensory functions are disturbed. Current treatment strategies aim to relieve symptoms, improve nutrition, accelerate gastric emptying, optimise glycaemic control, and support the patient psychologically, and a multidisciplinary approach is recommended. Gastric electrical stimulation appears promising in medically refractory cases, but more information is needed to determine who is likely to benefit.

NOTE:
This figure is included on page 91
of the print copy of the thesis held in
the University of Adelaide Library.

Figure 2.1. Relationship between postprandial glycaemia and gastric emptying after 75g oral glucose in healthy subjects and patients with type 2 diabetes (Adapted from Jones et. al. 1996)

Chapter 3:

Exocrine pancreatic insufficiency, gastric emptying, and glycaemic control in cystic fibrosis

3.1 Introduction

The human pancreas can be divided into exocrine and endocrine components. The secretion of glucagon and insulin from the pancreatic alpha- and beta-cells respectively, into the circulation, represents the major endocrine component; this process is regulated predominantly by the blood glucose concentration, but is also influenced by other factors such as the incretin hormones (GLP-1, GIP). The exocrine function is served by the secretion of digestive enzymes (amylase, lipase, trypsin, chymotrypsin), into the duodenum. Maldigestion only occurs when the postprandial secretion of pancreatic enzymes is below 10 % of normal (Lankisch 1993; Dominguez-Munoz et al. 2005), and pancreatic enzyme replacement is usually indicated when faecal fat excretion exceeds 15 g per day and/or when weight loss is present (Lankisch 1993).

The classic example of exocrine pancreatic insufficiency is cystic fibrosis (CF). CF has a high prevalence in western societies, with an estimated 1 in 2,500 live births being affected (Welsh MJ 1995; Wilson and Pencharz 1998). The disease is due to a genetic mutation occurring in the Cystic Fibrosis Transmembrane

Conductance Regulator (CFTR) Gene, inherited in an autosomal recessive fashion. More than 1,200 different mutations have been identified; however, the majority (70 %) of patients share the same mutation at the position of ΔF508 on chromosome 7, which is associated with more severe disease than other mutations (Rowe et al. 2005).

Mutation in the CFTR gene leads to the formation of defective chloride channels, typically affecting the lungs, pancreas, digestive tract, and reproductive organs, leading to multi-organ dysfunction. Approximately 95 % of all CF patients suffer from exocrine pancreatic insufficiency (Symonds et al. 2003; Baker et al. 2005), and fat malabsorption persists in 15 - 20 % despite pancreatic enzyme supplementation (Kalnins et al. 2005).

This chapter aims to explore the potential interactions between pancreatic enzyme supplementation, gastric emptying, and postprandial glycaemia, in patients with cystic fibrosis. A study that investigates these issues will subsequently be described in Chapter 11.

3.2 Pancreatic enzyme replacement therapy

The use of pancreatic enzyme replacement therapy (PERT) has resulted in dramatic improvements in nutrition, bodyweight, and linear growth, that are otherwise severely impaired in the majority of CF patients (Anthony et al. 1999).

The doses of pancreatic enzymes recommended by different centres have, however, been highly variable, and efforts have been made to standardise the dosing regimen. With the recognition that fibrosing colonopathy may be associated with high dose PERT (FitzSimmons et al. 1997; Ramsden et al. 1998), a maximum dose of 10,000 IU lipase per kilogram bodyweight per day is now generally recommended (Anthony et al. 1999).

Due to the potential for acid-mediated inactivation of pancreatic enzymes in the stomach, an enteric-coated formulation has become available, and has been demonstrated to be of equal, or superior, efficacy to the non-coated formulation in reducing faecal fat excretion (Dutta et al. 1983; Delchier et al. 1991). Compared to the non-coated formulation, enteric-coated enzymes do not increase fat absorption or free enzyme recovery in the proximal small intestine, as measured by aspiration, suggesting that enteric-coated enzyme release occurs more distally in the small intestine (Delchier et al. 1991). This has prompted the concept of adding non-coated enzymes to the enteric-coated pellets, in order to optimise food-enzyme interactions in the stomach and the proximal small intestine (Delchier et al. 1991; Meyer et al. 2001). However, such an approach was later shown to confer no additional benefit, in either fat absorption or daily energy intake (Kalnins et al. 2005). The size of the enzyme particles also influences the extent of mixing, and the synchronicity of gastric emptying, between food and enzymes. Microspheres with a diameter of about 1 mm are superior to larger particles (Meyer et al. 1988; Norregaard et al. 1996). Therefore, an enteric-coated

"mini-microsphere" represents the most frequently prescribed formulation for pancreatic enzyme supplementation (Dominguez-Munoz et al. 2005).

Despite the use of PERT, a significant proportion of CF patients still suffer from steatorrhoa, indicating inadequate digestion and absorption of fat (Meyer et al. 2001; Kalnins et al. 2005). In healthy subjects, there is a significant mismatch in the gastric emptying of dietary fat and pancreatic enzymes in mini-microspheres, with fat emptying rapidly initially and then more slowly, and enzymes emptying in the opposite pattern; for example, at 90 minutes after a meal, 50 % of oil but less than 25 % of enzymes have emptied from the stomach (Meyer and Lake 1997). Among CF patients, there is also a significant mismatch, with the enzymes generally emptying from the stomach more rapidly than a pancake and bean meal, but with large inter-individual variation (Taylor et al. 1999). More rapid gastric emptying of pancreatic enzymes has been shown to be associated with lower bodyweights (Taylor et al. 1999), suggesting an impact of the former on the adequacy of fat digestion.

It has long been demonstrated that pancreatic enzyme supplementation, when administered with meals, is as effective as hourly administration over the day in decreasing steatorrhoea (DiMagno et al. 1977). However, the ideal timing of administration in relation to a meal is still uncertain. Fat absorption was found to be no different when enteric-coated mini-microspheres of pancreatic enzymes were given before, during, and after a meal (Dominguez-Munoz et al. 2005);

however, the administration of enzymes in divided doses throughout the meal tended to improve fat absorption the most.

Excess faecal fat excretion in CF, as measured by the mixed triglyceride breath test, is not improved by the use of high-lipase versus conventional pancreatic enzyme supplementation (De Boeck et al. 1998). Potential explanations include: (a) the amount of lipase contained in existing pancreatic enzyme preparation is already adequate hence, any additional lipase will not improve fat digestion, as the predominant mechanism underlying persistent fat maldigestion is inadequate mixing between enzymes and food, and/or (b) the absorptive process for fat in the small intestine is defective in CF patients, as chloride channels are potentially involved in the preservation of small intestinal epithelial function (Gawenis et al. 2003; Simpson et al. 2005).

3.3   Gastric emptying in cystic fibrosis

There is limited information regarding gastric emptying in patients with CF. When compared to healthy subjects, fat given without pancreatic enzyme supplementation empties abnormally rapidly in CF patients with exocrine pancreatic insufficiency, presumably as a result of diminished feedback inhibition from the products of fat digestion in the small intestine (Carney et al. 1995). The available data about emptying of mixed meals is conflicting, with gastric emptying reported to be slowed (Cucchiara et al. 1996), accelerated (Collins et al.

1997), or unchanged (Symonds et al. 2003) in patients with CF, in the presence of pancreatic enzymes. The discrepancies are likely to reflect differences in meal composition, pancreatic enzyme regimen, posture, patient characteristics, and the technique used for measuring gastric emptying. Abnormal gastric myoelectric activity, measured by electrogastrography, appears to be more prevalent among CF patients (Schappi et al. 2004; Bentur et al. 2006), but whether this relates to gastric emptying remains uncertain.

3.4   Cystic fibrosis-related diabetes

3.4.1 Definition

The greatly improved life expectancy of CF patients, estimated to be over 40 years for those born in the 21st century (Frederiksen et al. 1996), has brought about a new clinical challenge – the management of their long-term co-morbidities, of which one of the most common and serious is cystic fibrosis-related diabetes (CFRD). Risk factors for the development of CFRD include older age, exocrine pancreatic insufficiency, ΔF508 mutation homozygosity, and female sex (Mackie et al. 2003; Marshall et al. 2005).

CFRD is generally attributed to a progressive decline in beta-cell function (Moran 2000; Lombardo et al. 2003), although peripheral insulin resistance also occurs later in the disease process (Hardin et al. 1999; Moran 2000). In the year 2000,

CFRD was estimated to affect 12 - 34 % of all CF patients, with a typical age of onset at about 20 years, and an increasing prevalence with age (Lanng et al. 1995; Moran 2000; Marshall et al. 2005). In addition, a similar number of patients had impaired glucose tolerance on standard oral glucose tolerance testing (Robert et al. 1992; Lanng et al. 1995; Moran 2000; Mackie et al. 2003). By 2008, however, these figures had increased substantially: impaired glucose tolerance was found to occur in 20 % of CF patients at age 10 years, 50 % at age 15 years, 75 % at age 20 years, and 82 % at age 30 years, whilst diabetes occured in >20 % at age 15 years, 45 % at age 20 years, and 75 % at age 30 years; insulin treatment was reported to be required in 30 % at age 20 years, and 40 % at age 30 years (Bismuth et al. 2008).

3.4.2 Pathogenesis

CFRD has long been recognised as a distinct clinical entity from type 1 or type 2 diabetes mellitus (Lanng et al. 1995; Moran et al. 1998; Moran et al. 1999). It is characterised by postprandial, rather than fasting, hyperglycaemia, and good glycaemic control is often difficult to achieve (Moran 2000).

The pathogenesis of CFRD is unclear, but progressive destruction of pancreatic beta-cells and subsequent pancreatic failure, caused by the CFTR gene, is believed widely to play an important role (Brennan et al. 2004). Histological samples collected during autopsy of CF patients have revealed increased fibrosis

and fatty infiltration in the pancreas, as well as a 50 % reduction in beta-cell numbers, and an increase in other non-beta type cells (Lohr et al. 1989). In one series, the autoantibody GAD65 (GADA), normally found in type 1 diabetes, was identified in 7 out of 8 CF patients (Stechova et al. 2005), suggesting an autoimmune contribution to the pathogenesis of CFRD. Conversely, in another study there was no association between the development of CFRD and the presence of the beta-cell autoantibodies GADA and IA-2A, or a family history of either type 1 or type 2 diabetes (Minicucci et al. 2005).

3.4.3 Glucose tolerance

The standard method for assessing glucose tolerance and diagnosing diabetes in the CF population is the 75 g oral glucose tolerance test (OGTT) (Dobson et al. 2004). Glycated haemoglobin has poor sensitivity for the diagnosis of CFRD, being within the normal range in >80 % of those subsequently confirmed to have diabetes on OGTT (Lanng et al. 1995), possibly due to reduced red cell life span and increased prevalence of iron deficiency anaemia (Brennan et al. 2004). Even in CF patients without diabetes, although conventional measures of glucose tolerance (HbA1c, fasting glucose, and blood glucose at 2h after OGTT) are similar to healthy subjects, overall glycaemia tends to be higher, as reflected by higher blood glucose concentrations at 30, 60, and 90 minutes, and a greater total area under the glucose curve (AUC), during an OGTT (Yung et al. 2002; Dobson et al. 2004). There is currently no consensus on a screening program for CFRD;

some have suggested annual screening for all CF patients from the age of 10 years (Lanng et al. 1995; Moran 2000; Mackie et al. 2003), while others advocate a more selected approach due to logistical limitations (Yung et al. 1999). The issue of screening is further complicated by fluctuating glucose tolerance, for example due to increased insulin resistance at times of infection, or during glucocorticoid therapy. Some have called for diabetes screening in all CF patients during episodes of acute illness (Moran 2000). However, in a Danish study, 58 % of CF patients with impaired glucose tolerance subsequently became normal at the following annual screening (Lanng et al. 1995), reflecting the fluctuating nature of glucose tolerance associated with this disease. It appears, therefore, that regular screening for diabetes should be performed when patients are well, in order to detect those requiring long term treatment and monitoring for diabetes-related complications, while additional evaluation of glucose tolerance should be performed during acute illnesses, to identify those requiring short term treatment, or adjustments to the existing treatment regimen.

3.4.4 Prognostic implications

The development of diabetes in CF patients is associated with an accelerated decline in clinical status and pulmonary function (Finkelstein et al. 1988; Lanng et al. 1992; Wilson DC 1996; Milla et al. 2000; Brennan et al. 2004; Marshall et al. 2005; Cawood et al. 2006), reduced bone mineral density (Cawood et al. 2006), increased rate of Pseudomonas aeruginosa respiratory infection (Cawood

et al. 2006), and an up to six-fold increase in mortality (Finkelstein et al. 1988; Moran et al. 1999; Liou et al. 2001; Mackie et al. 2003; Brennan et al. 2004). Earlier onset of glucose intolerance (< 18 years) is associated with higher rates of lung transplantation and mortality than those with later onset (Bismuth et al. 2008). When compared to non-diabetic CF patients, the rate of decline in forced-expiratory volume in 1 second (FEV-1) and functional vital capacity (FVC) is increased for up to 2 to 4 years preceding the diagnosis of CFRD (Brennan et al. 2004). Treating CFRD with insulin therapy has been reported to improve both lung function and nutritional status (Lanng et al. 1994; Moran 2000; Mohan et al. 2007), with FEV-1 being maintained at, or above, the pre-treatment level for a mean of 34 months, and an improvement in bodyweight for at least 3 years (Mohan et al. 2007). There is currently uncertainty over the likely long-term micro- and macro-vascular complications associated with CFRD. Limited data suggest that microvascular complications typically arise from 10 years after diagnosis (Mackie et al. 2003; Schwarzenberg et al. 2007). In one study, microvascular complications were only detected in those with CFRD for ≥10 years together with fasting hyperglycaemia (Schwarzenberg et al. 2007). Annual screening for microvascular complications after 5 years of diagnosis has, therefore, been suggested (Schwarzenberg et al. 2007). In contrast to type 1 and type 2 diabetes, the impact of macrovascular complications may be relatively less (Moran 2000), presumably reflecting the limited life-expectancy after diagnosis.

3.4.5 Treatment

Insulin is generally considered first-line treatment, and is targeted specifically at the control of postprandial hyperglycaemia (Moran 2000). However, the rationale for this approach is based largely on anecdotal evidence. Exogenous insulin aims to replace the underlying insulin deficiency, with the potential benefit of promoting weight gain, a desirable outcome in CF patients. Limited evidence, however, suggests that oral hypoglycaemic medications may be equivalent to insulin in terms of glycaemic control and long term outcome (Rosenecker et al. 2001; Onady and Langdon 2006). The only prospective study, involving 20 CFRD patients followed over 10 years, found no difference in glycated haemoglobin, FEV-1, or body weight, between groups who were either on insulin or oral hypoglycaemic agents (any combination of sulphonylurea, metformin, and thiazolidinedione) (Onady and Langdon 2006).

In CFRD patients on insulin, it has been recommended that blood glucose is measured at least four times per day, aiming for fasting values of 4.4 - 6.7 mmol/L, and values less than 10 mmol/L at 2 hours postprandially (Moran 2000). More frequent testing of blood glucose levels and titration of insulin doses are recommended during episodes of acute illness and corticosteroid treatment, as insulin requirements may increase by up to 4 times (Moran 2000).

Dietary management of CFRD is distinct from that of type 2 and some type 1 diabetics. Given the high risk of malnutrition, and the association between malnutrition and poor lung function and increased mortality, blood glucose should be controlled by adjusting insulin to balance the caloric intake, rather than by caloric restriction (Brennan et al. 2004).

3.5 Relationships between gastric emptying, pancreatic enzyme replacement, incretin hormone secretion, and glycaemic control

The breakdown of macronutrients into simple components is essential for stimulating small intestinal feedback to slow gastric emptying. Of all the macronutrient groups, fat appears to be the most potent inhibitor of gastric emptying (Horowitz et al. 1993). Blocking fat digestion with the specific lipase inhibitor, orlistat, is associated with accelerated gastric emptying (Pilichiewicz et al. 2003), reduced incretin hormone secretion (Feinle et al. 2003; Pilichiewicz et al. 2003), and greater postprandial glycaemic excursions (Pilichiewicz et al. 2003), indicating that fat needs to be digested in order to trigger neurohormonal mechanisms in the small intestine to slow gastric emptying. Fat emptying from the stomach is partially dependent on posture, as the upright, compared to the decubitus, posture, has been associated with a redistribution of oil to the proximal

stomach, and delayed emptying relative to the aqueous component (Horowitz et al. 1993; Boulby et al. 1997).

In CF patients with exocrine pancreatic insufficiency, the gastric emptying of oil consumed without pancreatic enzyme supplementation is more rapid than in healthy subjects, presumably as a result of fat maldigestion (Carney et al. 1995). The persistence of fat malabsorption in up to 20 % of CF patients (Kalnins et al. 2005), despite pancreatic enzyme supplementation, and the high prevalence of food-enzyme mismatch in their emptying from the stomach (Taylor et al. 1999), suggests that this persisting fat malabsorption may be secondary to the food-enzyme mismatch. The usual recommended diets for CF patients are high in fat and carbohydrates, due to the potential for malnutrition. Gastric emptying in CF patients is, therefore, likely to be dependent on the relative amounts of fat, protein, and carbohydrates in the meal, and be influenced by posture. Optimisation of pancreatic enzyme replacement therapy, with the aim of improving food-enzyme interaction, through coordinated emptying from the stomach may, therefore, improve fat digestion, and consequently slow gastric emptying and increase incretin hormone secretion.

Gastric emptying is an important determinant of postprandial glycaemia, accounting for approximately one-third of the variation in the initial postprandial rise in blood glucose (Jones et al. 1996). The incretin hormones, GLP-1 and GIP, play an important role in potentiating glucose-stimulated insulin secretion from

the pancreatic beta-cells (Elliott et al. 1993), whilst GLP-1 has the additional effects of suppressing glucagon production in the pancreatic alpha cells, and slowing gastric emptying. In the setting of CFRD, which is characterised by postprandial hyperglycaemia, optimisation of pancreatic enzyme replacement may therefore attenuate postprandial glycaemic excursions, ultimately leading to improved overall glycaemic control.

There is currently no published literature investigating the above concepts. The relationships between gastric emptying, incretin hormone secretion, and postprandial glycaemia in CF, will be addressed in Chapter 11.

3.6 Conclusion

As the life expectancy of patients with CF continues to improve, there will be a concomitant rise in the development of long term co-morbidities, and these will occupy an increasingly central place in their overall management. The prevalence of CFRD has risen dramatically in recent years, and this trend is likely to continue. Management of CFRD, despite recent accumulating evidence, is primarily anecdotal, and is non-standardised. Gastric emptying plays a fundamental role in the regulation of postprandial glycaemia, is frequently disturbed in CF, and should therefore be a focus in the management of CFRD, particularly as the latter is characterised by postprandial, rather than fasting, hyperglycaemia.

Chapter 4:

Techniques in assessing upper gut function

4.1   Introduction

Various techniques are available for the investigation of gastroduodenal function, each with its own advantages and disadvantages. The decision about which technique(s) to use is influenced by equipment availability, local expertise, desired outcome, cost, and the practicality of combining different methodologies. Combining more than one technique allows a more comprehensive assessment, and is often the preferred approach. All techniques discussed in this chapter have been employed in clinical studies included in this thesis.

4.2   Gastric emptying

4.2.1 Scintigraphy

Gamma camera scintigraphy is used extensively in both research and clinical settings, and is regarded as the "gold-standard" technique in evaluating gastric emptying of both solids and liquids (Horowitz and Dent 1991; Akkermans and van Isselt 1994; Camilleri et al. 1998; Horowitz et al. 2002; O'Donovan et al. 2003; Talley 2003; Rayner and Horowitz 2005). This test assumes that movement

of the radionuclide equates to emptying of the test meal. Therefore, it is imperative to ensure adequate mixing between the radionuclide and the test meal, and that the radionuclide stays bound to the meal particles whilst remaining in the gastrointestinal tract.

In diabetic patients, it has been suggested that solid emptying is more often delayed than liquid emptying and should therefore be the preferred test meal (Wright et al. 1985; Rayner and Horowitz 2005). However, this concept is based primarily on studies using low-, rather than high-nutrient, liquids (Horowitz et al. 2002), with the latter being equivalent to solids in terms of their rate of emptying (Gentilcore et al. 2003). Blood glucose levels should ideally be monitored during the study due to potential glycaemic influences on gastric emptying (Horowitz et al. 2002). Extending the duration of measurement from 90 minutes to at least 2, or even up to 4 hours, may improve specificity and accuracy (Park and Camilleri 2006).

In the studies described in Chapter 11, the test meal (either the glucose drink or mashed potato meal) was labeled with 20MBq of ^{99m}Technetium-sulfur colloid (^{99m}Tc). ^{99m}Tc has a short half life (6 hours), and is relatively low in cost and widely available, so was therefore chosen as the preferred radioisotope.

Scintigraphic images were acquired dynamically in the sitting position with the gamma camera positioned behind the subject, using 1-minute frames for the first

60 minutes, and 3-minute frames subsequently until 180 minutes. Data were corrected for subject movement, radionuclide decay, and for gamma ray attenuation Compton Scatter when necessary. A "region of interest" was outlined by tracing around the total stomach, followed by a separate line drawn across the mid-gastric band to divide the stomach into proximal and distal portions, with the proximal region corresponding to the fundus and the proximal body, and the distal region corresponding to the distal body and antrum (Collins et al. 1983). Gastric emptying curves were derived for total, proximal, and distal regions of the stomach, and expressed as a percentage of total initial count over time. The lag phase, defined as the time between completion of test meal ingestion and the first appearance of radioactivity in the duodenum, was also determined visually (Collins et al. 1983).

Limitations of scintigraphy include a lack of standardisation between centres in terms of the volume and composition of the test meal used, ideal posture of the subject, duration and frequency of data acquisition, and calculation of gastric emptying, although recent efforts have been made to reach a consensus in methodology (Abell et al. 2008).

4.2.2 Three-dimensional ultrasound

Ultrasound provides a non-invasive way of measuring gastric motor activity in real time, without any exposure to ionising radiation. Two-dimensional (2-D)

ultrasound has long been used to measure gastric emptying by taking serial images of a standardised parasaggital area of the antrum (Bolondi et al. 1985; Hausken et al. 1991). Calculating changes in antral area over time, as a measure of gastric emptying, has reasonable accuracy compared to the "gold-standard" technique of scintigraphy (Irvine et al. 1993; Hveem et al. 1996; Benini et al. 1999; Darwiche et al. 2003; Pedersen 2003). However, it remains less than ideal, due to potentially large errors arising from volume calculations based on major geometric assumptions regarding the shape of the stomach (Gilja et al. 1999), and does not take into account of variations in intragastric distribution.

More recently, 3-D ultrasound, with the aid of a magnetometer-based position and orientation measurement (POM) device, allows accurate estimation of gastric volume, by generating reconstructed 3-D images of the stomach based on sequential 2-D images (Gilja et al. 1994; Gilja et al. 1997; Gilja et al. 1998; Abraham et al. 2004). The use of 3-D ultrasound in the estimation of gastric fundic volume has been validated against the barostat balloon (Mundt and Samsom 2006). Estimated volumes of various other abdominal organs such as the stomach, kidney, or liver, have also been validated both in vitro (compared to actual organ volume) (Gilja et al. 1994; Gilja et al. 1998), and in vivo (compared to volume estimated by MRI) (Gilja et al. 1995). More recently, 3-D ultrasound has also been found to provide accurate measures of liquid gastric emptying in healthy individuals, as compared with scintigraphy (Gentilcore et al. 2006).

The process of volume measurement using 3-D ultrasound involves several steps, namely, data acquisition, digitisation, storage, processing, and display (Gilja et al. 1999). Acquisition of data is carried out by having a magnetic position sensor attached to the 2-D ultrasound scan-head, and obtaining sequential 2-D images during one continuous sweeping motion from the proximal to the distal stomach. Data processing involves the visual inspection of each 2-D image and manual selection of the area of interest.

3-D ultrasound allows separate analysis of the proximal and distal stomach. The proximal gastric volume is calculated using a dividing plane 10 cm distal to the diaphragm, perpendicular to the longitudinal axis of the stomach (Mundt and Samsom 2006). This distance was extrapolated from the mean maximum postprandial barostat bag volume of 500 ml (Mundt and Samsom 2006). The distal antral volume is calculated from an area starting from a plane perpendicular to the antral axis, where the antrum, liver, superior mesenteric vein, and the aorta can be seen simultaneously, to the pylorus (Mundt and Samsom 2006).

2-D and 3-D ultrasound share similar disadvantages, which include operator dependency, potentially compromised image quality when used on individuals who are obese or have large amounts of gas present in the bowels, and the limitation in meal composition, which requires the use of liquids.

