Gastrointestinal motility and glycaemic control in diabetes

A Thesis submitted by

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THESIS SUMMARY

Gastric emptying, and small intestinal glucose exposure and absorption, are potentially important determinants of postprandial blood glucose homeostasis and energy intake. The studies presented in this thesis were designed to provide novel insights into the interrelationships of upper gastrointestinal function with glycaemia and appetite in both health and type 2 diabetes. The issues which were addressed relate in particular to: (i) the physiology, regulation and measurement of gastric and small intestinal motility, (ii) the relationships between small intestinal glucose exposure, incretin hormone release, antropyloroduodenal motility and appetite, and (iii) the impact of gastric and small intestinal motility on glycaemia.

The study reported in chapter 4 evaluated the effect of variations in small intestinal glucose delivery on blood glucose, plasma insulin, and incretin hormone (GLP-1 and GIP) concentrations in healthy subjects. While initially rapid, and subsequently slower, duodenal glucose delivery potentiated incretin and insulin responses when compared to constant delivery of an identical glucose load, the overall glycaemic excursion was not improved. These observations add to the rationale for the use of dietary and pharmacological strategies designed to reduce postprandial glycaemic excursions in health and type 2 diabetes by slowing gastric emptying, rather than initially accelerating it.

Fat is a potent inhibitor of gastric emptying. In chapter 5, the acute effect of slowing gastric emptying by fat, on postprandial glycaemia in type 2 diabetes, has

been evaluated. Ingestion of a small amount of olive oil, as a 'preload' 30 min before a carbohydrate meal, was shown to markedly slow gastric emptying, affect intragastric meal distribution, delay the postprandial rises in blood glucose, plasma insulin, and GIP, and stimulate GLP-1. In contrast, the effects of including the same amount of oil within the meal, on gastric emptying, as well as glycaemic and incretin responses, were relatively modest. As blood glucose levels had not returned to baseline by 210 min (the end of each experiment), effects on the overall glycaemic (or insulinaemic) response could not be determined; this represents a priority for future studies.

The energy content of a meal is a major determinant of its rate of gastric emptying. The study reported in chapter 6 demonstrated that the substitution of an artificial sweetener ("diet" mixer) for sucrose ("regular" mixer) in a mixed alcoholic beverage has a major impact on the rate of gastric emptying and alcohol absorption in healthy adults. - A low calorie alcohol-containing drink (made with "diet" mixer) emptied from the stomach much more rapidly and resulted in higher blood alcohol concentrations when compared with a relatively high calorie alcoholic drink (made with "regular" mixer). These observations highlight the need for community awareness of factors, other than the alcohol content of a beverage, which should be taken into account in considering safe levels of consumption and the potential for inebriation.

Upper gastrointestinal motor function and incretin hormone (GLP-1 and GIP) secretion are known to be major determinants of postprandial glycaemia and

insulinaemia, however, the impact of small intestinal flow events on glucose absorption and incretin release has not been evaluated. In the study reported in chapter 7, intraduodenal pressures and impedance signals were recorded simultaneously in healthy humans, while glucose was infused into the duodenum in the presence and absence of the anticholinergic drug, hyoscine butylbromide. The frequency of duodenal flow events (evaluated by impedance) was suppressed by hyoscine much more than that of duodenal pressure waves, or propagated pressure wave sequences (evaluated by manometry). Blood glucose and plasma 3-OMG concentrations (the latter provide an index of glucose absorption) were lower during hyoscine than saline. Plasma insulin, GLP-1, and GIP concentrations were initially lower during hyoscine. The disparity between impedance measurements and manometry in detecting alterations in flow during hyoscine infusion was marked and, accordingly, supports the potential utility of small intestinal impedance monitoring to evaluate alterations in gastrointestinal transit in various disease states. The observations also indicate that the frequency of small intestinal flow events is a determinant of both glucose absorption and incretin release.

Intraduodenal administration of the local anaesthetic, benzocaine, has been shown to attenuate the release of cholecystokinin (CCK) by small intestinal lipid, and the perceptions of fullness, discomfort, and nausea induced by gastric distension during small intestinal lipid infusion, implying that local neural mechanisms may regulate CCK release in response to intraduodenal nutrients. In chapter 8, the effects of intraduodenal administration of benzocaine on: (i) blood glucose, incretin hormone and insulin concentrations (ii) antropyloroduodenal motility, and (iii) gut

sensations and appetite, in response to an intraduodenal glucose infusion, were evaluated in healthy subjects. Benzocaine attenuated the perceptions of abdominal bloating and nausea, but had no effect on antro-pyloro duodenal motility, blood glucose concentrations, or incretin responses. These observations indicate that the induction of sensations by small intestinal glucose is mediated by local neural pathways.

GLP-1 is released from L-cells whose density is greatest in the distal jejunum and ileum, GIP predominantly from duodenal K cells, and cholecystokinin (CCK) from I cells, which appear confined to the duodenum and jejunum. The study reported in chapter 9 evaluated the effects of infusion of glucose into different gut regions (mid-jejunal vs duodenal) on incretin hormones, CCK, appetite and energy intake in healthy subjects. There was no difference in the incretin responses between infusion at the two sites (85 cm apart), however the stimulation of CCK and suppression of hunger and energy intake, were greater with the duodenal compared to the jejunal infusion. These observations indicate that the site of small intestinal glucose exposure is a determinant of CCK release and appetite.

Both glucose and fat are known to be potent stimuli for incretin secretion, but the effect of protein is uncertain. Protein may also stimulate insulin secretion directly via absorption of amino acids. In the study reported in chapter 10, gastric emptying, and the blood glucose, insulin and incretin responses, alter a 300 mL drink containing 50 g glucose, 25 g protein, or both 50 g glucose and 25 g protein, were evaluated in healthy subjects. This study established that the addition of

protein to an oral glucose load improved the glycaemic response, predominantly by slowing gastric emptying. However, protein also stimulated incretin and insulin secretion. These observations have implications for the use of protein in the dietary management of type 2 diabetes.

The relationship between glycaemia, incretin hormones, appetite suppression and modulation of antropyloroduodenal motility with duodenal glucose delivery is poorly defined. In chapter 11, the effects of intraduodenal glucose infusions at different caloric rates (of 1 kcal/min, 2 kcal/min and 4 kcal/min, or control (saline)) on antropyloroduodenal motility, plasma GLP-1, GIP and CCK, appetite and energy intake have been evaluated in healthy subjects. While there was a rise in blood glucose in response to all the intraduodenal glucose loads, there was no significant difference in the response to infusions at 2 kcal/min and 4 kcal/min. An initial, transient, small rise in GLP-1 was evident, in response to all glucose loads, but a sustained and progressive rise only occurred with the 4 kcal/min infusion. In contrast, a load-dependent stimulation of GIP occurred in response to all glucose infusions. The stimulation of CCK was much greater in response to the 4 kcal/min infusion. While antral pressures were suppressed by all rates of glucose infusion, the stimulation of basal pyloric pressure was load-dependent. Energy intake was suppressed only by the 4 kcal/min infusion. This may potentially reflect the substantially greater stimulation of CCK, consistent with the observations reported in chapter 9. This study establishes that there is a substantial discordance in the acute effects of small intestinal glucose on glycaemia, incretin hormones, CCK, motility and appetite. It is planned to perform measurements of plasma insulin on

the stored samples - these results were, unfortunately, not available at the time of the submission of this thesis and are critical to the overall interpretation of the data.

STATEMENT OF ORIGINALITY

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

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Reawika Chaikomin

December 2006

DEDICATION

To my mother, my father, my grandmother, my father - in - law, my mother - in - law, my sisters, and my husband. - Without their support and ongoing encouragement this would not have been undertaken successfully.

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Completion of this thesis would not have been possible without the support and encouragement of many people. I am sincerely grateful to my supervisors, Professor Michael Horowitz, Dr Karen Jones, and Dr Chris Rayner for their sustained encouragement, patience and intellectual inspiration. All of them undertook the painstaking task of reading this thesis carefully, to minimise the number of errors in my English grammar and spelling, and their contributions to all aspects of my research programme and this thesis have been invaluable. As a result of their guidance, I have learned much about the process of research, as well as the importance of critical thinking and this has also been associated with personal development in many areas.

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This acknowledgement would be incomplete if I omitted to mention some of the numerous experiences (both positive and negative) that occurred during the 3 years and 6 months that I was a PhD student in Adelaide. I knew that the challenges associated with the decision to pursue a PhD and come to Adelaide would be substantial, particularly as I had been married for only 8 months. I was aware that to come to a new country, to interact with new people and in a new environment, successfully, without the support of my family and with relatively poor English skills, would be very difficult. I soon realised that this task would be much harder than I had imagined, but not impossible to achieve if I worked diligently. While a PhD student, I have been treated warmly and supported by many people. There have been unique professional opportunities, including an oral presentation during the 2005 Australian Gastroenterology Week meeting in Brisbane, which was

exciting and terrifying at the same time! It proved very difficult and stressful for me to obtain a visa to visit the USA, but in the end I was successful and was able to present some of my research during the Digestive Diseases Week meeting in Los Angeles and the American Motility Society meeting in Boston (both in 2006). It was very interesting to visit large American cities, but I would not want to live in them! - I had a delicious lobster, and my first clam chowder, in Boston which were very nice, but not as good as the big fish (bream) I caught by myself fishing in a river on Kangaroo Island. While in Adelaide, I also had the chance to go horse riding with my friend Alena, attend the opera (3 times!) and go to a number of classical music concerts with Michael and his family. I was surprised that I enjoyed the music so much, as if was very different to what I knew. I also went to the gym and played squash regularly with my friends, Yan and Niva. - So my life was never boring!

I plan to apply the knowledge and experience that I have gained as a PhD student, to develop my academic and personal skills further. My English has improved a lot (I now don't have difficulty in understanding the majority of jokes in English, which is a good test!) and I have promised myself to make it improve further. I am still very worried about giving lectures in English, but I also know that I can do it. I hope that Adelaide is a place that I can come back again …. and again.

As the American baseball coach/comedian, Yogi Berra, said: "Prediction is very hard, especially when it is about the future".

PUBLICATIONS ARISING FROM THIS THESIS

The material in this thesis formed the basis for the publications list below:

Chaikomin R, Doran S, Jones KL, Feinle-Bisset C, O'Donovan D, Rayner CK, Horowitz M. Initially more rapid small intestinal glucose delivery increases plasma insulin, GIP, and GLP-1 but does not improve overall glycemia in healthy subjects. *Am J Physiol Endocrinol. Metab*. 2005 Sep; 289(3): E504-7.

Gentilcore D, Chaikomin R, Jones KL, Russo A, Feinle-Bisset C, Wishart JM, Rayner CK, Horowitz M. Effects of fat on gastric emptying of and the glycemic, insulin, and incretin responses to a carbohydrate meal in type 2 diabetes. *J Clin Endocrinol Metab.* 2006 Jun; 91(6): 2062-7.

Wu KL, Chaikomin R, Doran S, Jones KL, Horowitz M, Rayner CK. Artificially sweetened versus regular mixers increase gastric emptying and alcohol absorption. *Am J Med*.2006 Sep; 119(9): 802-4.

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events, glucose absorption, and incretin hormone release. *Am J Physiol Gastrointest Liver Physiol*. 2007 Jan 4; [Epub ahead of print]

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Gastrointestinal motility

and glycaemic control in diabetes

CHAPTER 1:

GASTRODUODENAL MOTILITY IN HEALTH AND DIABETES - IMPACT ON APPETITE

1.1 INTRODUCTION

This chapter reviews current knowledge of gastroduodenal motor and sensory function in health and diabetes, and their relevance to the regulation of energy intake.

1.2 GASTRODUODENAL MOTILITY-PATTERNS, REGULATION AND MEASUREMENT

1.2.1 Patterns of gastroduodenal motility

The proximal region of the stomach is primarily concerned with storage of ingested food. During swallowing, there is a vagally-mediated, transient "receptive" relaxation, which is followed by a more prolonged relaxation, known as "accommodation". The contractions of the distal stomach are controlled by electrical signals ("slow waves") generated by a pacemaker region located on the greater curvature (Figure 1.1), which discharges at a rate of about 3/minute (Okuno et al. 1989; Collard and Romagnoli 2001). The generation of slow waves is dependent on the so-called interstitial cells of Cajal (Der-Silaphet et al. 1998), which are required for effective neurotransmission.

The proximal stomach generates tonic pressure which may facilitate emptying of liquids and 'liquefied' solids; the distal stomach grinds and sieves digestible solids and pumps chyme across the pylorus predominantly in a pulsatile manner, while phasic and tonic pyloric pressures, and duodenal contractile activity, act as a brake to gastric outflow. The timing of antral contractions is controlled by an electrical slow wave, which has frequency of about 3 per minute (Sanders 1996). During fasting, gastric motility is cyclical, with a periodicity of about 90 min, characterised by irregular contractions of increasing frequency (phase II), and a brief (5 - 10 min) period of regular contractions at a rate of ~3 /min (phase III) during which indigestible solids are emptied from the stomach, followed by motor quiescence (phase I) (40 - 60 min). As with the stomach, fasting small intestinal motility is also cyclical, and characterised by phases I to III, the latter occurring at a frequency of 9 to 12 /min (Figure 1.2). This so-called "migrating motor complex" (MMC) propagates aborally along the small intestine, and serves to "sweep" the lumen of indigestible debris. Meal ingestion disrupts the MMC, and causes distinct changes in gastric motility, characterized by an initial relaxation and a subsequent increase in tonic activity of the proximal stomach, irregular contractile activity in the antrum and an increase in tonic and phasic pyloric pressures. Antral contractions play the major role in grinding digestible solid food into small particles, generally < 2 mm in size; ingestion of a solid meal induces strong antral contractions. Phasic and tonic pyloric contractions play a major role in the regulation of gastric emptying (Horowitz et al. 1994) while duodenal contractions may potentially facilitate, or retard gastric emptying (Andrews et al. 2001).

There are complex interactions among the different segments of the gastrointestinal tract that are in contact with food. The delivery of unabsorbed nutrients to the distal small intestine activates the so-called “ileal brake”, whereby gastric and jejunal motility, gastric acid and pancreatic secretion are inhibited. These effects are mediated through hormonal and neural mechanisms (Giralt and Vergara 1999; van Dijk and Thiele 1999).

1.2.2 Neural regulation

Digestive functions, including gastric motility, are controlled by neural networks that are located in the wall of the gastrointestinal tract, prevertebral ganglia, spinal cord and brain, which are fundamental to the integration of the functions of the proximal and distal stomach, the pylorus and the duodenum (Hasler et al. 1995).

Gastric pacemaker

As discussed, the electrical stimulus or gastric (as well as small intestinal) smooth muscle contractile activity is provided by cells identified as a subtype of interstitial cells of Cajal (Faussone-Pellegrini 1992). The latter generate so called “slow waves”, or pacemaker potentials, and are located in the myenteric plexus (i.e. between the longitudinal and circular muscle layers) in the upper body (Sanders 1996). Slow waves are continuous rhythmic changes in the membrane potential, occurring in the human stomach at a frequency of about 3 /min, which *per se* do not initiate contractions. The latter are generated by action potentials (as a result of rapid membrane depolarisation), superimposed on the partial depolarisation of the slow wave (Hasler et al. 1995). Accordingly, phasic contractions in the antrum and

pylorus are always associated temporarily with the rhythm and propagation of the gastric pacemaker potential and the maximum contraction frequency is about 3 /min in the stomach. Both neural and hormonal inputs modulate the duration and the amplitude of the action potential, i.e. increase it above or maintain it below the threshold required for the triggering of a contraction.

Intrinsic innervation

The enteric nervous system (ENS), also termed the 'brain of the gut' since it has the capacity to function without input from the central nervous system, is located within the walls of the stomach and small intestine (Goyal and Hirano 1996). It consists of two systems of nerve plexi-the myenteric (or Auerbach) plexus, which is located between the circular and longitudinal muscles, and the submucosal (or Meissner) plexus, located beneath the mucosa (Goyal and Hirano 1996). Within the ENS, there are three major types of neurons. - Sensory neurons contain specialised receptors that detect changes in the environment (tension, contraction, chemical stimuli), which are then transmitted along sensory nerve fibres to other parts of the nervous system. Interneurones form networks that process sensory information and control the behaviour of motor neurones, which are the final common pathway for the transmission of signals to the effector systems (Goyal and Hirano 1996). Both excitatory (i.e. stimulating muscle contraction) and inhibitory (i.e. suppressing muscle contraction/stimulating muscle contraction) exist. The major excitatory transmitters are acetylcholine and substance P, while the major inhibitory neurotransmitters are vasoactive intestinal peptide (VIP) and nitric oxide (NO) (Schemann and Schaaf 1995). The enteric nervous system, thus, comprises local

circuitries for the performance of integrative function independent of extrinsic innervation.

Extrinsic innervation

The stomach, like other parts of the gastrointestinal tract, is innervated by extrinsic nerves-the sympathetic and parasympathetic parts of the autonomic nervous system and sensory nerves that project to the spinal cord (splanchnic and sacral afferents) and to the brain stem (vagal afferents). Gastric motility is controlled predominantly by the vagus nerve. Vagal afferents project to the nucleus tractus solitarius (NTS), where they form synapses with interneurones that project to the dorsal motor nucleus of the vagus (DMNV) and to higher brain centres (Sawchenko 1983). From the DMNV, efferent projections return to the stomach, modulating the activity of the muscle cells ('effector system') through activation of either inhibitory or excitatory motor neurons (Bligny et al. 2005). This circuit is termed a 'vago-vagal reflex'. Vago-vagal reflexes are also modulated by input from other brain regions, such as the forebrain and the area postrema (Sawchenko 1983).

1.2.3 Measurement of gastroduodenal motility

Several techniques may be used to assess gastroduodenal motor function in humans and these can be broadly divided into four categories: (i) measurement of gastric wall motion (ii) measurement of gastric relaxation (iii) measurement of intraluminal pressures or contractions; and (iv) measurement of gastric myoelectrical activity. In research studies a number of techniques are frequently used concurrently.

Measurement of gastric wall motion

Techniques which may be used to assess gastric wall motion include scintigraphy (discussed in 3.6.2), ultrasonograhy (discussed in 3.6.2) and magnetic resonance imaging (MRI). While MRI has been used to quantify contractile activity in different regions of the stomach (Marciani et al. 2001; de Zwart et al. 2006), due to its limited accessibility and the high costs involved, it is unlikely that MRI will be used clinically for this purpose in the foreseeable future.

Measurement of gastric relaxation

Until the advent of the barostat (Azpiroz and Malagelada 1985; Whitehead and Delvaux 1997), no technique was available to quantify relaxation of the proximal stomach. The barostat consists of a pressure transducer linked by an electronic relay to an air injection system. An infinitely compliant bag, positioned in the proximal stomach, is connected via a double-lumen tube to the barostat. Once a pressure is set in the system, frequently 2 mmHg above basal intragastric pressure, the barostat is capable of indirectly measuring gastric relaxation by monitoring changes in intragastric bag volume at a set pressure. Thus, when the stomach relaxes, air is injected into the gastric bag to maintain the pressure, while air is withdrawn when the stomach contracts. The barostat has significant limitations - it is uncomfortable for the subject and the presence of the air-filled bag in the proximal stomach disturbs normal physiology; i.e. the presence of the bag affects gastric emptying, intragastric meal distribution and antral motility (Mundt et al. 2002; Mundt et al. 2005). More recently, other techniques, including single photon emission computed tomography (SPECT) imaging (De Schepper et al. 2004;

Simonian et al. 2004), 3D ultrasound (Berstad et al. 1994; Hausken et al. 2001; Mundt and Samsom 2006) and MRI (Fraser et al. 1994; Pecchi et al. 2005; de Zwart et al. 2006) have been used to quantify proximal gastric motility in research studies. All of these techniques may potentially be used to evaluate the relaxation of the distal stomach-attempts have been made to use the basostat for this purpose (Ladabaum et al. 1998), but positioning of a barostat balloon in the distal stomach poses substantial logistical difficulties.

Measurement of intraluminal pressures or contractions

Intraluminal pressures or contractions can be measured by manometry (discussed in 3.6.1).

Measurement of gastric myoelectrical activity

Gastric electrical activity can be measured using surface electrodes attached to the skin, providing insight into the function of the gastric pacemaker (Smout et al. 1980). As discussed (1.2.2), the pacemaker generates the slow wave (electrical control activity), which in turn determines the frequency of contractions of the stomach musculature distal to it. Electrogastrography is only capable of measuring the electrical control activity and not the actual occurrence of contractions. Currently its application remains limited to the research sphere.

1.3 GASTRIC EMPTYING - PATTERNS, REGULATION AND MEASUREMENT

1.3.1 Patterns of gastric emptying

Patterns of gastric emptying are dependent on several characteristics of the ingested material, particularly nutritive and physical properties, so that solids, nutrient-liquids and non-nutrient liquids empty from the stomach at different rates (Malbert and Ruckebusch 1988; Horowitz and Dent 1991).

Liquids

Gastric emptying of liquids is critically dependent on volume ingested, as well as the osmolarity and nutrient content. Non-nutrient and low-nutrient liquids empty relatively rapidly from the stomach with an overall mono-exponential pattern i.e. the volume of liquid that enters the duodenum in a given time is approximately proportional to the volume remaining in the stomach (Hunt and Spurrell 1951) (Figure 1.2). Consequently, the rate of emptying is influenced by intragastric volume and posture. In contrast, high-nutrient containing liquids are retained in the distal stomach for longer periods and empty more slowly as a result of feedback from small intestinal receptors (Lin et al. 1989). Posture and intragastric volume appear to have minimal influence in the latter case. Gastric emptying of a nutrient liquid consists of an initial phase, during which emptying may be relatively faster, followed by a linear emptying phase when 2-3 kcal per minute are delivered into duodenum, essentially irrespective of the source of those calories (Elashoff et al. 1982; Horowitz et al. 1993a). In addition, to caloric density, characteristics of the

nutrient itself may influence the rate of emptying e.g. fructose is a less potent inhibitor of gastric emptying than glucose (Moran and McHugh 1981).

When evaluated a second-by-second basis emptying of the stomach content is predominantly a palsatile, rather than continuous, phenomenon - both antegrade and retrograde flow occur frequently and there is substantial variation in the characteristics of individual flow pulses (Malbert and Mathis 1994; Hausken et al. 1998). There is little information about the effect of variations in small intestinal flow on gastric and proximal small intestinal motility (this has been evaluated in the study reported in chapter 7).

Solids

The emptying of digestible solids from the stomach proceeds at a much slower pace than that of nutrient and non-nutrient liquids and is characterised by an initial "lag phase" during which little or no emptying occurs (Collins et al. 1983). The lag phase, which is usually 20 - 60 min in duration, is accounted for by an initial retention of the solid in the proximal stomach, followed by redistribution to the antrum (Figure 1.3). If the viscosity of the meal is increased sufficiently, the ability of the stomach to discriminate between large and small particles is impaired so that larger particles may be delivered into the duodenum (Russell and Bass 1985). Larger (> 3mm) indigestible solids were thought not to empty from the stomach until the return of migrating migratory complex, however, it is now clear that they may empty much earlier than this (Stotzer and Abrahamsson 2000). The amount of nutrient liquid consumed with the solid affects the rate of solid emptying (Heddle

et al. 1989a; Urbain et al. 1989). In a mixed meal, comprising solids and liquids, up to 80% of the liquid phase empties before the solid (Horowitz et al. 1989b), indicating that the stomach is capable of discriminating between solids and liquids.

Fat

Foods high in fat are handled differently by the stomach and can, therefore, be considered separately from liquids and solids. Fat represents a challenge as it is liquid at room temperature and coalesces to form large globules in the stomach. In the erect posture fat may float on other gastric contents because of its low density and be 'retained' in the uppermost part of the stomach (Edelbroek et al. 1992c; Horowitz et al. 1993b). Because of its high nutrient density of fat, it can markedly slow emptying of other meal components (Meyer et al. 1986). The rate of emptying of fats, as for digestible solids and aqueous nutrients liquids, is regulated by feedback from the small intestine triggered by fatty acids (Meyer et al. 1994b; Feinle et al. 2003a). Because of the requirement for digestion and the potential for 'layering' of fat on top of other, more dense, meal components it is likely that the slowing of gastric emptying induced by fat will be greater when it is ingested before, rather than with, a meal. This is addressed in the study reported in chapter 5.