4.3   Antropyloroduodenal motility

4.3.1 Manometry

Manometry is the most commonly employed tool in assessing motility in the upper gut. It involves the transnasal insertion of a flexible catheter (e.g. made of silicone), containing multiple lumens with each lumen terminating in the distal portion of the catheter, forming an array of multiple closely-spaced side-holes. The catheter also indorporates a sleeve sensor designed to span the pylorus; some catheters also have an extra side-hole distal to the sleeve sensor that allows infusion of a test nutrient directly into the proximal duodenum. The sleeve sensor (about 4.5 cm long) covers a relatively long section of the catheter, and is designed to record the tonic and phasic pressures generated by a very narrow (2 mm) contractile zone (Houghton et al. 1988; Houghton et al. 1988). The length of the sleeve sensor means that minor movements of the catheter relative to the pylorus can be tolerated without compromising its ability to measure pyloric pressures. The manometric catheter is constantly perfused with either degassed saline or water, at a constant rate of 0.15 ml/min; each lumen-occlusive contraction occludes one or more side-holes, generating a back-pressure to external sensors linked to a computer, and registering as a pressure wave. The frequency, amplitude, and organisation of pressure waves can then be recorded and subsequently analysed. The manometric catheter used in the studies in this thesis consisted of a 4 mm silicone tube containing a total of 21 lumens (but only

selected lumens were used, depending on the study design): sideholes 1 to 12 were situated in the duodenum, each 1.5 cm apart; sideholes 13 to 15 spanned the length of the sleeve, on the opposite side of the catheter, and were 1 cm apart; sideholes 16 to 20 were spaced 1 cm apart in the antrum, with channel 17 being connected to a 4 cm sleeve sensor; and an infusion sidehole was situated between sideholes 10 and 11 (Dentsleeve, Canada).

After an overnight fast of at least 10 hours, the manometric catheter is inserted transnasally and allowed to pass into the duodenum via peristalsis, which takes approximately 30 - 90 min in healthy humans. Correct positioning of the catheter is ensured by continuous monitoring of the transmucosal potential difference (TMPD) in the antrum and duodenum, that involves the subcutaneous insertion of a saline-filled needle in one forearm as a reference (Heddle et al. 1988). The antral reading is taken from the most distal antral channel immediately proximal to the sleeve sensor (channel 16), and duodenal reading from the most proximal duodenal channel immediately distal to the sleeve sensor (channel 12). Correct positioning of the catheter is indicated by an antral TMPD of <-20 mV, a duodenal TMPD of >-15 mV, and a difference between the two of >15 mV (Heddle et al. 1988). The antral and duodenal TMPD channels are perfused constantly with 0.9 % saline (degassed); both these and the saline-filled reference needle in the forearm are connected to calomel half cells (Ionode, Qld, Australia) via 2.2 M potassium chloride-filled electrodes (Heddle et al. 1988). This setup

creates an electric circuit enabling continuous measurement of TMPD across the pylorus (Sun et al. 1995).

Manometric pressure waves were digitised and recorded on either an Insight stationary system (Sandhill Scientific, Highlands Ranch, Colorado, USA) or a PC running commercially available software (Flexisoft; written by Assoc Prof G Hebbard, Royal Melbourne Hospital, Melbourne, Australia), for subsequent analysis. Antropyloroduodenal pressures were analysed using established software (written by Prof AJ Smout, Department of Gastroenterology, University Hospital Utrecht, The Netherlands). Pressure waves with an amplitude of ≥10 mmHg (Andrews et al. 2001), occurring in the antrum, pylorus, and the duodenum were counted and analysed for their frequency, amplitude, and motility index, the latter calculated according to the formula (Samsom et al. 1997): Motility index = ln [(sum of amplitudes x number of pressure waves) + 1]. Pressure wave sequences were defined as two or more temporally-related pressure waves, occurring in consecutive channels within ±3 s (in the duodenum) or ±5 s (in the antrum) of each other (Andrews et al. 2001).

In addition, pressure waves in the pylorus that occur in isolation (isolated pyloric pressure waves or IPPWs) were counted manually, and were defined as pressure waves ≥10 mmHg in amplitude, detected by either the sleeve sensor or one of the sideholes between the antral and duodenal channels, that were (i) not associated with a pressure wave of any amplitude within 5 seconds in the most distal antral

or most proximal duodenal channels and, (ii) not recorded by more than one of the sideholes between the antral and duodenal channels (Heddle et al. 1988). Basal pyloric pressures were calculated by subtracting the mean basal pressure recorded at the most distal antral side-hole from the mean basal pyloric pressure recorded by the sleeve sensor (Heddle et al. 1988), using custom written software (Prof AJ Smout, Utrecht, The Netherlands).

Disadvantages of manometry include its invasiveness and discomfort, which potentially interferes with upper gut function, the time required for the catheter to reach the correct position via peristalsis, and a lack of sensitivity for detecting non-lumen occlusive contractions.

4.4   Flow events

4.4.1 Impedance monitoring

The principle of impedance monitoring relies on measuring changes in the electrical conductance, or "impedance", across two electrodes situated inside the lumen of the gut. The catheter used to measure impedance consists of a series of paired electrodes mounted on a thin (2 mm diameter) plastic catheter, with each electrode connected to an impedance transducer outside the body via thin wires running within the catheter. The impedance measured between the paired electrodes is inversely proportional to the conductivity of the intraluminal

contents and the cross-sectional area of the lumen (Nguyen et al. 1999). Air has lower electrical conductivity, and its passage therefore causes an increase in impedance; conversely, electrolyte-containing fluids such as chyme have high electrical conductivity and lead to a drop in impedance (Nguyen et al. 1999). Passage of a bolus also leads to an increase in luminal diameter and results in a drop in impedance.

The impedance recording of the passage of a bolus typically yields five stages: (1) resting, (2) passage of air preceding a bolus, (3) passage of a bolus, (4) intestinal wall contraction associated with luminal occlusion and, (5) return to the resting state (Nguyen et al. 1999). Each stage yields a distinctive impedance pattern.

To date, impedance has largely been used for assessing gastroesophageal reflux disease, and has been validated against radiographic studies as having a high accuracy in intra-oesophageal fluid/air transport assessment (Nguyen et al. 1999). It is mostly used in combination with manometry and pH monitoring, and has proven to be valuable in the detection of non- or weakly-acidic reflux events; the height reached by the refluxate can also be measured.

The literature regarding the use of impedance monitoring to measure small intestinal flow is relatively limited. Compared to manometry, impedance monitoring shows greater concordance with the "gold-standard" of fluoroscopy, in the assessment of proximal small intestinal bolus transport (Imam et al. 2004);

in addition, impedance monitoring correlates well with transpyloric Doppler ultrasound in assessing flow events (Savoye et al. 2003; Savoye-Collet et al. 2003). The correlation between impedance and fluoroscopy for antegrade flow in the proximal small intestine ranged between 51.8 and 100 %, reflecting the fact that the intestinal lumen did not always completely clear its content, thus limiting the capability of impedance to detect subsequent flow events (Imam et al. 2004). Events recorded by impedance and manometry only correlated with each other in two-third of cases, indicating either flow events induced by changes in bowel tone without lumen-occlusive pressure waves, or pressure wave-induced flow events with incomplete luminal content clearance (Imam et al. 2004).

The combined manometry/impedance assembly used in the studies described in Chapters 8 and 9 comprised a multilumen silicone manometric catheter (external diameter 4 mm; 6 duodenal channels spaced at 3 cm intervals, with an extra side hole for infusion situated between the 2 most proximal channels) (Dentsleeve, Canada), and an impedance catheter (external diameter 2 mm; 8 electrodes spaced at 2 cm intervals) (Sandhill Scientific, Highland Ranch, Colorado, USA), bound in parallel with paraffin tape so that the manometry side holes and impedance electrodes spanned the same region of the duodenum. Once inserted transnasally, the combined manometry/impedance assembly was allowed to reach the desired position by peristalsis; correct positioning was ensured by continuous TMPD monitoring as described previously. Both manometry and impedance signals were recorded simultaneously at a sampling rate of 30 Hz (Insight stationary system,

Sandhill Scientific, Highlands Ranch, Colorado, USA) and stored on a hard disk for subsequent analysis. Impedance recordings were analysed by two independent observers (one of whom was the author), who were blinded to the study conditions. A flow event was defined as a transient decrease in impedance of ≥ 12 % from baseline (Imam et al. 2004) in at least 3 sequential electrode pairs (i.e. ≥ 6 cm) (Nguyen et al. 1995). The number, length, and direction (either antegrade or retrograde) of flow events were quantified for subsequent analysis. Observations of the two observers were compared, and consensus was reached for any discrepant interpretation.

Compared to other techniques for assessing small intestinal motor activity, such as manometry and fluoroscopy, impedance monitoring has the advantage of allowing direct measurement of intraluminal content movement, without exposure to ionising radiation. However, it does have its own limitations, such as a current lack of automated analysis, the presence of non-specific fluctuations in impedance of unknown significance, an inability to monitor large, hollow regions such as the stomach, and reduced sensitivity in the event of inadequately cleared intraluminal content from a previous flow event (Nguyen et al. 1999). Impedance monitoring is therefore best used in combination with other techniques, allowing a more comprehensive assessment of small intestinal motor activity, rather than being used in isolation.

4.5   Upper gut symptom questionnaire

4.5.1 Visual Analogue Scale (VAS)

The visual analogue scale is the most commonly used tool for assessing upper gut sensory function (Quan et al. 2003; Sturm et al. 2004). It consists of a horizontal line of a specified length, for each gastrointestinal symptom; one end of the line indicates that the subject experiences no such symptom at all, and the other end indicates maximum severity of the symptom. The subject is instructed to place a vertical mark across the line to reflect symptom intensity. The VASs used in this thesis were 100 mm long and had been previously validated (Quan et al. 2003; Sturm et al. 2004). Symptoms assessed included desire to eat, hunger, nausea, and fullness.

4.6   Conclusion

Various techniques were used in different combinations for each study. Gastric emptying was measured by either scintigraphy or 3-D ultrasound, antropyloroduodenal motility by manometry, duodenal flow events by impedance monitoring, and upper gut sensation by visual analogue scales. By combining various techniques, different aspects of upper gut motor and sensory function can be measured simultaneously, allowing a more comprehensive assessment of upper gut function.

Chapter 5:

Methodologies

5.1   Introduction

All techniques employed in the studies reported in this thesis have been validated and are considered either the best available or the most practical.

5.2   Subjects

Two major groups of subjects were recruited in the studies reported in this thesis:

(1) Healthy volunteers

(2) Patients with cystic fibrosis

Healthy volunteers (aged 18-50) were recruited by posting written flyers on notice boards around the local hospital and universities. Prior to enrolment, potential volunteers were screened either by direct face-to-face or over-the-phone interview, to exclude those with a past history of gastrointestinal disease or surgery (except uncomplicated appendicectomy), those taking medication(s) known to affect gastrointestinal function, and those with significant cardiac, respiratory, or renal disease, epilepsy, diabetes, or those who smoked more than

10 cigarettes per day or consumed excessive alcohol (>20 g per day for women and >40 g for men).

Patients with cystic fibrosis were recruited while they were inpatients by direct visits to the respiratory ward at the Royal Adelaide Hospital. Studies, however, were only performed subsequently as outpatients. Only those with documented pancreatic insufficiency and without significant organ impairment (forced expiratory volume in 1 second [FEV1] of < 30 %, Child-Pugh score > 6, previous gastrointestinal surgery), and those not on medication(s) with known gastrointestinal effects that could not be temporarily ceased, were recruited.

5.3   Ethical approval

All study protocols were first submitted to the Royal Adelaide Hospital Ethics Committee for assessment, and in cases where a drug was involved, also to the Drugs Subcommittee, followed by notification to the Australian Government Therapeutic Goods Administration. Where exposure of the subject to radiation was required, a radiation safety report was generated and submitted to the Environmental Protection Authority. Studies commenced only after all relevant approvals had been granted.

5.4 Study environment

All studies were performed in dedicated clinical study facilities in either i) The Discipline of Medicine, University of Adelaide, or ii) the Gastrointestinal Investigation Unit of the Department of Gastroenterology and Hepatology, or iii) where ionising radiation was involved, the Department of Nuclear Medicine, PET, and Bone Densitometry, all at the Royal Adelaide Hospital. Subjects assumed various positions during the studies according to specific protocol requirements. Generally, gastric emptying was measured in an upright sitting position, and manometry and impedance monitoring in the supine position.

5.5 Drugs

5.5.1 Metoclopramide

Metoclopramide hydrochloride is a dopamine antagonist that possesses both peripheral (prokinetic) and central (antiemetic) actions, and is a commonly used agent in clinical medicine. The usual therapeutic dose is 10 mg given either orally or parenterally. In the upper gut, metoclopramide accelerates gastric emptying, although its effect on the motor function of other parts of the gastrointestinal tract is poorly understood. Common side effects of metoclopramide include extrapyramidal symptoms and rarely (in approximately 1 % of recipients),

dystonic reactions requiring treatment with antidotes such as the dopamine agonist benztropine. However, the drug is generally well tolerated. A 10 mg intravenous dose of metoclopramide was therefore chosen as the prokinetic agent in the study described in Chapter 8.

5.6   Glycaemic clamp

In studies described in Chapters 9 and 10, the glycaemic clamp technique was used to maintain the subjects' blood glucose at desired levels. In chapter 9, subjects' blood glucose was kept at either 5 mmol/L or 9 mmol/L; in chapter 10, blood glucose concentrations were either 5 mmol/L or 15 mmol/L. Blood samples (~0.5 mL) were taken every 5 minutes throughout the duration of the glycaemic clamp, for immediate analysis of blood glucose using a portable glucometer (Medisense Optium, Bedford, MA, USA).

On the hyperglycaemic days, a loading dose of either 50 mL (glycaemic target 9 mmol/L) or 100 mL (glycaemic target 15 mmol/L) of 25 % glucose (Baxter Viaflex, Baxter Healthcare, Australia) was infused intravenously over 1 and 2 minutes respectively, followed by infusion initially at 150 mL per hour, with the rate adjusted subsequently according to blood glucose levels. A bag of insulin/gelofusine and a bag of 0.9 % normal saline were also connected, so that subjects could not distinguish between the study days.

On the euglycaemic days, the same volume of bolus dose was given but instead of 25 % glucose, 0.9 % normal saline (Baxter Viaflex, Baxter Healthcare, Australia) was used, and infused intravenously over 1 or 2 minutes, followed by a constant infusion at the rate of 150 mL per hour, throughout the remainder of the study. Insulin (Actrapid Penfill, 100 IU/mL, Novo Nordisk, Australia), made up of 100 IU (1 mL) of Actrapid in 499 mL of gelofusine (B. Braun, Australia), yielding a final concentration of 0.2 IU per mL, was infused to keep blood glucose at 5 mmol/L, and rate adjusted accordingly. An intravenous infusion of 25 % glucose (Baxter Viaflex, Baxter Healthcare, Australia) was started if blood glucose level fell below 5 mmol/L, and infusion rate adjusted accordingly.

5.7   Autonomic nerve function testing

Cardiovascular reflex tests are used widely as a surrogate marker for the function of the autonomic nerve supply to the gut, as no direct and accurate test of gastrointestinal autonomic nerve function is available. Autonomic nerve function testing evaluates both the sympathetic and parasympathetic systems, as sympathetic function tends to be impaired late compared to parasympathetic function (Clarke et al. 1979; Clarke and Ewing 1982). The presence of abnormal cardiovascular reflex tests in diabetic patients confers a predictive value in mortality, with 5-year mortality of 33 % if one test is abnormal, and up to 58 % if three tests are abnormal (Clarke and Ewing 1982). Recommended tests for parasympathetic function include the variation in heart rate (R - R interval) during

deep breathing, heart rate response to valsalva manoeuvre, and heart rate response to standing; tests for sympathetic function include the blood pressure response to sustained handgrip, and to standing (Clarke and Ewing 1982).

The tests adopted in this thesis include the heart rate R-R interval variation during deep breathing, heart rate response to standing, and blood pressure response to standing. Heart rate variation during deep breathing requires the subject to breathe deeply at the rate of 6 breaths per minute, and the difference between maximum and minimum heart rate is measured. The normal response is a difference of ≥ 15 beats-per-minute (bpm), with abnormal being ≤ 10 bpm. Heart rate response to standing is measured by using continuous ECG recording, and the length of the R - R interval at beats 15 and 30 after standing is measured to derive the 30:15 ratio; a ratio of >1.03 is normal and ≤ 1.00 is abnormal. The blood pressure response to standing is considered abnormal if the fall in systolic blood pressure is ≥ 30 mmHg (Clarke et al. 1979). Each test result is scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline, 2 = abnormal, for a total maximum score of 6; a score of ≥ 3 was considered to indicate definite autonomic dysfunction (Clarke and Ewing 1982; Piha 1991).

5.8 Biochemical/hormonal measurements

Blood samples were collected at specific time points via an intravenous cannula. After collection, hormone samples were placed immediately into ice-chilled tubes

containing EDTA and aprotinin (Bayer Australia, Pymble, Australia) at a concentration of 400 KIU/mL of blood; blood glucose samples were placed into S-monovette tubes (Sarstedt, Germany); 3-O-methylglucose and ^{14}C-3-O-methylglucose samples were placed into serum tubes (Sarstedt, Germany). All samples were centrifuged within 60 minutes of collection, at 3200 rpm (1,500 G) for 15 minutes at 4 ^{o}C, and stored at -70 ^{o}C until analysed.

5.8.1 Blood glucose

The blood glucose concentration (mmol/L) of whole blood was analysed immediately after collection using a portable glucometer (Medisense Optium, Abbot Laboratories, Bedford, MA, USA) using the glucose oxidase method. The accuracy of this method has been validated against the hexokinase technique (Horowitz et al. 1991).

5.8.2 Incretin hormones

5.8.2.1 GLP-1

In the study described in Chapter 7, plasma GLP-1 (pmol/L) was measured using radioimmunoassay; The intra-assay coefficient of variation (CV) was 17 %, the inter-assay CV 18 %, and the sensitivity was 1.5 pmol/L (Wishart et al. 1998). In the remaining chapters where plasma GLP-1 concentration was measured

(Chapters 8, 9, and 11), a newer GLP-1 kit was used for analysis (GLPIT-36HK, Linco Research, St. Charles, Missouri, USA); the intra-assay CV was 6.7 %, inter-assay CV 7.8 %, and the sensitivity was 3 pmol/L.

5.8.2.2 GIP

Plasma GIP (pmol/L) was measured using radioimmunoassay (Wishart et al. 1998). Intra- and inter-assay CVs were both 15 %, and the sensitivity was 2 pmol/L.

5.8.3 Insulin

Plasma insulin (mU/L) was measured by Enzyme-Linked Immunosorbant Assay (ELISA) (Diagnostics Systems Laboratories Inc., Webster, Texas, USA). The intra-assay coefficient of variation was 2.6 %, inter-assay coefficient of variation 6.2 %, and sensitivity 0.26 mU/L.

5.8.4 3-O-methylglucose

Approximately 30 % of glucose absorbed from the gut is metabolised by the liver (Bizeau and Pagliassotti 2005). 3-O-methylglucose (3-OMG) is a glucose analogue that is not metabolised by the liver, so its plasma levels serve as a more accurate measure of small intestinal glucose absorption (Campbell 1949; Csaky

and Glenn 1957; Fordtran et al. 1962). Following absorption, 3-OMG is excreted unchanged in the urine (Csaky and Glenn 1957). In rats, at 24 hours post ingestion of 3-OMG, traces of the analogue were found in the intestine, brain, and muscle, but not in blood or liver; after 48 hours, 95-100 % of the ingested dose was excreted in the urine (Csaky and Glenn 1957). In humans, approximately 90 % of ingested 3-OMG was excreted in the urine at 48 hours (Fordtran et al. 1962). 3-OMG is measured by high-performance liquid or gas chromatography (HPLC) (Kynaston et al. 1993).

5.8.5 ^{14}C-3-O-methylglucose

The analysis of plasma 3-OMG levels by HPLC is both labour-intensive and costly. The ^{14}C isotope of 3-OMG, plasma concentrations of which can be analysed by liquid scintillation, would provide a more rapid and less costly alternative. The study reported in Chapter 6 validated this technique by comparing plasma concentrations of ^{14}C-3-OMG to those of unlabelled 3-OMG, following oral ingestion of both isotopes.

5.9   Statistical analysis

All data were presented as mean values $\pm$ standard error of the mean (SEM). Two-way repeated measures of analysis of variance (ANOVA) was used to analyse gastric emptying rates, antropyloroduodenal pressures waves, duodenal

flow events, blood glucose, and plasma hormones, with treatment/patient group or time as factors. In the event of significant treatment/group x time interactions, post-hoc analysis by way of means comparisons was subsequently performed for each individual time point. Statview (SAS Institute Inc., Cary, NC, USA), SuperAnova version 1.11 (Acabus Concepts, Berkley, CA, USA), and SPSS version 15 (SPSS Inc., Chicago, IL, USA) were the software packages used to perform the statistical calculations. A P-value of <0.05 was considered significant.

5.10 Conclusion

The methodologies discussed in this chapter, and employed in the studies reported in this thesis, are well established and validated.

Chapter 6:

Validation of ^{14}C-3-O-methylglucose as a measure of intestinal glucose absorption in humans (Submitted for publication)

6.1 Summary

Labelling 3-O-methylglucose (3-OMG) with the ^{14}C isotope allows plasma ^{14}C-3-OMG activity to be analysed rapidly and inexpensively using liquid scintillation counting. This study aims to compare the plasma concentrations of ^{14}C-3-OMG to those of conventional 3-OMG, and establish the role of ^{14}C-3-OMG in measuring enteral glucose absorption. Eight healthy male volunteers consumed a drink comprising 50 g glucose, 30 mL of lemon juice, 3 g 3-OMG, and 111 kBq ^{14}C-3-OMG, made up to 300 mL with water. Plasma 3-OMG concentrations and plasma ^{14}C-3-OMG activities both peaked at 90 min after the drink, and the curves of mean plasma 3-OMG and ^{14}C-3-OMG showed a similar profile. There was a strong correlation between the two methods for the peak value (R = 0.89; P < 0.005) and area under the curve (R = 0.90; P < 0.005). It is concluded that plasma ^{14}C-3-OMG activity correlates closely with plasma 3-OMG concentration, and represents a more rapid and less costly alternative method for measuring enteral glucose absorption.

6.2   Introduction

Postprandial blood glucose concentrations are determined by multiple factors that affect glucose absorption or glucose disposal. Both the rate and the total amount of carbohydrate absorbed can impact on postprandial glycaemic excursions. The rate of gastric emptying is known to be a major determinant of glucose absorption and postprandial glycaemia (Jones et al. 1996; Jones et al. 2001; Gonlachanvit et al. 2003). A good correlation exists between gastric emptying and plasma 3-OMG concentrations, at 30 minutes after a glucose drink (Jones et al. 2001). Recent studies indicate that small intestinal motor function (Rayner et al. 2002; Schwartz et al. 2002), and the number and activity of mucosal glucose transporters (SGLT-1, GLUT-2) (Kellett and Brot-Laroche 2005) are also important.

The ability to measure small intestinal glucose absorption is important in studies of normal gut physiology, but is also potentially useful in evaluating small intestinal mucosal disorders and the "dumping syndrome" (Hasegawa et al. 1998). One established method of measuring glucose absorption involves the administration of the glucose analogue, 3-O-methylglucose (3-OMG). 3-OMG is absorbed from the small intestine by the same mechanism as glucose, but is not metabolised, so plasma concentrations of 3-OMG serve as a better index of glucose absorption than blood glucose concentrations, which are influenced by factors such as insulin, peripheral glucose uptake, and hepatic gluconeogenesis

(Fordtran et al. 1962). However, the analysis of plasma 3-OMG concentrations requires high-performance liquid, or gas, chromatographic methods (Kynaston et al. 1993), which are labour intensive and costly. By labeling 3-OMG with the ^{14}C radioisotope, plasma ^{14}C-3-OMG activity can be measured rapidly and relatively inexpensively by liquid scintillation counting, offering an alternative method for measuring intestinal glucose absorption.

This study aimed to validate the use of ^{14}C-3-OMG as a marker of intestinal glucose absorption by comparing its activity in plasma with the plasma concentration of unlabelled 3-OMG, after concurrent oral administration of both isotopes.

6.3   Methods

6.3.1 Subjects

Eight healthy male volunteers (mean age 26.7 ± 3.5 yr; mean body mass index [BMI] 24.8 ± 0.8 kg/m^2) were recruited by advertisement. No subject had diabetes, significant illness, or was taking medication known to affect gastrointestinal function.

6.3.2 Protocol

Each subject attended the laboratory at 0830h after an overnight fast of at least 10 hours for both solids and liquids. An intravenous cannula was inserted into a forearm vein for blood sampling.

At T = -2 minutes, subjects consumed a drink, consisting of 50 g glucose, 30 mL lemon juice, 3 g 3-OMG (Sigma-Aldrich Pty Ltd, Castle Hill, NSW, Australia), and 111 kBq ^{14}C-3-OMG (calculated effective dose 1.2 μSv), made up to 300 mL with water, over 2 minutes. Blood samples were obtained at T = -2, 15, 30, 45, 60, 90, 120, and 180 min, for measurement of blood glucose and plasma 3-OMG concentrations, and plasma ^{14}C-3-OMG activity.