1.3.2 Regulation of gastric emptying

Overall patterns of gastric emptying are critically dependent on the physical and chemical composition of a meal, so that there are substantial differences between solids, semi-solids, nutrient-liquids and non-nutrient liquids (Horowitz et al.

1989b). Gastric emptying is also influenced by both posture and intragastric volume. The major factor regulating gastric emptying of nutrients is, however, feedback inhibition triggered by receptors which are distributed throughout the small intestine (Lin et al. 1989). As a result of this inhibition, nutrient-containing liquids usually empty from the stomach at an overall rate of about 2 kcal per minute, after an initial emptying phase that may be somewhat faster (Horowitz et al. 1993b). Caeco-ileal reflux of short-chain fatty acids may also potentially contribute to the regulation of gastric emptying (Cuche and Malbert 1999). There is evidence that artificial sweeteners, such as aspartame, do not stimulate small intestinal feedback. Hence, it is likely that artificially sweetened drinks will empty relatively more rapidly from the stomach (chapter 6). Animal studies indicate that the extent of small intestinal feedback is dependent on both the length, and region, of small intestine which has been exposed (Lin et al. 1989) but this has not been evaluated in humans (chapter 9). While a number of studies have evaluated the motor correlates of small intestinal feedback (Heddle et al. 1988a; Heddle et al. 1989a; Rayner et al. 2001), there is relatively little information about the relationship between the small intestinal nutrient load and gastropyloroduodenal motility (Heddle et al. 1993) (chapter 11). The interaction of nutrients with the small intestine triggers the release of a number of hormones which may slow gastric emptying, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), amylin and peptide Y-Y (PYY). These hormones are discussed in some depth substantially, particularly in relation to their effects on energy intake (1.4.2). The studies reported in chapters 4, 7, 8, 9 and 11 were designed to provide insights into

the relationship between gut hormone release with both the small intestinal nutrient load and the region of small intestine stimulated.

1.3.3 Measurement of gastric emptying

Scintigraphy (discussed in 3.6.2)

Ultrasonography (discussed in 3.6.2)

Radioisotopic breath tests (discussed in 3.6.2)

Radiological measurement

Contrast studies with liquid barium sulphate have limited usefulness in the assessment of gastric emptying because of their non-physiological nature, high radiation exposure and inability to measure fractional stomach emptying. An abdominal x-ray taken 6 hr after ingestion of the radio-opaque markers has been reported as a sensitive technique for assessing gastric emptying of non-digestible solids (Feldman et al. 1984). However, this method probably only assesses whether phase III of the interdigestive myoelectrical complex is present in the stomach.

Electrical impedance

Measurement of electrical impedance can be used to quantify the volume of liquid in the stomach. A large volume drink is consumed and abdominal impedance recorded at regular intervals. The values obtained over time represent the emptying

of the liquid. The technique is non-invasive and does not employ radiation. Gastric emptying of solid meals, however cannot be evaluated reliably. Impedance techniques are not suitable for measuring rapid gastric emptying, as may occur after gastric resection (Miholic et al. 1991).

Magnetic resonance imaging

MRI allows detailed evaluation of gastric emptying (both total and regional) and intragastric distribution (Feinle et al. 1999). However as discussed, its role in clinical practice is limited as a result of its high cost and lack of accessibility.

Absorption kinetics of orally administered drugs

In humans, there is minimal gastric absorption of many orally administered drugs, hence, drug absorption is a measure of the rate of gastric emptying (Heading et al. 1973). This has been well established to be the case for both paracetamol (Heading et al. 1973; Nimmo et al. 1973; van Wyk et al. 1990; Cavallo-Perin et al. 1991) and alcohol (Holt 1981; Horowitz et al. 1989b). Hence, when gastric emptying is relatively slower peak blood alcohol concentrations are less (Horowitz et al. 1989b). In the study reported in chapter 6 examines the effects of artificial sweetening on the blood alcohol response to a drink. Estimation of the rate of gastric emptying by measurement of blood concentrations of intestinally absorbed solutes, such as paracetamol or alcohol, is, however, unsatisfactory for most circumstances where there is a need to measure gastric emptying precisely.

1.4 GASTROINTESTINAL MECHANISMS IN APPETITE REGULATION

The factors which control appetite and food intake in humans are complex; a central feeding drive is counterbalanced by the inhibitory influence of peripheral satiety signals, the latter arising predominantly from the gastrointestinal tract (Maus et al. 1988; Blundell et al. 1993; Bray 2000; Druce and Bloom 2003; Erlanson-Albertsson 2005; Beglinger and Degen 2006; Chaudhri et al. 2006). An understanding of the distinction between satiation and satiety is important in studying the factors that regulate appetite and food intake. - Satiation refers to the process that controls the size of a meal by terminating the period of eating. Satiety, on the other hand, has been defined as the inhibition of hunger and further eating as a consequence of food consumption, as reflected in the length of inter-meal interval and/or the amount of food consumed at the subsequent meal (Wardle 1987). Desire to eat, the onset of feeding and small intestinal digestion and absorption of nutrients trigger the so-called “satiety cascade”, in turn determines the onset of satiation and the duration of satiety.

1.4.1 Central control mechanisms

The central drive to eat is controlled primarily by the hypothalamus; a number of discrete nuclei are associated with the neural mechanisms that affect appetite, including the ventromedial nucleus, dorsomedial nucleus, paraventricular nucleus and lateral hypothalamus (Druce and Bloom 2003; Neary et al. 2004). Stimulation of the lateral hypothalamus results in overeating, hence the traditional terminology of the “feeding centre” while stimulation of the ventromedial hypothalamus results

in diminished food intake-the so called “satiety centre” (Neary et al. 2004). A number of neurotransmitters are involved in the central feeding drive including those which stimulate food intake; neuropeptide-Y (NPY), the agouti-related peptide (AGRP) and ghrelin (York 1999; Halford et al. 2004), and those which are inhibitory, including pro-opiomelanocortin (POMC) and cocaine-and-amphetamine-related transcript (CART) (York 1999; Halford et al. 2004). The paraventricular nucleus of the hypothalamus produces the stimulatory hormones, orexin and melanocyte concentrating hormone (MCH), and the inhibitory hormones, oxytocin and corticotrophin releasing hormone. These hormones, together with others from the periphery, converge on the brainstem to influence energy intake (Neary et al. 2004).

It has been proposed that carbohydrate metabolism is instrumental in the central regulation of food intake. The glucostatic hypothesis postulates that hunger is stimulated by changes in glucose utilisation (Mayer and Bates 1952), and more recently, in humans that transient falls in blood glucose levels trigger food intake (Melanson et al. 1999).

1.4.2 Peripheral mechanisms

The upper gastrointestinal tract, including the mouth and stomach, appear to have a major role in the short-term regulation of feeding, particularly the generation of signals which trigger satiation (Bray 2000). The interaction of nutrients with the small intestine is probably of primary importance in inducing satiety. Ingestion of a meal results in gastric distention, the interaction of nutrients with the small

intestine and the subsequent release of gastrointestinal peptides; these peripheral signals are relayed to the central feeding centres via hormonal and neural connections between the gut and brain. Animal studies indicate that the hormone leptin, which is released primarily from adipocytes, acts on the hypothalamus to inhibit neuropeptide-Y (NPY) and agouti-related peptide (AGRP) and stimulate cocaine-amphetamine-regulated transcript (CART) and melanocyte-stimulating hormone (MSH) production, plays an important role in the regulation of food intake and body weight (Rahmouni and Haynes 2001).

Gastric distension

Human studies indicate that gastric distension with intragastric volumes > 400 ml enhances sensations of fullness and satiation, while decreasing sensations of hunger and desire to eat (Geliebter 1988) as a result of the activation of mechanoreceptors within the wall of the stomach (Feinle et al. 1996). Gastric distension, per se, probably does not induce satiation, but rather an uncomfortable sensation of distension or bloating. - However, with concurrent small intestinal nutrient exposure this is converted to a “meal-like” sensation of fullness (Feinle and Read 1996). The sensations arising from gastric distension appear to be dependent on the site of distension and, by inference, intragastric meal distribution (Houghton et al. 1992). While most studies have focused on the role of the proximal stomach in inducing sensation, there is evidence that the distal stomach may play an important role. - For example, in healthy young and older subjects, the perception of postprandial fullness is directly related to, and subsequent food intake

inversely related to antral area and content (Jones et al. 1997a; Santangelo et al. 1998b; Sturm et al. 2004).

Small intestinal mechanisms

The interaction of nutrients with the small intestine is important in the regulation of appetite as well as gastric emptying. Small intestinal nutrient infusion, such as that of glucose or lipid, decreases hunger and subsequent food intake (Welch et al. 1988a; Lavin et al. 1996a). Indeed, enteral infusion of glucose suppresses appetite and subsequent energy intake much more than intravenous glucose, which results in comparable blood glucose concentrations (Lavin et al. 1996a), highlighting the importance of the interaction of nutrient with small intestinal receptors in the induction of satiety. The magnitude of the suppression of hunger appears to be related to a decline in the exposure of small intestine to nutrient stimuli (Sepple and Read 1989). The suppression of appetite by carbohydrate and fat is mediated by the products of nutrient digestion. For example, pharmacological inhibition of lipase activity with the lipase inhibitor, orlistat, attenuates the suppression of energy intake induced by intraduodenal triglyceride infusion (Feinle et al. 2001b). Animal studies suggest that the suppression of energy intake by enteral nutrients is load-dependent, as is the case for gastric emptying, as a result of a greater length of small intestine being exposed (Lavin and Read 1995). In rats, the suppression of subsequent food intake following nutrient infusions into the intestine is dependent on the length of exposure to nutrient (Meyer et al. 1998); these responses probably reflect a greater recruitment of receptors. The issue is addressed in humans the study reported in chapter 11.

Nutrients may also exhibit regional specificity in their affects on appetite; infusion of fat into the jejunum may result in a greater decrease in sensations of hunger and subsequent food intake when compared with identical infusions into terminal ileum (Welch et al. 1988a). The study reported in chapter 9 has evaluated the comparative effects of mid-jejunal compared to duodenal glucose infusion on perceptions of appetite and energy intake.

Mediators of nutrient feedback from the small intestine

Neural mechanisms

Mucosal nerve endings appear to be important in signaling the presence of nutrient in the small intestine. In pigs, topical local anaesthesia attenuates satiety induced by hyperosmolar solutions (Houpt et al. 1983). In humans, it has also been demonstrated that local anaesthesia abolishes the perception of nausea induced by intraduodenal lipid infusion (Feinle et al. 2001a). The study reported in chapter 8 has evaluated the effects of intraduodenal local anaesthesia on duodenal glucose sensing.

Hormonal mechanisms

The release of peptide hormones in response to the interaction of nutrient with the small intestine plays a major role in the regulation of appetite. Several hormones have been implicated, those with particular relevance to this thesis include cholecystokinin (CCK), glucagon like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), and insulin.

Cholecystokinin

CCK is released from the I-cells of the upper small intestine (McHugh et al. 1992). Circulating forms of CCK in humans include peptides of different amino acid lengths, CCK-33 and CCK-8 (which contains all of the biological activity) predominate (Rehfeld et al. 2003). In humans, circulating levels increase from fasting 1 ± 0.2 pmol/L to 5-10 pmol/L after a meal. Two CCK receptor subtypes have been identified; CCK-1 (previously known as CCK A) and CCK-2 (previously known as CCK B); - CCK-1 receptor are found predominantly in the periphery, including the pancreas, gallbladder, pylorus and in vagal afferent nerve fibres (Moran et al. 1987; Moriarty et al. 1997), but also in some areas of the CNS, and appears to be more important in appetite control (Asin et al. 1992). CCK-2 receptors are located mainly in the CNS including vagal afferents, the cerebral cortex, the olfactory bulb and hypothalamus (Moriarty et al. 1997).

Oral, intragastric and intraduodenal administration of fat and protein stimulate the release of CCK in mammals - glucose (and other carbohydrate) is a weaker stimulus for CCK release (Drewe et al. 1992). Studies in rats indicate that hydrolysis of triglycerides to free fatty acids is required for stimulation CCK; this also appears to be the case in humans (Matzinger et al. 2000; Feinle et al. 2003a). The chain length of fatty acids is also important; only chain lengths with greater than 12 carbon atoms stimulate CCK release (Matzinger et al. 2000; Feltrin et al. 2004a). Inhibition of chylomicron formation also blocks lipid-induced CCK release (Raybould et al. 1998). Because of the proximal site of CCK-releasing cells, it is likely that the stimulation of CCK by enteral nutrients will be region-dependent.

The study reported in chapter 9 has evaluated the effects of infusion of glucose into the duodenum and mid-jejunum on CCK release.

Central and/or peripheral administration of apparently physiological doses of CCK suppresses food intake in animals (Pappas et al. 1985). The satiating effects of CCK may be predominantly centrally mediated (Figlewicz et al. 1989). In humans, intravenous CCK (CCK 8 or CCK 33) decreases food intake (Lieverse et al. 1995b). While there is no difference in the satiating effect of CCK between lean and obese subjects (Lieverse et al. 1995a), this may be greater in healthy young adults (MacIntosh et al. 2001c). There is evidence that CCK administration may preferably reduce intake of fatty, rather than carbohydrate or protein-rich, foods (Degen et al. 2001) suggesting that the effects of CCK on food intake may be to some extent nutrient-specific. Studies employing the specific CCK antagonist, loxiglumide, have established that, at least acutely, CCK has a physiological role in the regulation of energy intake (Beglinger et al. 2001). Previous patterns of nutrient intake affect gastrointestinal function and hormone release, including that of CCK. In rats, exposure to a high-fat diet for 8 weeks resulted in increased stimulation of CCK and attenuation of the inhibition of food intake (Spannagel et al. 1996).

Glucagon-like peptide-1

GLP-1 is secreted from the L-cells of the distal small intestine and is present in the body in a number of forms, the biologically active of which is GLP-1 (7-36) (Holst 1999). GLP-1 immunoreactive nerve fibres and terminals are distributed widely throughout the brain with the highest density in the hypothalamus, thalamus, and

septal regions (Wei and Mojsov 1996b). GLP-1 receptors have also been identified in the lungs, pancreatic islets gastrointestinal tract, heart and kidney (Wei and Mojsov 1996a).

GLP-1 is released into circulation after meals containing carbohydrate, fat or protein in response to direct contact of nutrients with the gastrointestinal lumen (Holst 1999). Oral and intraduodenal nutrients stimulate a rise in GLP-1 from basal concentrations of 0.4-1.4 pmol/L to maximal postprandial concentrations of 10-12 pmol/L. The release of GLP-1 is biphasic, - it has been suggested that hormonal and neural inputs regulate the early release, while direct nutrient contact with the L-cells mediate later secretion (Schirra et al. 1996a). The relationship between GLP-1 (and GIP) release with the small intestinal nutrient load poorly defined. Schirra et al (1996) suggested that for GLP-1 a threshold of >1.8 kcal per minute needs to be exceeded to stimulate its release. However, this observation is inconsistent with recent study which reported that a duodenal glucose infusions as low as 1 kcal/min was sufficient for the stimulation of GLP-1 (O'Donovan et al. 2004b). GIP is released from duodenal K cells (Mortensen et al. 2003), there is little information regarding infusion of carbohydrate into different gut regions (duodenum/jejunum) in humans on the release of GLP-1 and GIP, and it may be expected that its release is load-dependent (O'Donovan et al. 2004b). These issues form a focus of the studies reported in chapter 4, 9 and 11.

Fat is a potent stimulant of GLP-1 release, the chain length and degree of saturation of fatty acids dictate the ability of fat to stimulate GLP-1 (Beysen et al. 2002).

Monounsaturated long-chain fatty acids (C16) are more effective than short chain or medium chain polyunsaturated or saturated fatty acids (Beysen et al. 2002). The study reported in chapter 5 examines the hypothesis that ingestion of a fat 'preload' will increase GLP-1.

GLP-1 acts centrally on the hypothalamus to reduce food intake in rats (Hwa et al. 1998); in rats intraperitoneal administration of GLP-1 does not suppresses food intake (Turton et al. 1996), in contrast, intravenous GLP-1 infusion enhances fullness and reduces energy intake in both healthy subjects and in type 2 diabetes in a dose-dependent manner (Gutzwiller et al. 1999). Exogenous administration of GLP-1 also results in slowing of gastric emptying (Holst 1999) and suppression of postprandial glucagons levels (Holst 1999), which may influence appetite.

High protein diets have generated increasing interest, particularly because of their potential impact on energy intake and body weight (Brinkworth et al. 2004; Simpson and Raubenheimer 2005). Protein may also stimulate insulin secretion directly via amino acids (Gannon et al. 1988). The effect of protein on GLP-1 and GIP release is controversial (chapter 10).

Insulin

The role of insulin in the regulation of appetite remains controversial. In animals, administration of intravenous glucose has been reported to decrease food intake (as well as induce hyperglycaemia and hyperinsulinaemia). Chronic central administration of insulin in the rat (Brief and Davis 1984) and peripheral

administration in the baboon may suppress food intake. However, recent observations that hyperinsulinaemia, of a level similar to that following a meal does not influence food intake in healthy volunteers when the blood glucose concentration is maintained in the euglycaemic range, argue against a significant role for insulin in the short-term regulation of appetite in humans (Chapman et al. 1998).

1.5 GASTRODUODENAL MOTILITY IN DIABETES MELLITUS

It has been established that gastric emptying is delayed in a substantial number of patients with type 1 or type 2 diabetes; this has substantial implications for an understanding of the etiology of gastrointestinal symptoms and glycaemic control in diabetes.

1.5.1 Prevalence

Cross-sectional studies, in most cases using radionuclide techniques to measure gastric emptying, have established that gastric emptying of solid, or nutrient liquid, meals is abnormally slow in some 30-50% of outpatients with longstanding type 1 (Meier et al. 2002b) or type 2 (Horowitz et al. 1989a; Annese et al. 1999; Samsom et al. 2003) diabetes. Early studies, using insensitive barium contrast techniques to quantify gastric emptying, clearly underestimated the prevalence substantially (Rundles 1950; Zitomer et al. 1968). The reported prevalence of delayed gastric emptying is highest when gastric emptying of both solid and nutrient-containing liquids (or semi-solids) is measured, either simultaneously, or on separate occasions (Horowitz et al. 1991a; Weytjens et al. 1998; De Block et al. 2002), as

there is a relatively poor correlation between gastric emptying of solids and liquids in diabetes (Wright et al. 1985; Horowitz et al. 1991b). In many cases, the magnitude of the delay in gastric emptying of solids or liquids is relatively modest. It is self-evident that the diagnosis of "gastroparesis" is critically dependent on the definition of the "normal range" for which there is a lack of consistency between studies, e.g. mean ± 2 SD (Horowitz et al 1991, Jones et al 1995), or mean ± 1.5 SD - in this thesis the term "gastroparesis" refers to emptying rates which are ≥ mean ± 2SD (Stacher et al. 1999).

The prevalence of delayed gastric emptying in patients with "brittle" type 1 diabetes is probably comparable to that which exists in patients with longstanding type 1 or type 2 diabetes (Lyrenas et al. 1997). It is now recognised that delayed gastric emptying also occurs frequently (perhaps in about 30%) in children and adolescents with type 1 diabetes (Reid et al 1992, Vaisman et al 1999). In contrast to some animal models of diabetes (Young et al. 1995; Green et al. 1997), gastric emptying is accelerated in only a minority (~5%) of patients with type 1 diabetes (Werth et al. 1992; Nowak et al. 1995; Lipp et al. 1997). There is evidence, albeit inconsistent (Jones et al. 1996a), that gastric emptying in patients with "early" type 2 diabetes, particularly that of nutrient liquids, is not infrequently abnormally rapid (Frank et al. 1995; Bertin et al. 2001); it has been suggested that this may predispose to the development of type 2 diabetes by leading to higher postprandial blood glucose concentrations (Bertin et al. 2001). No studies have evaluated the

prevalence of disordered gastric emptying in patients with recently diagnosed type 1 diabetes, nor in older people with type 2 diabetes.

Although it is recognised that disordered gastroduodenal contractile activity, as assessed by manometry, occurs frequently in diabetes (Kim et al 1991, Samsom 1996), there have been no population-based studies. It is, however, reasonable to assume that the prevalence of abnormal motility will be even higher than that of disordered gastric emptying - in a series of 84 type 1 and type 2 patients referred for evaluation of upper gastrointestinal symptoms (such as nausea, vomiting, and abdominal bloating), abnormal antral motility was evident in 83%. Diabetic gastroparesis is often associated with motor dysfunction in other areas of the gut e.g. oesophageal transit is delayed in some 50% of patients with longstanding diabetes (Horowitz et al. 1996c). However, there is a relatively poor relationship between transit in different regions (Iber et al. 1993) - for example, measurement of oesophageal transit cannot be used to predict the rate of gastric emptying.

1.5.2 Pathophysiology

The pathogenesis of disordered gastric emptying in diabetes is now recognised to be multifactorial factors which appear to be dominant are autonomic neuropathy and glycaemic control.

Autonomic neuropathy

In animal models of diabetes, a number of morphologic changes are evident in the autonomic nerves supplying the gut and the myenteric plexus, including a

reduction in the number of myelinated axons in the vagosympathetic trunk and neurons in the dorsal root ganglia, abnormalities in neurotransmitters, as well as a reduced number of interstitial cells of Cajal in the fundus and antrum (Belai et al. 1996; Ordog et al. 2000). In contrast, there is hitherto little evidence of a fixed pathological process in neural tissue of humans with diabetes. In a case report of a type 1 patient with gastroparesis, there was a marked decrease in the number of interstitial cells of Cajal in a jejunal biopsy taken at the time of performing a jejunostomy (He et al. 2001) - a reduction in interstitial cells of Cajal has also been evident in subsequent studies (Forster et al. 2005). Nitric oxide (NO) is a key transmitter in the regulation of gastrointestinal motor function (Russo et al. 1999). In some rodent models of diabetes, there is a marked reduction in NO-synthase expression in gastric myenteric neurons (Takahashi et al. 1997) which is associated with slow gastric emptying. The latter is normalised by administration of insulin or the cGMP-specific phosphodiesterase, sildenafil, which acts as an NO donor (Watkins et al 2000). It has been suggested that this has implications for the treatment of diabetic gastroparesis in humans (Watkins et al 2000).

Blood glucose concentration

Acute changes in the blood glucose concentration have a substantial, and reversible, effect on gastric motility, in both healthy subjects and patients with diabetes (Samsom et al. 1997; Rayner et al. 2001). Marked hyperglycaemia (blood glucose concentration ~15 mmol/L) affects motility in every region of the gastrointestinal tract (Rayner et al. 2001).

In both type 1 patients and healthy subjects, acute hyperglycaemia (blood glucose 16-20 mmol/L) slows gastric emptying of both solids and nutrient-liquids significantly, when compared to euglycaemia (blood glucose 5-8 mmol/L) (Fraser et al. 1990; Oster-Jorgensen et al. 1990). Cross-sectional studies suggest that hyperglycaemia also slows gastric emptying in type 2 patients. In healthy subjects (Schvarcz et al. 1995) and patients with type 1 diabetes with and without autonomic neuropathy (Schvarcz et al. 1995; Russo et al. 2005), gastric emptying is accelerated markedly during hypoglycaemia (blood glucose ~2.5 mmol/L). This is likely to be an important mechanism in the counterregulation of hypoglycaemia.

There is relatively little information about potential mechanisms mediating the effects of the blood glucose concentration on gut motor function. Animal studies have demonstrated the presence of glucose-responsive neurons in the central nervous system, which may modify vagal efferent activity (Mizuno and Oomura 1984; Song et al. 2001). The concept that the inhibitory effect of hyperglycaemia on gastric emptying is mediated in part by impaired vagal activity is also supported by animal studies (Shigushi et al 2002). Neurons responsive to glucose have also been identified in the rat small intestine (Liu et al. 1999) and, presumably, also exist in humans. Prostaglandins may be involved in the induction of abnormal gastric electrical rhythms by hyperglycaemia (Hasler et al. 1995).