6.3.3 Measurements

3-O-methylglucose (3-OMG)

Blood samples for plasma 3-OMG measurement were collected and centrifuged at 1,500 G for 15 minutes at 4 °C. Plasma was collected and stored at -70 °C until analysis by liquid chromatography-mass spectrometry (LC-MS). 3-OMG was extracted from plasma by protein precipitation extraction with methanol, under monitoring by an API3000^{TM} LC/MS/MS mass spectrometer (Applied Biosystems / MDS Sciex, Ontario, Canada). Data were acquired and processed

using an Analyst 1.4.2 system linked to the mass spectrometer. The range of detection was 2,000 to 200,000 ng mL^{-1}, when using a sample volume of 100 µL.

^{14}C-3-O-methylglucose (^{14}C-3-OMG)

Blood samples for plasma ^{14}C-3-OMG were collected and centrifuged at 1,500 G for 15 minutes at 4 °C. Plasma was collected and ^{14}C-3-OMG activities analysed within 24 hours of collection. 1.0 mL plasma (at room temperature) was solubilised with 20 mL Irga-Safe liquid scintillation counting cocktail (PerkinElmer Pty. Ltd., Rowville, Victoria, Australia). Samples were counted in a liquid scintillation counter (Packard 2100TR, Packard Instruments, Meriden, CT, USA) utilising the Direct DPM protocol with a coefficient of variation of 1 %. The colour and chemical quench results were expressed as disintegrations per minute (dpm) over background.

6.3.4 Statistical analysis

Correlations between peak value, area under the curve, and values at T = 15 and 60 min were examined by regression analysis using a statistical software package (SPSS 15.0, SPSS Inc, Chicago, IL, USA). Statistical significance was accepted as $P < 0.05$, and data are presented as means ± standard error of the mean (SEM).

6.4   Results

6.4.1 Blood glucose concentrations

Fasting blood glucose was 5.2±0.2 mmol/L, rising to a peak of 8.9±0.6 mmol/L at 45 minutes after the drink, before returning to baseline by 120 minutes. At 180 minutes, the blood glucose concentration (4.2±0.2 mmol/L) was slightly below that during fasting (Fig. 6.1).

6.4.2 Plasma 3-O-methylglucose (3-OMG) and ^{14}C-3-O-methylglucose (^{14}C-3-OMG)

Plasma 3-OMG concentrations reached a peak of 87.1±8.1 ng/L at 90 minutes, with an area under the curve (AUC) from 0-180 minutes of 11,158.0±561.7 ng/L min. (Fig. 6.1)

Plasma ^{14}C-3-OMG activity reached a peak of 195.4±14.6 dpm at 90 minutes, with an AUC from 0-180 minutes of 24,990.9±1,058.1 dpm min. (Fig. 6.1)

The profiles of mean plasma 3-OMG concentrations and mean plasma ^{14}C-3-OMG activities were similar. The 3-OMG and ^{14}C-3-OMG curves both showed a gradual rise and fall over 180 minutes; this contrasts with the blood glucose curve, where a much sharper rise and fall was observed (Fig. 6.1).

Both the peak value (R = 0.89; P <0.005) and area under the curve (R = 0.90; P < 0.005) showed excellent correlation between the two methods. (Fig. 6.2) Furthermore, excellent correlation was also found between 3-OMG and ^{14}C-3-OMG concentrations at 60 minutes (R = 0.95, P < 0.005).

6.4.3 Correlations between plasma 3-OMG concentrations, plasma ^{14}C-3-OMG activities, and blood glucose concentrations

Plasma 3-OMG and blood glucose concentrations showed good correlations, in terms of the peak value (R = 0.76, P < 0.05) and AUC (R = 0.81, P < 0.05). Similarly, plasma ^{14}C-3-OMG activities and blood glucose concentrations also showed good correlations, in terms of the peak value (R = 0.76, P < 0.05) and AUC (R = 0.79, P < 0.05). Furthermore, the initial rise (at 15 minutes after the drink) in blood glucose correlated well with both the plasma 3-OMG concentration (R = 0.94, P < 0.001) and plasma ^{14}C-3-OMG activity (R = 0.81, P < 0.05).

6.5   Discussion

To facilitate understanding of the physiology and absorption of glucose from the small intestine, and the impact of interventions on this parameter, a precise method of measuring glucose absorption which can be performed rapidly and

inexpensively is ideally required. We have shown that plasma activities of ^{14}C-3-O-methylglucose, measured by liquid scintillation counting (at 60 minutes, peak, and AUC), correlate closely with plasma 3-O-methylglucose concentrations, measured by liquid chromatography-mass spectrometry, in healthy subjects. We have also demonstrated that the shapes of the plasma concentration curves derived from the two techniques are very similar. Furthermore, the initial postprandial blood glucose concentration correlated well with the absorption of ingested 3-OMG, but later, factors affecting glucose disposal mean this is less the case.

The use of ^{14}C-3-OMG in the dose administered in this study involved a very small effective radiation dose of 1.2 µSv per study, indicating that the technique would potentially be safe for recurrent use in the same individual. Humans receive about 2 mSv of radiation from natural sources each year. Most of the 3-OMG and ^{14}C-3-OMG would be expected to be cleared from the body within 24 hours of administration, by renal elimination (Krecic et al. 2003). The long half-life of ^{14}C (5,720 years) means that analysis of plasma concentrations by liquid scintillation counting, if necessary, can be performed long after sample collection with negligible impact on the results.

This study has validated the use of ^{14}C-3-OMG as a rapid, simple, and inexpensive alternative to unlabelled 3-OMG, for the measurement of small intestinal glucose absorption.

(a)

(b)
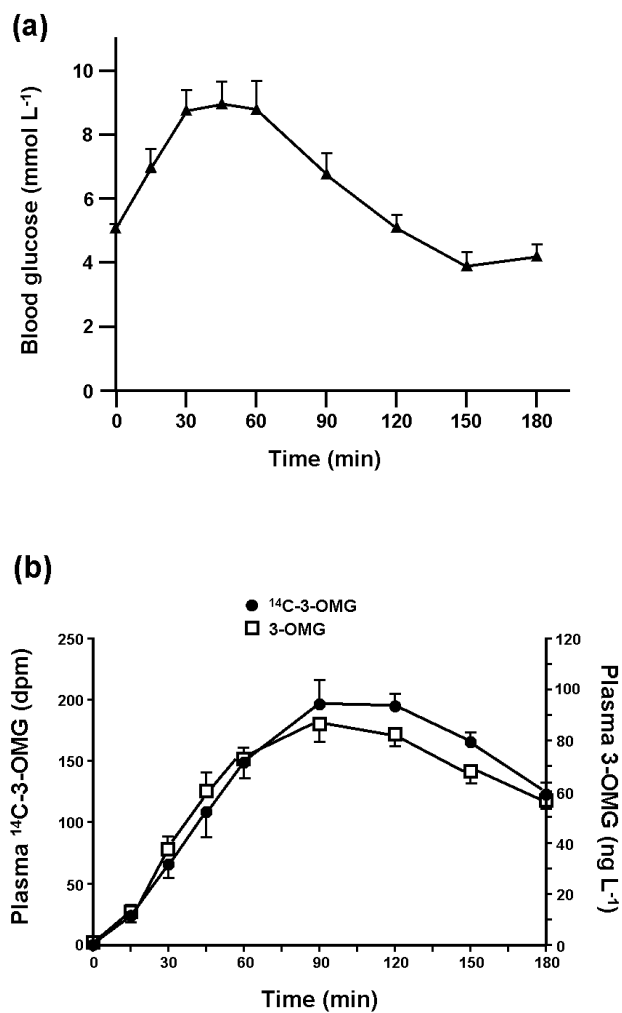
Figure 6.1

(a) Blood glucose, and (b) plasma 3-O-methylglucose (3-OMG) concentrations and plasma ^{14}C-3-O-methylglucose (^{14}C-3-OMG) activities. Values are means ± SEM. (n = 8).

(a)

Peak

R = 0.891
P < 0.005

3-OMG (ng L^{-1})

^{14}C-3-OMG (dpm)

(b)

AUC

R = 0.901
P < 0.005

3-OMG (x1,000ng L^{-1} min)
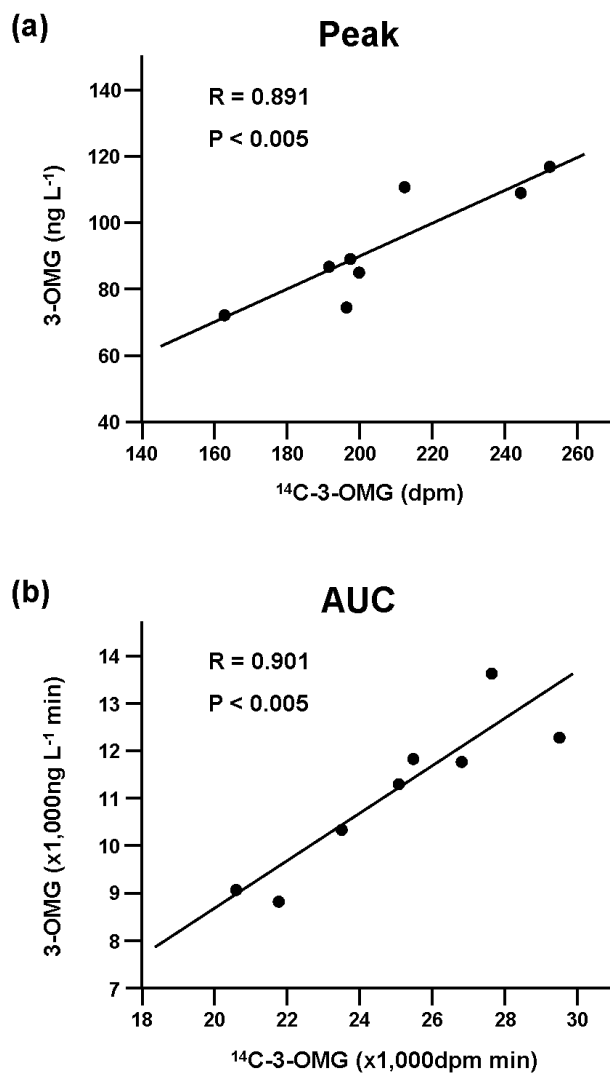
^{14}C-3-OMG (x1,000dpm min)

Figure 6.2

Relationships between plasma 3-O-methylglucose (3-OMG) concentrations and plasma ^{14}C-3-O-methylglucose (^{14}C-3-OMG) activities, for (a) peak value (R = 0.89; P < 0.005) and, (b) area under the curve over 180 minutes (R = 0.90; P = 0.005). (n = 8).

Chapter 7:

Transient, early release of glucagon-like peptide-1 during low rates of intraduodenal glucose delivery (Adapted from Kuo et al. Regulatory Peptides 2008;146:1-3)

7.1   Summary

The "incretin" hormones account for some 60% of the stimulation of insulin by oral glucose, but the determinants of their secretion from the small intestine are poorly understood. Cells which release GIP (K cells) are localised to the proximal small intestine, while GLP-1 releasing cells (L cells) predominate in the distal gut. It has been suggested that a threshold rate of duodenal glucose delivery (~1.8 kcal/min) needs to be exceeded for the stimulation of GLP-1 release. We performed a retrospective analysis on all studies performed in our laboratory involving healthy humans administered intraduodenal glucose at 1 kcal/min for 120 min, with the aim of establishing whether a low intraduodenal glucose load (1 kcal/min) has the capacity to stimulate GLP-1. A total of 27 healthy subjects (24 male; age 36 ± 3 years; BMI 25.2 ± 0.7 kg/m^2) were included. During intraduodenal glucose, plasma GLP-1 increased at 15 and 30 minutes ($P<0.001$ for both) and returned to baseline thereafter. In contrast, there were sustained increases in

plasma GIP (P<0.001), insulin (P<0.001), and blood glucose (P<0.001). It is concluded that, **i**n healthy subjects, there is early, transient stimulation of GLP-1 by glucose loads hitherto believed to be "sub-threshold". The mechanism underlying this effect, which could be attributed to initially rapid transit to jejunal L cells, or a duodeno-jejunoileal neural or hormonal loop, remains to be determined.

7.2   Introduction

In healthy subjects, orally ingested glucose elicits a much greater (~60 %) insulin response than an equivalent intravenous glucose load, a phenomenon termed the "incretin effect" (Efendic and Portwood 2004; Meier and Nauck 2004). This effect appears to be mediated primarily by the peptides glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are released from the small intestine in response to nutrients and augment glucose-induced insulin secretion from the pancreas (Efendic and Portwood 2004). GLP-1 is secreted by L cells located predominantly in the distal small intestine and colon, whilst GIP is secreted by K cells located predominantly in the duodenum (Deacon 2005).

In contrast to the load-dependent release of GIP, which is evident in response to small intestinal glucose loads of less than 1 kcal/min, it has been suggested that

the stimulation of GLP-1 secretion requires small intestinal glucose delivery at a rate exceeding 1.8 kcal/min (Schirra et al. 1996).

It has previously been shown that both GLP-1 and GIP, in response to intraduodenal glucose at 1 kcal/min, rise as early as at 15 min (O'Donovan et al. 2004; Chaikomin et al. 2005). While the pattern of their rise was not characterised, the levels of GLP-1 appeared to fall as early as at 45 min, as opposed to more sustained levels of GIP.

The rate of normal gastric emptying is controlled at 1 - 3 kcal/min (Horowitz et al. 1993). The pattern of GLP-1 and GIP release at 1 kcal/min may, therefore, be of physiological importance. To examine this issue, data were compiled from studies carried out previously in our laboratory, involving intraduodenal glucose infusion at 1 kcal/min, with the aim of characterising the patterns of GLP-1 and GIP release under low caloric stimulation.

7.3   Methods

7.3.1 Subjects

Three studies were performed during the preceding 4 years in which glucose was administered into the duodenum at 1 kcal/min for at least 120 min in healthy humans (O'Donovan et al. 2004; Chaikomin et al. 2005; Pilichiewicz et al. 2007).

These provided data from 27 healthy subjects (24 male; age 36±3 years; BMI 25.2±0.7 kg/m^2 [mean ± SEM]), who were all studied after an overnight fast of at least 10 hours. In the first study (12 subjects), glucose was given in water at 3ml per minute (463 mosmol/L) (Chaikomin et al. 2005); in the second study (6 subjects), glucose was given in saline (3 ml/min, 648 mosmol/L) (O'Donovan et al. 2004); in the third study (9 subjects), glucose was again given in saline (4 ml/min, 1390 mosmol/L) (Pilichiewicz et al. 2007). Three subjects from the original datasets, two in the second (O'Donovan et al. 2004) and one in the third (Pilichiewicz et al. 2007) study, were excluded, so that no subject was included more than once.

7.3.2 Protocol

In all studies, venous blood was collected at T = 0, 15, 30, 45, 60, 90 and 120 minutes (the intraduodenal infusion was commenced immediately after the baseline blood sample), and placed into ice-chilled tubes containing EDTA and 400 kIU/L aprotinin (Trasylol: Bayer Australia Pty. Ltd., Pymble, Australia). Blood glucose concentrations were determined immediately after each collection using a portable glucose meter (Medisense Precision QID; Abbott Laboratories, Bedford, MA). The accuracy of this method has been confirmed in our laboratory using the hexokinase technique. Plasma was separated by centrifugation (3200 rpm, 15 min, 4 ^{o}C) and stored at –70 ^{o}C for subsequent analysis of GLP-1, GIP, and insulin.

7.3.3 Measurements

Plasma GLP-1 concentrations were measured using radioimmunoassay (RIA) which detected total GLP-1 (O'Donovan et al. 2004). Plasma GIP concentrations were measured by RIA (O'Donovan et al. 2004). Plasma insulin concentrations were measured by Enzyme-Linked Immunosorbant Assay (ELISA) (Horowitz et al. 1996).

7.3.4 Statistical analysis

Blood glucose, GLP-1, GIP, and insulin concentrations were analysed using one way repeated measures analysis of variance (SuperANOVA, © 1989-1991 Abacus Concepts, Berkeley, CA 94704), followed by post-hoc comparisons with the baseline value in the event of a significant effect. Data are presented as mean ± standard error of the mean (SEM).

7.4 Results

7.4.1 Blood glucose, insulin, GLP-1 and GIP concentrations

Blood glucose and plasma GLP-1, GIP, and insulin all changed significantly during 120 minutes of intraduodenal glucose infusion (P < 0.001 for each). At 120 min, all but GLP-1 remained elevated compared with baseline. (Fig. 7.1)

Blood glucose increased significantly during the first 30 min (P < 0.001), and remained at a plateau thereafter. (Fig. 7.1)

Plasma insulin increased rapidly during the first 30 min (P < 0.001), followed by a smaller increase thereafter, eventually reaching a plateau by 90 min. (Fig. 7.1)

Plasma GIP also increased rapidly in the first 30 min (P < 0.001), and remained at a plateau thereafter. (Fig. 7.1)

In contrast, plasma GLP-1 showed an early peak at 15 min, remained significantly elevated until 30 min (P < 0.001 at both 15 and 30 min), but subsequently fell back to baseline level by 45 min. (Fig. 7.1)

7.5 Discussion

The use of intraduodenal, as opposed to oral, glucose in these studies allowed precise regulation of small intestinal glucose delivery. This study established that there is a transient, rapid stimulation of plasma GLP-1, in response to a small intestinal glucose load previously reported as "sub-threshold". This is in contrast to blood glucose, plasma insulin, and plasma GIP, which all remained significantly elevated until 120 min. Blood glucose was prevented from further increase after 30 min by the sustained increased levels of GIP and insulin. In contrast, the early and rapid fall in GLP-1 discounts its role as a major player in the incretin effect at the low caloric rate of 1 kcal/min.

The findings from this study contrast with those of Schirra et. al. (Schirra et al. 1996), where intraduodenal glucose delivery at rates below 1.8 kcal/min failed to release GLP-1. The smaller values reported for GLP-1 in their study, despite a higher caloric load, might indicate that differences in the GLP-1 assay account for the discrepancy.

Plasma GLP-1 is recognised to rise as early as 10 - 15 minutes after oral or intraduodenal glucose administration (Herrmann et al. 1995; Schirra et al. 1996; Rayner et al. 2000). This appears intuitively inconsistent with the concept of direct stimulation of the predominantly distally located L cells. There are four possibilities that could explain the early, transient rise: (a) One possibility is that

the existence of a "duodeno-jejunoileal loop", involving neural and/or hormonal signals, triggered by the presence of nutrient in the duodenum (Herrmann et al. 1995). (b) A second possibility is the release of GLP-1 directly from duodenal and proximal jejunal L cells (Theodorakis et al. 2006). Despite limited numbers, L cells in the duodenum and proximal jejunum may be capable of secreting enough GLP-1 to cause the small, early and transient peak that has been observed before the depletion of GLP-1 stores in these cells. (c) The transient nature of the rise in GLP-1, in response to low glucose loads, could be explained by the initial rapid transit of glucose in the small intestine, which is subsequently inhibited by the release of GLP-1; GLP-1 has been shown to slow small intestinal transit in rats (Tolessa et al. 1998), although there are no human data. In addition, time-related changes in absorption of glucose may account for the transient rise of GLP-1 from more distal L cells. There is evidence that small intestinal glucose absorption is enhanced by the insertion of GLUT2 transporters on the apical membranes of enterocytes, a process which is stimulated within minutes of exposure to glucose (Kellett and Helliwell 2000; Kellett and Brot-Laroche 2005). At the infusion rate of 1 kcal/min, the initial absorptive capacity of the Na^+/glucose co-transporter (SGLT-1) in the duodenum may be exceeded, leading to "spillage" of excess glucose into the jejunum which directly stimulates the release of GLP-1; as apical GLUT2 increases rapidly, the duodenum develops the capacity to absorb all of the glucose infused, leading to a loss of ongoing jejunal stimulation, and a subsequent fall in plasma GLP-1. A fourth possibility (d), that the transient rise in GLP-1 reflects feedback mechanisms that "down-regulate" the response of L cells to

ongoing nutrient stimulation, seems unlikely because hyperinsulinemia and hyperglycemia do not appear to mediate a negative feedback. For example, plasma GLP-1 levels in response to intrajejunal infusions of lipid and lactulose are not significantly different between euglycemic, hyperglycemic, and euglycemic hyperinsulinemic conditions (Byrne et al. 1998).

The recent observation that intraluminal glucose, infused at 3.5 kcal/min in healthy volunteers, released GLP-1 only when allowed access beyond the proximal 60 cm of small intestine (Little et al. 2006), appears to favor possibility (c), since there was no rise in GLP-1 when the 3.5 kcal/min glucose infusion was confined to the proximal 60 cm of small intestine.

The rapid fall in GLP-1 level, compared with the sustained elevation in GIP, suggests a much lesser contribution of GLP-1 towards the "incretin effect" than GIP, under low caloric (1 kcal/min) stimulation. This is in contrast to sustained elevations in both GLP-1 and GIP levels at higher caloric rates e.g. 3.5 kcal/min (Little et al. 2006), implying varying contributions from GLP-1 depending on the caloric load. A loss of GLP-1 response is therefore likely to be more detrimental during conditions of higher caloric load. Understanding the physiology of incretin release is likely to be important in type 2 diabetes, where GLP-1 secretion after an orally ingested meal is reduced compared with healthy subjects (Vilsboll et al. 2001), whilst secretion of GIP remains relatively intact.

Further studies are indicated to evaluate the mechanisms of GLP-1 release, and its relationship to gastric and small intestinal motility, and postprandial insulin and glycaemic responses, in both healthy and diabetic populations.
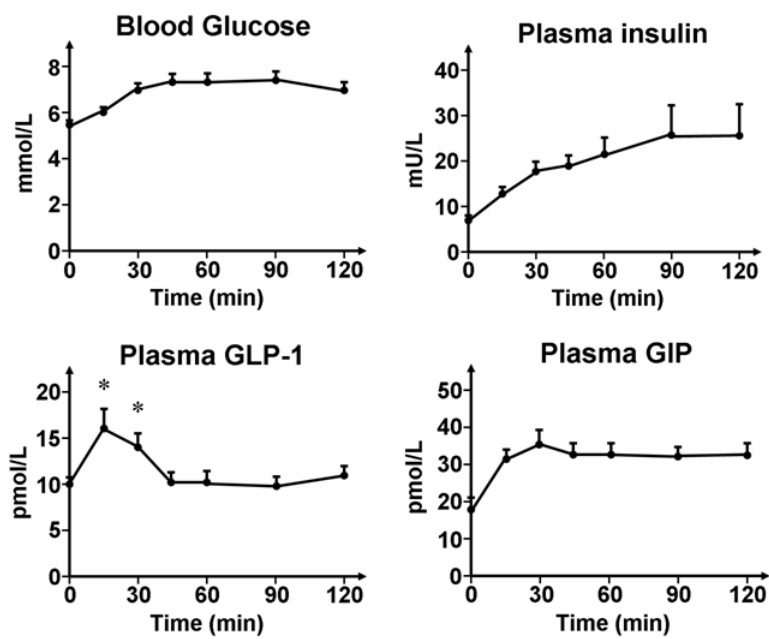
Figure 7.1

Blood glucose and plasma insulin, GLP-1, and GIP levels, during intraduodenal infusion of glucose at 1 kcal/min for 120 minutes. Blood glucose and plasma insulin, GLP-1, and GIP all increased significantly from baseline ($p < 0.001$). For GLP-1, only the values at 15 and 30 minutes differed significantly from baseline (*$p < 0.001$).

Chapter 8: Effects of metoclopramide on duodenal motility and flow events, glucose absorption, and incretin hormone release, in response to intraduodenal glucose infusion (Submitted for publication)

8.1 Summary

The contribution of small intestinal motor activity to nutrient absorption is poorly defined. A reduction in duodenal flow events after hyoscine butylbromide, despite no change in pressure waves, was associated with reduced secretion of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), and a delay in glucose absorption. Eight healthy volunteers (7 male; age 29.8±4.6 yr; BMI 24.5±0.9 kg/m^2) were studied twice in randomised order. A combined manometry and impedance catheter was used to measure pressure waves and flow events in the same region of the duodenum simultaneously. Metoclopramide (10 mg) or control was administered intravenously as a bolus, followed by an intraduodenal glucose infusion for 60 minutes (3 kcal/min) incorporating the ^{14}C-labelled glucose analogue 3-O

methylglucose (3-OMG). Metoclopramide was associated with more duodenal pressure waves and propagated pressure sequences than control ($P < 0.05$ for both), during intraduodenal glucose infusion. However, the number of duodenal flow events, blood glucose concentration, and plasma ^{14}C-3-OMG activity did not differ between the two study days. Metoclopramide was associated with increased plasma concentrations of GLP-1 ($P < 0.05$) and GIP ($P = 0.07$), but lower plasma insulin concentrations ($P < 0.05$). It is concluded that pharmacologically-induced changes in the frequency of duodenal pressure waves correlate poorly with both flow events and glucose absorption. Metoclopramide is associated with increased release of GLP-1 and GIP, possibly by enhancing mucosal contact with glucose.

8.2   Introduction

Upper gut motility has the potential to influence postprandial glycaemic excursions by altering the rate of nutrient delivery to the small intestinal mucosa. Differences in the rate of gastric emptying account for about one-third of the variation in the initial glycaemic response to a meal (Jones et al. 2001; Russo et al. 2003). However, the contribution of small intestinal motor activity to glucose absorption is poorly defined, partly because techniques for measuring small intestinal motor function are suboptimal.