Motor dysfunctions

Proximal gastric function is abnormal in many patients, with impairment of gastric relaxation induced by a meal (Samsom et al. 1995; Undeland et al. 1996; Undeland

et al. 1997; Samsom et al. 1998a). Fasting (reduced phase III activity), postprandial antral hypomotility (Camilleri and Malagelada 1984a; Samsom et al. 1996), and abnormal proximal small intestinal motor function (Nguyen et al. 1997; Samsom and Smout 1997) occur frequently in patients with diabetes.

Reports of an increased prevalence of gastric arrhythmias, particularly tachygastria, as assessed by cutaneous electrogastrography (Abell et al. 1991; Kawagishi et al. 1997; Qi et al. 2002), may be indicative of an effect of acute hyperglycaemia (Jebbink et al. 1994; Hasler et al. 1995; Kawagishi et al. 1997). In both healthy subjects and type 1 patients, acute hyperglycaemia (blood glucose ~15 mmol/L) induces a motor pattern associated with retardation of gastric emptying. The reduced number of propagated antral pressure waves during hyperglycaemia is associated with less frequent episodes of retrograde pyloric flow (Kawagishi et al. 1994).

1.5.3 Clinical significance

Upper gastrointestinal symptoms are the most widely recognised complication of disordered gastric motility in diabetes. It is, however, now clear that there may be a number of other effects.

Gastrointestinal symptoms

There is a high prevalence of upper gastrointestinal symptoms in both type 1 (Schvarcz et al. 1996) and type 2 (Bytzer et al. 2001) diabetes and that these effect quality of life adversely.

A recent longitudinal study in predominantly type 2 patients indicates that there is a significant fluctuation in both upper and lower gastrointestinal symptoms over a 3 year period with a relatively constant prevalence (Talley et al. 2002). In a study of outpatients with longstanding type 1 diabetes, Schvarcz et al (1996) reported that the prevalence of postprandial fullness was 19%, compared to 8.5% in control subjects. In a study from Australia, which focused on type 2 diabetes, all upper and lower gastrointestinal symptoms evaluated were more common in community-dwelling people with diabetes than controls (Bytzer et al. 2001). As a group there is only a relatively weak correlation between gastrointestinal symptoms and the rate of gastric emptying in diabetes (Horowitz et al. 1991a). The perception of fullness/abdominal bloating (but not nausea or vomiting) is predictive of delay in solid emptying, but not strongly (Jones et al. 2001b).

Acute changes in the blood glucose concentration also affect the perception of sensations arising from the stomach and duodenum, although this issue has been studied less comprehensively than the effect of hyperglycaemia on gastric motility (Rayner et al. 2001). - For example, in patients with type 1 and type 2 diabetes, the perception of postprandial fullness is related to the blood glucose concentration (Jones et al. 1997b; Rayner et al. 2001).

Impaired oral drug absorption

Gastric emptying is potentially an important determinant of oral drug absorption (Hebbard et al. 1995). Delayed gastric emptying (particularly that of tablets or capsules which are not degraded easily in the stomach) and a reduction in phase 3

activity, may potentially lead to fluctuations in the serum concentrations of orally administered drugs. There is relatively little information about drug absorption in patients with diabetic gastroparesis (Hebbard et al. 1995) and additional studies are required.

Impaired glycaemic control (discussed in chapter 2)

Postprandial hypotension

Postprandial hypotension, defined as a fall in systolic blood pressure of greater than, or equal to, 20 mmHg within 2 hour of a meal, is an important clinical problem (Puisieux 2003). Postprandial hypotension occurs more frequently than orthostatic hypotension and is particularly common in older type 2 patients (O'Donovan et al. 2002). Recent studies suggest that the rate of gastric emptying is a significant factor in postprandial hypotension. - In type 2 patients the magnitude of the fall in blood pressure after a glucose drink is related to the rate of gastric emptying (Jones et al. 1998). Moreover, slowing of gastric emptying (and the rate of small intestinal glucose absorption), by the addition of guar gum to the glucose attenuates the fall in blood pressure (Jones et al. 2001a). These observations suggest that therapies which slow carbohydrate absorption may be effective in the treatment of postprandial hypotension in diabetes and that the magnitude of the postprandial reduction in blood pressure may be less in those patients to have delayed gastric emptying.

1.5.4 Diagnosis of disordered gastric emptying

In any diabetic patient who presents with upper gastrointestinal symptoms, a comprehensive history and examination should be performed, followed by appropriate investigation to identify other causes of upper gastrointestinal symptoms. Upper gastrointestinal endoscopy is usually required to exclude gastric outlet, or duodenal obstruction, as well as mucosal disorders (Parkman and Schwartz 1987). Occasionally, the vomitus contains food that has been eaten many hours earlier, which is highly suggestive of gastroparesis. It should be recognised that there are many causes of gastroparesis apart from diabetes (Horowitz et al. 1994).

As discussed, scintigraphic measurement of gastric emptying is the most accurate and, arguably, the only clinically useful assessment of gastric motility at present, although other techniques, particularly ultrasound and carbon breath tests, show promise. Scintigraphy should ideally be performed during euglycaemia, but at a minimum with regular blood glucose monitoring. Unfortunately, there is a lack of standardisation of scintigraphic techniques.

1.5.5 Treatment

Management of diabetic patients who have significant gastrointestinal symptoms can be challenging. The emphasis of treatments is on pharmacological approaches.

Non-pharmacological

In the treatment of type 2 diabetes mellitus, dietary modifications potentially represent a more attractive and cost-effective approach than drugs and, accordingly, warrant increased attention. A number of dietary strategies may slow carbohydrate absorption. For example, in patients with type 2 diabetes, an increase in dietary fibre benefits glycaemic control; the magnitude of this improvement, which is likely to be primarily attributable to soluble, rather than insoluble, fibre, is comparable to that achieved by oral hypoglycaemic agents (Chandalia et al. 2000). Slowing of gastric emptying is likely to be important in mediating this effect (Torsdottir et al. 1984). Fat is a potent inhibitor of gastric emptying, and its effects may be dependent on posture (Horowitz et al. 1993b). As discussed in 2.3.2 there is the potential for relatively small quantities of fat given immediately before consumption of, or with, a meal to slow gastric emptying of other meal components (Stacher et al. 1991), so that the postprandial rise in blood glucose is minimised (Cunningham and Read 1989). It is not known whether fat slows gastric emptying in patients with longstanding type 2 diabetes in whom there is a high prevalence of gastroparesis. This issue is evaluated in the study reported in chapter 5.

Pharmacological

The use of prokinetic drugs designed to improve gastric emptying form the mainstay of current treatment. The drug of first choice for oral administration was probably cisapride. While cisapride well tolerated in clinical trials, reports of cardiac arrhythmias, including deaths have led to marked restrictions in its

availability (Hoover et al. 1996; Evans and Krentz 1999). Metoclopramide appears to be less effective in accelerating gastric emptying than cisapride (McHugh et al. 1992), although it has the advantage of being available for parenteral use and having central anti-emetic properties. Furthermore, many patients taking metoclopramide experience central nervous system adverse-effects (Ganzini et al. 1993). It would be not surprising if the use of domperidone increases, as it has been shown to improve quality of life in diabetic patients with upper gastrointestinal symptoms, and is better tolerated than metoclopramide because of the reduced risk of central nervous system side-effects (Silvers et al. 1998).

New therapies

There is a need for novel therapeutic options. Dopamine antagonists and 5-HT 4 agonists which do not affect cardiac function are currently in development. There may be therapeutic advantages in combining drugs which have different mechanisms of action. Therapies designed to relax the proximal stomach, such as sumatriptan and clonidine, could potentially be beneficial if symptoms reflect an increase in the sensitivity of gastric mechanoreceptors. The dopamine D2 receptor antagonist levosulpiride has been reported to improve symptoms in diabetic gastroparesis (Melga et al. 1997). Tegaserod, which is a partial 5-HT4 agonist, warrants evaluation. Ghrelin has recently been shown to accerelate gastric emptying in symptomatic patients with diabetes, at least acutely (Murray et al. 2005). There is renewed interest in the potential role of gastric electrical stimulation, using either neural electrical stimulation at a high frequency, or gastric electrical pacing, in which electrical stimulation of cholinergic motor neurons

approximates the physiological frequency (McCallum et al. 1998). A device for electrical stimulation has been approved by the FDA.

1.6 CONCLUSIONS

Gastroduodenal motility is complex; the stomach receives a meal, mixes the ingesta with secretions, grinds solids into small particles, and delivers its contents into the duodenum in a controlled manner. Feedback arising from the interaction of nutrients with the small intestine is central to the regulation of gastric emptying and energy intake; these interrelated effects are modulated by both humoral and neural mechanisms, which are in turn likely to be dependent on both the length and site of small intestinal exposed to nutrient. Studies conducted by the author were designed to address the following issues:

(i) the effect of variations in duodenal glucose delivery on glycaemia and incretin hormone release in healthy subjects (chapter 4)

(ii) the effects of fat on gastric emptying of, and the glycaemic, insulin and incretin responses to, oral carbohydrate meal in type 2 diabetes (chapter 5)

(iii) the effect of artificially sweetened, compared to regular mixers, on gastric emptying and alcohol absorption from mixed alcoholic beverages (chapter 6)

(iv) the effects of hyoscine on duodenal motility and flow events, glucose absorption, and incretin hormone release (chapter 7)

(v) the effects of intraluminal local anaesthetic on duodenal motility, blood glucose, hormone response and gastrointestinal symptoms (chapter 8)

(vi) the effects of mid-jejunal, compared to duodenal, glucose infusion on peptide hormone release, small intestinal motility, and appetite (chapter 9)

(vii) the effects of protein on gastric emptying, blood glucose and insulin response to oral glucose in healthy subjects (chapter 10)

(viii) the effects of variations in small intestinal glucose load on antropyloroduodenal motility, gastrointestinal hormones, appetite and energy intake (chapter 11).

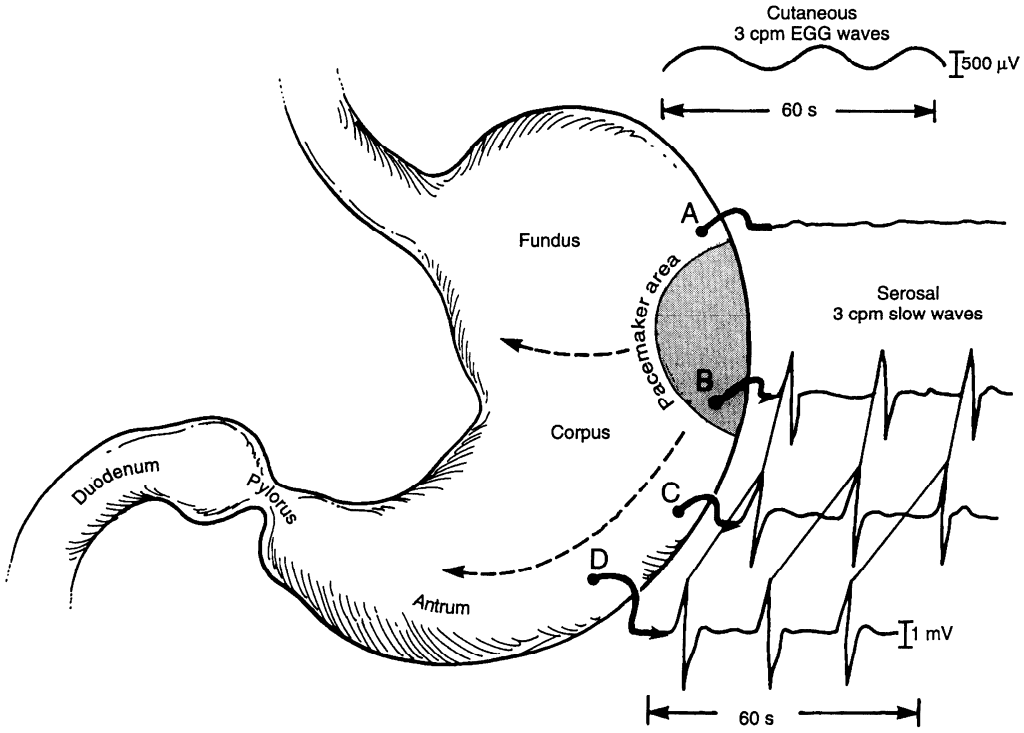

Figure 1.1

Schematic representation of the anatomical regions of the stomach, and the position of the gastric pacemaker.

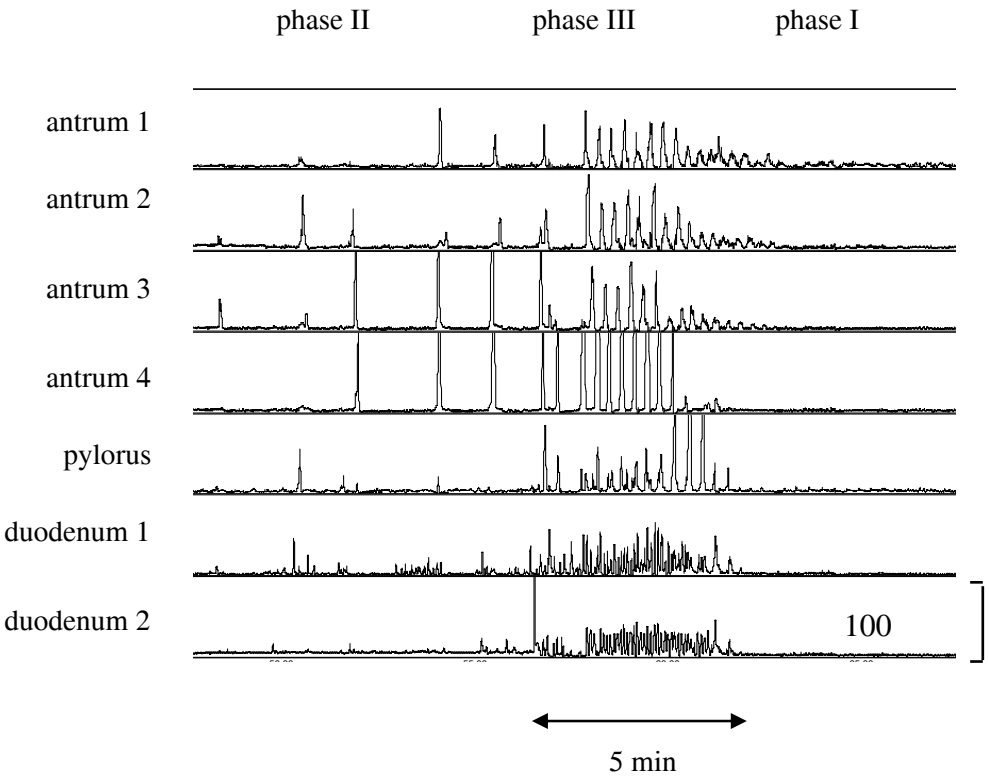

Figure 1.2

Manometry recording showing the three phases of the migrating motor complex in a healthy human.

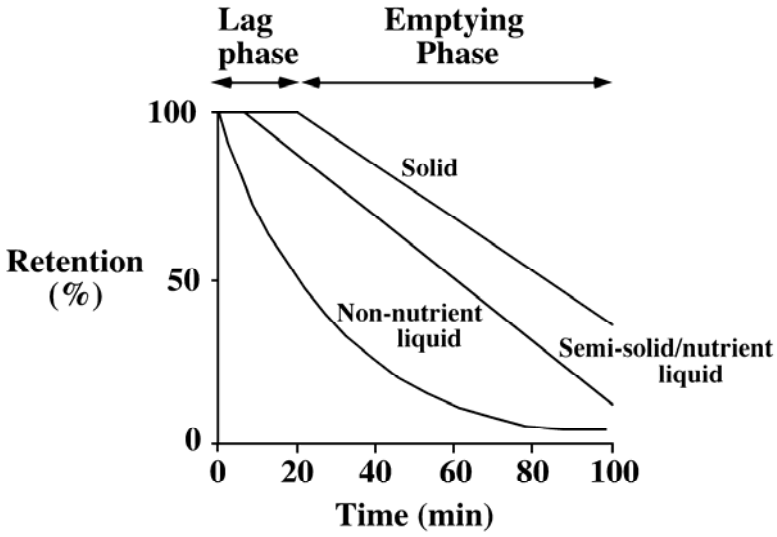

Figure 1.3

Scintigraphic gastric emptying curves for solid (pancake) and nutrient liquid (10% glucose). Solid empty in linear fashion following a lag phase while nutrient liquids empty in a monoexponential manner with minimal lag.

CHAPTER 2:

IMPACT OF GASTRIC EMPTYING AND SMALL INTESTINAL MOTILITY ON POSTPRANDIAL GLYCAEMIA

2.1 INTRODUCTION

Diabetes and its associated long-term complications, including cardiovascular, renal, neurologic, and ophthalmic disease, represent a major cause of morbidity and mortality throughout the world (Mokdad et al. 2000). The prevalence of both type 1 (insulin-dependant) and type 2 (non insulin-dependant) diabetes is increasing, the latter dramatically and as a consequence of obesity - in the US, some 29 million people and 14% of adults have diabetes or impaired fasting glucose, of whom about a third are undiagnosed (Centers for Disease Control and Prevention. 2003). Similar figures are evident throughout the developed world (Dunstan et al. 2002).

Hyperglycaemia is central to the pathogenesis of diabetic micro- and macro-vascular complications (Nathan et al. 2005). There is increasing evidence that postprandial hyperglycaemia is the major determinant of "average" glycaemic control as assessed by glycated haemoglobin (Home et al. 2002; El-Kebbi et al. 2004), and represents an independent risk factor for macrovascular disease, even in people without diabetes (Del Prato 2002). While the relative importance of individual determinants of the blood glucose response to a meal remains to be clarified precisely, it is clear that upper gastrointestinal motility has a major impact that has generally been overlooked. Moreover, postprandial glycaemic control has, in turn, a profound effect on the motor function of the upper gut. Hence, the blood

glucose concentration is both determined by, as well as a determinant of, gastric and small intestinal motility (Rayner et al. 2001). This chapter summarise current knowledge relating to the impact of gastric and small intestinal motility on glycaemia in both health and diabetics, with a particular focus on the regulation of postprandial glycaemia.

2.2 IMPACT OF POSTPRANDIAL GLYCAEMIA ON HEALTH

The DCCT/EDIC and UKPDS trials have established that the onset and progression of microvascular, and probably macrovascular, complications of diabetes are related to "average" glycaemic control, as assessed by glycated haemoglobin (DCCT 1993; UKPDS Group 1998; Nathan et al. 2005), providing a rationale for the widespread use of intensive therapy directed at the normalisation of glycaemia in patients with diabetes. In the recently reported DCCT/EDIC study involving type 1 patients, a period of intensive, as opposed to conventional, therapy for 6.5 years between 1983 and 1993 was shown to be associated with a reduction in the risk of a subsequent cardiovascular event by 42% (Nathan et al. 2005). While glycated haemoglobin is potentially influenced by both fasting and postprandial blood glucose concentrations, given that the healthy stomach empties ingested nutrients at a closely regulated overall rate of ~ 6-12 kJ (1.5 - 3 kcal) per minute (Brener et al. 1983a; Hunt et al. 1985a) (as discussed in chapter 1), and humans ingest around 10,000 kJ (2500 kcal) daily, it is clear that most individuals spend the majority of each day in either the "postprandial" or "post-absorptive" phases, with a duration of true "fasting" limited to perhaps three or four hours before breakfast (Monnier 2000). The duration of the postprandial period is likely to be even greater

in diabetics, given that gastric emptying is frequently delayed. Hence, the traditional focus on the control of “fasting” blood glucose in diabetes management appears inappropriate.

It is well established that postprandial hyperglycaemia precedes elevation of fasting blood glucose in the evolution of type 2 diabetes (Lebovitz 1998). Furthermore, it appears to be the better predictor of coronary artery (Balkau et al. 1998) and cerebrovascular (Yamasaki et al. 1995) disease, even in the general population, without known diabetes (de Vegt et al. 1999). For example, the blood glucose level two hours after an oral glucose load is a better predictor of mortality than fasting blood glucose (1999). Furthermore in patients with type 2 diabetes improvement in postprandial, as opposed to fasting, glycaemia is associated with a substantial reduction in cardiovascular risk (Hanefeld et al. 1996). For example, patients with impaired glucose tolerance treated with the α-glucosidase inhibitor, acarbose, which reduces postprandial, but not fasting, glycaemia, experienced a reduction in cardiovascular risk of about a third when compared to placebo during a mean of three years’ follow-up (Chiasson et al. 2003). Postprandial blood glucose concentrations correlate well with glycated haemoglobin in the setting of mild to moderate hyperglycaemia (El-Kebbi et al. 2004), with fasting blood glucose only assuming greater importance at higher glycated haemoglobin values (Monnier et al. 2003) (Figure 2.1). There is also evidence that therapy directed towards lowering postprandial blood glucose concentrations may have a greater impact on glycated haemoglobin than does attention to fasting blood glucose (Bastyr et al. 2000).

2.2.1 Factors mediating the effects of hyperglycaemia on vascular disease

The mechanisms mediating the adverse effects of postprandial hyperglycaemia on micro- and macrovascular disease are poorly defined. Hyperglycaemia potentially has diverse effects on blood vessels. In the short term, hyperglycaemia is associated with activation of protein kinase C, which affects endothelial permeability, cell adhesion, and proliferation in the vessel wall. Over the longer term, non-enzymatic glycosylation of proteins may leads to atherosclerosis (Haller 1998). Elevated postprandial blood glucose concentrations are associated with an increase in plasma biochemical markers of oxidative stress (Ceriello 1997; Ceriello 1998); to what degree hyperglycaemia *per se* accounts for this effect, as opposed to concurrent elevations of non-esterified fatty acids and triglycerides, remains to be elucidated (Heine and Dekker 2002).

2.3 DETERMINANTS OF GLYCAEMIA

2.3.1 Introduction

Postprandial blood glucose levels are potentially affected by a number of factors, including the pre-prandial blood glucose concentration, food properties such as viscosity, fibre content, and quantity and type of carbohydrate, gastric emptying, small intestinal delivery and absorption of nutrients, insulin secretion, hepatic glucose metabolism, and peripheral insulin sensitivity (Horowitz et al. 2002b). The relative importance of these factors is likely to vary with time after a meal, and between healthy subjects and patients with type 1 or type 2 diabetes. While it is logical that the gastrointestinal tract, which controls the rate at which ingested

carbohydrate is absorbed, and releases peptides that stimulate insulin secretion, should have a major impact on postprandial glycaemia, its role has frequently been overlooked and generally underestimated in the past. The rate of gastric emptying is now established as a major contributor to variations in glycaemia, while the influence of small intestinal motor function represents a current research focus.

2.3.2 Role of gastric emptying and incretin hormones

Gastric emptying accounts for at least 35% of the variance in the initial rise in blood glucose, as well as the peak blood glucose level, after oral glucose in both healthy individuals (Horowitz et al. 1993a) and patients with type 2 diabetes (Jones et al. 1996a) (Figure 2.2). Pharmacological slowing of gastric emptying by morphine reduces the postprandial glycaemic response to a mixed meal in type 2 patients, while acceleration of gastric emptying by erythromycin increases postprandial blood glucose concentrations (Figure 2.3); the impact of modulation of gastric emptying appears to be more marked on peak blood glucose than the area under the blood glucose curve (Gonlachanvit et al. 2003). There is little information about the 'dose-response' relationship of glycaemia and insulinaemia with small intestinal glucose delivery; this is evaluated in the study reported in chapter 11. In type 1 patients, the glycaemic response to a meal, and therefore the requirement for exogenous insulin, is also critically dependent on the rate of gastric emptying - when emptying is slower, the initial insulin requirement to achieve euglycaemia is less (Ishii et al. 1994). As discussed in chapter 1, in health, gastric emptying is modulated by feedback arising from the interaction of nutrients with the small intestine, so that the overall rate of gastric emptying is closely regulated

at about 6 - 12 kJ (1.5 - 3 kcal) per minute, tending to be slower for fat (Horowitz et al. 1993b) than carbohydrate (Brener et al. 1983a). Infusion of a caloric load directly into the small intestine slows gastric emptying by mechanisms that include relaxation of the gastric fundus, suppression of antral motility, and stimulation of phasic and tonic pressures localised to the pylorus (Heddle et al. 1989a; Heddle et al. 1993); the latter act as a brake to gastric outflow. The length of small intestine exposed to nutrient appears to be the primary determinant of the magnitude of the feedback response (Lin et al. 1989; Lin et al. 1990). Glucagon-like peptide-1 (GLP-1), suppresses antral and duodenal motility and stimulates pyloric contractions induced by the presence of glucose in the small intestine (Schirra et al. 2000; Horowitz and Nauck 2006; Schirra et al. 2006), probably represents one such humoral mediator, as will be discussed (p 122) the slowing of gastric emptying by this peptide appears likely to be the major mechanism by which its exogenous administration improves postprandial glycaemia in healthy subjects and patients with type 2 diabetes (Nauck et al. 1997b; Little et al. 2006a) discussed in chapter 5.

In considering the potential impact of gastric emptying on postprandial glycaemia, the initial rate of glucose entry into the small intestine (so-called “early phase” of gastric emptying) may be particularly important (O'Donovan et al. 2005b). Type 2 diabetes is characterised by reduced “early”, and frequently increased “late”, postprandial insulin responses. Studies in rodents have established the potential importance of “early” insulin release is determining postprandial glucose excursions, in that a small, “early”, increase in portal vein/ peripheral blood insulin is more effective than a larger, “later”, increase in reducing blood glucose levels

(de Souza et al. 2001a). A recent study, established that modest variations in the initial rate of small intestinal glucose entry, designed to be within the physiological range, have major effects on the subsequent glycaemic, insulin and incretin responses (Figure 2.4) (O'Donovan et al. 2004b) - healthy subjects and type 2 patients received an intraduodenal glucose infusion of ~120 kcal over 2 hours on two separate days. On one day, the infusion rate was 3 kcal/min for 15 min followed by 0.71 kcal/min until 120 min, whereas on the other day the rate was constant at 1 kcal/min. While initially rapid, and subsequently slower, duodenal glucose delivery boosted incretin and insulin responses when compared to constant delivery of an identical glucose load, the overall glycaemic excursion was, if anything, greater (O'Donovan et al. 2004b). This may potentially have been because the "early" phase of gastric emptying was underestimated (chapter 4), as the latter approximate 6 kcal/min (Chaikomin et al. 2005). As discussed, the overall "dose-response" relationship between glycaemia with duodenal glucose delivery remains to be determined (chapter 11). As discussed both fat and protein slow gastric emptying (Welch et al. 1987; Cunningham and Read 1989) - these effects may be more marked when given before, rather than with, a meal and should be associated with a reduction in the 'glycaemic' response (Gentilcore et al. 2006a). Protein may also stimulate insulin secretin directly and both fat and protein probably release incretin hormones (chapter 5 and 10). The studies reported in chapter 5 and 10 extend previous observations characterising these effects. This information is fundamental to the application of dietary and pharmacological strategies designed to minimise postprandial glycaemic excursions in healthy subjects and diabetics by modulating gastric emptying.

It has long been recognised that oral or enteral administration of glucose results in a much greater insulin response than an equivalent intravenous glucose load (Elrick et al. 1964; McIntyre et al. 1965; Perley and Kipnis 1967; Creutzfeldt 1979), a phenomenon referred to as the "incretin" effect. The putative incretin peptides, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), are released from the small intestine in response to nutrients (Holst and Gromada 2004), apparently in a load-dependent fashion (Schirra et al. 1996a). Accordingly, the rate of delivery of carbohydrate from the stomach into the small intestine is likely to be critical in determining not only the rate of glucose absorption, but also the incretin response. This issue is evaluated in the studies reported in chapters 4 and 11. Although GIP is the more potent of the two incretin hormones in healthy individuals (Meier et al. 2002a), the insulinotropic effect of GIP appears to be markedly diminished in patients with type 2 diabetes. In contrast, the insulin response to GLP-1 is maintained (Holst and Gromada 2004), forming a rationale for the therapeutic use of GLP-1 and its analogueues in the management of type 2 diabetes (discussed on p 62). There is limited evidence that type 2 diabetes is associated with an impaired GLP-1 response to oral glucose (Toft-Nielsen et al. 2001), but to what degree delayed gastric emptying, which occurs frequently in type 2 patients (Horowitz et al. 1989a), accounts for this decrease is uncertain.

2.3.3 Role of small intestinal glucose absorption and region of small intestine

The small intestine is the site of absorption of glucose from the external environment (ie. the gut lumen) into the body, as well as being the source of the

incretin peptides that, as discussed, drive much of the postprandial insulin response. Therefore, it is logical that variations in the small intestinal function should be a major determinant of postprandial glycaemia. Nevertheless, there is little information about the impact of small intestinal motility and absorptive function on glycaemia, at least in part because of the technical demands in studying this region of gastrointestinal tract (Rayner et al. 2001; Schwartz et al. 2002).

The large surface area of the small intestine is well suited to absorption of water and solutes. Perfusion studies in healthy humans have established that the proximal jejunum has a maximal absorptive capacity for glucose of approximately 0.5 g per minute per 30 centimeters (Holdsworth and Dawson 1964; Modigliani and Bernier 1971; Duchman et al. 1997). Small intestinal mucosal hypertrophy occurs in animal models of diabetes, with concomitant increases in glucose absorption, but this is rapidly reversed by insulin treatment (Fedorak et al. 1987). However, acute hyperglycaemia does result in a transient increase in intestinal glucose absorption in rodents (Csaky and Fischer 1977; Csaky and Fischer 1981; Fischer and Lauterbach 1984). The few studies performed in humans with type 1 (Gottesburen et al. 1973), “insulin-requiring” (Costrini et al. 1977), or type 2 (Gulliford et al. 1989) diabetes have not demonstrated increased small intestinal glucose absorption, other than one report of increased absorption at high luminal glucose concentrations. Attention was paid to maintaining euglycaemia in at least one of these studies (Costrini et al. 1977). There is a recent report of increased expression of monosaccharide transporters in humans with type 2 diabetes (Dyer et al. 2002), the clinical significance of which remains to be clarified. One human study failed

to demonstrate an effect of marked hyperglycaemia (14 mmol/L) on jejunal glucose absorption in healthy subjects (Costrini et al. 1977), although a relationship has subsequently been observed between more physiological postprandial blood glucose concentrations (less than 10 mmol/L) and the absorption of the glucose analogueue, 3-O-methylglucose, in healthy subjects and type 1 diabetics (Rayner et al. 2002). Hence, in view of these discrepant observations the effect of acute hyperglycaemia on small intestinal glucose absorption remains uncertain.

Given that an upper limit exists for absorption of glucose across the small intestinal mucosa, it is logical that patterns of intestinal motility that serve to spread luminal glucose over a large surface area could promote glucose absorption. Furthermore, certain motor patterns could facilitate mixing of complex carbohydrates with digestive enzymes, and their exposure to brush border disaccharidases. Thus, when glucose is infused directly into the duodenum, its rate of absorption increases with the number of duodenal pressure waves and propagated pressure wave sequences (Rayner et al. 2002; Schwartz et al. 2002). These observations are likely to be of relevance to patients with type 1 diabetes mellitus (Rayner et al. 2002), as discussed (p 154), demonstrate an increased frequency of small intestinal pressure waves in the postprandial state. Further insights into the effects of luminal flow on glucose absorption are likely to require novel techniques, such as intraluminal impedance measurement (chapter 7).

As discussed (1.4.2) the region of small intestine that is exposed to carbohydrate is also likely to be a determinant of the glycaemic response. - GLP-1 is released from

intestinal L cells, whose concentration is greatest is the distal jejunum, with fewer L cells located in the proximal jejunum, ileum, and colon (Eissele et al. 1992). In humans, it is unclear whether nutrients must interact directly with L cells to stimulate GLP-1 release; a neural or endocrine loop between the duodenum and the more distal small bowel has been postulated (Holst 1994), but remains unproven. It is however, clear that GLP-1 release by glucose in humans is dependent on the length of small intestinal exposed (Little et al. 2006a). GLP-1 responses to meals are enhanced following intestinal bypass procedures that promote access of nutrients to more distal small intestine (Lauritsen et al. 1980; Andrews and Irving 1992; Mason 1999; Jeppesen et al. 2000a), while inhibition of sucrose digestion in the proximal small intestine with acarbose increases the GLP-1 response, presumably by facilitating more distal interaction of the intestine with glucose (Gentilcore et al. 2005). It follows that dietary modifications which favor exposure of more distal small intestinal segments to glucose could reduce glycaemic excursions by stimulating GLP-1 release. This issue is evaluated in the study reported in chapter 9. Furthermore, a major action of GLP-1 is to retard gastric emptying, thus slowing further entry of carbohydrate to the small intestine (Wen et al. 1995).

2.4 IMPACT OF GLYCAEMIA ON UPPER GUT MOTILITY

Acute changes in the blood glucose concentration are now recognised to have a major, reversible, impact on the motor function of every region of the gastrointestinal tract. This may account, in part at least, for the poor correlation of gastrointestinal dysfunction in diabetes with evidence of ‘irreversible’ autonomic

neuropathy, to which it has traditionally been attributed (Horowitz et al. 1991b). When compared to euglycaemia (4 - 6 mmol/L), gut motility is modulated through the range of blood glucose concentrations from marked hyperglycaemia (≥ 12 mmol/L) (Fraser et al. 1990; Oster-Jorgensen et al. 1992), through "physiological" blood glucose elevation (8 - 10 mmol/L) (Schvarcz et al. 1997), to insulin-induced hypoglycaemia (≤ 2.5 mmol/L) (Fraser et al. 1991a; Schvarcz et al. 1993; Russell et al. 2001), and effects are observed rapidly (within min), although the thresholds of response may differ between gut regions (Verhagen et al. 1999; Rayner et al. 2001). The mechanisms mediating the effects of acute changes in the blood glucose concentration are poorly defined, and the potential impact of chronic, as opposed to acute, variations in glycaemia on gastrointestinal motility has hitherto received little attention. Nevertheless, it is clear that gut motor function and postprandial glycaemia are highly interdependent variables.

2.4.1 Gastric emptying and gastroduodenal motility

Marked hyperglycaemia (16 - 20 mmol/L) slows both solid and nutrient liquid emptying in heathy subjects (MacGregor et al. 1976; Oster-Jorgensen et al. 1990) patients with type 1 diabetes (Fraser et al. 1990) when compared to euglycaemia; in type 2 patients, cross-sectional data also indicate an inverse relationship between the blood glucose concentration and the rate of gastric emptying (Horowitz et al. 1989b). Conversely, gastric emptying is accelerated by acute hypoglycaemia induced by insulin (~2.5 mmol/L) in healthy subjects (Schvarcz et al. 1995) and type 1 patients, even when emptying is slower than normal during euglycaemia (Russo et al. 2005). In patients with type 1 diabetes, as well as healthy volunteers,

elevation of blood glucose to “physiological” postprandial levels (8 mmol/L) also slows gastric emptying when compared to euglycaemia (4 mmol/L) (Schvarcz et al. 1997) (Figure 2.5). The magnitude of the effect of glycaemia on the rate of gastric emptying is substantial, and has implications for absorption of orally administered medications, including oral hypoglycaemic agents (Groop et al. 1989), as well as impacting on carbohydrate absorption.

As discussed in chapter 1, the rate of gastric emptying is determined by the coordinated activity of various regions of the stomach and proximal small intestine (Horowitz and Dent 1991). Acute hyperglycaemia is associated with diminished proximal gastric tone (Hebbard et al. 1996a; Rayner et al. 2000d), suppression of both the frequency and propagation of antral pressure waves in health (Barnett and Owyang 1988; Bjornsson et al. 1994; Hasler et al. 1995), and type 1 diabetes (Samsom and Smout 1997), as well as, stimulation of pyloric contractions (Fraser et al. 1991b) – a motor pattern associated with slowing of gastric emptying. The frequency of the gastric slow wave is also disturbed (Jebbink et al. 1994; Hasler et al. 1995; Hebbard et al. 1997). The suppression of antral motility is observed at blood glucose concentrations as low as 8 mmol/L (Barnett and Owyang 1988; Hasler et al. 1995); the threshold for proximal gastric relation appears higher (Verhagen et al. 1999). The suppression of gastric (antral) phase III activity, evident at modest blood glucose elevations, would favour the development of gastric bezoars, a well recognised, albeit rare, complication of diabetic gastroparesis. Hyperglycaemia also attenuates the prokinetic effects of erythromycin in both healthy subjects and type 1 patients (Jones et al. 1999;

Petrakis et al. 1999), probably at least in part, by inhibiting the stimulation of antral waves and coordinated antroduodenal pressure sequences (Rayner et al. 2000c). The action of other prokinetic drugs is also likely to be impaired during hyperglycaemia (Horowitz et al. 2002a), although this issue has not been specifically examined.

2.4.2 Small intestinal motility

There is less information relating to the effects of acute changes in the blood glucose on small intestinal, than gastric, motility. In healthy subjects during hyperglycaemia (10 mmol/L), the duodenum becomes less compliant (more "stiff") to balloon distension, while distension stimulates a greater number of phasic pressure waves, when compared to euglycaemia (Lingenfelser et al. 1999); both phenomena could contribute to a duodenal "brake" to gastric emptying. More marked hyperglycaemia (12-15 mmol/L) reduces the cycle length of the MMC (Oster-Jorgensen et al. 1992) the frequency of, duodenal (Lingenfelser et al. 1999) (Figure 2.6) and jejunal pressure waves, and the duration of the postprandial period (early return of phase III activity), and slows small intestinal transit (Russo et al. 1996). These alterations in function could have implications for absorption of nutrients and medications, bowel habit, and the occurrence of small intestinal bacterial overgrowth in diabetes (Virally-Monod et al. 1998). Other than suppression of proximal duodenal wave frequency (Samsom et al. 1997), there is limited information about the effects of hyperglycaemia on small intestinal motor function in diabetic patients, as opposed to healthy volunteers.

2.4.3 Mechanisms mediating the effects of hyperglycaemia and hypoglycaemia on gastric and small intestinal motility

Most information about the aetiology of gastrointestinal dysfunction in diabetes relates to the effects of longstanding diabetes, rather than acute, reversible, changes that could result from transient fluctuations in the blood glucose concentration (Rayner and Horowitz 2006). Rodent models of diabetes have demonstrated marked apoptosis of enteric neurons (Fregonesi et al. 2001), affecting nitrergic (inhibitory) neurons in particular (Watkins et al. 2000), and loss of interstitial cells of Cajal (Ordog et al. 2000); the latter are also deficient in humans with diabetes and severe gut symptoms (Forster et al. 2005). Hyperglycaemia appears to be responsible for apoptosis of enteric neurons in animal models (Anitha et al. 2006), but the latter would seem unlikely to mediate changes that are evident within min, rather than days or weeks. Enteric neurons sensitive to changes in glucose have been identified (Liu et al. 1999), although their responsiveness to systemic, as opposed to luminal, glucose remains unclear. Vagal nerve function is reversibly inhibited by acute hyperglycaemia (Lam et al. 1993; Yeap et al. 1996), and this may account for some of the observed phenomena. Hyperinsulinaemia is unlikely to explain the observed effects, particularly as they are seen in type 1 (insulin deficient) as well as type 2 and healthy subjects. Studies are indicated to determine whether reversible changes in nitrergic or serotonergic neurotransmission occur with variations in glycaemia.

2.4.4 Effects of glycaemia on gastrointestinal sensation

Both acute physiological and epidemiological studies support the concept that acute changes in the blood glucose concentration modulate the perception of sensations arising from the gut. The prevalence of symptoms referable to the upper gastrointestinal tract, such as early satiation or nausea, is greater in type 1 and type 2 diabetic patients with poor glycaemic control, as assessed by glycated haemoglobin (Schvarcz et al. 1996) or self report (Bytzer et al. 2002). In a cross-sectional study of type 1 diabetic patients, the perception of postprandial fullness was related to the blood glucose concentration (Jones et al. 1997b). In healthy subjects, marked hyperglycaemia (blood glucose ~ 15 mmol/L) increases perceptions, such as nausea and fullness, during proximal gastric distension, both in the fasted state and during intraduodenal lipid infusion (Hebbard et al. 1996a; Hebbard et al. 1996b) and in response to duodenal distension (Lingenfelser et al. 1999), as well as, the threshold for initial perception of oesophageal distension (Boeckxstaens et al. 1997). Although a study in patients with type 1 diabetes failed to demonstrate an effect of acute hyperglycaemia on the perception of gastric distension in the fasted state, the sensitivity to gastric distension was substantially greater than that in healthy control subjects during euglycaemia (Rayner et al. 2000d), which may have made any further increase in sensitivity difficult to detect.

2.5 POTENTIAL THERAPEUTIC STRATEGIES TO MINIMISE POSTPRANDIAL GLYCAEMIA

The major impact of gastrointestinal function on the glycaemic response to meals, as outlined, suggests a number of logical, and in many cases complimentary,

strategies to lower postprandial blood glucose concentrations. These include (1) minimising the carbohydrate content, or substituting low- for high-glycaemic index foods in meals, (2) slowing gastric emptying, even in those individuals who have a modest delays in emptying, provided they remain free of symptoms, (3) inhibiting the absorption of carbohydrate from the small intestine, or delaying its absorption to more distal small intestinal segments, and (4) augmenting the incretin response. Many approaches fulfill a number of these aims concurrently. Most studies relating elevated postprandial glycaemia to cardiovascular risk have evaluated blood glucose 2 hr after a meal (Ceriello et al. 2004), suggesting that lowering peak blood glucose may be an appropriate target. Nevertheless, glycated haemoglobin relates closely to the integrated, mean blood glucose (ie. area under the curve) over 24 hr, albeit in a curvilinear, rather then linear, fashion (Hassan et al. 2006). Accordingly, reducing the total area under the blood glucose excursion over several hours after a meal may also be an important goal. It should be noted that strategies for individuals with impaired glucose tolerance or type 2 diabetes managed without exogenous insulin, particularly those involving slowing of gastric emptying, may not be applicable to type 1 and insulin-requiring type 2 patients, in whom the goal should be to optimise the coordination between the absorption of carbohydrate with the action of exogenous insulin; the latter may potentially involve accelerating gastric emptying, if the latter is already delayed (Ishii et al. 1997).

2.5.1 Carbohydrate content and glycaemic index

Low-carbohydrate diets represented the mainstay of treatment for diabetes in the pre-insulin era (Westman et al. 2006). The outcome of the Nurses' Health Study

suggests that there is a relationship between both cardiovascular risk and the incidence of diabetes with dietary glycaemic load (Liu et al. 2000). Short-term studies indicate the potential for low-carbohydrate diets to improve 24 hr glycaemia and glycated haemoglobin in patients with type 2 diabetes (Boden et al. 2005), including patients who have failed conventional treatment with diet and a sulfonylurea (Gutierrez et al. 1998). In medium- to long-term studies, the substitution of protein for carbohydrate improved glycaemia in overweight, hyperinsulinaemic subjects (Farnsworth et al. 2003), while a low-carbohydrate diet improved fasting glucose over a period of 6 months in type 2 patients, with glycaemic benefits maintained at 1 year, when compared to a low-fat diet (Samaha et al. 2003; Stern et al. 2004). The magnitude of the observed decreases in glycated haemoglobin were small (e.g. mean of 0.6% in the latter study), but likely to be clinically relevant. In addition to the reduction in carbohydrate load, protein itself might improve glycaemia by stimulating insulin release directly (Gannon et al. 1988), although this phenomenon is less apparent in medium- versus short-term studies (Gannon et al. 2003). Protein is also known to stimulate incretin hormone secretion (Deacon 2005) and will, of course, slow gastric emptying (Hellstrom and Naslund 2001). The relative important of these effects is poorly defined (chapter 10).

As discussed, (p 19) the interaction of nutrients with small intestinal receptors regulates both gastric emptying and appetite, and stimulates the release of gastrointestinal hormones, including cholecystokinin (CCK) (Fried et al. 1991) and glucagon-like peptide-1 (GLP-1) (MacIntosh et al. 2001a). In healthy subjects,

intraduodenal infusion of fat slows gastric emptying (Heddle et al. 1989a); these effects are, at least in part, mediated by CCK (Fried et al. 1991). The slowing of gastric emptying by small intestinal nutrients is associated with a reduction in proximal gastric tone (Feinle et al. 1996), suppression of antral pressure waves (Heddle et al. 1989a) and stimulation of tonic and phasic pyloric pressures (Fone et al. 1989). The increase in pyloric motility may be the most important of these mechanisms, as the stimulation of phasic and tonic pyloric pressure is associated with cessation of transpyloric flow (Tougas et al. 1992). Studies in animals (Meyer et al. 1994a) and humans (Carney et al. 1995; Feinle et al. 2003a) suggest that the slowing of gastric emptying, suppression of appetite and stimulation of CCK secretion by fat are dependent on lipolysis of triglyceride to fatty acids.

Rather than trading carbohydrates for alternative macronutrients, another approach is to substitute low-, for high-glycaemic index, carbohydrates. The glycaemic index (GI) compares the blood glucose response of a test food with that of a standard carbohydrate, either glucose or white bread (Wolever 1990). Foods may be low GI by virtue of a relative delay in gastric emptying and/or small intestinal glucose absorption (Ludwig 2002; Bjorck and Elmstahl 2003). For example, spaghetti (low GI) empties from the stomach much slower than potato (high GI) from about 60 min after a meal, although their glycaemic profiles diverge earlier (Mourot et al. 1988), indicating that slowing of small intestinal glucose absorption is important. Both the physical properties of the carbohydrate (such as enclosed kernels) and its chemical composition (such as a high amylose : amylopectin ratio) influence small intestinal carbohydrate digestion and absorption (Hallfrisch et al. 2000; Bjorck and

Elmstahl 2003). Glycaemic index tends to vary inversely with the content of dietary fibre in meals (Wolever 1990); dietary fibre *per se* potentially slows gastric emptying (Benini et al. 1995) and small intestinal carbohydrate absorption (Cherbut et al. 1994), the latter by a mechanism that includes modification of small intestinal motility from a stationary (favouring mixing), to a propulsive, pattern. The beneficial effect on the glycaemic response of adding guar gum to an oral glucose load appears to be achieved mainly by slowing gastric emptying (Leclere et al. 1994; Jones et al. 2001a). Nevertheless, guar also slows small intestinal glucose absorption, probably by inhibiting diffusion of glucose out of the luminal contents (Blackburn et al. 1984); this is reflected by the observation that both GLP-1 and insulin responses are less when guar is added to an enteral glucose load (O'Donovan et al. 2005a).

Low GI foods may also stimulate insulin release, through the incretin effect, or other mechanisms (Nilsson et al. 2004). Furthermore, they may enhance satiation and reduce energy intake at a subsequent meal (Anderson and Woodend 2003; Roberts 2003). Additional information about the potential for these beneficial effects for different classes of low GI foods is needed. Fructose has been advocated as a low GI substitute for glucose in the diabetic diet, since it results in a much lower glycaemic excursion than an equivalent glucose load (Uusitupa 1994). In addition, some investigators have found that fructose ingestion suppresses food intake more than glucose (Spitzer and Rodin 1987; Rayner et al. 2000a), although this issue is controversial (Vozzo et al. 2002) - effects may well be dependent on the load, and timing, of fructose ingestion in relation to the subsequent meal. Most

medium- to long-term studies of low GI diets indicate a benefit for glycaemic control (Miller 1994); typically the effect is modest (about 9% reduction in glycated haemoglobin), but of comparable magnitude to the improvement in glycaemic control achieved by pharmacological agents (Brand-Miller et al. 2003).