Several techniques are available for measuring motor activity in the small intestine, each with its own limitations. Manometry can provide information on

the spatial and temporal arrangements of lumen-occlusive contractions, but the functional significance of manometric events in terms of chyme movement is often uncertain. Fluoroscopy is generally considered the "gold-standard" technique for measuring movement of intraluminal contents (Imam et al. 2004), but radiation exposure precludes prolonged use of this modality in humans. Intraluminal impedance monitoring represents an alternative method of monitoring movement of contents in the proximal small intestine. Typically, the passage of a fluid bolus results in a fall, whilst an air bolus results in an increase, in impedance (Nguyen et al. 1995). By detecting transient drops in impedance, recorded between serial pairs of electrodes situated along a catheter, flow patterns of intraluminal contents can be inferred. Despite the growing use of impedance recordings in the oesophagus, particularly for the evaluation of gastroesophageal reflux events (Sifrim et al. 2001), impedance recording has not yet been adopted widely for use in the small intestine. A limited number of studies have used the technique to characterise antropyloroduodenal flow events in healthy humans (Nguyen et al. 1995; Savoye et al. 2003; Savoye-Collet et al. 2003) and patients with diabetic gastroparesis (Nguyen et al. 1997). When compared to manometry, impedance monitoring in humans shows higher concordance with fluoroscopy, for the assessment of chyme transport in the proximal small intestine (Imam et al. 2004).

 "Prokinetic" drugs, commonly used to treat delayed gastric emptying, have variable effects on small intestinal motility, and little is known about their effects

on glucose absorption. Metoclopramide is one of the most commonly used prokinetic agents, and has been shown to accelerate small intestinal transit as well as gastric emptying (Thompson et al. 1982; Holgate and Read 1983; Prokop et al. 1988; Erbas et al. 1993), as measured by scintigraphy, but has uncertain effects on nutrient absorption (Holgate and Read 1983); for example, one study reported that metoclopramide reduced small intestinal transit time but had little effect on the absorption of all macronutrients after the ingestion of a solid meal (Holgate and Read 1983).

The incretin hormones, which enhance glucose-induced insulin release from the pancreas, are important in the regulation of postprandial glycaemia (Nauck et al. 1986). At present, there is little information about the impact of small intestinal flow events on incretin hormone secretion. One recent study, involving hyoscine butylbromide, showed that the suppression of duodenal flow events by hyoscine was associated with delayed release of both GLP-1 and GIP, presumably reflecting slowing of the spread of glucose over the full length of GIP-bearing mucosa in the duodenum and proximal jejunum, and postponing the arrival of glucose to the GLP-1-bearing mucosa in the more distal jejunum and ileum (Chaikomin et al. 2007).

This study aimed to investigate the effects of metoclopramide on duodenal flow events and motility, and the impact on glucose absorption and incretin hormone release.

8.3   Methods

8.3.1 Subjects

Nine healthy human volunteers were recruited by advertisement. No subject was taking medication known to affect gastrointestinal function. One male subject experienced agitation 5 minutes after receiving metoclopramide, and was withdrawn from the study, without requiring the administration of the antidote benztropine. Accordingly, 8 subjects were included in the analysis (7 males; mean age 29.8 ± 4.6 yr; mean body mass index [BMI] 24.5 ± 0.9 kg/m^2).

8.3.2 Protocol

Each subject underwent 2 studies, separated by at least 3 days, in single-blinded, randomised order. Subjects attended the laboratory at 0900 h after an overnight fast (12 h for solids, 10 h for liquids). A combined manometry and impedance catheter was inserted transnasally into the stomach and allowed to pass into the duodenum by peristalsis. The position of the catheter was determined by transmucosal potential difference (TMPD), monitored continuously throughout the study, using established criteria (Heddle et al. 1988) (antral TMPD < -20 mV, duodenal TMPD > -15 mV, difference > 15 mV), which necessitated the subcutaneous insertion of a 20-gauge saline-filled cannula into one forearm as a

reference. The assembly comprised a multilumen silicone manometric catheter (external diameter 4 mm; 6 duodenal channels spaced at 3-cm intervals, with an extra side hole for infusion situated between the two most proximal channels) (Dentsleeve International Ltd, Mississauga, Ontario, Canada), and an impedance catheter (external diameter 2 mm; 8 ring electrodes spaced at 2-cm intervals) (Sandhill Scientific, Highland Ranch, CO, USA), bound in parallel with paraffin tape so that the manometry side holes and impedance electrodes spanned the same region in the duodenum. The manometric catheter was constantly perfused with degassed water and 0.9 % saline (for the TMPD channels), to allow measurement of pressure waves.

Once the combined catheter was positioned correctly, a cannula was inserted into a forearm vein for the administration of metoclopramide or control, and subsequent blood sampling. Fasting motility was observed until the onset of duodenal phase II activity. At this time (t = -5 min), 10 mg of metoclopramide hydrochloride (AstraZeneca, North Ryde, NSW, Australia), made up to 10 mL with 0.9 % saline, or 10 mL 0.9 % saline alone (as control), was infused intravenously as a bolus over 1 minute. At t = 0 min, an intraduodenal (ID) infusion containing 45 g glucose, and 111 kBq of ^{14}C-labelled 3-O-methylglucose (^{14}C-3-OMG), made up to 200 ml with water, was given via the infusion channel in the manomety catheter over 60 min (i.e. 3 kcal/min) (t = 0 to 60 min). Manometry and impedance recording continued from t = 0 to 180 min.

Venous blood was sampled every 5 min from t = -5 to 60 min, and then at t = 70, 80, 90, 105, 120, 150, and 180 min, for measurement of blood glucose and plasma ^{14}C-3-OMG. Plasma insulin, GIP, and GLP-1 concentrations were measured on blood samples obtained every 10 minutes from t = 0 to 90 min, and then at t = 105, 120, 150, and 180 min.

8.3.3 Measurements

Both the manometric and impedance signals were recorded at a sampling rate of 30 Hz (Insight stationary system, Sandhill Scientific, Highlands Ranch, CO, USA) and stored on a hard disc for subsequent analysis.

Manometric analysis

Manometric data were analysed in automated fashion using established software (Chaikomin et al. 2007). The number of duodenal waves with amplitudes ≥10 mmHg (total number in all 6 duodenal channels per 10 min) and number of propagated sequences of duodenal waves (total number per 10 min, each recorded in at least 2 channels) were analysed, assuming a propagation velocity between 0.9 and 16 cm/s. The mean amplitudes of these waves were calculated as the average per 10 minutes across all 6 channels. Phase III-like activity was defined as pressure waves occurring at a frequency of 10 - 12 per minute, for ≥2 minutes.

Impedance analysis

Impedance recordings were analysed by two independent observers (P. K. and C. K. R.) who were blinded to the study conditions. A flow event was defined as a transient decrease in impedance of ≥ 12 % from baseline in at least three sequential electrode pairs (i.e. ≥ 6 cm) (Chaikomin et al. 2007). Flow events were classified as either antegrade or retrograde. The detection of flow events was compared between the two observers, and consensus was reached over discrepant observations.

Blood glucose, plasma insulin, GIP and GLP-1 concentrations

Blood samples for measurement of plasma insulin, GIP and GLP-1 concentrations were collected in ice-chilled tubes containing EDTA and 400 kIU aprotinin (Trasylol; Bayer Australia, Pymble, Australia) per litre of blood. Plasma was separated by centrifugation (1,500 G for 15 min, at 4 °C) and stored at -70 °C for subsequent analysis. Blood glucose concentrations were determined immediately using a portable glucometer (MediSense Optium, MediSense Inc., Waltham, MA, USA). Plasma total GLP-1 concentrations were measured using radioimmunoassay (RIA) (GLPIT-36HK, Linco Research, St. Charles, Missouri, USA). Plasma GIP concentrations were measured by RIA (Wishart et al. 1992).

Plasma insulin concentrations were measured by Enzyme-Linked Immunosorbant Assay (ELISA) (Diagnostics Systems Laboratories Inc., Webster, TX).

Plasma ^{14}C-3-OMG activity

3-OMG is a glucose analogue that is absorbed through the same mechanism as glucose but is not metabolised; plasma concentrations of 3-OMG are therefore used as an index of glucose absorption (Chaikomin et al. 2007). Blood samples for plasma ^{14}C-3-OMG activity were collected in serum tubes, followed by centrifugation (1,500 G for 15 min, at 4 °C) and plasma collection, and analysed within 24 hours. Results are expressed as disintegrations per minute (dpm) over the background count. We have validated this method of measuring 3-OMG absorption against chromatographic analysis of unlabelled 3-OMG concentrations in plasma (Kuo et al. 2009).

8.3.4 Statistical analysis

Data were evaluated by repeated-measures ANOVA with treatment and time as factors, with post hoc means comparisons for individual time points in the event of significant treatment-by-time interactions. Area under the curve was also calculated using the trapezoidal rule for plasma ^{14}C-3-OMG and hormone concentrations, and compared using paired T tests. A statistical software package (SPSS 15.0, SPSS Inc, Chicago, IL, USA) was used for all analyses. Statistical

significance was accepted as P < 0.05, and data are presented as means ± standard error of the mean (SEM).

8.4   Results

The eight subjects who completed the protocol tolerated the study well and could not discriminate between the two study conditions.

8.4.1 Duodenal pressure waves

There was a gradual decline in the number of both duodenal pressure waves and propagated pressure wave sequences over the 60 minutes of ID glucose infusion, on both study days. During this period, metoclopramide was associated with a greater number of duodenal pressure waves and propagated sequences than saline (treatment-by-time interaction with significant differences at the first 3 time points; P = 0.01 for both). (Fig. 8.1a,b) Propagated sequences were almost all antegrade. The mean duodenal wave amplitude was not different between the two study days (data not shown). There was a non-significant trend for more episodes of duodenal phase III-like activity during the entire study period of 180 minutes with metoclopramide (1.25±0.27), compared to saline (0.63±0.28) (P = 0.09).

8.4.2 Duodenal flow events

There was also a gradual decrease in the number of flow events detected over the 60 minutes of ID glucose infusion on both study days. The number of duodenal flow events, expressed either as the total number over 60 minutes (31.9±7.2 [metoclopramide] vs. 28.8±7.6 [saline]), or as frequency of events per 10 minutes over the same period, did not differ between the two study days, despite the impression of a greater number of flow events with metoclopramide during the first 10 minutes. (Fig. 8.1c)

8.4.3 Blood glucose and ^{14}C-3-OMG concentrations

Blood glucose concentrations rose rapidly during the intraduodenal glucose infusion on both days, reaching a peak at 50 minutes, followed by a rapid fall, returning to baseline values by 90 minutes. There was no difference in the area under the blood glucose curve (AUC) between the two study days, during either the first 60 minutes (483.3±12.7 mmol/L.min [metoclopramide] vs. 488.5±14.6 mmol/L.min [saline]; P = N.S.), or during the entire study (0 - 180 min) (1142.8±33.3 mmol/L.min [metoclopramide] vs. 1152.6±25.3 mmol/L.min [saline]; P = N.S.). (Fig. 8.2a)

Plasma ^{14}C-3-OMG concentrations also rose rapidly during the intraduodenal glucose infusion on both days, and continued to rise thereafter, reaching a peak at

80 minutes, followed by a gradual decline, and remained at much higher values than baseline at 180 minutes. There was no difference in the plasma ^{14}C-3-OMG profile and area under the ^{14}C-3-OMG curve between the two study days, during either the first 60 minutes (4247.2±325.8 dpm.min [metoclopramide] vs. 3640.3±277.9 dpm.min [saline]; P = N.S.), or during the entire study (0 - 180 min) (27413.8±2123.3 dpm.min [metoclopramide] vs. 25707.2±1514.6 dpm.min [saline]; P = N.S.). (Fig. 8.2b)

8.4.4 Plasma insulin, GIP, and GLP-1 concentrations

Insulin

Plasma insulin concentrations rose rapidly after the intraduodenal glucose infusion on both days, reaching a peak at 50 minutes with metoclopramide, and 60 minutes with saline, followed by a rapid decrease, returning to baseline values by 120 minutes on both days. After commencement of the intraduodenal glucose infusion, metoclopramide was associated with lower levels of plasma insulin, compared to saline, as a treatment-by-time effect (P < 0.005). However, the area under the insulin curve did not differ significantly between the 2 study days, during either the first 60 minutes (5423.4±641.9 IU.min [metoclopramide] vs. 5636.0±933.7 IU.min [saline]), or the entire study (0 - 180 min) (9493.8±940.4 IU.min [metoclopramide] vs. 12054.3±1747.7 IU.min [saline]). (Fig. 8.3a)

GIP

Plasma GIP concentrations rose rapidly after the intraduodenal glucose infusion on both days, reaching a peak at 60 minutes, followed by a gradual decline, returning to baseline values by 180 minutes. Metoclopramide was associated with higher levels of plasma GIP, compared to saline, as a treatment-by-time effect ($P < 0.001$). During the first 60 minutes, the area under the GIP curve was higher with metoclopramide, compared to with saline (3950.8 ± 444.7 pmol/L.min [metoclopramide] vs. 3268.3 ± 232.3 pmol/L.min [saline], $P < 0.05$), and a similar trend was observed over the entire study (8605.2 ± 860.4 pmol/L.min [metoclopramide] vs. 7848.1 ± 539.3 pmol/L.min [saline], $P = 0.07$). (Fig. 8.3b)

GLP-1

Plasma GLP-1 concentrations rose rapidly after the intraduodenal glucose infusion with metoclopramide, but more slowly with saline, with the peak concentration reached at 50 and 60 minutes respectively, followed by a rapid decrease, returning to baseline values by 80 minutes on both days. Metoclopramide was associated with higher plasma GLP-1 concentrations, compared to saline, as a treatment-by-time effect ($P < 0.01$). A similar trend for area under the GLP-1 curve was also observed over the first 60 min (1478.1 ± 290.9 pmol/L.min [metoclopramide] vs. 1060.4 ± 210.8 pmol/L.min [saline]; $P = 0.12$), but not over the entire study (0 - 180 min) (2824.5 ± 517.1

pmol/L.min [metoclopramide] vs. 2483.5±462.1 pmol/L.min [saline]; P = N.S.).
(Fig. 8.3c)

8.4.5 Relationships between flow events, pressure waves, glucose absorption, and plasma hormones

No correlation existed between the total number of flow events and either the total number of pressure waves or propagated pressure wave sequences, during 60 minutes of ID glucose infusion.

There was no correlation between the total number of flow events during 60 minutes of ID glucose infusion and i) the incremental blood glucose concentration at 60 minutes, ii) plasma ^{14}C-3-OMG activity at 60 minutes, iii) area under the plasma GIP curve (0 - 60min), and iv) area under the plasma GLP-1 curve (0 - 60 min); however, there was a correlation between the total number of flow events during 60 minutes of ID glucose infusion and area under the plasma insulin curve (0 - 60 min) (R = 0.53, P < 0.05).

There was a good correlation between the total number of pressure waves during 60 minutes of ID glucose infusion and area under the curve (0 - 60 min) of both GIP (R = 0.59, P < 0.05) and GLP-1 (R = 0.56, P < 0.05), and similarly, between the total number of propagated sequences and area under the curve of GIP (R = 0.46, P = 0.07) and GLP-1 (R = 0.51, P < 0.05), but not with insulin, incremental

blood glucose concentration at 60 minutes, and plasma ^{14}C-3-OMG activity at 60 minutes.

8.5   Discussion

In this study, the administration of metoclopramide stimulated duodenal pressure waves but did not alter the number of duodenal flow events, nor glucose absorption, in response to an intraduodenal glucose infusion. These findings are consistent with previous observations using hyoscine butylbromide, where duodenal flow events were identified as a more important determinant of glucose absorption than pressure waves.

Multiple factors potentially influence the rate of glucose absorption from the gut, including the rate of gastric emptying, small intestinal transit, and the activity of the glucose transporters in the small intestinal mucosa. Their relative contributions in health and diabetes are currently poorly defined. The effect of gastric emptying on postprandial glycaemia is well established. For example, slowing the rate of gastric emptying by administering morphine delays glucose absorption in healthy subjects and patients with type 2 diabetes (Gonlachanvit et al. 2003), as indicated by changes in the postprandial glycemic excursions and area under the blood glucose curve; conversely, accelerating the rate of gastric emptying using erythromycin has the opposite effect. An impact of activity of the small intestinal glucose transporters is also apparent, at least in rodent models

(Kellett and Helliwell 2000; Kellett and Brot-Laroche 2005). These transporters include the Na^+/glucose co-transporter SGLT-1, and the GLUT-2 transporter, with the former maintaining constant activity and exhibiting saturable absorptive capacity, whilst the expression of the latter on the apical membrane is up-regulated within minutes of exposure to luminal glucose (Kellett and Helliwell 2000), increasing the glucose absorptive capacity of the GLUT-2 mechanism to three-times that of SGLT-1 in a rodent model (Kellett and Brot-Laroche 2005). In contrast, there is little information as to how patterns of small intestinal flow influence glucose absorption. In the current study, a combined manometry/impedance catheter was used to measure both pressure and impedance signals concurrently from the same region of the duodenum. By infusing glucose intraduodenally, at a rate similar to that of physiological gastric emptying (Horowitz et al. 1996), variations in the delivery of nutrient to the small intestine between individuals were avoided. A single, intravenous dose of 10 mg metoclopramide was chosen, as this represents the usual dose used clinically as a prokinetic drug (Kuo et al. 2007).

For the measurement of bolus transit in the duodenum, impedance monitoring correlates more closely with the "gold-standard" of fluoroscopy, than does manometry (Imam et al. 2004). However, the length of our manometry/impedance assembly spanned only a segment of the duodenum. Sensitivity for the detection of flow events by impedance also decreases if there is incompletely cleared luminal content from a previous event (Imam et al. 2004). In addition, we only

counted a flow event if impedance drops spanned at least 3 channels (6 cm); it is possible that shorter events, which cannot be distinguished from background "noise" by current techniques, may be of importance in the transport and absorption of duodenal contents.

Glucose absorption occurs predominantly in the proximal and mid-jejunum (Buchman et al. 2003). It appears intuitive that an increased rate of transit of chyme in the duodenum will lead to increased transit in the jejunum, and will accordingly increase the rate of glucose absorption by spreading carbohydrate over a larger absorptive surface, but data to support this concept are limited. A recent study showed that hyoscine butylbromide was associated with fewer duodenal flow events and delayed glucose absorption compared to saline, despite having minimal impact on the frequency of duodenal pressure waves (Chaikomin et al. 2007). This suggested that duodenal flow events, as determined by impedance analysis, correlate better with glucose absorption than the number of pressure waves and propagated sequences, perhaps by influencing the rate of transit to the jejunum. Such a concept is in keeping with observations made in the current study, where metoclopramide, despite inducing more duodenal pressure waves and propagated sequences, failed to generate more flow events, and subsequently had no impact on glucose absorption, as measured by plasma ^{14}C-3-OMG activity. The marked disparity between the effects of metoclopramide on events detected by manometry and impedance further reinforces their complimentary characteristics, and strengthens the rationale for using both

techniques in combination.

The observation that the number of duodenal pressure waves and flow events during intraduodenal glucose infusion declined over time on both study days, may be attributable to several mechanisms including hyperglycaemia, hyperinsulinaemia, or increased secretion of incretin hormones, particularly GLP-1. Hyperglycaemia, even in the physiological range (8 mmol/L), slows gastric emptying (Fraser et al. 1990; Schvarcz et al. 1997), but its effect on small intestinal motor activity is uncertain. In the current study, blood glucose concentrations peaked at just below 10 mmol/L on both study days, and may have influenced duodenal motor function. Hyperinsulinaemia is perhaps less likely to play a role as it has no effect on gastric motility (Hasler et al. 1995). Conversely, exogenous GLP-1 slows gastric emptying (Nauck et al. 1997), inhibits duodenal motility in healthy humans (Schirra et al. 2000), and inhibits small intestinal motility and transit in rats (Tolessa et al. 1998; Tolessa et al. 1998).

In this study, the lack of change in the number of flow events with metoclopramide contrasts with previous studies where metoclopramide increased small intestinal transit, as measured by scintigraphy (Holgate and Read 1983; Prokop et al. 1988). However, this may be due to subject and methodological differences. For example, Holgate et. al. (Holgate and Read 1983) studied patients with terminal ileostomies, who ingested a mixed solid meal; while in the study by Prokop et. al. (Prokop et al. 1988), subjects ingested lactulose.

The relatively lesser impact of the frequency of duodenal pressure waves compared to flow events on glucose absorption, both in this and a previous study (Chaikomin et al. 2007), contrasts with other reports that the frequency of small intestinal pressure waves does influence glucose absorption. In pigs, absorption of glucose (Rayner 1991) and xylose (Fioramonti et al. 1982) was increased when infused into the lumen during periods of increased motor activity (phases II and III of the migrating motor complex), when compared to periods of motor quiescence (phase I). In healthy humans, increased numbers of antegrade propagated pressure wave sequences, particularly those over short distances, were associated with increased absorption of a small intestinal glucose load, possibly by optimising the surface area of mucosal contact with glucose (Schwartz et al. 2002). In type 1 diabetic patients, the small intestinal absorption of the glucose analogue, 3-OMG, was also increased with increasing frequency of duodenal pressure waves and antegrade propagated sequences (Rayner et al. 2002). In contrast, the amplitude of duodenal pressure waves has been reported not to affect 3-OMG absorption (Schwartz et al. 2002). Nevertheless, in none of these studies were duodenal flow events recorded directly. The lack of difference in glucose absorption observed in the current study, despite greater numbers of duodenal pressure waves and propagated sequences associated with metoclopramide, may be partly explained if glucose absorption in healthy volunteers is already optimal, making any further increase difficult to achieve. It would therefore be of interest to repeat the current study in patients with impaired baseline glucose absorption,

such as those who are critically ill (Hadfield et al. 1995).

In our study, metoclopramide was associated with higher plasma GIP and GLP-1, but lower plasma insulin concentrations, in response to intraduodenal glucose. The higher GIP and GLP-1 concentrations are likely to reflect the increased duodenal motility resulting in enhanced interactions between glucose and proximal small intestinal K and L cells respectively, as evidenced by the good correlations found between the total number of pressure waves and propagated sequences, and area under the GIP and GLP-1 curves, during intraduodenal glucose infusion, despite no increase in flow events that could be detected by impedance. Exogenous dopamine has been consistently demonstrated to increase insulin secretion (Martin et al. 1993; Martin et al. 1994; Contreras et al. 2008). However, the effect of the dopamine antagonist, metoclopramide, on insulin secretion is less consistent. During fasting, one study reported a reduction in serum insulin with the administration of intravenous metoclopramide (Morricone et al. 1990), while other studies found metoclopramide by itself had no effect on insulin secretion, but prevented the stimulation of insulin release by exogenous dopamine (Martin et al. 1993; Martin et al. 1994). Whether metoclopramide attenuates glucose-induced secretion of insulin is, however, unknown, but if this were the case it may, at least in part, explain the lower plasma insulin concentrations observed in the current study. The lack of difference in blood glucose concentrations between the 2 study days, despite lower insulin levels with metoclopramide, cannot be readily explained; there is currently no information

regarding any potential effect of metoclopramide on peripheral glucose uptake or glucagon secretion, and glucagon concentrations were not measured in our study.

Understanding the determinants of small intestinal glucose absorption has considerable importance for the management of diabetes, and is an area that has, to date, received inadequate attention. Previous studies have established the benefits of modulating the rate of gastric emptying as a therapeutic strategy to improve postprandial glycaemia in type 2 diabetes; for example, this may well represent the major mechanism of action of GLP-1 analogues, such as exenatide (Linnebjerg et al. 2008). More studies are required to define the role of small intestinal chyme transport in glucose absorption, and how it affects incretin hormone release and postprandial glycaemia, in health and diabetes.
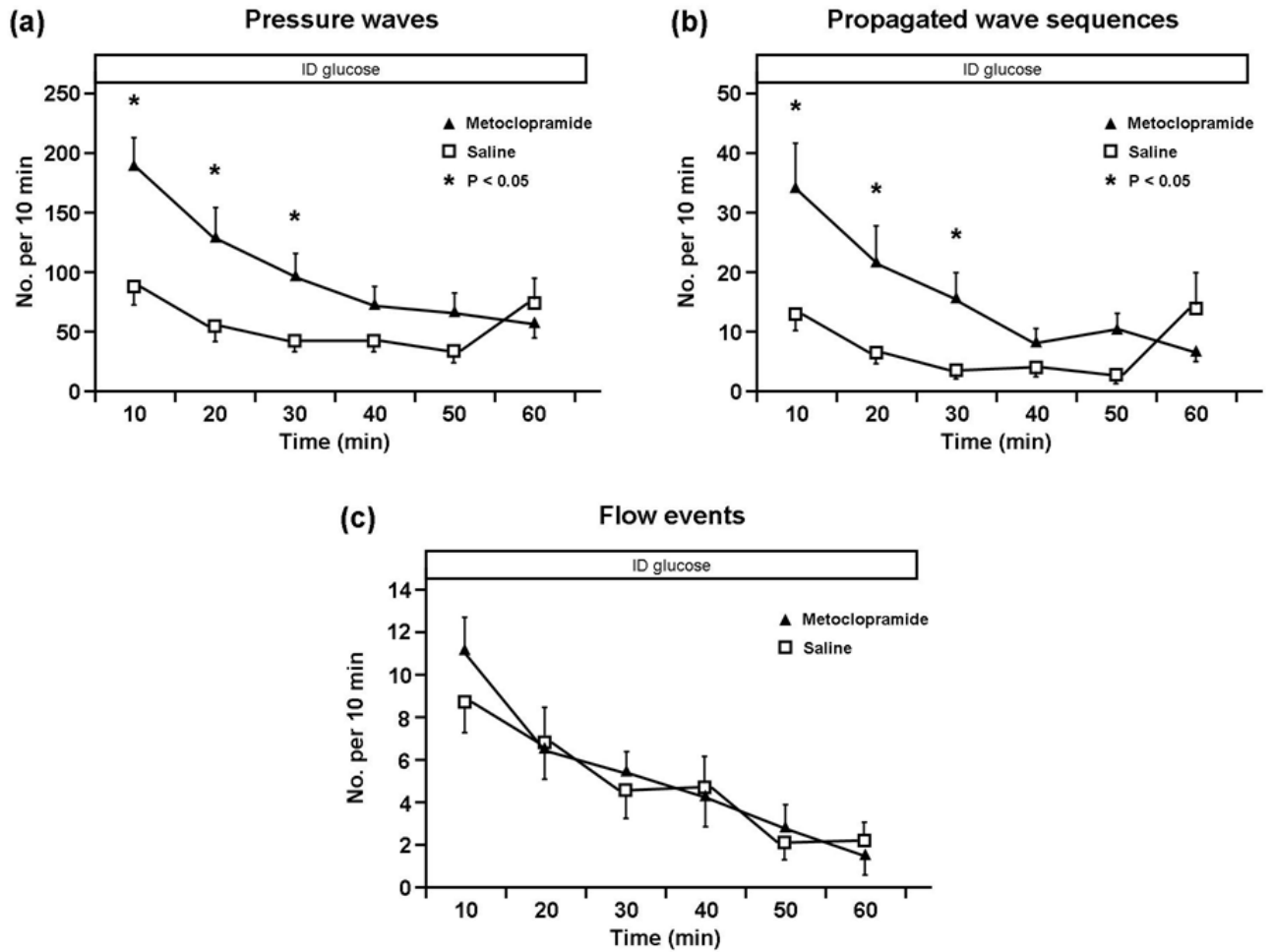
Figure 8.1

Total number of (a) duodenal pressure waves, (b) total propagated wave sequences, and (c) flow events, during 60 minutes of intraduodenal glucose infusion. Values are expressed as number per 10 minutes ± SEM. (n = 8). In the event of a significant treatment-by-time interaction, time points with $P < 0.05$ were marked by *.

Figure 8.2

Plasma (a) glucose and (b) ^{14}C-3-O-MG concentrations during the entire study (t = 0 to 180 min). Values are means ± SEM. (n = 8).
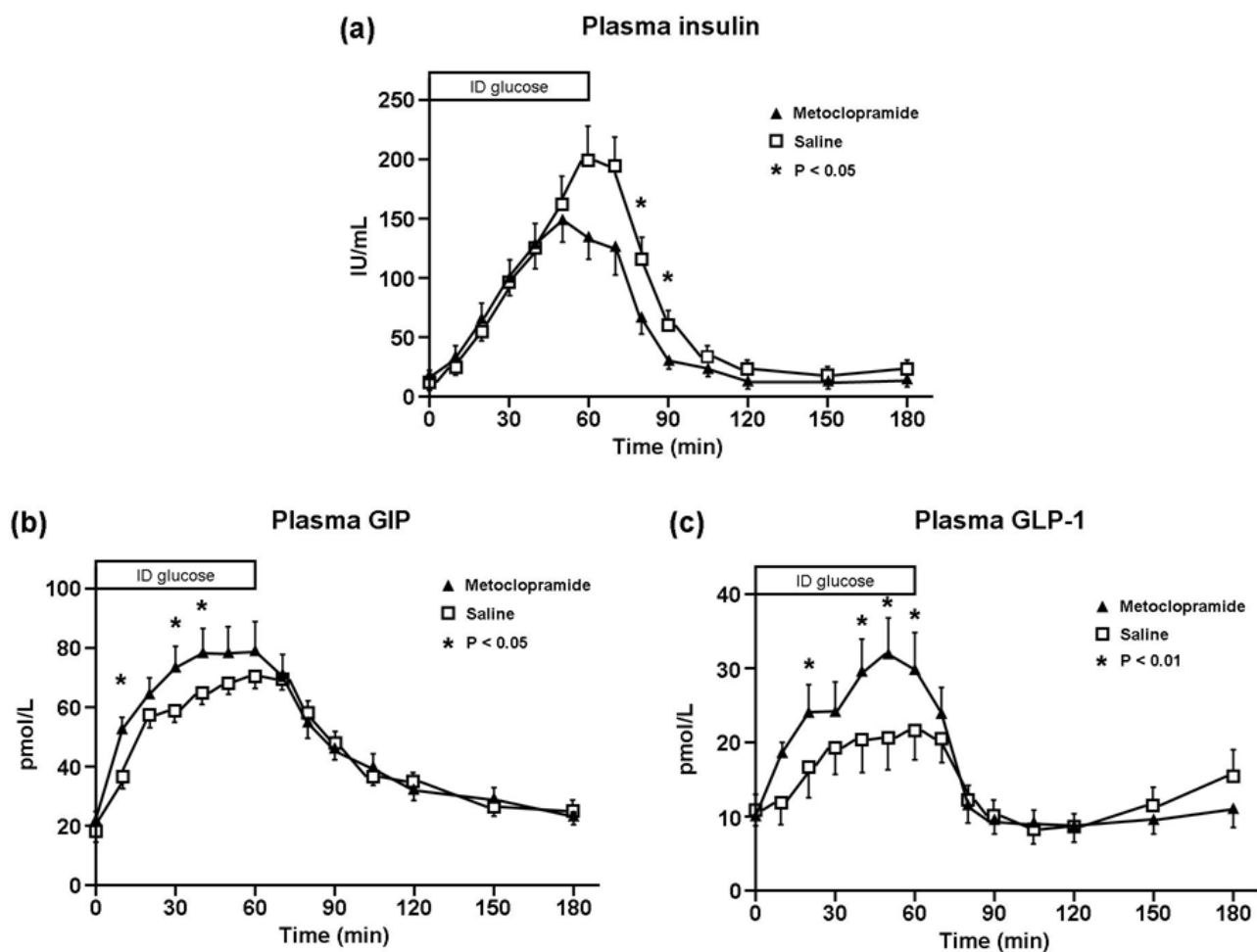
Figure 8.3

Plasma (a) insulin, (b) glucose-dependent insulintropic polypeptide (GIP), and (c) glucagon-like peptide-1 (GLP-1) concentrations during the entire study (t = 0 to 180 min). Values are means ± SEM. (n = 8). In the event of a significant treatment-by-time-interaction, time points with P < 0.05 were marked by *.
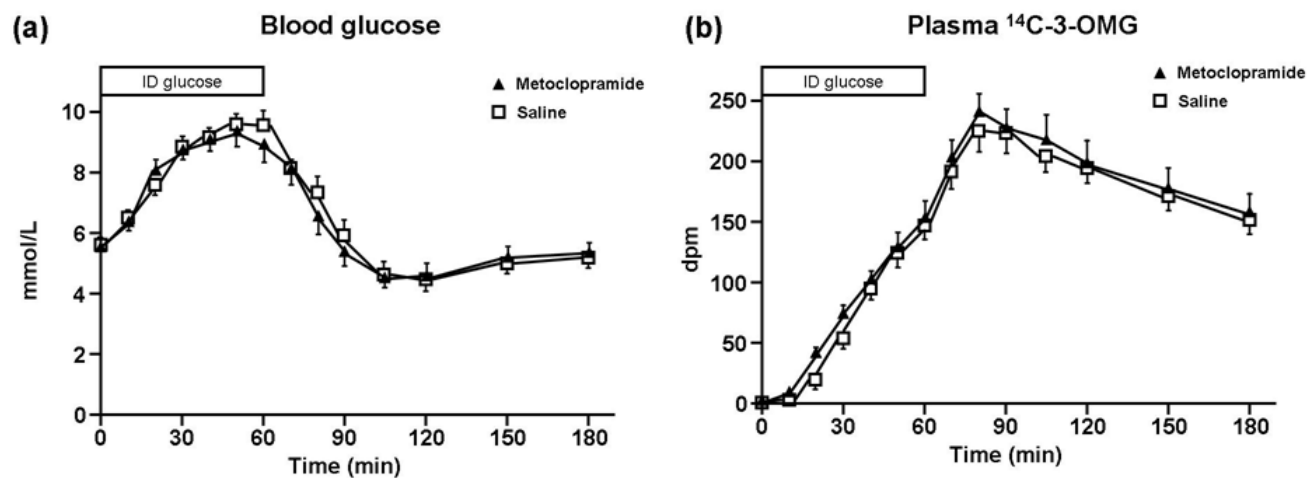
Chapter 9: Effects of physiological hyperglycaemia on duodenal motility and flow events, glucose absorption, and incretin hormone secretion in healthy humans (Submitted for publication)

Chapter 10:

The nitric oxide (NO) synthase inhibitor, NG-nitro-L-arginine-methyl-ester (L-NAME), attenuates the delay in gastric emptying induced by hyperglycaemia in healthy humans

(Adapted from Kuo et al. Neurogastroenterol Motil 2009;21:1175-e103)

10.1 Summary

Acute hyperglycaemia exerts a number of reversible effects on the motor function of the upper gut, such as slowing of gastric emptying, suppression of antral pressure waves, and stimulation of pyloric pressure waves. Nitric oxide (NO) is an important inhibitory neurotransmitter in the gut, and may be involved in this process. This study aimed to determine whether the NO synthase inhibitor, L-NAME, reverses the effects of acute hyperglycaemia on gastric emptying and antropyloroduodenal (APD) motility, in healthy humans. Seven healthy volunteers (4 male; age 30.3±3.8 yr; BMI 23.6±1.2 kg/m^2) were recruited, and each attended 4 visits. Study days were randomised in a 4-way crossover

(hyperglycaemia vs. euglycaemia; L-NAME vs. placebo) fashion, and performed under double-blinded condition. After positioning a transnasal manometry catheter across the pylorus, the blood glucose concentration was maintained at either 15 mmol/L or 5 mmol/L using a glucose/insulin clamp. An intravenous infusion of L-NAME (180 μg/kg/h) or placebo (0.9 % saline) was commenced (T = -30 min) and continued for 150 min. At T = -2 min, subjects ingested a drink containing 50 g glucose made up to 300 mL with water. Gastric emptying was measured using 3-D ultrasound, and APD motility using manometry. Hyperglycaemia slowed gastric emptying ($P < 0.05$), and this effect was abolished by L-NAME. L-NAME had no effect on gastric emptying during euglycaemia. Hyperglycaemia suppressed fasting antral motility (motility index: 3.9±0.8 [hyperglycaemia] vs. 6.5±0.6 [euglycaemia]; $P < 0.01$); L-NAME suppressed postprandial antral motility (motility index: 3.6±0.2 [L-NAME] vs. 5.1±0.2 [placebo]; $P < 0.001$). Postprandial basal pyloric pressure was higher during hyperglycaemia ($P < 0.001$), and lower with L-NAME ($P < 0.001$). We conclude that the slowing of gastric emptying induced by hyperglycaemia is mediated by NO, and may involve the modulation of tonic pyloric activity.

10.2 Introduction

Although delayed gastric emptying occurs in 30-50 % of outpatients with longstanding type 1 and type 2 diabetes (Horowitz et al. 2002), its pathogenesis is poorly understood. Acute changes in the blood glucose concentration have major,

reversible, effects on gastrointestinal motor function in both healthy individuals and patients with diabetes (Fraser et al. 1990; Hebbard et al. 1996; Samsom et al. 1997; Schvarcz et al. 1997). Hyperglycaemia is associated with slowing of gastric emptying (Fraser et al. 1990; Samsom et al. 1997; Schvarcz et al. 1997), reduced proximal gastric tone (Hebbard et al. 1996; Rayner et al. 2000), inhibition of antral pressure waves (Barnett and Owyang 1988; Hasler et al. 1995), and stimulation of tonic and phasic pyloric contractions (Fraser et al. 1991). However, the mechanisms mediating these effects are unknown.

Nitric oxide (NO) is a major inhibitory neurotransmitter in the gastrointestinal tract, and appears to act as the final common pathway mediating enteric smooth muscle relaxation (Takahashi 2003). In healthy subjects, an increase in NO availability has been reported to slow gastric emptying (Konturek et al. 1995; Sun et al. 1998; Konturek et al. 1999). There is, however, no information as to whether nitrergic mechanisms mediate the effects of hyperglycaemia on gastric emptying and antropyloroduodenal motility.

This study aimed to determine the effects of the NO synthase inhibitor, L-NAME, on the delay in gastric emptying and changes in antropyloroduodenal motility, associated with hyperglycaemia in healthy humans. Since glucose-induced insulin secretion is influenced by NO availability in vitro and in animal models (Nunemaker et al. 2007; Abaraviciene et al. 2008; Mosen et al. 2008), the effects

of L-NAME on the secretion of insulin and the incretin hormone glucose-dependent insulinotropic polypeptide (GIP) were also evaluated.

10.3 Methods

10.3.1 Subjects

Seven healthy volunteers (4 males; age 30.3 ± 3.8 years; body mass index 23.6 ± 1.2 kg/m^2 [mean ± SEM]) were recruited by advertisement. No subject was taking medication known to affect gastrointestinal function.

10.3.2 Protocol

Each subject was studied on 4 occasions, each separated by at least 3 days, in a single-blinded, randomised cross-over design (euglycaemia [5 mmol/L] vs. hyperglycaemia [15 mmol/L]; L-NAME vs. placebo). Subjects attended the laboratory at 0830h after an overnight fast (12 hours for solids, 10 hours for liquids). A manometric catheter (Dentsleeve International Ltd, Mui Scientific, Mississauga, Ontario, Canada) was inserted transnasally into the stomach and allowed to pass into the duodenum by peristalsis. The correct position of the catheter was determined by measurement of transmucosal potential difference (TMPD), monitored continuously throughout the study, using established criteria (Heddle et al. 1989). The manometric catheter incorporated 3 antral sideholes (at

1.5 cm intervals), 6 duodenal sideholes (3 cm intervals), and a 4.5 cm pyloric sleeve sensor. The most distal antral and most proximal duodenal sideholes were perfused with saline (0.9 %) to measure TMPD (Pilichiewicz et al. 2006). All other channels were perfused with degassed water.

Once the catheter was positioned correctly, 100 mL of either 25 % dextrose (Baxter Healthcare Pty. Ltd., Old Toongabbie, NSW, Australia) or 0.9 % saline (Baxter Healthcare Pty. Ltd., Old Toongabbie, NSW, Australia), was infused intravenously over 2 minutes, followed by an infusion of the same solution commencing at 150 mL/h, and adjusted every 5 minutes according to the glucometer readings (Medisense Optium, Medisense Inc, USA) on the "hyperglycaemic" days, or remaining at 150 mL/h on the "euglycaemic" days, for the remainder of the study. In addition, insulin (Actrapid Penfill, Novo Nordisk Pharmaceuticals Pty. Ltd., Baulkham Hills, NSW, Australia) in 4 % gelatin solution (Gelofusine, B. Braun Australia Pty. Ltd., Bella Vista, NSW, Australia), made up to 0.2 IU/mL, was infused intravenously according to a sliding scale (Russo et al. 1996).

Once the blood glucose concentration had been stabilised for 30 minutes, and during phase II activity of the migrating motor complex (MMC), an intravenous infusion of either L-NAME (180 µg/kg/h) (Clinalfa, Merck Pty Ltd, Kilsyth, VIC, Australia) or placebo (0.9 % saline) was commenced (T = -30 min) and continued for 150 minutes (i.e. until T = 120 min) (Gentilcore et al. 2005). At T = -2 min,

subjects consumed a drink containing 50 g glucose monohydrate (170 kcal), made up to 300 mL with water, over 2 minutes.

Gastric emptying was quantified by three-dimensional (3D) ultrasound (Gilja et al. 1997): images of the stomach were acquired at T = -30, -15, -2 (immediately before the glucose drink), 0 (immediately after the drink), 15, 30, 45, 60, 90, 120, and 150 min, while antropyloroduodenal motility was recorded between T = -30 and 150 min.

Venous blood was sampled every 15 min from T = -30 to 60 min, and then at 30 min intervals until T = 150 min, for measurement of blood glucose, plasma insulin, and plasma GIP concentrations.

Blood pressure and heart rate were recorded every 15 minutes, using an automated device (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA), and mean values calculated for the entire study duration.

10.3.3 Measurements

Gastric emptying and intragastric distribution.

Images of the stomach were acquired using a Logiq^{TM}9 Ultrasound System (GE Healthcare Technologies, IL, USA) with Truscan Architecture (Gentilcore et al.

2006). The 3D positioning and orientation measurement (POM) was performed with a magnetic sensor (Gentilcore et al. 2006). Scans were performed in the sitting position, during breath holding in inspiration. Images were later transferred to a Microsoft Windows workstation, and processed using EchoPAC3D software[®] (GE Vingmed Sound, Horten, Norway) (Gentilcore et al. 2006).

Changes in gastric volume over time were used to generate total gastric emptying curves, expressed as percentage retention relative to the initial postprandial volume at T = 0 min. The stomach was separated into proximal and distal portions by a sagittal plane joining the incisura to the greater curvature (Tefera et al. 2002). Changes in proximal and distal gastric volumes over time were calculated as percentages of the initial postprandial total gastric volume.

Manometric analysis.

Manometric signals were recorded using commercially available software (Flexisoft, Version 3, Assoc Prof GS Hebbard, Royal Melbourne Hospital, Melbourne, Australia, written in LabVIEW 3.1.1 [National Instruments Australia, North Ryde, NSW, Australia]) (Pilichiewicz et al. 2007).

Manometric data were analysed using established software (Professor AJ Smout, Utrecht, The Netherlands) (Pilichiewicz et al. 2007), to calculate the: (a) number of antral pressure waves ≥ 10 mmHg, (b) basal pyloric pressure, (c) number of

duodenal pressure waves ≥ 10 mmHg, (d) number of propagated antral and duodenal wave sequences, and (e) antral and duodenal wave amplitudes (Pilichiewicz et al. 2007), during 10 minute periods between T = -30 and T = 150 min. The number of isolated pyloric pressure waves (IPPWs) per 10 minutes was determined visually, according to established criteria (Heddle et al. 1988). Propagated wave sequences were further divided into those that propagated over short (< 4.5 cm) and long (≥ 4.5 cm) distances, and these were analysed separately (Samsom et al. 1997).

Antral and duodenal motility indices were calculated using the formula:

motility index = ln [(sum of amplitudes x number of pressure waves) + 1] (Samsom et al. 1997).

Blood glucose, plasma insulin, and GIP concentrations

Blood glucose concentrations were determined immediately using a portable glucometer (MediSense Optium, MediSense Inc., Waltham, MA, USA). Blood samples for hormone measurements were collected in ice-chilled tubes containing EDTA and 400 kIU aprotinin (Trasylol; Bayer Australia, Pymble, Australia) per litre of blood. Plasma was separated by centrifugation (3200 rpm, 15 min, 4 °C) and stored at -70 °C for subsequent analysis. Plasma GIP was measured by radioimmunoassay (O'Donovan et al. 2004), and insulin by Enzyme-Linked

Immunosorbant Assay (Diagnostics Systems Laboratories Inc., Webster, TX, USA) (Horowitz et al. 1996).

10.3.4 Statistical analysis

Data were evaluated by repeated-measures analysis of variance (ANOVA) with "glycaemic state" (euglycaemia or hyperglycaemia), "treatment" (L-NAME or placebo), and "time" as within-subject factors. Post hoc means comparisons were performed for individual time points in the event of significant interactions between either "glycaemic state" or "treatment", and "time". The presence of a significant interaction between "glycaemic state" and "treatment" indicates a differential effect of L-NAME depending on "glycaemic state". In the event of no interaction, a significant effect of either "glycaemic state" or "treatment" indicated an overall difference between hyerglycaemia and euglycaemia, or L-NAME and placebo, averaged over both conditions of the other factor. In such case, a single mean value for each condition was obtained. A statistical software package (SPSS 15.0, SPSS Inc, Chicago, IL, USA) was used for all analyses. Statistical significance was accepted at $P < 0.05$, and data are presented as mean values ± standard error of the mean (SEM).

The primary endpoint was the rate of emptying from the total stomach. Subject numbers were based on power calculations performed on previous similar studies (Samsom et al. 1997; Gentilcore et al. 2006).

10.4 Results

All subjects tolerated the study well. The volume of glucose or saline solutions infused intravenously was greater on the hyperglycaemic, compared to euglycaemic, days (P < 0.05), but did not differ between the two hyperglycaemic, or between the two euglycaemic, days (945.3±116.9 mL [hyperglycaemia/L-NAME], 864.0±150.2 mL [hyperglycaemia/placebo], 479.8±10.2 mL [euglycaemia/L-NAME], 485.0±12.0 mL [euglycaemia/placebo]).

10.4.1 Blood pressure and heart rate

Systolic blood pressure was not affected by either L-NAME or hyperglycaemia. However, L-NAME was associated with a modest increase in the mean diastolic blood pressure, not specific to any "glycaemic state" (65.6±1.8 mmHg [L-NAME] vs. 62.8±1.7 mmHg [placebo]; P < 0.05), and hyperglycaemia was associated with a slight decrease in the mean diastolic blood pressure, not specific to any "treatment" (63.5±1.7 mmHg [hyperglycaemia] vs. 64.9±1.6 mmHg [euglycaemia]; P < 0.05). The mean heart rate was lower with L-NAME (63.0±6.0 bpm [L-NAME] vs. 69.0±5.7 bpm [placebo]; P < 0.01), but was unaffected by glycaemic state.

10.4.2 Gastric emptying and intragastric distribution

Total gastric emptying

Gastric emptying on the hyperglycaemia/placebo day was slower compared to the remaining 3 days ($P < 0.05$). L-NAME abolished the effect of hyperglycaemia on gastric emptying, so that on the hyperglycaemia/L-NAME day, gastric emptying was not different from the euglycaemic days. There was an interaction between "glycaemic state" and "treatment" ($P < 0.05$) i.e. L-NAME affected gastric emptying during hyperglycaemia ($P < 0.05$), and not during euglycaemia ($P =$ N.S.). (Fig. 10.1a)

Proximal gastric retention

During hyperglycemia, proximal retention was less with L-NAME than placebo ($P < 0.05$), while L-NAME had no effect on proximal retention during euglycaemia. (Fig. 10.1b) The overall emptying patterns from the proximal stomach resembled those from the total stomach.

Distal gastric retention

There was no effect of either "glycaemic state", or "treatment", on retention in the distal stomach. (Fig. 10.1c)

10.4.3 Antral pressure waves

Fasting

The number (10.1±3.7 [hyperglycaemia] vs. 21.6±3.8 [euglycaemia]; P = 0.01), mean amplitude (35.7±10.4 mmHg [hyperglycaemia] vs. 67.2±9.8 mmHg [euglycaemia]; P < 0.05), and mean motility index (MI) (3.9±0.8 [hyperglycaemia] vs. 6.5±0.6 [euglycaemia]; P < 0.01) of antral waves, were less during hyperglycaemia than euglycaemia (Fig. 10.2). Propagated wave sequences were suppressed during hyperglycaemia (7.6±3.3 compared to 17.3±4.0; P < 0.05), particularly over short distances (< 4.5 cm, P < 0.01; ≥ 4.5 cm, P = 0.09) (Samsom et al. 1997). (Fig. 11.2) L-NAME had no effect on fasting antral motility.

Postprandial

After the drink, antral motility was suppressed on all study days, when compared to fasting. There were fewer short propagated antral sequences during hyperglycaemia (11.3±1.8) than euglycaemia (34.3±7.3) (P < 0.05), but no other effect of hyperglycaemia was observed on antral waves.

L-NAME, compared to placebo, was associated with the following effects: fewer

antral waves (10.8±2.9 vs. 18.5±2.9; P = 0.05), a reduction in amplitude (23.5±2.0 mmHg vs. 32.8±2.6 mmHg; P < 0.01) and motility index (3.6±0.2 mmHg vs. 5.1±0.2 mmHg; P < 0.001), and fewer propagated antral sequences (32.6±11.5 vs. 67.2±11.6; P < 0.05), particularly over long distances (14.1±5.9 vs. 40.1±9.2; P < 0.05). (Fig. 10.3) These were overall effects not specific to any "glycaemic state".

10.4.4 Duodenal pressure waves

Fasting

There was no effect of "glycaemic state" on duodenal motility. L-NAME was associated with a small reduction in the duodenal motility index (8.2±0.2), compared to placebo (8.8±0.2) (P < 0.01).