2.5.2 Gastric emptying

Given the relationship between the degree of postprandial glycaemia and the rate of gastric emptying in both healthy subjects (Horowitz et al. 1993b) and type 2 patients (Jones et al. 1996a) discussed previously, it is logical that dietary and/or pharmacological interventions which slow gastric emptying should be effective in reducing postprandial glycaemia in type 2 diabetes. In addition to the effects of dietary fibre in retarding gastric emptying, slowing of emptying by either an oral proteinase inhibitor (Schwartz et al. 1994), adding a solid non-carbohydrate meal to an oral glucose load (Berry et al. 2003), or combining fat (the most potent macronutrient for slowing gastric emptying (Horowitz et al. 1993b)) with carbohydrate (Cunningham and Read 1989), all reduce postprandial blood glucose and insulin responses in either healthy subjects and/or patients with type 2 diabetes. The underlying concept, that the presence of nutrients in the small intestine will both delay gastric emptying and stimulate GLP-1 and GIP secretion and has the potential advantage of simplicity when compared to pharmacological strategies, which also appear to act predominantly by slowing gastric emptying. As discussed, chapter 5 reports a study which evaluated the effects of consumption of oil, as a ‘preload’, on gastric emptying of, and the glycaemic and incretin responses to, a carbohydrate meal in type 2 patients. The study reported in chapter 10 attempts to

define the mechanisms (i.e. slowing of gastric emptying, stimulation of insulin and incretin hormones) by which protein improves the glycaemic response to a meal.

Slowing of gastric emptying appears integral to the beneficial effects of recently developed pharmacological therapies for diabetes (Horowitz et al. 2002b). The improvement in postprandial glycaemia associated with exogenous administration of GLP-1 and its analogues appears to be related to slowing of gastric emptying, rather than enhancement of insulin secretion (Nauck et al. 1997a; Little et al. 2005); the latter is in fact reduced due to a decrease in the rate of entry of carbohydrate into the small intestine. The amylin analogue, pramlintide, also slows gastric emptying (Kong et al. 1996; Samsom et al. 2000), and its use is associated with an improvement in overall glycaemic control, as assessed by glycated haemoglobin, in type 1 and type 2 patients (Thompson et al. 1997; Thompson et al. 1998; Hollander et al. 2003; Ratner et al. 2004). Pramlintide has the additional advantage of promoting weight loss, probably by suppressing energy intake (Chapman et al. 2005).

2.5.3 Modulation of intestinal absorption of glucose

The α-glucosidase inhibitors, including acarbose, delay carbohydrate absorption in the proximal small intestine (Bischoff 1994); the resultant exposure of more distal intestinal segments to glucose may result in enhanced, and prolonged, GLP-1 secretion in healthy subjects (Seifarth et al. 1998; Gentilcore et al. 2005), with consequent slowing of gastric emptying (Ranganath et al. 1998). The magnitude of

these effects is likely to be dependent on meal content (i.e. disaccharide load); furthermore they do not account for all the therapeutic effects of acarbose. - Acarbose failed to stimulate GLP-1, or slow gastric emptying after a mixed meal in type 2 patients, although it still reduced postprandial glycaemia in this group (Hucking et al. 2005), presumably by impairing carbohydrate absorption. Inhibition of glucose entry into enterocytes may represent an additional mode of action of acarbose (Hirsh et al. 1997). It is, accordingly, clear that the mechanisms leading to improvement in postprandial glycaemia frequently overlap. For example, slowed absorption of glucose, as discussed, is also a feature of low GI and high fiber diets.

2.5.4 Augmentation of the incretin response

The effect of the dietary strategies already discussed on GLP-1 concentrations, and the observed potentiation of GLP-1 secretion and associated improvement in glycaemic control after bariatric surgery (Valverde et al. 2005), point to the value of augmenting the incretin response in minimising postprandial glycaemia. As discussed, GLP-1 is metabolised rapidly by the enzyme dipeptidyl peptidase IV (DPP-IV) and is, therefore, unsuitable for therapeutic administration in diabetes. Instead, longer lasting agonists have been developed, including both albumin-bound analogues of GLP-1, and exendin-4, a peptide derived from the saliva of the Gila monster lizard, which is structurally similar to GLP-1 and shares several biological properties, but may be a more potent insulinotropic agent (Horowitz et al. 2002b). Subcutaneous administration of these drugs has been shown to reduce postprandial glycaemia in type 2 patients (Fineman et al. 2003) and exendin-4 has been shown to be a potent inhibitor of gastric emptying in type 2 patients

(Linnebjerg H 2006). Resistant analogueues of GLP-1, along with DPP-IV inhibitors, appear to have a promising role in the therapy of diabetes (Holst and Deacon 2004). Inhibitors of DPP-IV have shown efficacy and tolerability when used to control the hyperglycaemia of noninsulin-dependent animal models and human type 2 diabetes (Green et al. 2006). These DPP-IV inhibitors prolong active incretin hormone concentrations (Green et al. 2006) as well as stimulate insulin levels, and reduce glucagon levels in type 2 diabetes (Ahren et al. 2004). The effect of DPP-IV inhibitors on gastric emptying has hitherto, not been reported and would be of interest, particularly given that the elevation of GLP-1 is 'physiological' rather than 'pharmacological'.

2.6 CONCLUSIONS

The risk of macro-and microvascular complications of diabetes is closely related to average glycaemia, as assessed by a glycated haemoglobin which is, in turn, highly dependent on postprandial glycaemic excursions. Postprandial hyperglycaemia per se probably plays a distinct role in the pathogenesis of macrovascular disease in individual who do not have diabetes.

While the rate of gastric emptying is an important determinant of postprandial blood glucose concentrations by determining both the delivery of carbohydrate to the small intestine and the release of incretin hormones, there is limited information about the relationship between glycaemia and incretin hormone levels with the rate of small intestinal carbohydrate entry. Similarly the impact of dietary modulation of gastric emptying on incretin hormone secretion and glycaemia has

not been well characterised. Studies conducted by the author were designed to address the following issues:

(i) the effect of variations in duodenal glucose delivery on glycaemia and incretin hormone release in healthy subjects (chapter 4)

(ii) the effects of fat on gastric emptying of, and the glycaemic, insulin and incretin responses to, oral carbohydrate meal in type 2 diabetes (chapter 5)

(iii) the effect of artificially sweetened, compared to regular mixers, on gastric emptying and alcohol absorption from mixed alcoholic beverages (chapter 6)

(iv) the effects of hyoscine on duodenal motility and flow events, glucose absorption, and incretin hormone release (chapter 7)

(v) the effects of intraluminal local anaesthetic on duodenal motility, blood glucose, hormone response and gastrointestinal symptoms (chapter 8)

(vi) the effects of mid-jejunal, compared to duodenal, glucose infusion on peptide hormone release, small intestinal motility, and appetite (chapter 9)

(vii) the effects of protein on gastric emptying, blood glucose and insulin response to oral glucose in healthy subjects (chapter 10)

(viii) the effects of variations in small intestinal glucose load on antropyloroduodenal motility, gastrointestinal hormones, appetite and energy intake (chapter 11).

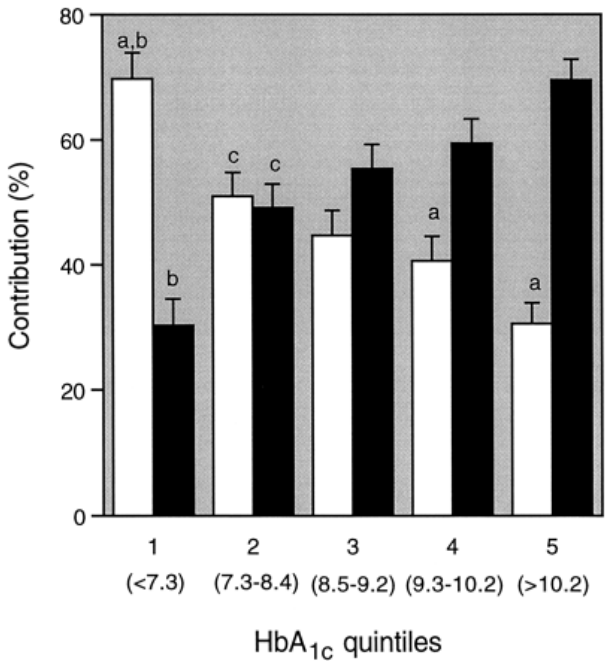

Figure 2.1

Relative contributions of postprandial (▯) and fasting (▯) hyperglycaemia (%) to the overall diurnal hyperglycaemia in 290 non-insulin- and non-acarbose-using patients with type 2 diabetes, over quintiles of glycated haemoglobin (HbA_{1c}) a, significant difference was observed between fasting and postprandial plasma glucose (paired t test); b, significantly different from all other quintiles (ANOVA); c, significantly different from quintile 5 (ANOVA) adapted from (Monnier et al. 2003).

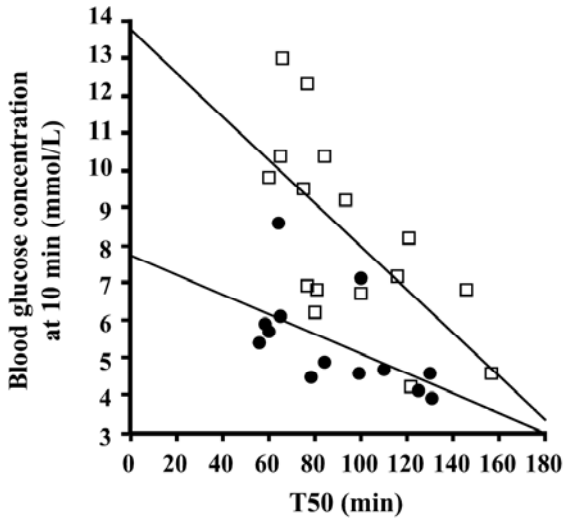

Figure 2.2

Relationship between the blood glucose concentration 10 min after consuming 75 g glucose in 300 mL water, and the gastric half-emptying time (T50), in patients with type 2 diabetes (n = 16) (open squares, r = -0.67, P < 0.005) and healthy subjects (n = 13) (filled circles, r = -0.56, P < 0.05). Adapted from (Jones et al. 1996b).

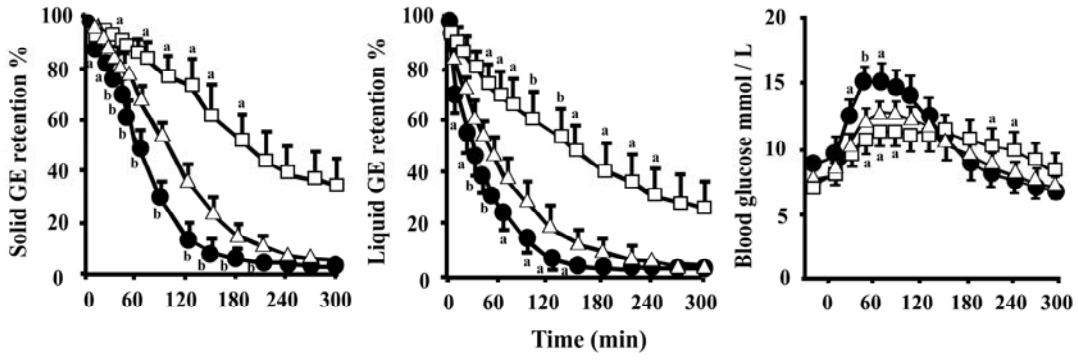

Figure 2.3

Effects of erythromycin (200 mg iv, filled circles), morphine (8 mg iv, open squares) and placebo (open triangles) on solid and liquid gastric emptying (two eggs, a toasted white bagel, a pat of butter, a half cup of fruit cocktail, and 300 ml orange juice) and blood glucose concentrations in 9 type 2 patients. The result showed that, erythromycin accelerated and morphine delayed solid- and liquid-phase gastric emptying compared to placebo ($P < 0.05$). $^{a}P < 0.05$ vs placebo, $^{b}P < 0.01$ vs placebo. Adapted from (Gonlachanvit et al. 2003).

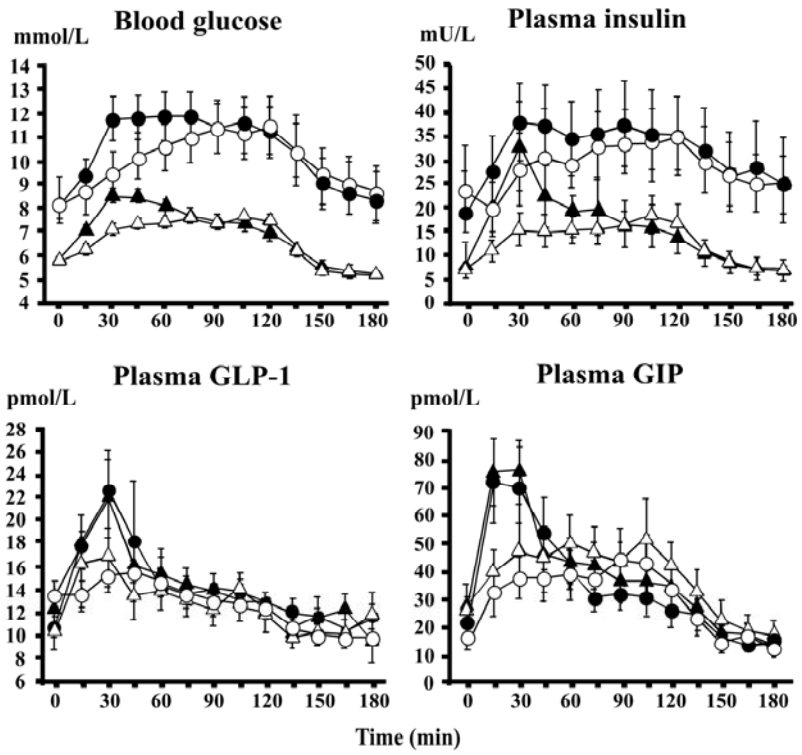

Figure 2.4

Effect of an initially more rapid intraduodenal glucose infusion (~12.6 kJ/min or 3 kcal/min between t = 0 and 15 min and ~3.0 kJ/min or 0.71 kcal/min between t = 15 and 120 min) (closed symbols) compared to constant infusion (~4.2 kJ/min or 1 kcal/min between t = 0 and 120 min) (open symbols) in healthy subjects (n = 8) (triangles) and type 2 patients (n = 8) (circles) on blood glucose, plasma insulin, plasma GLP-1, and plasma GIP. Each pair of curves differs between 0 and 30 min for variable vs. constant intraduodenal infusion (P < 0.05), showing that, modest variations in the initial rate of duodenal glucose entry may have profound effects on subsequent glycaemic, insulin, and incretin responses. Adapted from (O'Donovan et al. 2004b).

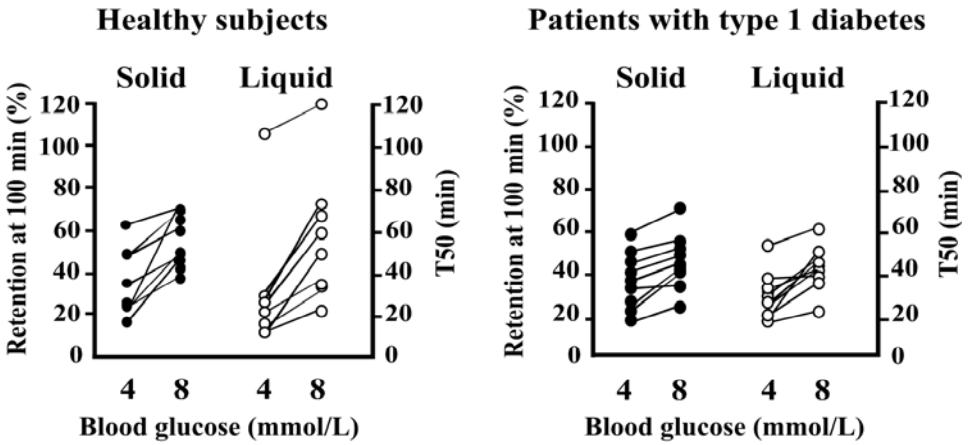

Figure 2.5

Solid and liquid gastric emptying in healthy subjects (n = 8) and type 1 patients (n = 9) during euglycaemia (blood glucose 4 mmol/L) and "physiological" hyperglycaemia (blood glucose 8 mmol/L). This study demonstrated that emptying of solids and liquids is slower during hyperglycaemia. Adapted from (Schvarcz et al. 1997).

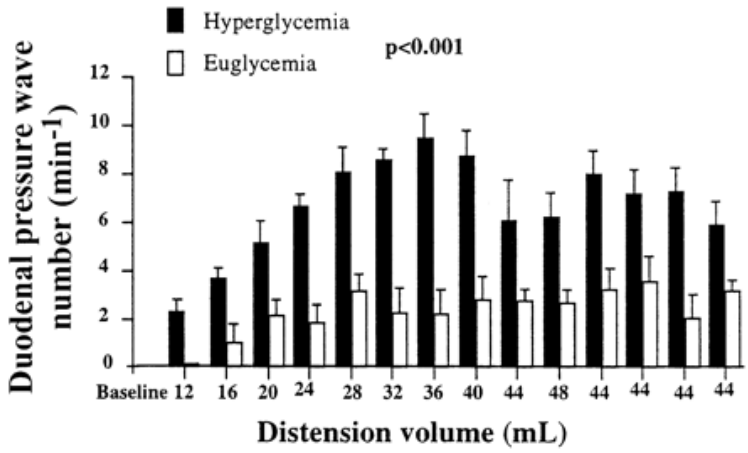

Figure 2.6

Effects of duodenal distension on the number of duodenal pressure waves in healthy subjects (n = 9) during hyperglycaemia (blood glucose - 10 mmol/L) and euglycaemia (blood glucose - 4 mmol/L). Duodenal distension stimulated duodenal pressure waves (P < 0.01), the response being volume related (P < 0.05) and greater (P < 0.001) during hyperglycaemia than euglycaemia (by ANOVA).Data are means ± SE. Adapted from (Lingenfelser et al. 1999).

CHAPTER 3:

COMMON METHODOLOGIES

3.1 INTRODUCTION

The techniques used by the author are all validated and well accepted methods to assess antropyloroduodenal motility, gastric emptying and appetite, with the exception of the use of impedance monitoring to evaluate duodenal flow (discussed in chapter 7).

3.2 SUBJECTS AND TYPE 2 DIABETIC PATIENTS

Healthy subjects

Healthy subjects (aged 18 - 65 yr) were recruited by the placement of information flyers on hospital and local university bulletin boards. Prior to enrole, volunteers were screened to exclude those with evidence of gastrointestinal disease, previous gastrointestinal surgery, and those who were taking medication known to affect gastrointestinal motility and/or appetite. Those with significant respiratory or cardiovascular disease, diabetes, epilepsy, who smoked > 10 cigarettes/day, or consumed > 20 g alcohol/day, were excluded.

Patients with type 2 diabetes

Patients with type 2 diabetes, as defined by World Health Organisation (WHO) criteria (WHO 1999) (studies reported in chapters 5), were recruited by the placement of information flyers on hospital notice boards and from community-based referrals attending the Royal Adelaide Hospital Diabetes Services. Prior to

enrolment subjects were screened to exclude those with significant respiratory or cardiovascular disease, epilepsy, who smoked > 10 cigarettes/day, or consumed > 20 g alcohol/day. All type 2 patients were managed by diet alone; those requiring oral hypoglycaemic therapy and/or insulin were excluded.

3.3 ETHICAL APPROVAL

All study protocols were approved by the Royal Adelaide Hospital Research Ethics Committee, the University of Adelaide, and, where appropriate, the Royal Adelaide Hospital Investigational Drug Sub-Committee, prior to their initiation.

3.4 STUDY ENVIRONMENT

The majority of the studies were performed in either the clinical research rooms of the Discipline of Medicine, University of Adelaide, or the Gastrointestinal Investigation Unit of the Department of Gastrointestinal and General Medicine, both at the Royal Adelaide Hospital; those involving radioisotopic measurement of gastric emptying were performed in the Department of Nuclear Medicine PET and Bone Densitometry at the Royal Adelaide Hospital. The posture of the subject during studies varied and this is specified for each study - most studies were performed with the subject sitting when gastric emptying was measured, and while lying supine when manometry and/or intestinal infusion were performed (chapters 4, 7, 8, 9 and 11). Where appetite was an outcome measure (chapter 9 and 11) subjects were not permitted to wear a watch, or listen to the radio, in order to minimise the influence of external cues.

3.5 DRUGS

Hyoscine butylbromide (Buscopan, Boehringer Ingelheim Australia), made up to 10 mL in normal saline, was infused intravenously in a dose of 20 mg over 2 min as a bolus, followed by a continuous infusion (20 mg made up to 60 mL with normal saline) over 60 min (study reported in chapter 7).

Benzocaine (Subcutin N; comprising benzocaine 0.75%, polyoxyl-40-hydrogenated castor oil 9.25%, propylene glycol 20%, distilled water 70%) (Ritsert Inc, Eberbach, Germany) was infused intraduodenally as an initial 10 mL bolus, followed by a continuous infusion over 90 min (study reported in chapter 8).

3.6 ANTROPYLORODUODENAL MOTILITY, GASTRIC EMPTYING AND DUODENAL FLOW MEASUREMENT AND ANALYSIS TECHNIQUES

3.6.1 Antropyloroduodenal manometry

The use of perfusion manometry to measure intraluminal pressures provides an accurate assessment of antral, pyloric and duodenal pressures (Camilleri et al. 1998; Rayner et al. 2002; Rayner et al. 2004). Transducers linked to a manometric catheter allow the concurrent recording of luminal pressures at multiple points along the gastrointestinal tract. Intraluminal pressures exhibit significant variation even over short distances, therefore, closely spaced (1.5 cm apart) pressure sensors are essential (Sun et al. 1995; Camilleri et al. 1998). The pylorus has a narrow contractile zone (~2 mm), hence optimal measurement of pyloric motility requires

the incorporation of a sleeve sensor (usually 4.5 cm) into the design of the catheter (Houghton et al. 1988b); the development of the pyloric sleeve sensor is an adaptation of the sensor developed originally for lower oesophageal sphincter manometry (Dent 1976). A schematic representation of a typical manometric catheter is shown (Figure 3.1).

The position of the catheter was monitored by continuous measurement of the antroduodenal transmucosal potential difference (TMPD) gradient (Heddle et al. 1988b); this is essential to correct for antegrade and retrograde movement of the catheter. The secretions of the stomach (primarily hydrochloric acid) and proximal duodenum (bicarbonate ions) produce electrically negative and electrically neutral charges respectively, when compared to the reference point (the forearm). Using TMPD recordings, measured via voltage transducers connected to these two channels, the position of the sleeve sensor across the pylorus can be maintained. The TMPD between the stomach and duodenum was measured using side-holes located at the oral and orad margins of the sleeve sensor (i.e. the most distal antral side-hole and the most proximal duodenal side-hole). The channels corresponding to these side-holes were perfused independently of each other, and the other pressure channels, with 0.9% degassed saline; 2.2 M potassium chloride electrodes (Heddle et al. 1988a; Heddle et al. 1988b) connected each of these side-holes to calomel half cells (Ionode, Qld, Australia). A common reference electrode (a sterile saline filled 21 G cannula placed subcutaneously in the forearm, also connected to a calomel half-cell), established an electric circuit enabling continuous measurement of TMPD across the stomach and small intestine (Sun et al. 1995).

Data were subsequently only analysed when TMPD measurements indicated correct positioning across the pylorus (Figure 3.2). The criteria used for this were that the antral TMPD potential was < -20mV, the duodenal TMPD potential was > -15mV (Heddle et al. 1988b). Studies involving manometry used either 4 mm (outer diameter), or 4.5 mm, silicone rubber manometric assemblies (Dentsleeve, Wayville, South Australia) which incorporated 2-4 antral side-holes, a pyloric sleeve and between 3-7 duodenal side-holes (chapters 7, 8, 9 and 11). All side-holes were perfused at a rate of 0.15 ml/min; the TMPD side-holes with degassed 0.9% saline and the manometric side-holes with degassed distilled water (Sun et al. 1996). After an overnight fast (12 hr for solids and 10 hr for liquids) the manometric assembly was inserted via an anaesthetised nostril into the stomach, and allowed to pass by peristalsis into the duodenum; this took between 20 - 150 min.

Manometric pressures were digitised using an NBM1016H data acquisition board, and recorded on a computer-based system (PowerMac 7100/75; Apple Computer, Cupertino, CA, USA) running commercially available software (HAD; written by Assoc Prof G Hebbard, Royal Melbourne Hospital, Melbourne, Australia in Labview 3.0.1 (National Instruments) and Flexisoft; written by Assoc Prof G Hebbard, Royal Melbourne Hospital, Melbourne, Australia)) and then stored for later analysis. Antropyloroduodenal pressures were analysed using software (written by Prof A Smout, Department of Gastroenterology, University Hospital Utrecht, The Netherlands). All manometric analysis was done manually by two investigators (one of whom was the author) blinded to the specific study

interventions. Parameters assessed included isolated waves in the antrum, pylorus and duodenum, isolated antral, pyloric waves and duodenal waves with an amplitude ≥ 10 mmHg were analysed (Andrews et al. 2001). Pressure wave sequences and basal pyloric pressure were also assessed; waves were characterised as either isolated, when they occurred in only one channel, or as part of a pressure sequence, when they occurred in at least two channels. A pressure wave sequence was defined as two or more temporally-related pressure waves. Pressure waves in adjacent channels were regarded as temporally related if they had onsets within ± 3 s (in the duodenum) or 5 s (in the antrum) of each other (Andrews et al. 2001). In addition, isolated waves were characterized by their amplitudes, and pressure wave sequences by the distance travelled. Basal pyloric pressure ('tone') was calculated for each minute by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side-hole from the mean basal pressure recorded at the sleeve (Heddle et al. 1988b), using custom written software (MAD, Prof C-H Malbert, Institut National de la Recherche Agronomique, Rennes, France).

3.6.2 Gastric emptying

Three techniques were used to assess gastric emptying, scintigraphy (chapter 5), ultrasonography (chapter 6) and a C13 breath test (chapter 10). In the studies reported in chapters 6 and 10 only gastric emptying of liquid was quantified.

Scintigraphy

For the measurement of gastric emptying, radionuclide markers are incorporated into liquid, solid, or mixed liquid/solid meals. All tests assume that the gastric emptying of the radionuclide adequately represents the behaviour of the test meal, and since the liquid and solid phases of a mixed liquid/solid meal may empty at different rates, the precise identification of each phase is necessary for accurate definition of the emptying of either phase, or of the total meal (Collins et al. 1983). The isotope used in the studies presented in this thesis was ^{99m}Technetium. -^{99m}Tc is favoured due to its short half life (6 hr), relatively low cost and wide availability. In all studies radio-isotopic data were acquired in 1 min frames for the first 60 min and in 3 min frames thereafter. Data were corrected for subject movement, radionuclide decay and, where appropriate, gamma ray attenuation Compton Scatter. A region-of-interest was drawn for the total stomach, which was subsequently divided into proximal and distal regions, with the proximal region corresponding to the fundus and proximal corpus and the distal region corresponding to the distal corpus and antrum (Collins et al. 1983). Gastric emptying curves were derived for total, proximal and distal regions and expressed as percent retention over time. For the total stomach, the duration of the lag phase (determined visually as the time before any radioactivity appeared in the proximal small intestine) was also derived (Collins et al. 1983).

Ultrasonography

The use of ultrasonography provides a non-invasive method for the study of gastric function in real time and potentially in 3-dimensions without the need for radiation

exposure. Gastric emptying can be measured with ultrasound using a variety of techniques (Scarpignato and Galmiche 1990; Gilja et al. 1999). The most common of these is to image a standardised parasagittal area in the antrum with both the aorta and the superior mesenteric vein in the field of view (Bolondi et al. 1985). Using a built in measurement programme, included on all modern ultrasound machines, the circumference of the antrum can be outlined and the area then calculated. The area recorded during the fasting state is subtracted from the subsequent measurements made after a meal. Gastric emptying is expressed at any time as: $A_{C(t)} = 100-((A_{(t)} / A_{max})\times 100)$ where $A_{C(t)}$ = corrected antral area at a time point, $A_{(t)}$ = area measured at a given time and A_{max} = maximum antral area recorded after meal ingestion (Hveem et al. 1994). The technique is operator dependent, technically demanding in obese subjects and in the presence of large amounts of bowel gas, and can only be used to measure meals of specific composition, mainly liquids. Measurement of gastric emptying of liquids by ultrasonography in this way has been shown to correlate closely with scintigraphic assessment (Hveem et al. 1996). In the study reported in chapter 6 gastric emptying of an alcohol-containing drink was assessed by ultrasound by measurement of antral area. More recently, 3-dimensional ultrasonography imaging, both of the proximal and distal stomach, has been used to quantify gastric emptying (Gilja et al. 1997; Gentilcore et al. 2006b).

Radioisotopic breath test

The use of isotopic breath tests to evaluate gastric emptying is based on the principle that gastric emptying is the rate-limiting step in the absorption of ^{13}C-

octanoate from the small intestine and its metabolism to $^{13}CO_2$ (de Meer et al. 1999; Jackson and Bluck 2005). In healthy subjects there is a good correlation between scintigraphic and breath test measurements of gastric emptying (Jackson et al. 2004). A major advantage of breath tests is their relatively low cost, simplicity and non-invasive nature (with the use of stable isotopes). In the study reported in chapter 10, breath samples were analysed for $^{13}CO_2$ concentration using an isotope ratio mass spectrometer (Europa Scientific, ABCA model 20/20, Crewe, UK). Samples were considered to be end-expiratory if the CO_2 concentration was > 1 %. The $^{13}CO_2$ concentration of each sample was expressed relative to the international standard (PDB Limestone), which has the highest natural enrichment of ^{13}C. The values obtained were converted to the percent dose recovery (PDR) per hour from the baseline and used to determine the cumulative percent dose recovery (cPDR) per hour during the 6 hr period following administration (Sun et al. 2003).

3.6.3 Impedancometry

Intraluminal impedance recording provides a means of determining flow events by monitoring changes in electrical impedance between pairs of electrodes positioned within the gut lumen. The intraluminal electrical impedance between two electrodes is inversely proportional to the electrical conductivity of the luminal contents and the cross-sectional area. When compared with the muscular wall, air has a lower electrical conductivity and yields an increase in impedance. In contrast, saliva or nutrients, have a higher conductivity and, therefore, yield drop in impedance at the corresponding measurement segments. The passage of a fluid bolus results in a fall in impedance, while an air bolus increases impedance; the

transit of the bolus along a gut segment can be monitored by recordings from sequential electrode pairs (Figure 3.3). Impedance monitoring has now been used widely to evaluate oesophageal motility (Nguyen et al. 1999). In the study reported in chapter 7, the assembly incorporated an impedance catheter with 6 electrode pairs spaced at 3 cm intervals (external diameter 2 mm) (Sandhill Scientific Inc, Highlands Ranch, Colorado, USA) in parallel with a multilumen silicone manometry catheter (external diameter 4 mm) (Dentsleeve, Wayville, Australia) with 6 duodenal side-holes spaced at 3 cm intervals. The location of the manometric side-holes corresponded to the midpoint of each electrode pair, so that measurement of duodenal pressure waves, propagated pressure sequences and flow events could be obtained concurrently. Both the manometric and impedance signals were recorded 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 (of whom one was the author), who were blinded to the study conditions. A flow event was defined as a transient decrease in impedance of $\geq$ 12% from baseline (Imam et al. 2004) in at least 3 sequential electrode pairs (i.e. $\geq$ 6 cm) (Nguyen et al. 1995) and flow events were classified as either antegrade or retrograde (Nguyen et al. 1995; Imam et al. 2004). The detection of a flow event was compared between the two observers, and consensus was then reached over discrepant interpretations.

3.7 APPETITE AND ENERGY INTAKE MEASUREMENT AND ANALYSIS TECHNIQUES

Assessments of appetite and energy intake were performed in chapters 9 and 11 using a number of methods. In all studies those subjects with clinical evidence of an eating disorder were excluded.

3.7.1 3 - Factor eating questionnaire

The three factor eating questionnaire, developed by Stunkard and Messick (1985) (Stunkard and Messick 1985) is the most widely used screening method for detecting 'abnormal' eating behaviour and was used in the studies reported in chapters 9 and 11. It was designed to measure three factors related to human feeding habits: 1) cognitive restraint of eating, 2) disinhibition and 3) hunger. Cognitive restraint, described as the tendency of individuals to restrict their food intake in order to control their body weight, is the most relevant to the studies presented in this thesis, as the latter were designed to evaluate the effects of a specific intervention on appetite sensations and food intake. The Stunkard and Messick (1985) questionnaire demonstrated a final mean score for dietary restraint (± SEM) of 14.3 ± 3.6 amongst 'dieters' and 6.0 ± 5.5 amongst 'free-eaters'. Accordingly, in all the studies presented in this thesis a score < 11 for dietary restraint was used as an exclusion criterion. This arbitrary value has been used in previous studies to recruit 'unrestrained' eaters (Rolls et al. 1988; Rolls et al. 1990).

3.7.2 Visual Analogue Scales (VAS)

Visual analogue scales (VAS) are the most common form of assessment of sensations of appetite including hunger, fullness, prospective consumption and desire to eat (Sepple and Read 1989; Chapman et al. 1999; Chapman et al. 2005). They consist of horizontal lines (of specified length) with words describing two extremes of sensation anchored at each end. Subjects make a vertical mark along the line corresponding to the strength with which they are experiencing a particular sensation at any given time. The VAS used in this thesis were in the form of a validated questionnaire (Sepple and Read 1989) with the extremes of a particular sensation written at either end of a 10 cm horizontal line. Subjects were instructed to place a vertical mark, indicating the strength of the nominated sensation.

3.7.3 Energy intake

Arguably, the most effective way of measuring energy intake is to quantify food intake at an *ad libitum* test meal. A standard, or modified, buffet meal was employed for this purpose (Lavin et al. 1996a). This was offered to the subjects at a fixed time interval after a study intervention and subjects were then given 30 min to eat until they felt comfortably full. Food items included in the cold buffet meals were whole meal bread, white bread, sliced ham, sliced chicken, sliced cheese, sliced tomato, lettuce, sliced cucumber, strawberry yoghurt, fruit salad, chocolate custard, apple, banana, unsweetened orange juice, iced coffee, water, margarine and mayonnaise. The quantity of food that was available was in excess of what the subject would normally be expected to eat (Lavin et al. 1996a). All food items were weighed before and after the meal. Energy intake and macronutrient distribution

(%) were calculated from the amount of food consumed during the buffet meal using Foodworks Nutritiona software (Version 3.1, Xyris Software, Highgate Hill, Qld, Australia).

3.8 AUTONOMIC NERVE FUNCTION

Autonomic nerve function was evaluated in the studies reported in chapter 5 using standardised cardiovascular reflex tests (Ewing and Clarke 1982). Parasympathetic function was evaluated by the variation (R-R interval) of the heart rate during deep breathing and the immediate heart rate response to standing ("30:15" ratio). Sympathetic function was assessed by the fall in systolic blood pressure in response to standing. Each test result was scored according to age-adjusted pre-defined criteria as 0 = normal, 1 = borderline or 2 = abnormal, for a total maximum score of 6. A score $\geq$ 3 was considered to indicate definite autonomic dysfunction (Ewing and Clarke 1982; Piha 1991).

3.9 BIOCHEMICAL/HORMONAL MEASUREMENTS

Cannulae for blood sampling were inserted into a forearm vein and blood samples drawn at specific time points. Blood samples were immediately placed into ice-chilled EDTA dipotassium-treated tubes containing 400 KIU/ml blood aprotonin (Bayer Australia Ltd, Pymple, Australia). Plasma was isolated within 60 min of blood sample collection by centrifugation at 3200 rpm for 15 min at 4ºC and stored at -70ºC until assayed.

3.9.1 Blood glucose and gut peptides

Blood glucose

Glucose concentration (mmol/L) of whole blood was measured at the time of collection with a portable glucometer (Medisense Inc, Bedford, MA, USA) using the glucose oxidase method; the accuracy of which has previously been confirmed using the hexokinase technique (Horowitz et al. 1991b).

Plasma insulin

Plasma insulin (mU/L) was measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc, Webster, Texas). The sensitivity of the assay was 0.26 mU/L; the intra assay coefficient of variation was 2.6 %, and the inter-assay coefficient of variation was 6.2% (Horowitz et al. 1996a).

Glucagon-like peptide-1 (GLP-1)

Plasma GLP-1 (pmol/L) was measured by radioimmunoassay using an adaptation (Wishart et al. 1998) of a previous method (Orskov et al. 1991). Standards were prepared in charcoal-stripped plasma and extracted in 66% ethanol along with the samples. Extracts were dried down under N_2 and resuspended in assay buffer (0.1 M phosphase, 3.9 g/L ETDA, 1 g/L HAS, 0.6 mM thimmerosol, 1.3 g/L aminocaproic acid, pH 7.4). Antibody did not cross-react with glucagon, glucose-dependent insulinotropic polypeptide (GIP), or other gut or pancreatic peptide, and has been demonstrated by chromatography to measure intact GLP-$1_{(7\text{-}36)}$ amide. $_{125}$I-labeled GLP-1 was prepared using the lactoperoxidase method and purified by HPLC for use as tracer. Incubation was for 48 hr at 4ºC. The antibody-bound

fraction was separated by the addition of dextran-coated charcoal containing gelatin (0.015 g gelatin, 0.09 g dextran, 0.15 g charcoal in 30 ml assay buffer) and the radioactivity determined in the supernatants following centrifugation. The intra-assay coefficient of variation was 17 %, and the inter-assay coefficient of variation was 18 %. Sensitivity was 1.5 pmol/L.

Glucose-dependent insulinotropic polypeptide (GIP)

Plasma GIP was measured by radioimmunoassay, with some modification of a published method (Wishart et al. 1992). The standard curve was prepared in buffer, rather than extracted charcoal stripped serum, and the radioiodinated label was supplied by ProSearch International (Victoria, Australia). Intra- and inter-assay coefficients of variation were both 15%.

Cholecystokinin (CCK)

Plasma CCK (pmol/L) was determined after ethanol extraction by radioimmunoassay, as described previously (MacIntosh et al. 2001c). A commercially available antibody (C2581, Sigma Chemical, St Louis, MO, USA), raised in rabbits against synthetic sulphated CCK-8, was employed. This antibody binds to all CCK peptides containing the sulphated tyrosine residue in position 7, shows 26% cross-reactivity with un-sulphated CCK-8, less than 2% cross-reactivity with human gastrin, and does not bind to structurally unrelated peptides. The intra-assay coefficient of variation was 9.5% at 50 pmol/L and the inter-assay coefficient of variation was 27% with a sensitivity of 1 pmol/L.

3.9.2 3-O-methylglucose (3-OMG)

3-OMG is an analogue of glucose, which uses the same intestinal active transport mechanism. Unlike glucose, 3-OMG is not metabolised by the liver and is renally cleared. Plasma concentrations of 3-OMG have, therefore, been used as an index of glucose absorption (Fordtran et al. 1962). In the study reported in chapters 7, plasma 3-OMG concentrations were measured by gas liquid chromatography (Rayner et al. 2002).

3.9.3 Plasma alcohol

In the study reported in chapter 6, plasma alcohol concentrations were determined by gas chromatography (Cooper 1971).

3.10 STATISTICAL ANALYSIS

All data were expressed as mean values ± standard error of mean (SEM). In general, antropyloroduodenal pressures, gastric emptying rates, appetite ratings, blood glucose and plasma hormone concentrations were analysed using two-way repeated measures analysis of variance (ANOVA) (with treatment, patient group or time as the two factors). Post-hoc analysis was performed when the initial comparison was found to be significant. Student's t-test was employed to compare means for energy intake and macronutrient composition when these data were distributed normally. Correlations were performed using linear regression analysis. Statview (SAS Institute Inc., Cary, NC, USA) and SuperAnova Version 1.11 (Acabus Conceots, Berkley, CA, USA) software packages were used to perform the analyses. A P-value of < 0.05 was considered significant.

3.11 CONCLUSIONS

The studies reported in this thesis employed a number of diverse, and in many cases complimentary, techniques. Manometry was used to evaluate antropyloroduodenal motility (chapters 7, 8, 9 and 11) and impedance monitoring to evaluate duodenal flow (chapter 7). Scintigraphy was used to assess gastric emptying of a solid meal (chapter 5) and ultrasonography (chapter 6) and a radioisotopic breath test (chapter 10) used to evaluate liquid emptying. Perceptions of appetite were evaluated by visual analogue questionnaires and energy intake by the consumption at a buffet meal (chapters 9 and 11). Blood samples were obtained for measurement of blood glucose and hormonal responses in all studies. In type 2 diabetic patients, autonomic function was evaluated by cardiovascular reflex testing (chapter 5).

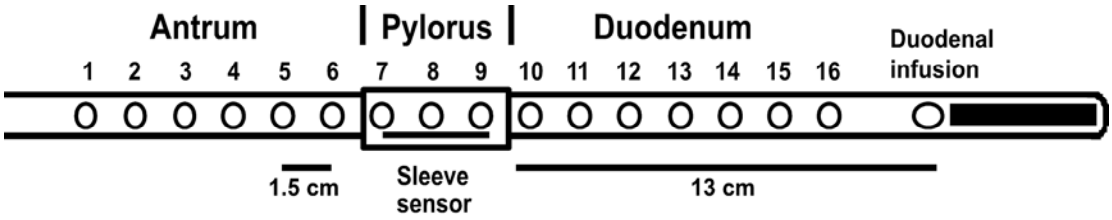

Figure 3.1

Schematic representation of a manometric catheter used to measure antropyloroduodenal pressures.

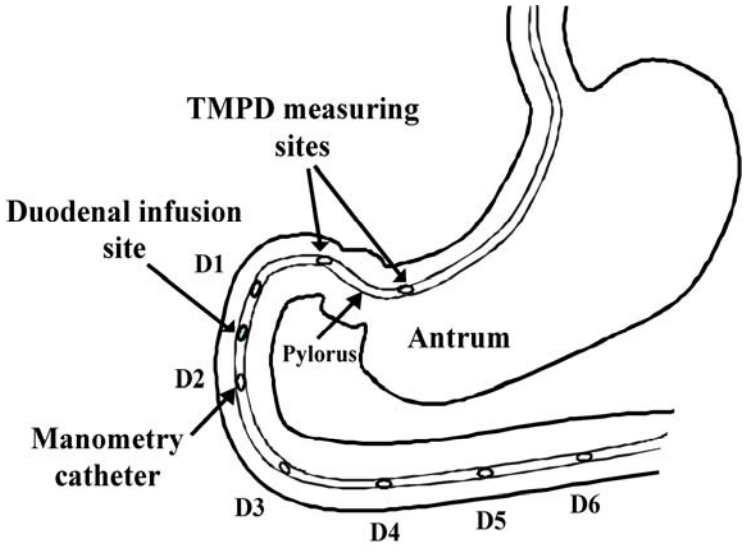

Figure 3.2

Schematic representation of the placement of a manometric catheter across the pylorus using measurement of the antroduodenal tranmucosal potential difference (TMPD).

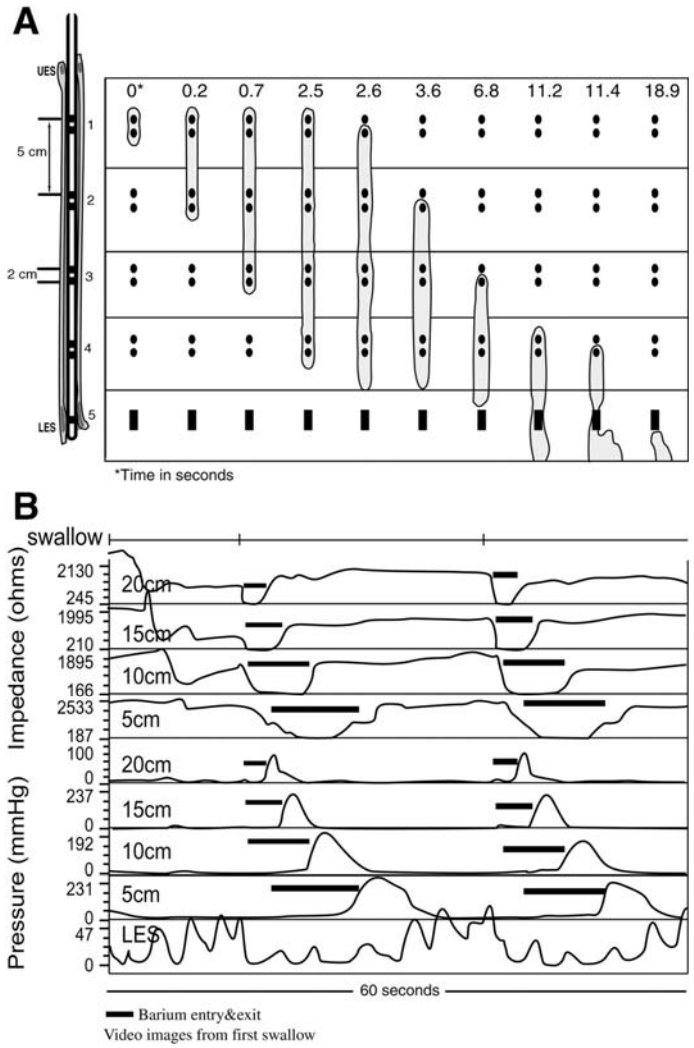

Figure 3.3

Simultaneous recording of oesophageal manometry-impedance shows normal bolus entry and exit by impedance with both swallows, and normal oesophageal peristalsis with a contraction amplitude of >30 mmHg at all sites (Imam et al. 2005).

CHAPTER 4:

INITIALLY MORE RAPID SMALL INTESTINAL GLUCOSE DELIVERY INCREASES PLASMA INSULIN, GIP AND GLP-1, BUT DOES NOT IMPROVE OVERALL GLYCAEMIA IN HEALTHY SUBJECTS

4.1 SUMMARY

The rate of gastric emptying of glucose-containing liquids is a major determinant of postprandial glycaemia. The latter is also dependent on stimulation of insulin secretion by glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). While overall emptying of glucose approximates 1-3 kcal/min, the "early phase" of gastric emptying is usually more rapid. The hypothesis that increased stimulation of incretin hormones and insulin by a more rapid initial rate of small intestinal glucose delivery would reduce the overall glycaemic response to a standardised enteral glucose load was evaluated. 12 healthy subjects were studied on two separate days on which they received an intraduodenal (ID) glucose infusion for 120 min; on one day the infusion rate was variable, being more rapid (6 kcal/min) between t = 0-10 min and slower (0.55 kcal/min) between t = 10-120 min, whereas, on the other day, the rate was constant (1 kcal/min) from t = 0-120 min, i.e. on both days 120 kcal were given. Between t = 0-75 min plasma insulin, GIP and GLP-1 were higher with the variable infusion. Despite the increase in insulin and incretin hormones, blood glucose levels were also higher. Between t = 75-180 min blood glucose and plasma insulin were lower with the variable infusion. There was no difference in the area under the curve (AUC) 0-180 min for blood glucose. In conclusion the stimulation of incretin hormone and insulin

release by a more rapid initial rate of ID glucose delivery does not lead to an overall reduction in glycaemia in healthy subjects.

4.2 INTRODUCTION

While the relative importance of the factors which determine postprandial blood glucose concentrations is controversial (Gerich 2003), It has been established that in both healthy subjects (Horowitz et al. 1993a; Berry et al. 2003) and patients with diabetes mellitus (Schwartz et al. 1994; Jones et al. 1996a; Rayner et al. 2001) the rate of carbohydrate entry into the small intestine is important. The concept that postprandial glycaemia is a major determinant of average glycaemic control, as assessed by glycated haemoglobin (Avignon et al. 1997; El-Kebbi et al. 2004), and is likely to represent an independent risk factor for cardiovascular disease (Chiasson et al. 2003; Gerich 2003), has focussed attention on dietary and pharmacological strategies aimed at reducing postprandial glycaemic excursions, including by modulating the rate of gastric emptying (Rayner et al. 2001).

It is well established that enterally administered glucose stimulates insulin secretion more than a comparable amount of glucose given intravenously (Creutzfeldt 1979). This so-called 'incretin" effect is due to the secretion of gut hormones, including GIP and GLP-1 (Creutzfeldt 1979). Type 2 diabetes is characterised by a reduced 'early' (and frequently increased 'late') postprandial insulin response. In animals, a small 'early' increase in portal vein/peripheral blood insulin levels is more effective than a larger, 'later' increase in reducing blood glucose levels (de Souza et al. 2001b). Furthermore, the release of GIP and GLP-1

is related to the rate of carbohydrate entry into the small intestine (Schirra et al. 1996a). Therefore, it is possible that an initially more rapid rate of small intestinal delivery of a standardised glucose load would be associated with a reduction in the overall glycaemic response.