Postprandial

The mean amplitude of duodenal waves was slightly greater during hyperglycaemia (29.5±1.0 mmHg) than euglycaemia (26.4±0.5 mmHg) (P < 0.01), although the number of waves and propagated sequences, and the motility index, did not differ. L-NAME was associated with a reduction in long propagated sequences (≥ 4.5 cm), only during hyperglycaemia (31.7±9.6 [L-NAME] vs. 127.4±25.9 [placebo]; P < 0.01), but had no other effect on postprandial duodenal motility.

10.4.5 Isolated pyloric pressure waves and basal pyloric pressures

Fasting

During fasting, the number of isolated pyloric pressure waves and the basal pyloric pressure did not differ with "glycaemic state" or "treatment". (Fig. 10.4)

Postprandial

The number of isolated pyloric pressure waves was not different between the 4 study days. Basal pyloric pressure was higher during hyperglycaemia (4.5 ± 0.4 mmHg) than euglycaemia (1.9 ± 0.6 mmHg) ($P < 0.001$), and was less during L-NAME administration (1.6 ± 0.6 mmHg) than placebo (4.8 ± 0.4 mmHg) ($P < 0.001$), not specific to any "glycaemic state". (Fig. 10.4)

10.4.6 Plasma insulin and GIP concentrations

Insulin

During fasting, plasma insulin levels were higher during hyperglycaemia (75.6 ± 15.7 IU), compared to euglycaemia (16.1 ± 1.8 IU) ($P = 0.01$), but were not affected by L-NAME.

Postprandially, the incremental area under the insulin curve was greater during hyperglycaemia (11793.9±1273.8 IU.min), compared to euglycaemia (5234.1±1201.1 IU.min) ($P < 0.01$). During hyperglycaemia, L-NAME was associated with a smaller area under the insulin curve, compared to placebo (9287.1±1628.1 IU.min vs. 14300.7±1381.6 IU.min; $P < 0.05$); however, there was no difference during euglycaemia (5610.8±1593.5 IU.min [L-NAME] vs. 4857.5±861.4 IU.min [placebo]; P = N.S.). (Fig. 10.5a)

GIP

Plasma GIP concentrations increased substantially after the drink on all study days ($P < 0.001$), but were not affected by either the "glycaemic state" or "treatment". (Fig. 10.5b)

10.5 Discussion

This study has confirmed that acute hyperglycaemia delays gastric emptying of a nutrient liquid, and has established for the first time that this effect is mediated by nitric oxide.

The dose of L-NAME used was based on previous studies (Su et al. 2001; Gentilcore et al. 2005) and, as previously observed (Su et al. 2001; Gentilcore et

al. 2005), changes in cardiovascular function were consistent with nitric oxide synthase inhibition. However, the possibility that some of the effects of L-NAME observed were not related to nitrergic mechanisms cannot be excluded (Das et al. 1999). Three-dimensional ultrasound was chosen to measure gastric emptying due to its superior accuracy over 2D ultrasound (Gilja et al. 1997), and non-invasive nature, and because no ionising radiation is required, unlike scintigraphy (Gentilcore et al. 2006). 3D ultrasound has also been validated against scintigraphy for the measurement of gastric emptying of a liquid meal in healthy subjects (Gentilcore et al. 2006). Although this method could potentially over-estimate retention, as it is unable to differentiate between the liquid meal and gastric secretions, L-NAME was unlikely to have had any major effect on gastric secretions, since the gastric volume was not significantly different between the two euglycaemic days. It should be acknowledged that only healthy subjects were studied, and that these observations may not necessarily apply to patients with diabetes, in whom there may be changes to gastrointestinal signaling pathways secondary to either chronic hyperglycaemia or diabetes per se.

In this study, the marked slowing of gastric emptying observed with hyperglycaemia is consistent with previous reports (Fraser et al. 1990; Samsom et al. 1997; Schvarcz et al. 1997; Jones et al. 1999). The observation that proximal, but not distal, gastric retention, closely resembled total gastric emptying probably reflects the fact that the majority of the drink was retained, at least initially, in the proximal stomach. It should be recognised that these findings may not apply to

the emptying of solid meals, although after ingestion of a mixed solid/liquid meal, solids also tend to remain in the proximal stomach until the majority of the liquid has emptied (Houghton et al. 1988). The increased proximal gastric retention associated with hyperglycaemia in this study may indicate the suppression of fundic tone as a potential mechanism, although tone was not measured directly, which would have required the use of a barostat (Azpiroz and Malagelada 1987). Observations regarding the effect of hyperglycaemia on fasting antral motility are also largely in keeping with the existing literature (Barnett and Owyang 1988; Fraser et al. 1991; Hasler et al. 1995). Postprandially, however, only short propagated antral wave sequences were suppressed during hyperglycaemia. This observation may reflect the fact that antral motility is normally suppressed after nutrient liquid ingestion (Heddle et al. 1989; Samsom et al. 1997; Pilichiewicz et al. 2007), making any further difference between varying glycaemic states difficult to demonstrate. Although no effect of hyperglycaemia on the number of isolated pyloric pressure waves was observed, in contrast to a previous report (Fraser et al. 1991), there was an increase in basal pyloric pressure associated with hyperglycaemia postprandially, which has hitherto not been reported. Information on the effect of hyperglycaemia on proximal small intestinal motility is limited and inconsistent (Bjornsson et al. 1994; Russo et al. 1996; Byrne et al. 1998; Lingenfelser et al. 1999), but this study has not identified any major effect of hyperglycaemia on duodenal motility.

The role of NO in the gastropyloroduodenal region has been controversial. In humans, several studies involving either NO donors, such as intravenous nitroglycerin (Sun et al. 1998) or sublingual glyceryl trinitrate (Gilja et al. 1997), or inhibitors of NO production such as NG-monomethyl-L-arginine (L-NMMA) (Konturek et al. 1999), indicate that NO mediates relaxation of the gastric fundus (Gilja et al. 1997), slows gastric emptying (Konturek et al. 1995; Sun et al. 1998; Konturek et al. 1999; Shah et al. 2000; Calatayud et al. 2002; Wang et al. 2002; Abraham et al. 2004; Patil et al. 2005), and decreases antral (Konturek et al. 1995; Konturek et al. 1999) and pyloric (Sun et al. 1998) contractions. Conversely, another study reported that L-NAME had no effect on antropyloroduodenal motility (Su et al. 2001), whilst in the healthy elderly, L-NAME did not influence gastric emptying of a glucose drink (Gentilcore et al. 2005). In animals, pyloric relaxation is impaired in neuronal nitric oxide synthase (nNOS)-deficient mice (Watkins et al. 2000), and the relaxation of the rat gastric antrum appears to be dependent on nNOS expression (Gangula et al. 2007), indicating an inhibitory effect of NO. However, other animal studies suggest that L-NAME is associated with slowing of gastric emptying (Calatayud et al. 2002; Lefebvre et al. 2005). Furthermore, there may be sex-dependent differences in the contribution of nitrergic mechanisms to gastric motor function, with female rats having greater levels of nitrergic activity during health, and a greater propensity for disordered gastric motor function during diabetes, than male rats (Gangula et al. 2007).

In this study, the administration of L-NAME abolished the slowing of gastric emptying induced by hyperglycaemia, but had no effect on gastric emptying during euglycaemia, suggesting that the actions of nitric oxide may be glucose-dependent, which may partly account for the discrepancies in the literature regarding its actions, and indicates that in future studies the glycaemic state should be specified. The lack of effect of L-NAME on gastric emptying during euglycaemia, however, does not completely discount a potential role of nitric oxide in the normal feedback mechanism that regulates gastric emptying after a meal, particularly as the "physiological" postprandial hyperglycaemia that normally occurs after a meal was prevented by the euglycaemic insulin/glucose clamp. In this study, there was no effect of L-NAME on antral motility during the fasting period, although the duration of observation was relatively short (i.e. 30 minutes). However, L-NAME suppressed all measures of postprandial antral motility. This contrasts with previous reports that increased NO availability was associated with a decrease, rather than an increase, in the postprandial antral motility index (Konturek et al. 1995; Konturek et al. 1999), although methodological differences may account for the discrepancy. For example, other studies either used a different NO synthase inhibitor (L-NMMA) (Konturek et al. 1999) or a NO donor (Konturek et al. 1995), and one study used a semi-liquid, rather than a liquid, meal (Konturek et al. 1999). The fact that L-NAME was associated with a suppression of postprandial antral motility, but not with a slowing of gastric emptying, suggests that antral motility probably does not play a major role in the emptying of a liquid meal. The number of isolated pyloric

pressure waves was unaffected by L-NAME during both the fasting and postprandial periods. However, basal pyloric pressure was reduced by L-NAME postprandially, with a similar trend during fasting, indicating that L-NAME may have a suppressive effect on tonic, but not phasic, pyloric contractions. This could represent the dominant effect through which L-NAME abolishes the delay in gastric emptying induced by hyperglycaemia. Duodenal motility appeared little affected by L-NAME, suggesting limited involvement of nitrergic mechanisms in this region. Volunteers in this study were predominantly male, and in future studies relating to nitric oxide, it would be interesting to evaluate male and female responses separately.

The higher plasma insulin levels on the hyperglycaemic, compared to euglycaemic days, were anticipated. The lower incremental area under the insulin curve on the hyperglycaemia/L-NAME, compared to the hyperglycaemia/placebo, day, is modest, but is consistent with the previous observation that L-NAME is associated with less stimulation of insulin after oral glucose in healthy elderly subjects, compared to placebo (Gentilcore et al. 2005), and implies that NO plays a role in mediating insulin secretion. The mechanism of this effect is unknown, but appears unlikely to involve the incretin hormone GIP, the levels of which did not differ between the study days, nor did the previous study indicate that glucagon-like peptide-1 (GLP-1) was involved (Gentilcore et al. 2005). Given the latter observation, GLP-1 was not measured in the current study. The involvement of NO in insulin secretion warrants further exploration, particularly in patients

with diabetes, as both hyperglycaemia (Ding et al. 2000) and diabetes (Angulo et al. 2003) per se have been associated with impaired nitrergic activity. There are well established links between NO and insulin release in animal and in vitro models, with one study suggesting that NO may contribute to glucose-stimulated insulin release (Nunemaker et al. 2007), but others supporting an inhibitory effect of NO on beta-cell function (Abaraviciene et al. 2008; Mosen et al. 2008). The lack of difference in plasma GIP between the 4 study days is somewhat surprising, given the markedly slower rate of gastric emptying on the hyperglycaemia/placebo day, compared to the other three days. A logical explanation can not be provided for this. Hyperglycaemia and hyperinsulinaemia are unlikely to be involved in the secretion of GIP, given that levels on the hyperglycaemia/L-NAME day did not differ from the two euglycaemic days. Nitric oxide is also unlikely to be involved in GIP release, given the lack of difference in GIP levels between the euglycaemia/L-NAME and euglycaemia/placebo days.

In summary, this study showed that the slowing of gastric emptying of a nutrient liquid, induced by acute hyperglycaemia, was abolished by the NO synthase inhibitor, L-NAME. Basal pyloric pressure was elevated during hyperglycaemia, and reduced by L-NAME. Insulin secretion appeared to be influenced by NOS inhibition. Further studies relating to the role of NO on gastric emptying and insulin release are now warranted in patients with diabetes.
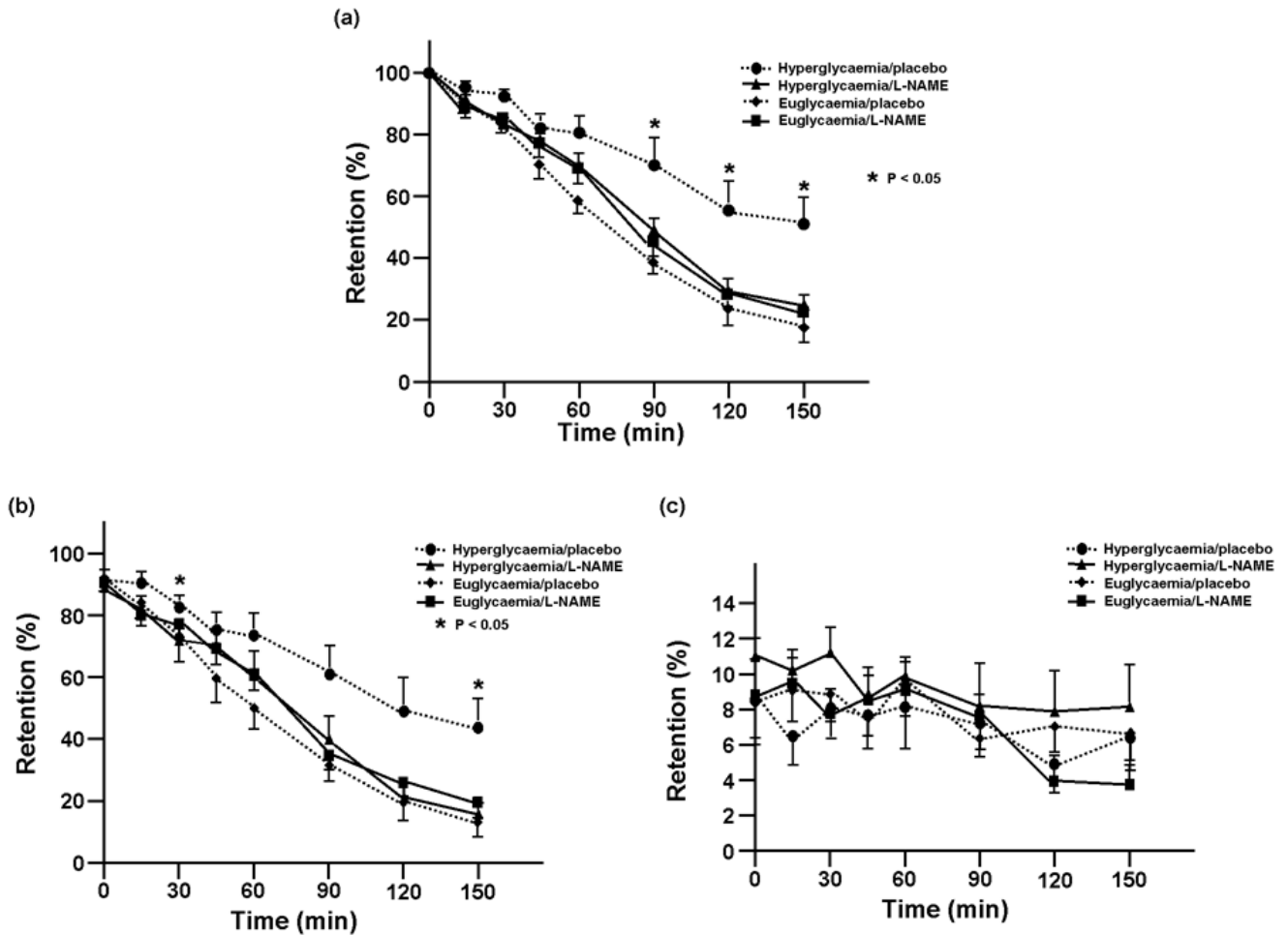
Figure 10.1

(a) Total gastric emptying, (b) proximal retention, and (c) distal retention, expressed as a percentage of the total gastric volume immediately after the glucose drink (T = 0 min). L-NAME was associated with an acceleration of total gastric emptying and a reduction in proximal gastric retention only during hyperglycaemia, but had no effect during euglycaemia. * indicate time points that are significantly different between the hyperglycaemia/placebo day and each of the remaining 3 days. Data are mean±SEM. (n = 7).
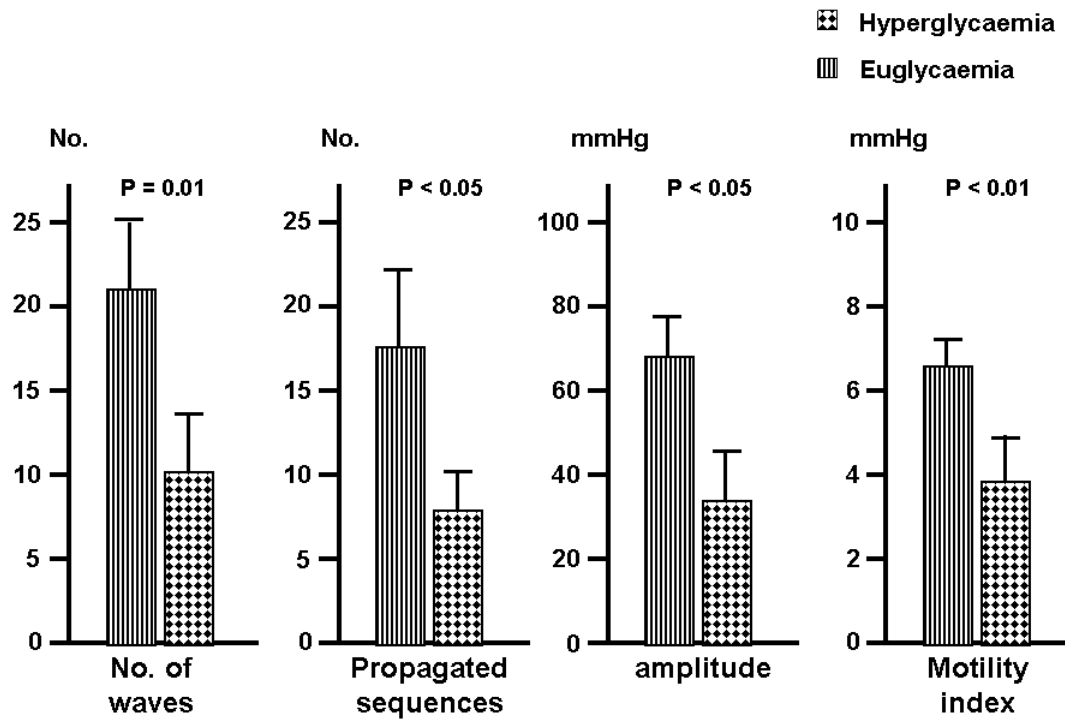
Figure 10.2

Effects of hyperglycaemia on fasting antral motility. Number of waves and propagated sequences are the mean of the number per 10 min, ±SEM, between T = -30 min and T = 0 min. Amplitude and motility index are the mean values ± SEM, between T = -30 min and T = 0 min. (n = 7).
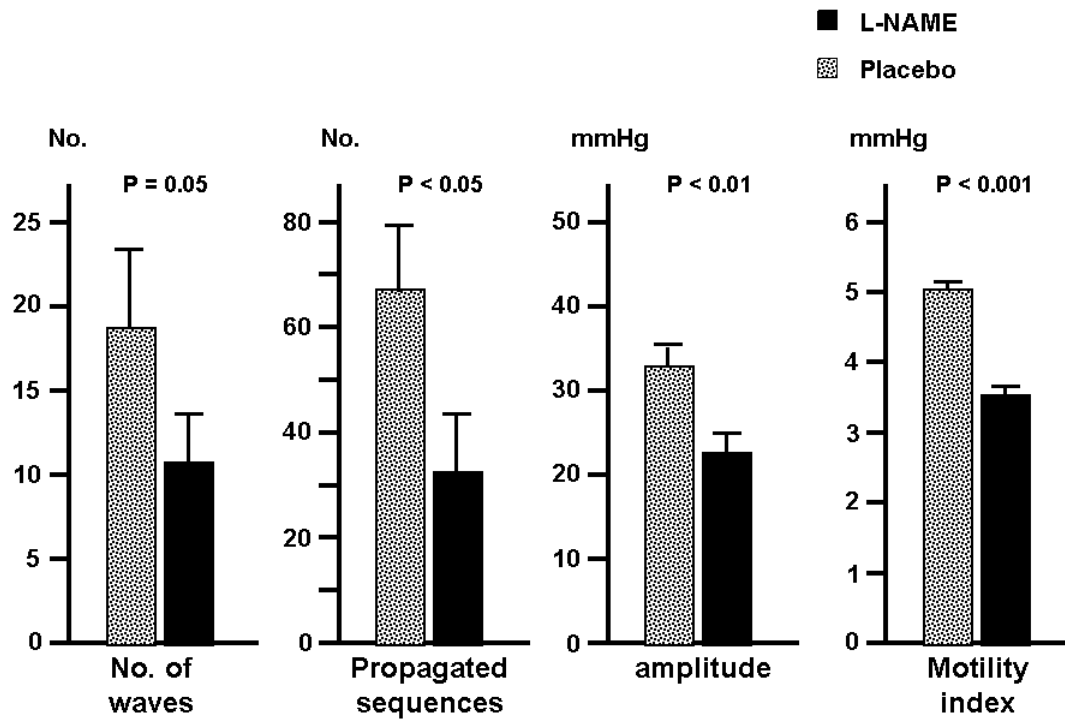
Figure 10.3

Effects of L-NAME on postprandial antral motility. Number of waves and propagated sequences are the mean of the number per 10 min, ±SEM, between T = 0 min and T = 150 min. Amplitude and motility index are the mean values ± SEM, between T = 0 min and T = 150 min. (n = 7).
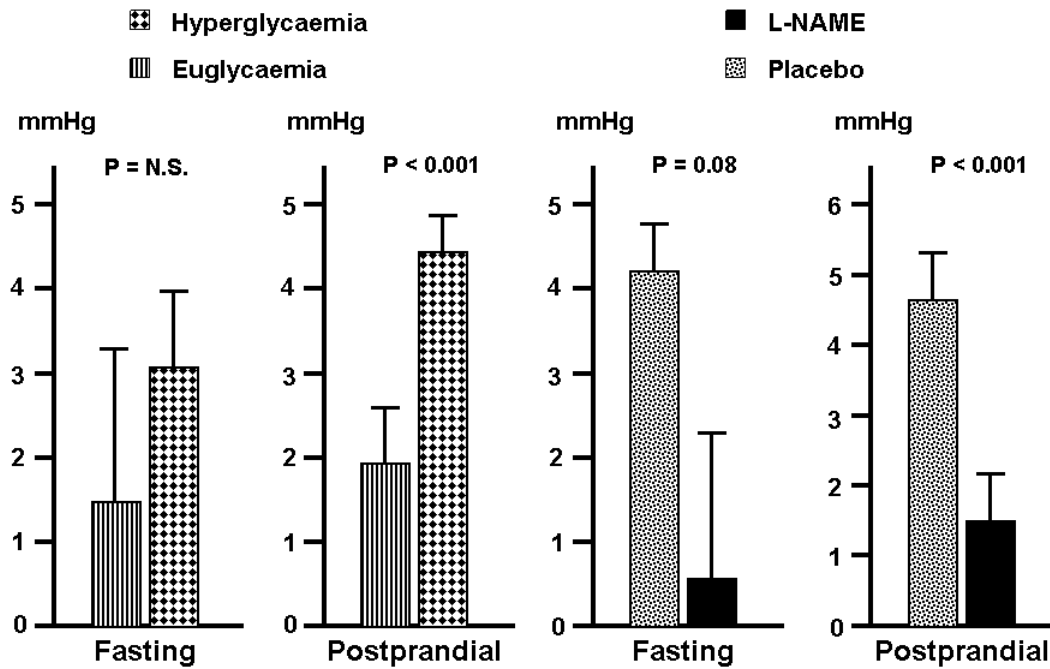
Figure 10.4

Basal pyloric pressure. Values are the mean over either the entire fasting (T = -30 min to T = 0 min), or the entire postprandial (T = 0 min to T = 150 min), period. Data are mean ± SEM. (n = 7).
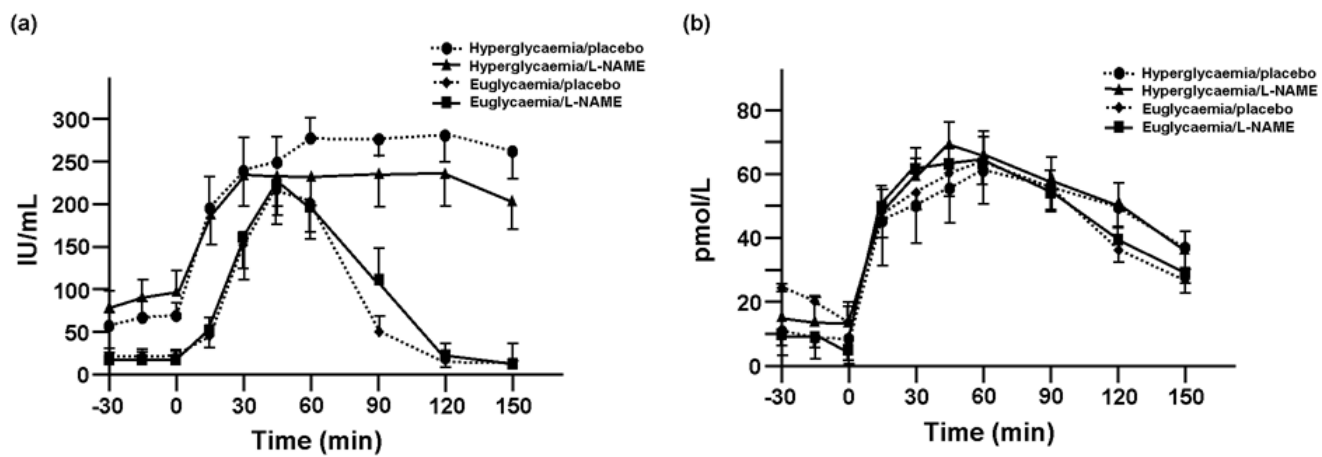
Figure 10.5

Plasma concentrations of (a) insulin, and (b) glucose-dependent insulinotropic polypeptide (GIP). Data are mean ± SEM. (n = 7).

Chapter 11:

Gastric emptying, incretin hormone secretion, and postprandial glycaemia in cystic fibrosis – effects of pancreatic enzyme supplementation (Submitted for publication)

11.1 Summary

Postprandial hyperglycaemia is common among cystic fibrosis (CF) patients, despite the absence of diabetes. The rate of gastric emptying and the secretion of incretin hormones are major determinants of postprandial glycaemia. The breakdown of fat into free fatty acids is critical in slowing gastric emptying and stimulating incretin hormone release. 95 % of CF patients have exocrine pancreatic insufficiency leading to fat maldigestion and potentially rapid gastric emptying. This study therefore aimed to evaluate the effects of pancreatic enzyme supplementation on gastric emptying, incretin hormone secretion, and postprandial glycaemia in CF. Five non-diabetic CF patients (3 male; age 25.8±1.0 yr; BMI 20.2±1.1 kg/m^2) with exocrine pancreatic insufficiency, and 6 healthy subjects, were studied in a randomised, placebo-controlled fashion. All subjects consumed a 75 g glucose drink on their first visit. Subsequently, CF

patients consumed a radiolabelled mashed potato meal on 2 separate days, together with 4 capsules of Creon Forte (100,000 IU lipase) or placebo, in double-blinded, randomised order. Healthy subjects consumed the meal once. Gastric emptying was measured using scintigraphy and blood was sampled frequently for glucose concentrations, GLP-1, GIP, and insulin. CF patients had more rapid gastric emptying ($P < 0.001$), impaired secretion of GLP-1 ($P < 0.01$) and GIP ($P < 0.001$), and greater postprandial glycaemic excursions ($P < 0.001$), compared to healthy subjects; pancreatic enzyme supplementation either normalised (gastric emptying and GLP-1) or substantially reversed (blood glucose, GIP, and insulin-to-glucose ratio) these changes. There was an excellent correlation between gastric emptying and blood glucose concentration at 60 minutes ($R = 0.75$, $P = 0.01$). It is concluded that postprandial hyperglycaemia is common in non-diabetic CF, and may be partly due to rapid gastric emptying and impaired incretin hormone secretion. Optimising fat digestion represents a novel approach to managing CF-related diabetes.

11.2 Introduction

Cystic fibrosis (CF) is becoming increasingly prevalent due to the dramatic improvement in life expectancy with this condition. However, this is accompanied by a rapid increase in cystic fibrosis-related diabetes (CFRD), which affects ~75 % of all CF patients by the age of 30 (Bismuth et al. 2008). CFRD is distinct from type 1 and type 2 diabetes (Lanng et al. 1995; Moran et al. 1999), and is

characterised by postprandial, rather than fasting, hyperglycaemia (Lanng et al. 1995; Moran et al. 1999). Postprandial hyperglycaemia is frequently evident in CF patients who are considered to have "normal glucose tolerance" as assessed by oral glucose tolerance testing (Yung et al. 2002). CFRD is associated with a worse clinical outcome (Liou et al. 2001; Marshall et al. 2005; Cawood et al. 2006). Treating CFRD with insulin has been shown to improve lung function and nutritional status (Mohan et al. 2007).

Approximately 90 % of all CF patients have exocrine pancreatic insufficiency (Symonds et al. 2003; Baker et al. 2005), and the gastric emptying of oil in these patients is faster than in healthy subjects (Carney et al. 1995), probably due to reduced fat digestion (Pilichiewicz et al. 2003), since the digestive products of fat are potent inhibitors of gastric emptying (Borovicka et al. 2000). The rate of gastric emptying is a major determinant of postprandial glycaemia, accounting for one-third of the variation in the initial rise in blood glucose (O'Donovan et al. 2004). The frequent occurrence of postprandial hyperglycaemia in CF patients may, therefore, be partly attributable to persistent fat maldigestion, which occurs in 15 - 25 % of CF patients taking pancreatic enzyme supplementation (Kalnins et al. 2005), and its associated rapid gastric emptying.

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are secreted from intestinal L and K cells respectively, upon simulation by luminal nutrient (Deacon 2005). These

hormones augment the release of insulin in response to elevated blood glucose, the so-called "incretin effect" (Elliott et al. 1993), and account for approximately two-thirds of the postprandial rise in insulin (Meier and Nauck 2004). Fat is a potent stimulator of incretin hormone release (Feinle et al. 2003; Pilichiewicz et al. 2007), but this effect is dependent on fat digestion to yield free fatty acids (Pilichiewicz et al. 2003). Fat maldigestion in CF patients could therefore result in reduced incretin hormone secretion, contributing to postprandial hyperglycaemia.

This study therefore aimed to investigate the rate of gastric emptying, and the glycaemic and incretin hormone responses, to a high fat, high carbohydrate meal in CF patients compared to healthy subjects, and whether any differences would be abolished by pancreatic enzyme supplementation.

11.3 Methods

11.3.1 Subjects

Five non-diabetic CF patients with exocrine pancreatic insufficiency who normally took pancreatic enzyme supplements with meals (3 males; mean age 25.8±1.0 yr; mean body mass index [BMI] 20.0±1.1 kg/m^2), and 6 healthy subjects (3 males; mean age 21.7±1.3 yr; mean BMI 21.3±0.5 kg/m^2), were studied. One CF patient was diagnosed with impaired glucose tolerance, whilst all others had normal glucose tolerance, as assessed by a 75 g oral glucose tolerance

test performed within one year prior to the study. Other than diabetes, exclusion criteria for CF patients were: the presence of severe pulmonary disease (forced expiratory volume in 1 second [FEV-1] < 30 % predicted for age), significant liver disease (Child-Pugh score > 6), previous gastrointestinal surgery (apart from uncomplicated appendicectomy), and pregnancy or lactation. In CF patients, all medications known to affect gastrointestinal function were withheld on the morning of each study day. No healthy subject was taking any medication known to affect gastrointestinal function.

11.3.2 Protocol

CF patients were each studied on 3 days, and healthy subjects on 2 days; all studies were separated by at least 3 days. On each study day, subjects attended the laboratory at 0900h after an overnight fast (12h for solids, 10h for liquids), and an intravenous cannula was inserted into a cubital fossa vein for blood sampling. On the first visit, all subjects consumed a radiolabelled glucose drink (75 g glucose made up to 300 mL with water, and 20 MBq of ^{99m}Tc sulfur-colloid). On the two subsequent study days, CF patients consumed a mashed potato meal (60 g olive oil, 65 g dry potato powder, 20 g glucose, 20 MBq ^{99m}Tc sulfur-colloid, and 250 mL water), together with either 4 Creon Forte capsules (100,000 U lipase in total) (Solvay Pharmaceuticals, Pymble, NSW, Australia) or placebo, in double-blinded, randomised fashion. CF patients were instructed to take one capsule immediately before, two capsules half-way through, and one immediately after the meal, in

order to optimise mixing between food and enzymes. On the second study day, healthy subjects consumed the same mashed potato meal without enzyme or placebo capsules. The glucose drink was consumed within 5 minutes, and the mashed potato meal within 10 minutes. Subjects were seated with their backs against a gamma camera, and gastric emptying was measured by scintigraphy for 3 hours. After each gastric emptying study, subjects remained in the laboratory for a further 3 hours, for collection of blood samples.

Venous blood was sampled at frequent intervals for measurement of blood glucose, and plasma concentrations of insulin, GLP-1, and GIP.

Gastrointestinal symptoms were assessed using validated visual analogue questionnaires (Sturm et al. 2004), at the same intervals as the blood samples.

All CF patients also underwent standardised tests of autonomic nerve function at the end of the last study day (Clarke and Ewing 1982).

11.3.3 Measurements

Gastric emptying

Radioisotopic data were acquired for 180 minutes (1-minute frames in the first 60 minutes, and 3-minute frames thereafter) (Jones et al. 2005). Data were corrected

for subject movement, radionuclide decay, and γ–ray attenuation (Collins et al. 1983). Regions-of-interest were outlined manually on each frame, initially for the total, and then the proximal and distal regions, and gastric emptying was expressed as percentage retention over time (Jones et al. 2005). The amount of the meal remaining in the total, proximal, and distal stomach, was calculated, and the time for 50 % of the radioactivity to leave the stomach (T50), was also determined (Collins et al. 1983).

Blood glucose and plasma insulin, GLP-1, and GIP

Blood glucose concentrations were determined immediately using a portable glucose meter (MediSense Optium, MediSense Inc., Waltham, MA, USA). We have validated the accuracy of this method against the hexokinase technique (Horowitz et al. 1991). Blood samples for measurement of plasma insulin, GLP-1, and GIP were collected in ice-chilled tubes containing EDTA and 400 kIU aprotinin (Trasylol; Bayer Australia, Pymble, Australia) per litre of blood. Plasma was separated by centrifugation (1,500 G, 4 °C, 15 min) and stored at -70 $^{\circ}$C for subsequent analysis.

Plasma insulin concentrations were measured by Enzyme-Linked Immunosorbent Assay (ELISA) (Diagnostics Systems Laboratories Inc., Webster, TX, USA) (Chaikomin et al. 2007). Plasma total GLP-1 concentrations were measured by

radioimmunoassay (RIA) (GLPIT-36HK, Linco Research, St. Charles, Missouri, USA). Plasma GIP concentrations were measured by RIA.

Gastrointestinal symptoms

Symptoms of hunger, desire to eat, fullness, and nausea were measured using validated visual analogue questionnaires. (Sturm et al. 2004)

Autonomic nerve function

Autonomic nerve function was determined using a combination of 3 standardised cardiovascular reflex tests, including the variation in heart rate during deep breathing, "30:15" ratio, and postural blood pressure change (Jones et al. 2005). Results were scored according to pre-defined criteria for each test as: 0 = normal, 1 = borderline, 2 = abnormal, for a total maximum score of 6. A score of ≥3 was considered to indicate definite autonomic dysfunction (Clarke and Ewing 1982).

11.3.4 Statistical analysis

Data were evaluated by repeated-measures ANOVA with "treatment" (or "group" when comparing between CF and healthy) and "time" as factors, with post hoc means comparisons at individual time points in the event of significant "treatment"/"group"-by-"time" interactions. Student's t-tests were used to analyse

gastric half-emptying time (T50), and peak values for blood glucose and hormone concentrations. Linear regression analysis was used to evaluate relationships between variables. Insulin-to-glucose ratio was calculated using plasma insulin (in mU/L) divided by plasma glucose (in mmol/L). A statistical software package (SPSS 15.0, SPSS Inc, Chicago, IL, USA) was used for all analysis. The number of subjects required was calculated based on data from a previous similar study (Carney et al. 1995). Statistical significance was accepted as $P < 0.05$, and data are presented as mean $\pm$ standard error of the mean (SEM).

11.4 Results

All subjects tolerated the studies well. CF patients could not discriminate between the Creon Forte and placebo capsules. None of the CF patients had definite autonomic nerve dysfunction; one (with impaired glucose tolerance) had a score of 2/6, indicating possible autonomic dysfunction, whilst the other 4 patients scored 0.

11.4.1 Gastric emptying

75g glucose drink

Total gastric emptying

In both CF and healthy subjects, gastric emptying of glucose approximated a linear pattern, with a trend for slower emptying in CF patients compared to healthy subjects, as a group-by-time interaction (P = 0.06). (Fig. 11.1a) The half-emptying time (T50) was, however, not different between the groups (123.8±13.0 [CF] vs. 114.2±9.5 min [healthy]; P = N.S.).

Intragastric distribution

In CF patients, more of the drink was retained in the distal stomach (P = 0.07), and less in the proximal stomach (P < 0.05), compared to healthy subjects. (Fig. 11.1b,c)

Mashed potato meal

Total gastric emptying

Without pancreatic enzymes, total gastric emptying in CF patients approximated

an exponential pattern and was faster than in healthy subjects (P < 0.001), as a group-by-time interaction. (Fig. 11.1a) Pancreatic enzyme supplementation slowed gastric emptying substantially in CF patients (P < 0.001), so that the rate became comparable to that of healthy subjects, and approximated a more linear pattern. (Fig. 11.1a) The T50 in CF patients without enzymes tended to be less compared to healthy subjects (68±6.8 [CF] vs. 130.2±16.8 min [healthy]; P = 0.08), and enzyme supplementation tended to prolong the T50 in CF patients compared to placebo (115.0±17.3 min; P = 0.09).

Intragastric distribution

Without enzyme supplementation, a similar proportion of the meal was retained in the proximal stomach in CF patients, compared to the healthy subjects (P < 0.01), and this phenomenon was not affected by enzyme supplementation. No difference in distal retention was observed between the 3 groups. (Fig.11.1b,c)

11.4.2 Blood glucose

75g glucose drink

Fasting blood glucose did not differ between CF and healthy subjects. After the drink, blood glucose concentrations rose sharply in both groups, but in CF patients, a much higher peak was reached at 60 minutes and remained

substantially greater than baseline until ~180 minutes, compared to an earlier and more modest peak at 45 minutes followed by a rapid fall back to baseline by ~150 minutes in healthy subjects (peak blood glucose: 13.4±0.8 [CF] vs. 10.7±0.9 mmol/L [healthy]; P = 0.05). Blood glucose concentrations were higher in CF patients (P < 0.001), as a group-by-time interaction. (Fig 11.2a)

Mashed potato meal

Fasting blood glucose did not differ between the three groups. After the mashed potato meal, CF patients taking placebo capsules had the greatest rise in blood glucose, reaching a marked peak at 60 minutes, followed by a rapid fall, and return to baseline by ~150 minutes; in CF patients taking pancreatic enzymes, the rise was moderate, peaking at 75 minutes, followed by a more gradual return to baseline by ~210 minutes; conversely, the rise in blood glucose in the healthy subjects was slow and modest, reaching a much lower peak at 105 minutes, and returning to baseline by ~180 minutes (peak blood glucose: 13.7±0.9 [CF placebo] vs. 8.2±0.4 mmol/L [healthy]; P < 0.001). Without pancreatic enzyme supplementation, the blood glucose concentrations (P < 0.001, group-by-time effect) were substantially higher in CF patients, compared to healthy subjects. (Fig. 11.2a) Among CF patients, pancreatic enzyme supplementation lowered the blood glucose concentrations substantially, as a treatment-by-time interaction (P < 0.001), but did not significantly alter the peak blood glucose concentration (11.6±0.6 [Creon] vs. 13.7±0.9 mmol/L [placebo]; P = 0.09), compared to

placebo. When compared to healthy subjects, CF patients with pancreatic enzyme supplementation still had higher blood glucose concentrations (P < 0.05) and peak blood glucose (11.6±0.6 [CF Creon Forte] vs. 8.2±0.4 mmol/L [healthy]; P < 0.01). (Fig. 11.2a)

Combining data from the Creon and placebo day, there was excellent correlation between the percentage of total gastric retention and blood glucose concentration at 60 minutes, in CF subjects (r = 0.75, P = 0.01). (Fig. 11.3)

11.4.3 Plasma insulin, GLP-1, and GIP concentrations

Insulin

75g glucose drink

Fasting insulin did not differ between CF patients and healthy subjects. After the glucose drink, plasma insulin concentrations rose sharply in healthy subjects, reaching a peak at 45 minutes and returning to baseline by ~180 minutes; conversely, the rise in insulin was modest and delayed in CF patients, reaching a substantially lower peak at 120 minutes and returning to baseline by ~210 minutes. CF patients had lower plasma insulin concentrations than healthy subjects (P < 0.01), as a group-by-time interaction. (Fig. 11.2b)

The fasting insulin-to-glucose ratio did not differ between CF patients and healthy subjects. After the glucose drink, the insulin-to-glucose ratio was substantially less in CF patients than healthy subjects (P < 0.001). (Fig. 11.2c)

Mashed potato meal

Fasting insulin did not differ between the groups. Postprandially, plasma insulin concentrations rose rapidly in all three, reaching a peak between 60 and 120 minutes, and returned to baseline by ~240 to 300 minutes. Insulin concentrations were not different between the three groups. (Fig. 11.2b)

Again, the insulin-to-glucose ratio during fasting did not differ between CF patients and healthy subjects. Postprandially, CF patients had a lower insulin-to-glucose ratio than healthy subjects (P < 0.05), which was not improved by enzyme supplementation. (Fig. 11.2c)

GLP-1

75g glucose drink

CF patients tended to have lower fasting plasma GLP-1 concentrations compared to healthy subjects (9.5±3.1 [CF] vs. 15.7±3.1 pmol/L [healthy]; P = 0.05). After the drink, there was a rapid rise in GLP-1 in both groups, peaking at 30 minutes,

followed by a similarly rapid decline back to baseline by ~60 minutes. There was a trend for lower GLP-1 concentrations (treatment-by-time interaction; P = 0.09), and a lower peak GLP-1 (P = 0.05), in CF patients compared to healthy subjects. (Fig. 11.4a)

Mashed potato meal

Fasting GLP-1 concentrations tended to be lower in CF patients compared to healthy subjects (P = 0.09 with enzymes and P = 0.07 without). (Fig. 11.4a) Postprandially, plasma GLP-1 rose rapidly, reaching a peak by 30-45 minutes, which was lower in the CF patients than the healthy subjects (P < 0.01). CF patients also had a more rapid return to baseline by ~150 minutes, compared to healthy subjects, in whom GLP-1 remained elevated until 360 minutes. Enzyme supplementation in CF patients abolished any difference in GLP-1 concentrations compared to healthy subjects. (Fig. 11.4a)

GIP

75g glucose drink

Fasting GIP concentrations did not differ between CF patients and healthy subjects. After the glucose drink, there was a rapid rise in GIP in both groups, reaching a peak at 30 minutes, followed by a gradual decline back to baseline

after ~240 minutes, without any difference between the groups. (Fig. 11.4b)

Mashed potato meal

Fasting GIP concentrations were again similar between CF patients and healthy subjects. Postprandially, plasma GIP concentrations rose rapidly in all three groups. CF patients, however, had lower GIP concentrations ($P < 0.001$, treatment-by-time interaction) and a lower peak value ($P < 0.05$), compared to healthy subjects. Enzyme supplementation increased GIP secretion in CF patients ($P < 0.001$), but remained lower than in healthy subjects ($P < 0.01$). (Fig. 11.4b)

11.4.4 Gastrointestinal symptoms

75g glucose drink

Sensations of desire to eat, hunger, fullness, and nausea were not different between CF patients and healthy subjects, either when fasting or after the glucose drink (data not shown).

Mashed potato meal

There were no differences in any sensation between CF patients and healthy subjects during fasting. After the potato meal, the increase in fullness, and

reduction in hunger and desire to eat, appeared less in CF patients compared to healthy subjects, regardless of enzyme supplementation, but these differences did not reach statistical significance. (Fig. 11.5) Nausea scores were relatively low in studies, but were lower in CF patients than healthy subjects (P < 0.05), and this difference was abolished by enzyme supplementation. (Fig. 11.5)

11.5 Discussion

This study has established that without pancreatic enzyme supplementation, patients with cystic fibrosis have more rapid gastric emptying after a high fat, high carbohydrate meal, compared to healthy subjects, and this is accompanied by profound impairments in the secretion of the incretin hormones GLP-1 and GIP, a deficient insulin response when allowing for the glycaemic state, and exaggerated postprandial glycaemic excursions. Pancreatic enzyme supplementation in these patients slowed gastric emptying, and substantially improved postprandial glycaemia, and insulin and GIP secretion, while normalising GLP-1 concentrations. In contrast, a glucose drink emptied relatively normally in CF patients and was followed by an intact incretin hormone response, but insulin secretion was still deficient, and the postprandial glycaemic excursion was greater than in healthy subjects, confirming that postprandial hyperglycaemia is common among CF patients without diabetes. Gastrointestinal sensory impairment may also occur in CF, leading to blunted postprandial sensations, and is not attributable to autonomic neuropathy. The above findings were evident despite the

small sample size, because of the marked and consistent differences between CF patients and healthy subjects.

The mashed potato meal was designed to be high in fat and carbohydrate, consistent with the usual recommended diet for CF patients. As anticipated, the rate of gastric emptying in CF patients without enzyme supplementation was more rapid than in healthy subjects, presumably due to reduced fat digestion and therefore diminished small intestinal feedback. This is likely also to explain the loss of the normal linear pattern of solid emptying in CF patients without enzymes. Digestion of fat to liberate free fatty acids is essential to slow gastric emptying, and stimulate incretin hormone release, as demonstrated with the use of the specific lipase inhibitor, olistat, in patients with type 2 diabetes (Pilichiewicz et al. 2003; O'Donovan et al. 2004). In an attempt to optimise pancreatic enzyme supplementation in this study, we gave twice the usual recommended dose of Creon Forte, and divided the dose throughout the meal. It has been suggested that distributing pancreatic enzymes throughout a meal may improve their efficacy, compared to administration solely before or after food (Dominguez-Munoz et al. 2005), although formulations containing a higher dose of lipase have been reported not to improve fat digestion, compared to conventional preparations (De Boeck et al. 1998). In our CF patients, pancreatic enzyme supplementation normalised both gastric emptying and GLP-1 release. In contrast, GIP secretion improved, but did not return to normal, suggesting incomplete meal digestion in the proximal small intestine, where GIP-secreting K cells exist most densely, but

more complete digestion by the time the meal reached the distal small intestine and colon, where GLP-1-secreting L cells are predominantly located. Inadequate mixing between pancreatic enzymes and nutrients has been demonstrated in a number of studies involving both healthy subjects (Meyer and Lake 1997) and CF patients (Taylor et al. 1999), and could potentially explain why digestion in the proximal small intestine may be incomplete. The failure of even high-dose enzyme supplementation to improve fat absorption is also consistent with the concept that mixing between the meal and the enzymes is suboptimal (De Boeck et al. 1998). Therefore, strategies to optimise mixing, or to stimulate incretin hormone release without the need for digestion, such as by adding free fatty acids to the meal, represent novel approaches to the management of postprandial hyperglycaemia and diabetes in CF patients.

The fact that the rate of gastric emptying of the glucose drink did not differ between CF patients and healthy subjects indicates that the small intestinal feedback mechanisms controlling gastric emptying remain relatively intact in CF. However, CF patients retained less of the drink in the proximal,, and more in the distal, part of the stomach, indicating subtle differences in gastric mechanics, such as impaired fundic or enhanced antral relaxation. However, fundic tone was not measured, which would have required the use of a barostat, and there are currently no published data about gastric accommodation or meal distribution in CF patients. After the glucose drink, despite a similar rate of gastric emptying and incretin hormone secretion between CF patients and healthy subjects, CF patients

displayed a much lower insulin-to-glucose ratio, supporting the notion that impaired insulin secretion makes an important contribution to postprandial hyperglycaemia in this group. The fact that their fasting insulin concentrations were not elevated argues against the presence of insulin resistance, although the possibility of increased secretion of glucagon can not be excluded.

In this study, it was observed that fasting GLP-1 concentrations in CF patients were lower than in healthy subjects. One potential explanation is that chronic impairment of fat digestion in CF patients leads to down-regulation of the GLP-1 secretory mechanism, but not that of GIP, given that fat has a greater impact on GLP-1 secretion (Gentilcore et al. 2006); however, this explanation is inconsistent with the normalisation of GLP-1 concentrations after the mashed potato meal with enzyme supplementation.

In patients with type 2 diabetes, the postprandial secretion of GLP-1 in response to a mixed meal is reduced, and the secretion of GIP is relatively preserved (Toft-Nielsen et al. 2001); furthermore, the insulinotropic effect of exogenous GLP-1 is intact, whilst sensitivity to exogenous GIP is lost (Elahi et al. 1994). These findings have led to the recent development of various GLP-1 analogues, such as exenatide, for the treatment of type 2 diabetes. Cystic fibrosis-related diabetes (CFRD) is currently managed with either insulin or oral hypoglycaemic agents, but evidence to support such treatment approaches is limited, and good glycaemic control remains difficult to achieve (Rosenecker et al. 2001). The results from this

study suggest the mechanism of postprandial hyperglycaemia in CF patients may well be different from type 2 diabetes, as there was a persistent abnormality in GIP, but not GLP-1, secretion, despite pancreatic enzyme supplementation, and this was associated with postprandial hyperglycaemia, suggesting that reduced GIP secretion could be an important determinant of postprandial hyperglycaemia, in addition to impaired insulin secretion. It is not known whether exogenous GIP has any therapeutic effect in CF patients, but the potential therapeutic use of a GIP analogue in the management of postprandial hyperglycaemia or diabetes in CF, warrants further investigation. Conversely, exogenous GLP-1 may have a lesser impact on postprandial glycaemia in CF, given the normalisation of GLP-1 and the rate of gastric emptying by pancreatic enzymes. DPPIV inhibitors, which increase the concentrations of active GIP as well as GLP-1, may also represent an alternative therapeutic option.