Gastric emptying of glucose is closely regulated (Moran and McHugh 1981; Hunt et al. 1985a; Lin et al. 1989) so that in humans, duodenal glucose delivery remains relatively constant at a rate of 1-3 kcal/min over a wide range of concentrations. This regulation is due to small intestinal feedback, the extent of which is dependent on the length of small intestine contacted by glucose (Lin et al. 1989). The initial rate of gastric emptying of liquids, sometimes referred to as "gastric emptying during gastric fill" (Kaplan et al. 1992), is, however, usually more rapid than the subsequent, overall linear, delivery, presumably, at least in part, because of the time taken to initiate effective small intestinal inhibitory feedback. This "early phase" of emptying is usually 5-15 min in duration, is influenced by intragastric drink volume, and associated with duodenal glucose delivery of the order of 6 kcal/min (Brener et al. 1983a; Hunt et al. 1985a; Kaplan et al. 1992; Horowitz et al. 2002b).

A recent study sought to determine the impact of changes in the rate of glucose entry into the small intestine on the secretion of GIP, GLP-1 and insulin, and blood glucose (O'Donovan et al. 2004b). Healthy subjects and patients with type 2 diabetes received an identical intraduodenal glucose load over 120 min on 2 days; on one day the infusion rate was variable, being more rapid initially (3 kcal/min for

15 min), and subsequently slower, whereas on the other day, the infusion was constant (1 kcal/min). This modest variation in the initial rate of small intestinal glucose entry, within the physiological range, resulted in marked early differences in glycaemic, insulin and incretin responses. However, any subsequent reduction in glycaemia on the "variable" infusion day was minimal, and the overall area under the blood glucose curves did not differ (O'Donovan et al. 2004b). From the above discussion it is apparent that this negative outcome may have reflected the fact that the initial rate of glucose delivery with the "variable" glucose infusion (3 kcal/min) was less than may occur physiologically. In the current study the author has, accordingly, evaluated the effects of a more rapid rate of intraduodenal glucose infusion (6 kcal/min for 10 min) which is still within the physiological range for the "early" phase of gastric emptying.

4.3 METHODS

4.3.1 Subjects

12 healthy male subjects (age 33 ± 3 years, body mass index 24 ± 1.0 kg/m^2) were recruited by advertisement. No subject had a history of gastrointestinal disease or surgery, significant respiratory or cardiac disease, alcohol abuse, or epilepsy, smoked more than 10 cigarettes/day, or was taking medication known to affect gastrointestinal function.

4.3.2 Protocol

Each subject underwent paired studies, separated by an interval of 4 - 7 days, in randomised order. Following an overnight fast (14 hours for solids and 12 hours

for liquids), the subject attended the laboratory at 0900h. A manometric assembly (diameter 4 mm) was inserted into the stomach via an anaesthetised nostril, and allowed to pass into the duodenum by peristalsis (O'Donovan et al. 2004b). The assembly included an infusion channel with a port located 10 cm distal to the pylorus, and two other channels, positioned in the antrum and duodenum respectively, perfused with saline at 0.15 ml/min (O'Donovan et al. 2002). The position of the assembly was monitored by measurement of the antroduodenal transmucosal potential difference, using a reference electrode (a 20G intravenous cannula filled with sterile saline) placed subcutaneously in the forearm (Cook et al. 1997b). An intravenous cannula was inserted in a forearm vein for blood sampling; the forearm was heated (using a heat pad) to obtain "arterialised" samples. The subject was then allowed to rest comfortably in the recumbent position for ~20 min.

At time t = 0 min, an intraduodenal infusion of 50% glucose, or a mixture of 50% glucose and water, was infused at a rate of 3 ml/min between 0 - 120 min. On one day the rate of energy delivery was 6 kcal/min between 0 - 10 min and 0.55 kcal/min from 10 - 20 min; on the other day the energy delivery was maintained at 1 kcal/min from 0 - 120 min i.e. on both days a total of 360 ml and 120 kcal of glucose was infused intraduodenally. Furthermore, the volumes infused from t = 0-10 and t = 10 - 120 min were the same on both days. At t = 240 min the manometric assembly was removed. Blood samples (~20 ml volume) were obtained immediately before the commencement of the intraduodenal infusions (t = 0) and subsequently at 2, 4, 6, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150,

165 and 180 min for measurement of glucose, insulin, GLP-1 and GIP, using established methods (O'Donovan et al. 2002).

4.3.3 Measurements

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

Blood samples for determination of plasma insulin, GLP-1, and GIP were collected in ice-chilled tubes containing EDTA and 400 kIU aprotonin (Trasylol; Bayer Australia Ltd., Pymple, Australia) per liter blood. Plasma was separated by centrifugation and stored at -70 ºC for subsequent analysis.

Blood glucose concentrations were determined immediately using a portable glucose meter (Medisense Precision QID, Abbott Laboratories, Bedford, MA). Plasma insulin concentrations were measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc., Webster, TX).

Plasma GLP-1 and GIP concentrations were measured by RIA: (Wishart et al. 1998; O'Donovan et al. 2004b).

4.3.4 Statistical analysis

Data were evaluated for two time periods (0 - 75 min and 75 - 180 min) using repeated measures ANOVA with treatment and time as factors. Areas under the curves (AUCs 0 - 180 min) for blood glucose and plasma GLP-1, GIP and insulin were calculated using the trapezoidal rule, and compared using Student's t-test.

Statistical significance was accepted at $P < 0.05$, and data are presented as mean values ± SE.

4.4 RESULTS

All subjects tolerated the study well. Fasting concentrations of glucose, insulin, GIP and GLP-1 did not differ between the two days.

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

Blood glucose

Blood glucose increased from baseline during each infusion ($P < 0.05$). Between 0-75 min blood glucose concentrations were greater during the variable, compared with the constant, infusion ($P < 0.05$). In contrast, between 75-180 min, blood glucose concentrations were less ($P < 0.05$) during the variable infusion. There was no difference in the AUC (0 - 180) of blood glucose between the two study days (1164 ± 44 vs 1124 ± 46) (Figure 4.1a).

Plasma insulin

Plasma insulin concentrations increased from baseline during each infusion ($P < 0.05$). Between 0-75 min plasma insulin concentrations were higher during the variable, compared with the constant, infusion ($P < 0.05$). Between 75 - 180 min, plasma insulin was less ($P < 0.05$) during the variable infusion. The AUC 0 - 180 min for plasma insulin was greater (5153 ± 609 vs 3462 ± 493, $P < 0.05$) during the variable infusion (Figure 4.1b).

Plasma GLP-1

Plasma GLP-1 concentrations increased from baseline during each infusion ($P < 0.05$). Between 0 - 75 min plasma GLP-1 concentrations were greater during the variable, when compared with the constant, infusion ($P < 0.05$). Between 75 -180 min, there was no difference in plasma GLP-1 between the two infusions. The AUC 0 - 180 min for plasma GLP-1 was greater (2796 ± 238 vs 1807 ± 148, $P < 0.05$) with the variable infusion (Figure 4.1d).

Plasma GIP

Plasma GIP concentrations increased from baseline during each infusion ($P < 0.05$). Between 0 - 75 min plasma GIP concentrations were higher ($P < 0.05$) during the variable, compared with the constant, infusion. Between 75 - 180 min, there was no difference in plasma GIP between the two infusions. The AUC 0-180 min for plasma GIP was greater (5838 ± 623 vs 4580 ± 640, $P < 0.05$) with the variable infusion (Figure 4.1c).

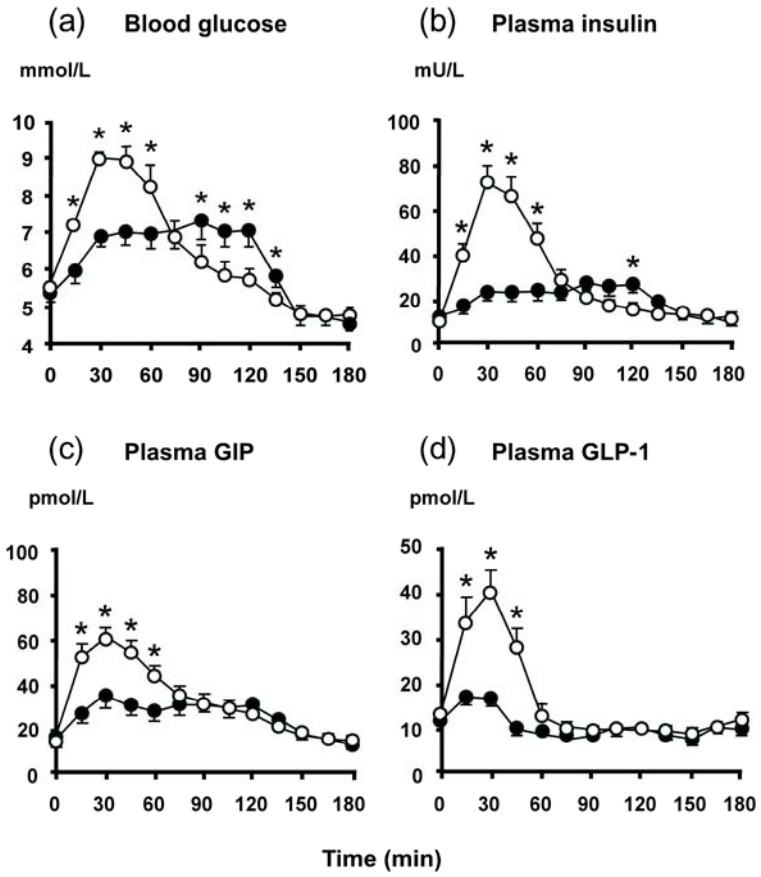

Figure 4.1

Effects of a variable intraduodenal glucose infusion (6 kcal/min between t = 0 - 10 min and 0.55 kcal/min between t = 10 - 120 min) (open symbols) and a constant infusion (1 kcal/min between t = 0 - 120 min) (closed symbols) in healthy subjects on (a) blood glucose, (b) plasma insulin, (c) plasma GLP-1 and (d) plasma GIP. Data are mean values ± S.E.M.

4.5 DISCUSSION

These observations indicate that the stimulation of incretin hormones and "early" insulin release by a more rapid initial rate of duodenal glucose delivery, designed to approximate the early phase of liquid gastric emptying, does not lead to an overall reduction in the glycaemic response to a standardised enteral glucose load given over a fixed time in healthy subjects.

The current study represents a logical development from a recent report, which evaluated the effects of more minor variations in the pattern of small intestinal delivery of glucose on glycaemic, insulin and incretin hormone responses in both healthy subjects and patients with type 2 diabetes (O'Donovan et al. 2004b). In that study the rates of intraduodenal glucose infusion (0.71 - 3 kcal/min) were selected to be within the reported range observed for the linear emptying phase of glucose-containing liquids in healthy subjects (Hunt et al. 1985a; Berry et al. 2003). Hence, the impact of the normal, initially more rapid, emptying phase was not evaluated. In comparing the two studies, there were substantial differences in the patterns of blood glucose, insulin and incretin hormone responses. In the initial study the variable glucose infusion was 3 kcal/min between 0 - 15 min followed by 0.71 kcal/min between 15 - 120 min; the current study employed 6 kcal/min between 0 - 10 min, followed by 0.55 kcal/min from 10 - 120 min. When compared with the previous study: (i) the magnitude of the initial rises in blood glucose, plasma insulin and plasma GLP-1, but not GIP, in response to the variable infusion, were substantially greater; (ii) during the variable infusion, blood glucose concentrations between 75 - 180 min were significantly less compared with those

which resulted from the constant infusion; and (iii) the overall glycaemic response was the same with the variable when compared with the constant infusion, rather than greater.

The substantial initial increase in blood glucose in response to the variable infusion is not surprising; even in healthy subjects, relatively minor differences in duodenal glucose delivery may have a major effect on the initial glycaemic response to carbohydrate meals (Horowitz et al. 1993a; Nauck et al. 1993a; Schwartz et al. 1994; Beckoff et al. 2001; O'Donovan et al. 2005b). The increased stimulation of insulin can be accounted for by the greater rise in blood glucose and GLP-1. The study confirms that there is modest stimulation of GLP-1 in response to a 1 kcal/min duodenal glucose infusion (O'Donovan et al. 2004b), which apparently conflicts with the report by Schirra et al (1996) suggesting that a threshold of duodenal glucose delivery in excess of ~1.8 kcal/min is required for GLP-1 release. It has been assumed that the stimulation of GLP-1 by enteral glucose is a load-, rather than concentration-dependent phenomenon, as has been documented to be the case for the regulation of gastric emptying of glucose (Hunt et al. 1985a; Lin et al. 1989), but this has not been formally evaluated and may account for the discrepancy. Nevertheless, the stimulation of GLP-1 secretion by the 6 kcal/min infusion was substantially greater than the response to 3 kcal/min, which is not surprising (Schirra et al. 1996a). Further studies are indicated to define the small intestinal glucose load which results in maximum stimulation of GLP-1 release.

In contrast, there was no difference in the initial GIP response between the two variable infusions in the current and previous study, suggesting that stimulation by 3 kcal/min may have already been maximal. In the study by Schirra et al. (1996), the GIP response to intraduodenal glucose infusion at 2.2 kcal/min was greater than that to 1.1 kcal/min. A difference in the effect of small intestinal glucose on GIP and GLP-1 is not unexpected - GIP is released from duodenal K cells, whereas GLP-1 is released from L cells whose concentration is greatest in the distal jejunum.

While the lowering of blood glucose between 75 - 180 min is likely to be attributable to the greater stimulation of plasma insulin which preceded it, it should be recognised that the rate of duodenal glucose delivery during this time was less with the variable, compared with the constant, infusion. The author did not employ glucose tracer techniques in this study, which may have clarified which of these phenomena made the greater contribution.

The study was designed to provide insights potentially relevant to the management of postprandial glycaemia in patients with type 2 diabetes. While the author did not study type 2 patients, it is well documented that first phase insulin secretion is impaired in this group, as is the incretin effect (Nauck et al. 1986). The latter appears to reflect impaired secretion of GLP-1 (Toft-Nielsen et al. 2001; Vilsboll et al. 2001) and a reduced insulinotropic effect of GIP (Nauck et al. 1993b; Vilsboll et al. 2002). Hence, these observations in healthy subjects add to the rationale for the use of dietary and pharmacological strategies designed to reduce postprandial

glycaemic excursions by slowing gastric emptying in this group, rather than initially accelerating it (Rayner et al. 2001).

CHAPTER 5:

EFFECTS OF FAT ON GASTRIC EMPTYING OF AND THE GLYCAEMIC INSULIN AND INCRETIN RESPONSES TO, A CARBOHYDRATE MEAL IN TYPE 2 DIABETES

5.1 SUMMARY

Gastric emptying (GE) is a major determinant of postprandial glycaemia. As the presence of fat in the small intestine inhibits GE, ingestion of fat may attenuate the glycaemic response to carbohydrate. This study evaluates the effect of patterns of fat consumption on GE, and glucose, insulin, GLP-1 and GIP concentrations, after a carbohydrate meal in type 2 diabetes. 6 male type 2 diabetics were studied in randomised order. On each day, subjects ingested (i) 30ml water, 30 min before the mashed potato ('water'), (ii) 30ml olive oil, 30 min before the mashed potato ('oil') and (iii) 30ml water, 30 min before the mashed potato meal which contained 30ml ('water and oil'). Gastric half-emptying time measured by scintigraphy was much slower with 'oil' compared with both 'water' ($P < 0.0001$) and 'water and oil' ($P < 0.05$), and slower following 'water and oil' compared with 'water' ($P < 0.01$). The postprandial rise in blood glucose was markedly delayed ($P = 0.03$) and peak glucose occurred later ($P = 0.04$) with 'oil' when compared to the two other meals. The rises in insulin and GIP were attenuated ($P < 0.0001$), whereas the GLP-1 response was greater ($P = 0.0001$), after 'oil'. In conclusion, ingestion of fat before a carbohydrate meal markedly slows GE and attenuates the postprandial rises in glucose, insulin and GIP, but stimulates GLP-1, in type 2 diabetes.

5.2 INTRODUCTION

It has been recognised, albeit only relatively recently, that humans are predominantly in the 'postprandial', rather than 'fasted', state (Monnier et al. 2003) simply because the rate at which nutrients, including glucose, are delivered from the stomach into the small intestine in healthy subjects approximates 2 - 4 kcal/min, after an initial emptying phase that may be slightly faster (Brener et al. 1983a; Jones et al. 2005). Hence, it is not surprising that 'postprandial' glycaemia is probably the major determinant of 'overall' glycaemia, as assessed by glycated haemoglobin (El-Kebbi et al. 2004) the traditional marker for the development and progression of diabetic microvascular complications. The extent of postprandial glycaemic excursions probably represents an independent risk factor for macrovascular disease, even in individuals who do not have diabetes (Ceriello et al. 2004). Accordingly, there is substantial interest in dietary and pharmacologic (eg short acting insulin analogues, α-glucosidase inhibitors, GLP-1 and its analogues, and pramlintide) strategies directed at the control of postprandial blood glucose excursions, particularly in type 2 diabetes (Rayner et al. 2001; Ceriello et al. 2004). Gastric emptying is a major determinant of postprandial glycaemia, as attested to by the relationship between the rise in blood glucose after oral carbohydrate with gastric emptying (Horowitz et al. 1993a; Schwartz et al. 1994; Jones et al. 1996a; Rayner et al. 2001) and the effects of modulation of gastric emptying on postprandial glucose and insulin concentrations (Cunningham and Read 1989; Schwartz et al. 1994; Berry et al. 2003; Gonlachanvit et al. 2003; Pilichiewicz et al. 2003; O'Donovan et al. 2004a). Even minor variations in the initial rate of small

intestinal carbohydrate delivery may have major effects on the glycaemic response (O'Donovan et al. 2004b; Chaikomin et al. 2005). Enteral administration of glucose also stimulates the secretion of the 'incretin' hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP); the 'incretin effect' accounts for ~50% of the rise in plasma insulin after oral glucose (Nauck et al. 2004). Hence, interventions which result in slowing of gastric emptying have the potential to improve postprandial glycaemic control (Rayner et al. 2001), and may be more effective if incretin hormone secretion is also stimulated.

The interaction of nutrients with the small intestine plays the dominant role in the regulation of gastric emptying (Brener et al. 1983a; Heddle et al. 1989a; Lin et al. 1990); the extent of small intestinal feedback is related to the length and, possibly, region of small intestine exposed to nutrient (Lin et al. 1990). Of the macronutrients, fat generates the most potent feedback, primarily because of its high caloric density and, possibly, because its absorption rate is relatively slower (Lin et al. 1996). In healthy young subjects when fat is incorporated into a carbohydrate-containing drink (Houghton et al. 1990), a solid meal (Cunningham and Read 1989), or administered directly into the small intestine (Welch et al. 1987), gastric emptying is slowed, and the blood glucose and insulin responses, attenuated (Welch et al. 1987; Cunningham and Read 1989). Fat also a stimulates GLP-1 (Feinle et al. 2003a) and (possibly to a lesser extent) GIP (Herrmann et al. 1995), secretion. The slowing of gastric emptying (Carney et al. 1995; Schwizer et al. 1997; Pilichiewicz et al. 2003; O'Donovan et al. 2004a), and stimulation of

GLP-1 and GIP (Feinle et al. 2003a), are dependent on digestion of fat to fatty acids. Accordingly, acute administration of the lipase inhibitor, orlistat, accelerates gastric emptying of carbohydrate-containing meals which have a high fat content (Pilichiewicz et al. 2003; O'Donovan et al. 2004a), while attenuating the incretin, and exacerbating the glycaemic, responses (Pilichiewicz et al. 2003; O'Donovan et al. 2004a).

Despite the theoretical benefits of fat on postprandial glycaemia, its effects on gastric emptying and the blood glucose and incretin responses to carbohydrate in type 2 diabetes have, to our knowledge, not been evaluated. Given that (i) fat digestion is required to inhibit gastric emptying (Carney et al. 1995; Schwizer et al. 1997; Pilichiewicz et al. 2003; O'Donovan et al. 2004a) and stimulate incretin hormones (Feinle et al. 2003a), (ii) the slowing of gastric emptying by fat is dependent on the length of small intestine exposed to lipolytic products (Lin et al. 1990), and (iii) intracellular and homogenised fat empty from the stomach with other meal components (Meyer et al. 1986; Edelbroek et al. 1992c), the effects of fat on the glycaemic response to oral carbohydrate were likely to be greater if the fat were consumed by itself and before, rather than with, a carbohydrate-containing meal. The author has now, evaluated the effects of fat consumption, including the timing of ingestion, on gastric emptying, blood glucose and plasma insulin, GLP-1 and GIP concentrations after a carbohydrate meal in patients with type 2 diabetes.

5.3 METHODS

5.3.1 Subjects

Six males with type 2 diabetes, managed by diet alone (median age of 56 years (range 46 - 65) and body mass index (BMI) 26.1 kg/m^2 (range: 21.9 - 28.9)), were recruited by advertisement. None had a history of significant gastrointestinal, respiratory, renal, hepatic or cardiac disease, chronic alcohol abuse or epilepsy, was a smoker, or was taking medication known to influence blood pressure or gastrointestinal function. The mean duration of known diabetes was 2.6 ± 1.4 years and glycated haemoglobin at the time of the study was 6.2 ± 0.3% (normal < 6.0%).

5.3.2 Protocol

Each subject was studied on three occasions, each separated by at least seven days, in random order. On each day, subjects attended the Department of Nuclear Medicine, PET and Bone Densitometry at 0830h, following an overnight fast (14 hr for solids; 12 hr for liquids) (Jones et al. 2005). A cannula was placed in a right antecubital vein for blood sampling and subjects were seated with their back against the gamma camera. Concurrent measurements of gastric emptying, blood glucose, plasma insulin, GIP and GLP-1 concentrations were performed following ingestion, on three separate days of: (i) 30ml of water, 30 min before a mashed potato meal ('water'), (ii) 30ml of olive oil (Faulding Healthcare Pty Ltd, Rydalmere, NSW, Australia), 30 min before a mashed potato meal ('oil') and (iii) 30ml of water, 30 min before a mashed potato meal which contained 30ml of olive

oil ('water and oil'). Each meal consisted of 65g powdered potato (Deb Instant Mashed Potato, Continental Brand Foods, Epping, NSW, Australia), reconstituted with 250ml water and 20g glucose and labelled with 20MBq 99mTc-sulfur colloid (O'Donovan et al. 2004a). The energy content of the potato was 1263 kJ (total carbohydrate 61g) and the olive oil contained 1010 kJ (i.e. 27.3g). Subjects consumed the meal between t = -5 - 0 min; t = 0 min was considered the time of meal completion. The olive oil or water was swallowed as one mouthful at t = -30 min. At t = 210 min the iv cannula was removed, and the subject allowed to leave the laboratory. On one of the three days cardiovascular autonomic nerve function was evaluated after the completion of the gastric emptying measurement (Jones et al. 2005).

5.3.3 Measurements

Gastric emptying and intragastric meal distribution

Radioisotopic data were acquired for 180 min (60 s frames for the first 60 min and 3 min 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 drawn around the total stomach, which was subsequently divided into proximal and distal regions, and gastric emptying curves (expressed as % retention over time) derived (Jones et al. 2005). The lag phase was defined visually as the time before any radioactivity had entered the proximal small intestine (Collins et al. 1983). The amount of the meal remaining in the total, proximal and distal stomach at t = 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165

and 180 min was calculated and the 50% emptying time (T50) determined (Collins et al. 1983). For the 'water and oil' and 'water' meals the emptying rate for both the total meal (ie mashed potato and oil for 'water and oil' and mashed potato alone for 'water') and the carbohydrate component (ie only the mashed potato for 'water and oil') was also calculated as kJ on the basis of the T50.