Gastrointestinal symptoms did not differ between CF patients and healthy subjects after the glucose drink, presumably reflecting the lack of difference in the rate of gastric emptying or incretin hormone secretion. Differences in intragastric distribution of the drink did not appear to have affected symptoms. There was a trend for CF patients to respond to the mashed potato meal with less suppression of hunger and desire to eat, a smaller increase in fullness, and less nausea compared to healthy subjects, but nausea was the only sensation to achieve a statistically significant difference, probably due to the small sample size. These findings are consistent with our previous study showing a lack of suppression of

hunger in CF patients after an oily drink (Carney et al. 1995), and another study demonstrating that lipase inhibition diminished the increase in nausea in response to intraduodenal fat in healthy volunteers (Feinle et al. 2003). In our CF patients, the failure of pancreatic enzyme supplementation to enhance the symptomatic response to food suggests that their impaired gut sensation is not related to gastric emptying, fat digestion, or incretin hormones. Rather, they might have a deficiency of gut receptors or afferent pathways, perhaps as an adaptation to life-long consumption of a high fat, high carbohydrate diet, or for other reasons. There is currently little information about gastrointestinal sensory function in CF patients, and further investigations would be indicated.

In summary, pancreatic enzyme supplementation in CF patients normalised the rate of gastric emptying and secretion of GLP-1 after a high fat, high carbohydrate solid meal, but while the secretion of GIP and postprandial glycaemia improved, these were not normalised. The therapeutic potential of strategies to optimise mixing between nutrients and enzymes, or the use of exogenous GIP or DPPIV inhibitors, in the management of postprandial hyperglycaemia and diabetes in CF, warrant further investigation.
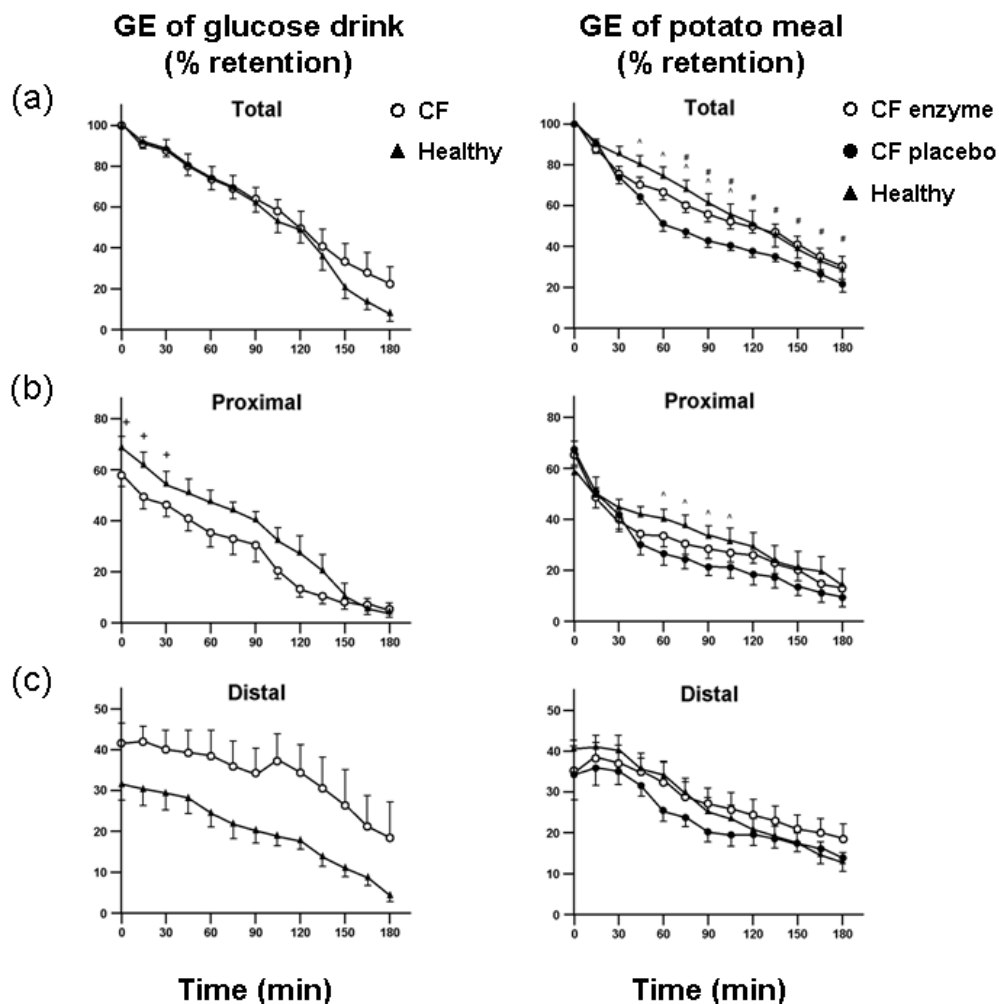
Figure 11.1

Gastric emptying and intragastric distribution of 75g glucose drink (left) and mashed potato meal (right), over 180 minutes, in healthy subjects and CF patients (with and without pancreatic enzymes): (a) total, (b) proximal, and (c) distal. Results are means ± SEM. Symbols signify time points where P < 0.05 (+ CF vs. healthy; * CF enzyme vs. healthy; ^ CF placebo vs. healthy; # CF enzyme vs. CF placebo).
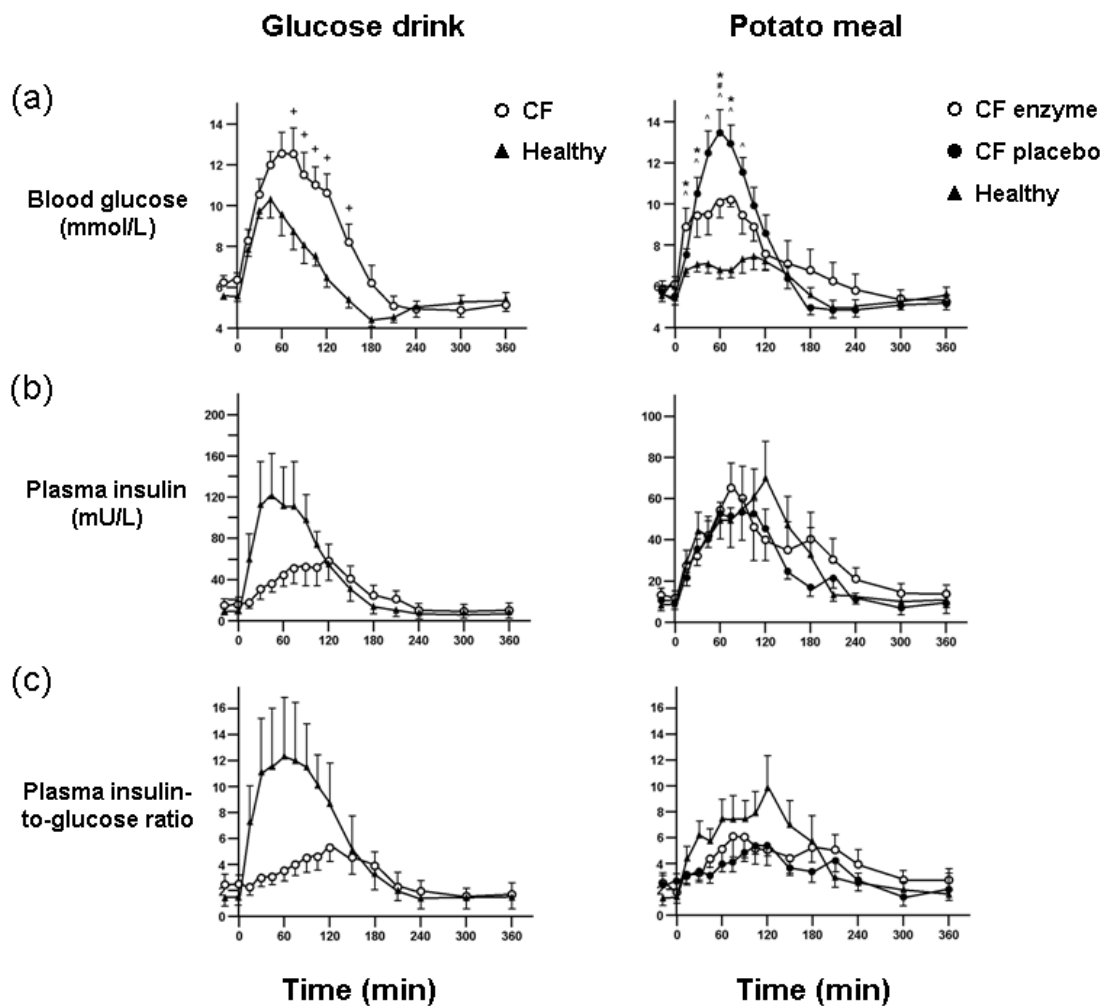
Figure 11.2

(a) Blood glucose, (b) plasma insulin, and (c) plasma insulin-to-glucose ratio, before and after 75g glucose drink (left) and mashed potato meal (right). Results are means ± SEM. Symbols signify time points where P < 0.05 (+ CF vs. healthy; * CF enzyme vs. healthy; ^ CF placebo vs. healthy; # CF enzyme vs. CF placebo).
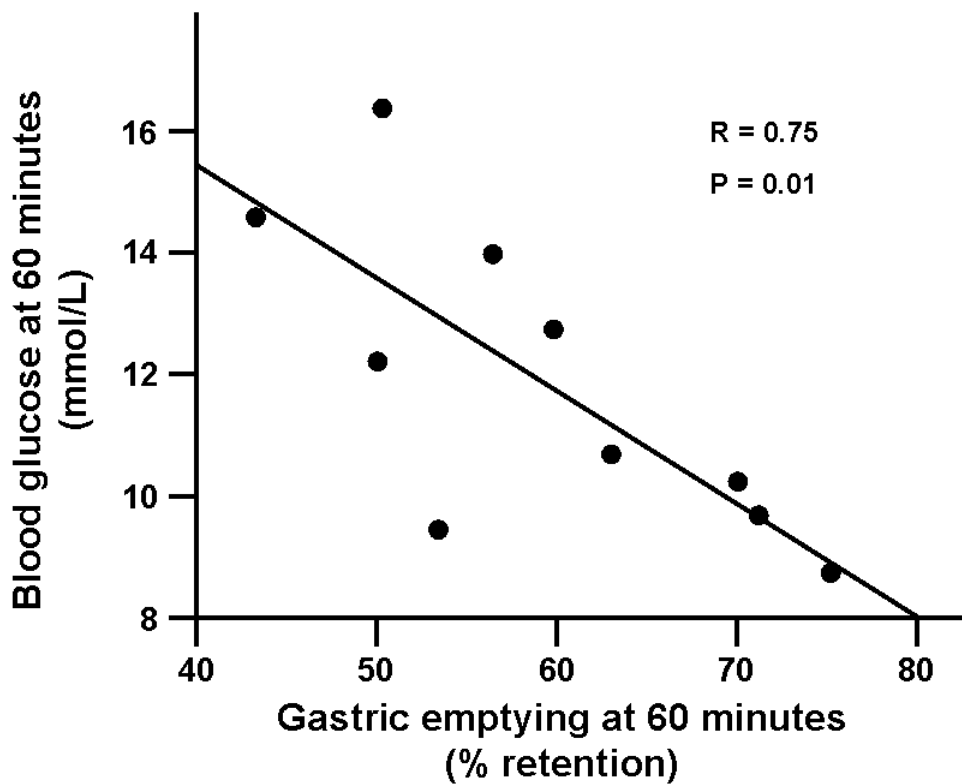
Figure 11.3

Relationship between blood glucose concentration and gastric emptying (expressed as intragastric retention) at 60 minutes in CF patients (with and without pancreatic enzymes).
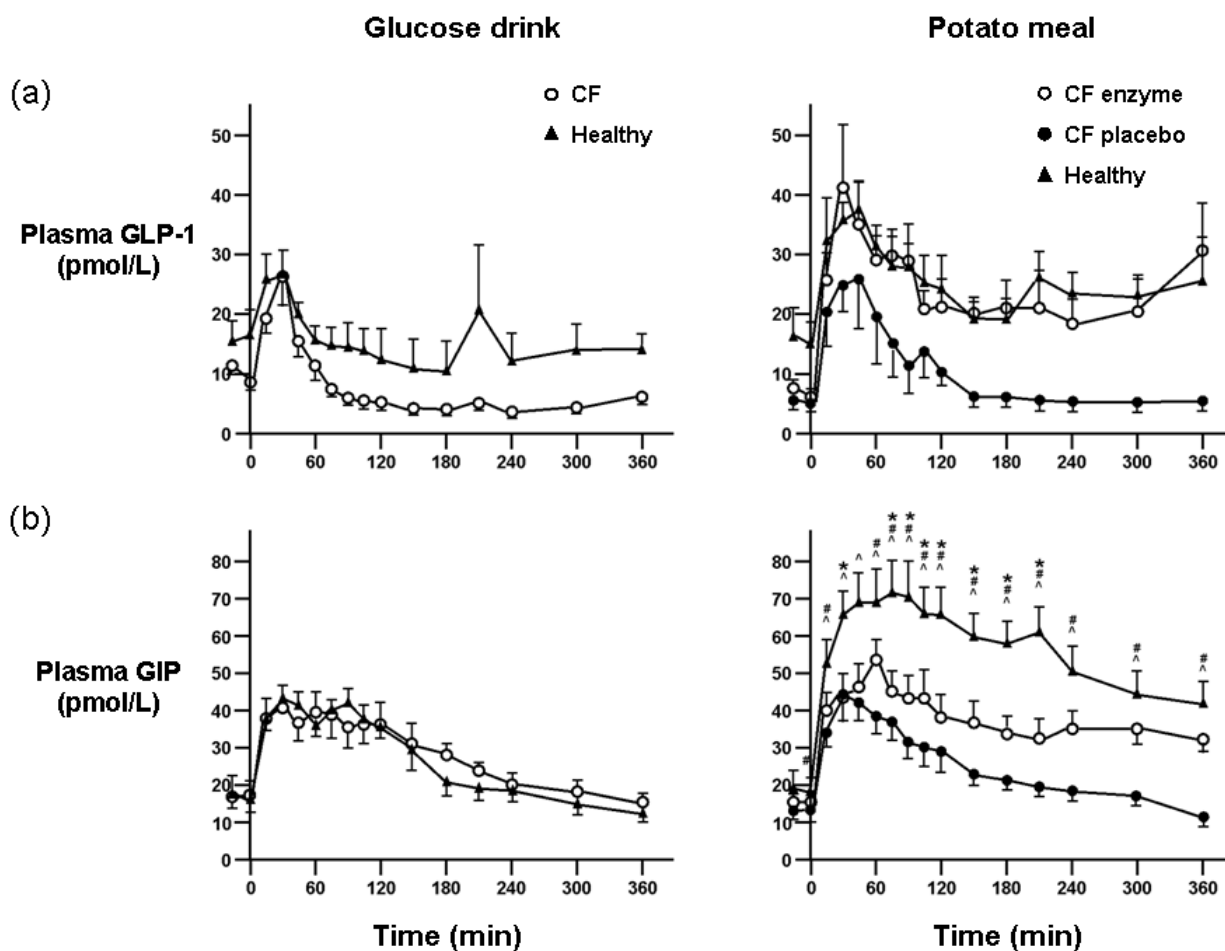
Figure 11.4

(a) Plasma glucagon-like peptide-1 (GLP-1) and (b) glucose-dependent insuloinotropic polypeptide (GIP) concentrations, before and after a 75g glucose drink and mashed potato meal. Results are means ± SEM. Symbols signify time points where $P < 0.05$ (+ CF vs. healthy; * CF enzyme vs. healthy; ^ CF placebo vs. healthy; # CF enzyme vs. CF placebo).
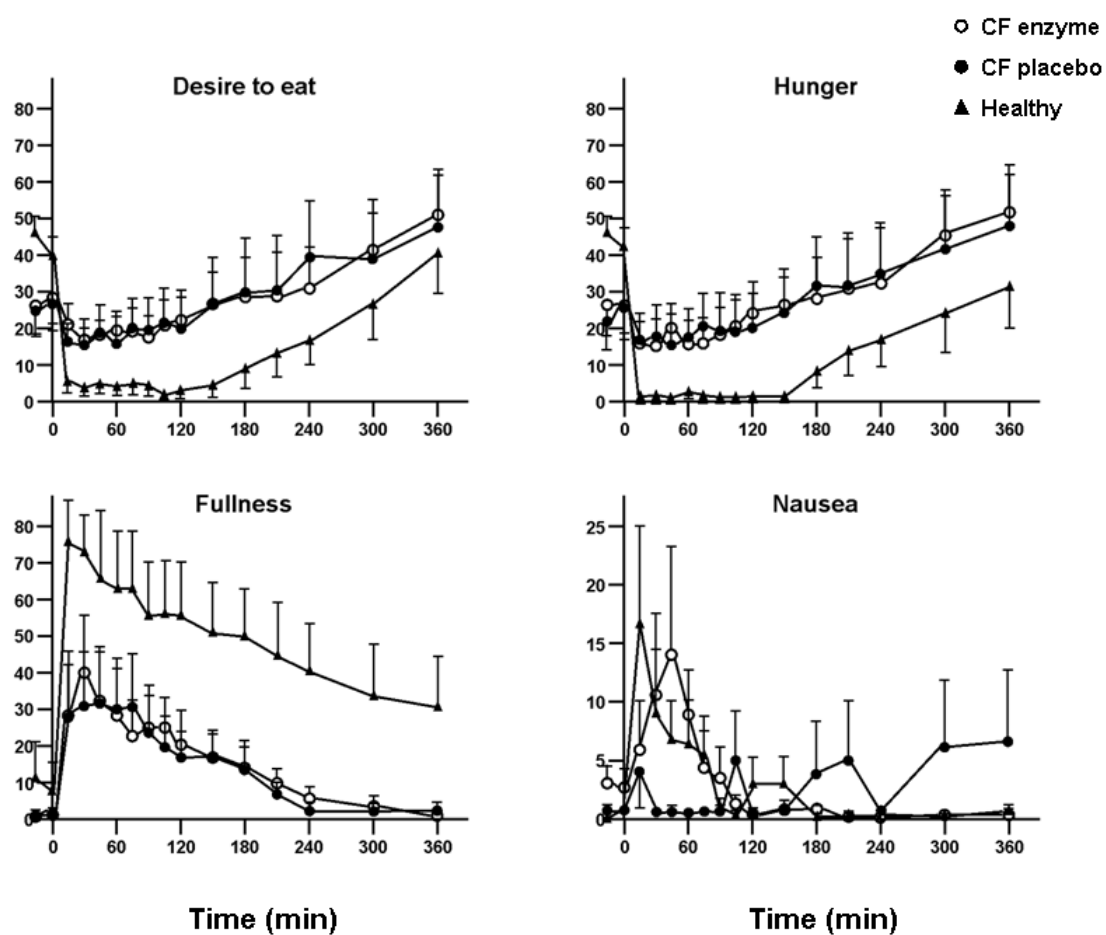
Figure 11.5

Perception of desire to eat, hunger, fullness, and nausea, in CF patients after a mashed potato meal. Results are means ± SEM. Symbols signify time points where P < 0.05 (+ CF vs. healthy; * CF enzyme vs. healthy; ^ CF placebo vs. healthy; # CF enzyme vs. CF placebo).

Chapter 12:

Conclusions

This thesis is comprised of studies that provide insights into the motor function of the gastroduodenal region in the human digestive tract, and how it relates to glucose absorption, incretin hormone secretion, and postprandial blood glucose regulation.

In the study reported in Chapter 6, the use of ^{14}C-3-OMG was validated as a rapid and cost-effective method of measuring enteral glucose absorption. The plasma activity of ^{14}C-3-OMG, as measured by scintillation counting, closely correlated with plasma concentrations of unlabelled 3-OMG, measured by chromatography, in terms of the profile of the absorption curve, peak value, and area under the curve. The great savings in time and cost from scintillation counting, compared to chromatography, and the minimal radiation exposure associated with ^{14}C-3-OMG, allowed the incorporation of this technique into studies described in Chapters 8 and 9.

The study reported in Chapter 7 demonstrated an early, transient, rise in plasma GLP-1, in response to intraduodenal glucose administered at 1 kcal/min, in contrast to a previous report that the rate of nutrient delivery into the small intestine needed to exceed 1.8 kcal to trigger the release of GLP-1. Plasma GLP-1

concentrations reached an early peak at 15 minutes after the intraduodenal glucose infusion commenced, and returned to baseline by 45 minutes, despite continuing glucose infusion. The potential mechanisms involved include an initial rapid transit of nutrient to the distally located L cells, the existence of a duodeno-jejunoileal loop, release of GLP-1 from the proximal small intestinal L cells, and down-regulation of the responsiveness of L cells to ongoing glucose stimulation. The significance of this finding is uncertain, but suggests that further investigation is warranted into the mechanism of GLP-1 release.

The rate of gastric emptying contributes to about one-third of the variations in the initial rise in postprandial blood glucose concentration after oral glucose, but the contribution made by small intestinal motor function is poorly understood. By using the prokinetic agent, metoclopramide, the study reported in Chapter 8 demonstrated an increase in duodenal pressure waves in association with metoclopramide, but no change in the number of duodenal flow events and glucose absorption. These findings are consistent with those from a previous study using hyoscine butylbromide, and support the notion that duodenal pressure waves correlate poorly with flow events and glucose absorption, and that flow events are probably more important a determinant of glucose absorption than pressure waves. The study also supports the benefit of combining impedance monitoring with manometry, thus allowing a more comprehensive assessment of small intestinal motor function.

Another study, using the same combined manometry and impedance monitoring technique, found that physiological hyperglycaemia (~9 mmol/L) had no effect on duodenal pressure waves and flow events compared to euglycaemia (~5 mmol/L), but was associated with lower fasting plasma GLP-1 concentrations, and increased GIP secretion and glucose absorption after intraduodenal glucose administration. Limited evidence suggests that hyperglycaemia reduces incretin hormone secretion after an orally ingested meal, but this study is the first to evaluate an intraduodenal nutrient load, and the results indicate that previous findings were almost certainly due to the effects of hyperglycaemia on gastric emptying. There is also evidence, albeit mostly in animals, that hyperglycaemia is associated with increased glucose absorption. The mechanisms mediating these effects are unknown, but could potentially involve complex interactions between the incretin secreting L and K cells, glucose transporters SGLT-1 and GLUT2, and the sweet taste receptors in the small intestine (Cheeseman 1997; Au et al. 2002; Mace et al. 2007). Limited evidence suggests that different regions of the gut possess their own glycaemic thresholds, above which an effect on motor function is observed (Bjornsson et al. 1994; Hasler et al. 1995; Hebbard et al. 1996; Hebbard et al. 1996; Verhagen et al. 1999). It would therefore be of interest to repeat similar studies using either a higher glycaemic target, for example 15 mmol/L instead of 9 mmol/L, or greater glycaemic differentials, for example 12 mmol/L vs. 4 mmol/L.

Nitric oxide is an inhibitory neurotransmitter in the gut, but its influence on gastric emptying is controversial, and its potential role in mediating hyperglycaemia-induced slowing of gastric emptying has not been reported. The study described in Chapter 10 showed that the specific nitric oxide synthase inhibitor, NG-nitro-L-arginine-methylester (L-NAME), attenuated the slowing of gastric emptying induced by hyperglycaemia, implying that such an effect is indeed mediated by nitric oxide. Furthermore, hyperglycaemia was associated with an increase, and L-NAME with a decrease, in pyloric tone, providing a potential mechanism for the observed changes in gastric emptying. Insulin secretion was suppressed with L-NAME on the hyperglycaemic days, suggesting potential involvement of nitric oxide in glucose-induced pancreatic insulin secretion. However, this study examined the effects of acute hyperglycaemia in healthy subjects, and whether nitric oxide has a similar function in the setting of chronic hyperglycaemia and/or diabetes, remains to be established.

The study presented in Chapter 11 demonstrated that: (1) the rate of gastric emptying was not different between CF patients and healthy subjects after a glucose drink, indicating that the feedback mechanisms from the small intestine that regulate gastric emptying are relatively intact in CF, (2) there is an underlying defect in glucose handling in CF patients, as indicated by greater glycaemic excursions after the glucose drink, despite being classified as having "normal" glucose tolerance according to the standard oral glucose tolerance test, and (3) without pancreatic enzyme supplementation, CF patients with exocrine pancreatic

insufficiency emptied a high fat, high carbohydrate solid meal from their stomachs more rapidly, but had reduced GLP-1 and GIP secretion, and greater postprandial glycaemic excursions, compared to healthy subjects, and these abnormalities were either substantially improved or normalised by pancreatic enzyme supplementation. The partial correction of GIP secretion with enzyme supplementation could suggest inadequate mixing of enzymes with food in the proximal small intestine, possibly also contributing to the incomplete improvement in postprandial hyperglycaemia. These observations suggest that strategies to improve mixing between enzymes and food in the proximal small intestine, or the administration of a GIP analogue, may represent novel approaches in the management of postprandial hyperglycaemia and diabetes in CF patients.

Gastric and small intestinal motor function is fundamental in determining glucose absorption, incretin hormone secretion, and postprandial glycaemia. Various techniques are available for evaluating gastric and small intestinal motor function, although a combined approach is likely to offer the most comprehensive assessment. The importance of postprandial hyperglycaemia to overall glycaemic control is now being recognised, and should become a major focus of diabetes management in the future. Modulation of gastroduodenal motor function, therefore, represents a promising novel approach in the management of diabetes, and further research in this area is warranted.

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