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

Venous blood samples (~ 15 ml) were obtained immediately before ingestion of the water or oil at t = -30 min, prior to the commencement of the meal at t = -7 min and then at 15 minute intervals after meal completion until t = 210 min. Blood glucose concentrations were determined immediately using a portable blood glucose meter (Medisense Companion 2 Meter, Medisense Inc., Waltham, MA, USA) (Horowitz et al. 1993a). The peak blood glucose was defined as the greatest increment above baseline. Blood samples for plasma insulin, GIP and GLP-1 were collected in ice-chilled EDTA-treated tubes containing 400 kIU aprotinin (Trasylol; Bayer Australia Ltd, Pymble, Australia) per millilitre blood. Peak insulin was defined as the greatest increment above baseline. For both blood glucose and plasma insulin the rate of increase between t = -7 min and peak levels was also calculated. Plasma was stored at -70˚C for subsequent analysis and measurements performed on blood samples obtained at t = -30, -7, 15, 30, 45, 60, 90, 120, 150, 180 and 210 min. Plasma insulin was measured by ELISA immunoassay (Diagnostic System Laboratories, Inc, Webster, Texas) (O'Donovan et al. 2004a). Plasma GLP-1 and GIP were measured by RIA (O'Donovan et al. 2004a).

Cardiovascular Autonomic Function

Autonomic nerve function was evaluated using three standardised cardiovascular reflex tests (Jones et al. 2005). Each test result was scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline and 2 = abnormal, for a total maximum score of 6. A score $\geq$ 3 was considered to indicate autonomic dysfunction (Jones et al. 2005).

5.3.4 Statistical analysis

Data were evaluated using mixed model Repeated Measures 2 way Analysis of Variance (ANOVA) with post-hoc comparisons in the event of a 'treatment x time' interaction. Relationships between variables were assessed using linear regression analysis. All analyses were performed using Statview (version 5.0; Abacus Concepts, Berkeley, CA, USA) and SuperANOVA (version 1.11, Abacus Concepts, Berkeley, California, USA). Data are shown as mean values ± SEM and a P-value <0.05 was considered significant in all analyses.

5.4 RESULTS

All subjects tolerated the study well. The median score for autonomic nerve dysfunction was 1.7 (range: 0 - 4); one of the six subjects had definite autonomic dysfunction.

5.4.1 Gastric emptying and intragastric distribution

Total stomach

Gastric emptying commenced after a short lag phase; the latter was longer when oil was consumed before the meal ('water': 4.8 ± 2.5 min vs 'oil': 11.2 ± 3.3 min vs 'water and oil': 4.3 ± 2.2 min; P = 0.007). After the lag phase, emptying approximated a linear pattern for 'water and oil' and a monoexponential pattern for 'oil' and 'water'. There was a treatment x time effect (P = 0.0001) for gastric emptying on the three study days. Gastric emptying was slower between t = 15 - 180 min with 'oil' when compared with 'water' (P < 0.0001); between t = 15 - 165 min with 'oil' when compared with 'water and oil' (P < 0.05), and between t = 30 - 180 min with 'water and oil' when compared with 'water' (P < 0.01). The T50 was longer for 'oil' than both 'water' (P = 0.002) and 'water and oil' (P = 0.02) (T50 'water': 43.0 ± 2.4 min vs 'oil': 107.3 ± 16.9 min vs 'water and oil': 66.2 ± 9.9 min) (Figure 5.1a). Gastric emptying of both the oil and carbohydrate for 'water' and 'water and oil' meals (expressed as kJ/min on the basis of the T50) were not different at 14.9 ± 0.9 kJ/min and 18.9 ± 2.5 kJ/min ('water' vs 'water and oil'), respectively. In contrast, the rate of emptying of carbohydrate alone was less with 'water and oil' (10.5 ± 1.4 kJ/min) than 'water' (14.9 ± 0.9 kJ/min) (P = 0.02). In the one subject with autonomic neuropathy, gastric emptying of all three meals was within the range observed in the remainder of the group.

Intragastric distribution

There was a modest increase in meal retention in the proximal stomach between t = 0 - 150 min with 'oil' when compared with 'water' ($P < 0.05$), between t = 0 - 60 min with 'oil' when compared with 'water and oil' ($P < 0.01$), and at t = 0 and between t = 30 - 135 min following 'water' when compared with 'water and oil' ($P < 0.05$) (Figure 5.1b). In contrast, there was a marked increase in meal retention in the distal stomach between t = 15 - 180 min with 'oil' when compared with 'water' ($P < 0.05$) and between t = 0 - 180 min with 'oil' when compared with 'water and oil' ($P < 0.05$). At t = 0 and between t = 90 - 120 min meal retention in the distal stomach was greater following 'water and oil' when compared with 'water' ($P \leq 0.05$) (Figure 5.1c).

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

There was no significant difference in baseline (ie t = -30 min) blood glucose (6.7 ± 0.3 mmol/L vs 7.1 ± 0.4 mmol/L vs 7.4 ± 0.4 mmol/L), plasma insulin (10.2 ± 1.8 mU/L vs 10.0 ± 1.8 mU/L vs 13.0 ± 0.7 mU/L), plasma GIP (13.5 ± 3.5 pmol/L vs 11.2 ± 3.3 pmol/L vs 11.9 ± 2.1 pmol/L) or plasma GLP-1 (9.2 ± 1.5 pmol/L vs 8.0 ± 1.5 pmol/L vs 7.1 ± 2.0 pmol/L) between the three days ('water' vs 'oil' vs 'water and oil' respectively). Similarly, there was no difference in blood levels at t = -7 min nor any significant change between t = -30 min - t = -7 min (data not shown).

There was a significant treatment x time effect ($P = 0.0001$) for blood glucose - while there was a postprandial rise in blood glucose on all days ($P < 0.0001$), this

was significant from t = 30 min after both 'water' (P = 0.0001) and 'water and oil' (P = 0.04), and substantially later at t = 75 min (P = 0.03) after 'oil'. Between t = 30 - 105 min, blood glucose concentrations were less (P < 0.01), and between t = 165 - 210 min, were greater (P < 0.01), with 'oil' when compared with both 'water' and 'water and oil'. Blood glucose concentrations were less between t = 45 - 60 min (P < 0.05), and were greater at t = 150 min (P < 0.05), with 'water and oil' when compared with 'water'. While there was no significant difference in peak blood glucose concentrations (14.0 ± 0.9 mmol/L, 13.2 ± 0.7 mmol/L and 12.8 ± 0.9 mmol/L for 'water', 'oil', and 'water and oil' respectively) (P = 0.28), peak blood glucose tended (P = 0.07) to be less with 'water and oil' than 'water'. The time of peak blood glucose was much later for 'oil' (140 ± 19 min) when compared with 'water' (75 ± 7 min) (P = 0.005) and 'water and oil' (98 ± 6 min) (P = 0.04). The rate of increase in plasma blood glucose was different on the three days (P = 0.001) and was slower for 'oil' (P = 0.0005) and 'water and oil' (P = 0.003) when compared with 'water' (0.10 ± 0.0 mmol/L/min, 0.05 ± 0.0 mmol/L/min and 0.06 ± 0.0 mmol/L/min for 'water', 'oil', and 'water and oil' respectively) At t = 210 min blood glucose was higher than at baseline (ie t = -30 min) with 'oil' (P = 0.03) but not with the 'water' (P = 0.38) and 'water and oil' (P = 0.84) meals (Figure 5.2a).

There was a prompt rise in plasma insulin on the three days (P < 0.0001). This was significant from t = 15 min after 'water' (P = 0.04), t = 30 min after 'water and oil' (P = 0.001) and from t = 60 min after 'oil' (P = 0.05). There was a significant treatment x time effect (P = 0.0001) for plasma insulin on the three days - between

t = 30 - 120 min, plasma insulin was much lower with ‘oil’ when compared with ‘water’ ($P = 0.05$) and ‘water and oil’ ($P < 0.01$). In contrast, plasma insulin was greater ($P < 0.01$) between t = 180 - 210 min with ‘oil’ when compared with ‘water’ and greater ($P = 0.003$) at t = 210 min with ‘oil’ when compared with ‘water and oil’. At t = 150 min, plasma insulin was less ($P = 0.05$) with ‘water’ when compared with ‘water and oil’. There was no difference in peak plasma insulin concentrations (72.0 ± 13.3 mU/L, 69.1 ± 10.5 mU/L and 70.9 ± 10.6 mU/L for ‘water’, ‘oil’, and ‘water and oil’ respectively) ($P = 0.89$), but the time of peak plasma insulin was much later for ‘oil’ (160 ± 15 min) when compared with ‘water’ (85 ± 14 min) ($P = 0.0001$) and ‘water and oil’ (90 ± 17 min) ($P = 0.0002$). The rate of increase in plasma insulin was not significantly different for the three days (0.37 ± 0.1 mU/L/min, 1.0 ± 0.5 mU/L/min and 0.88 ± 0.4 mU/L/min for ‘water’, ‘oil’, and ‘water and oil’ respectively) ($P = 0.19$). At t = 210 min, plasma insulin was markedly higher than baseline with ‘oil’ ($P = 0.01$) and, higher with ‘water’ ($P = 0.02$) but not with ‘water and oil’ ($P = 0.08$) (Figure 5.2b).

There was a prompt rise in plasma GIP on the three days ($P < 0.0001$), which was significant from t = 15 min, and a significant treatment x time effect ($P = 0.004$). Plasma GIP concentrations were less ($P < 0.05$) between t = 30 - 120 min and t = 180 - 210 min with ‘oil’ when compared with ‘water and oil’. Between t = 150 - 180 min, plasma GIP was greater ($P < 0.05$) with ‘oil’ when compared with ‘water’. Plasma GIP was less ($P < 0.001$) between t = 120 - 210 min with ‘water’

when compared with 'water and oil'. At t = 210 min plasma GIP was higher than baseline ($P \leq 0.05$) after all three meals (Figure 5.2c).

There was a rise in plasma GLP-1 on the three days ($P = 0.0002$), which was rapid after 'oil' ($P = 0.0001$) ie significant from t = 15 min. There was a significant treatment x time effect ($P = 0.05$) for plasma GLP-1 on the three days so that plasma GLP-1 was much greater between t = 15 - 150 min with 'oil' when compared with both 'water' ($P < 0.05$) and 'water and oil' ($P < 0.05$). There was no significant difference in plasma GLP-1 concentrations with 'water' when compared with 'water and oil'. At t = 210 min plasma GLP-1 concentrations were not significantly different than baseline after any of the meals (Figure 5.2d).

5.4.3 Relationships between blood glucose, plasma insulin, GLP-1, and GIP concentrations with gastric emptying

When data from the three study days was pooled, the magnitude of the postprandial rise in blood glucose from baseline was inversely related to the T50 (e.g. at t = 60 min; $r = -0.80$, $P < 0.0001$ (Figure 5.3) and t = 90 min; $r = -0.71$, $P < 0.0009$). There were no significant relationships between gastric emptying and plasma insulin, GIP or GLP-1.

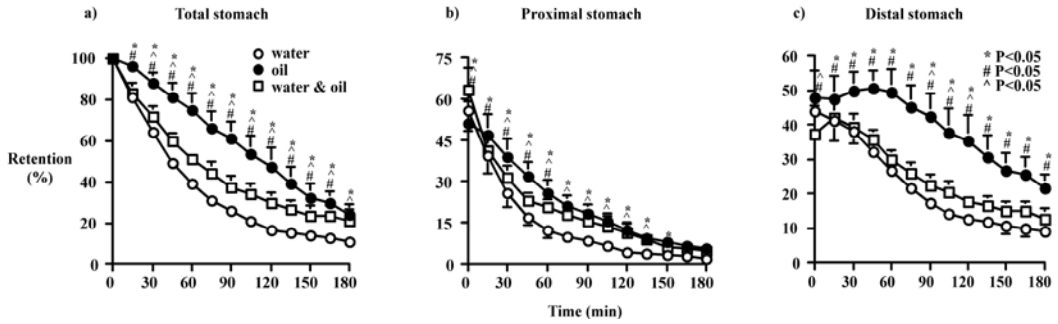

Figure 5.1

*Gastric emptying (a) and intragastric distribution (b) and (c) of a mashed potato meal when either 30ml of olive oil was consumed before the meal ('oil'), 30ml of water was consumed before the meal ('water'), or 30ml of water was consumed before the meal which also contained 30ml olive oil ('water and oil') in type 2 patients. Data are mean values ± S.E.M. *P < 0.05 'oil' vs 'water'; #P < 0.05 'oil' vs. 'water and oil'; ^P < 0.05 'water' vs 'water and oil'.*

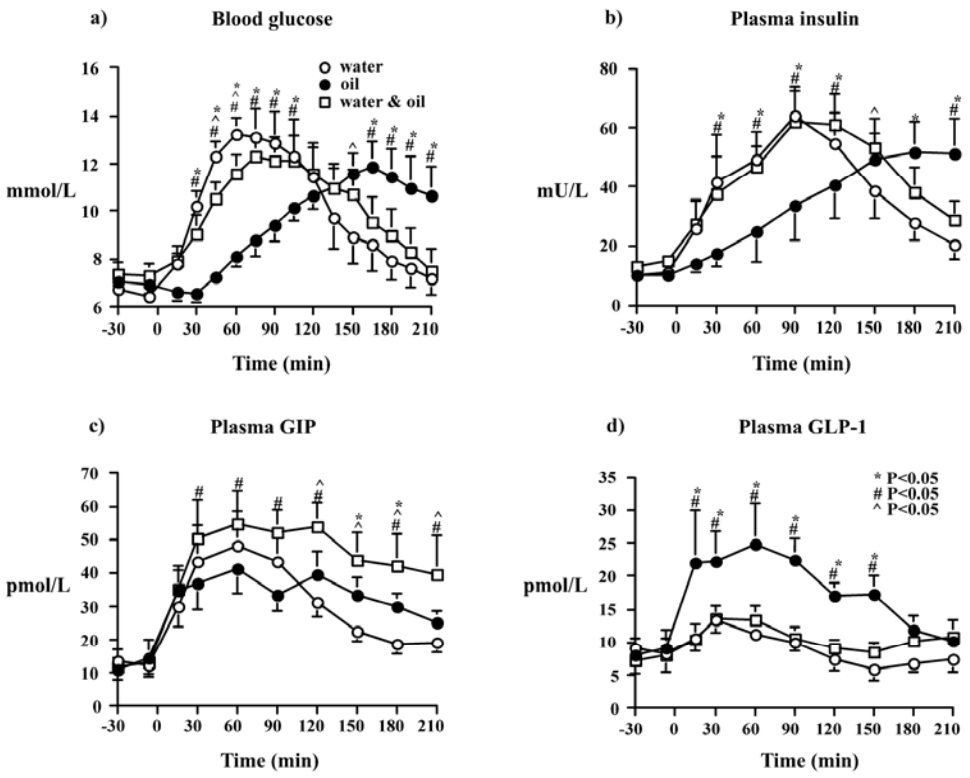

Figure 5.2

*Blood glucose concentrations (a), plasma insulin concentrations (b), plasma GIP concentrations (c) and plasma GLP-1 concentrations (d) after ingestion of a mashed potato meal when either 30ml of olive oil was consumed before the meal ('oil'), 30ml of water was consumed before the meal ('water'), or 30ml of water was consumed before the meal which also contained 30ml olive oil ('water and oil') in type 2 patients. Data are mean values ± S.E.M. *P < 0.05 'oil' vs 'water'; #P < 0.05 'oil' vs 'water and oil'; ^P < 0.05 'water' vs 'water and oil'.*

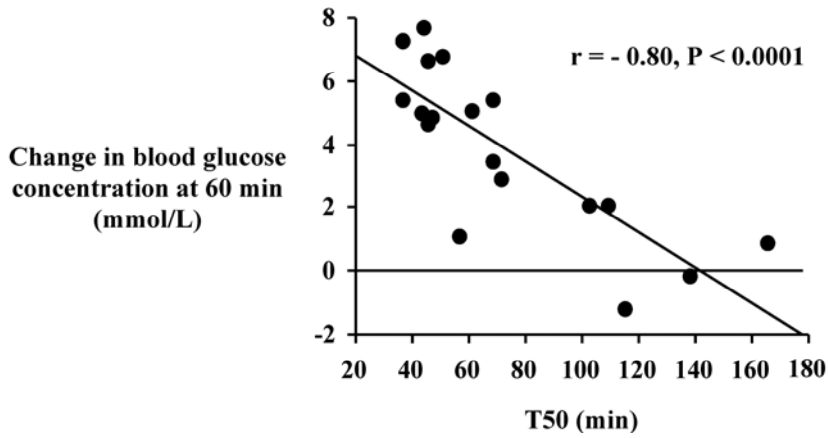

Figure 5.3

Relationship between the change in blood glucose concentration from baseline at t = 60 min after all three meals (i.e. 'water', 'oil' and 'water and oil') and the gastric 50% emptying time (T50).

5.5 DISCUSSION

This study establishes that, in type 2 patients managed by diet, ingestion of a relatively small amount of olive oil as a 'preload' 30 min before a carbohydrate meal, markedly slows gastric emptying, affects intragastric meal distribution, delays the postprandial rises in blood glucose, plasma insulin and GIP, and stimulates the secretion of GLP-1. In contrast, the effects of including the same amount of oil in an identical carbohydrate meal on gastric emptying, and glycaemic and incretin responses, were relatively modest. These observations are consistent with the concept, initially suggested by Cunningham and Read (1989), that the effects of fat on gastric emptying and glycaemia will be dependent on whether fat is given independently of carbohydrate, or mixed with it, and have significant implications for dietary strategies to minimise postprandial glycaemic excursions in type 2, and possibly type 1, diabetes.

The slowing of gastric emptying (Carney et al. 1995; Schwizer et al. 1997; Pilichiewicz et al. 2003; O'Donovan et al. 2004a), stimulation of a number of gut hormones (including GLP-1, GIP, peptide YY (PYY), cholecystokinin (CCK), and pancreatic polypeptide (PP)) (Feinle et al. 2003a; Feinle-Bisset et al. 2005) and suppression of ghrelin (Feinle-Bisset et al. 2005) by fat are dependent on lipolysis of triglycerides to fatty acids. The author reasoned that the magnitude of the slowing of gastric emptying was likely to be greater when oil was given before, rather than with, a carbohydrate-containing meal given that it takes some time (~ 30 - 40 min) for small intestinal feedback mechanisms induced by fat to become established (Heddle et al. 1989a; Fone et al. 1990a; Feinle et al. 2003a; Feinle-

Bisset et al. 2005) (probably reflecting the time required to generate sufficient fatty acids to induce these responses) and administration of oil before a meal ensures that it would empty from the stomach preferentially, so that both digested and non-digested fat would be in the small intestine when the remainder of the meal is consumed and caudal spread of oil and lipolytic products facilitated. It would be expected that at the time of ingestion of the meal ~ 40% of the oil 'preload' would have emptied from the stomach (Hunt and Knox 1968; Edelbroek et al. 1992c). The slowing of gastric emptying by the oil 'preload' was associated with changes in intragastric meal distribution, with increased retention in both the proximal and distal stomach. Intragastric meal distribution may influence gastrointestinal symptoms and appetite; in healthy subjects the perception of fullness is greater, and energy intake less, when antral volume is relatively greater (Sturm et al. 2004). The slowing of gastric emptying induced by incorporation of fat into the meal was much less marked and attributable to the higher energy density, as attested to by the comparable emptying rates of the meals with and without oil, when expressed as kJ/min. It is appropriate to note that when oil is consumed concurrently, but not mixed with, a meal consumed in the seated or erect posture, it 'layers' on top of other meal components because of its lower density (Edelbroek et al. 1992c; Horowitz et al. 1993b) and would be expected to have little effect on gastric emptying of carbohydrate.

Because slowing gastric emptying of carbohydrate has a profound influence on postprandial glycaemia (Schwartz et al. 1994; Rayner et al. 2001; Gonlachanvit et al. 2003), the 'incretin effect' is an important determinant of the postprandial

insulin response (Nauck et al. 2004), and fat stimulates the secretion of GLP-1 and GIP (Herrmann et al. 1995; Feinle et al. 2003a), the author reasoned that a fat 'preload' had the potential to attenuate the glycaemic response to carbohydrate. When oil was given as a 'preload', there was a marked delay in the onset of the postprandial rise, as well as the rate of increase, in blood glucose and a trend for a reduction in the peak blood glucose level. In contrast to glucose, elevations in plasma insulin, GIP and GLP-1 occurred promptly after the three meals, presumably reflecting the emptying of the meal and, in the case of the fat 'preload', the stimulation of incretin hormone secretion by the presence of fat in the small intestine (Feinle et al. 2003a). The secretion of GIP from duodenal K cells (Nauck et al. 2004) reflects the rate of carbohydrate entry into the small intestine (Horowitz et al. 1996a); which may account for the diminished GIP response after the fat 'preload', but not the statistically greater GIP response to the carbohydrate meal which contained fat, for which the author has no ready explanation. As the capacity of GIP to stimulate insulin secretion is diminished in type 2 diabetes (Nauck et al. 2004), and the observed differences were modest, they are unlikely to be clinically relevant. In contrast, there was a rapid, and marked, rise in GLP-1 immediately after the carbohydrate meal following the fat 'preload', which probably reflects the interaction of lipolytic products with L cells in the distal jejunum and ileum (Feinle et al. 2003a), although this is controversial (Herrmann et al. 1995). The stimulation of GLP-1 may have contributed to the reduction in glycaemia by both slowing gastric emptying and stimulating insulin secretion (Nauck et al. 2004; Meier et al. 2005). However, while it is not possible to quantify the relative effects of slowing of gastric emptying and the 'incretin effect' on glycaemia, that the latter

is likely to be of lesser importance, particularly as the glycaemic response and gastric emptying were related, and plasma insulin increased when there was a rise in plasma glucose but a fall in plasma GLP-1.

In interpreting these observations it should be recognised that the number of subjects studied was relatively small and only the acute effects of modifications in fat intake were evaluated. Adaptive changes in gastrointestinal function, including gastric emptying, may occur in response to changes in dietary fat (Cunningham et al. 1991; Boyd et al. 2003). The energy content of the oil 'preload' was comparable to that of the meal and it would be of interest to evaluate the effects of smaller triglyceride loads. Patients with uncomplicated type 2 diabetes of short duration were studied; longstanding type 2 diabetes is associated with a high prevalence of gastroparesis (although the relationship to upper gastrointestinal symptoms is poor) (Horowitz et al. 2002b) and impaired insulin secretion; the effects of fat on gastric emptying and glycaemia in such patients warrant evaluation. Because blood glucose levels had not returned to baseline by 210 min, effects on the overall glycaemic (or insulinaemic) response could not be determined, which, represents a priority for future studies, despite evidence that this is reduced by small intestinal and oral fat in healthy subjects (Welch et al. 1987; Cunningham and Read 1989). Irrespective of these limitations, the observations establish the capacity for the administration of a relatively small quantity of fat before a carbohydrate-containing meal to minimise glycaemic excursions and potentiate GLP-1 secretion in type 2 diabetes. It would not be surprising if the dominant effect of pharmacological therapies, including GLP-1 and its analogues, and pramlintide, on postprandial

glycaemia, in type 2 diabetes, is also mediated by the slowing of gastric emptying (Meier et al. 2005).

CHAPTER 6:

ARTIFICIALLY-SWEETENED VERSUS REGULAR MIXERS INCREASE GASTRIC EMPTYING AND ALCOHOL ABSORPTION

6.1 SUMMARY

Mixed alcoholic drinks are increasingly being consumed in “diet" varieties. This study evaluates the hypothesis that such drinks will empty more rapidly from the stomach and, thereby, increase the rate of alcohol absorption when compared to “regular” versions containing sugar. 8 healthy males were studied twice in randomized order. On each day, they consumed an orange-flavoured vodka beverage (30 g alcohol in 600 mL), made with either a “regular” mixer containing sucrose (total 478 kcal), or a “diet” mixer (225 kcal). Gastric half-emptying time measured by ultrasound was less for the “diet” than the “regular” drink (21.1 ± 9.5 vs 36.3 ± 15.3 min, $P < 0.01$). Both the peak plasma alcohol concentration (0.053 ± 0.006 vs 0.034 ± 0.008 g%, $P < 0.001$) and the area under the plasma alcohol concentration curve between 0 - 180 min (5.2 ± 0.7 vs 3.2 ± 0.7 units, $P < 0.001$) were greater with the “diet” drink. In conclusion, substitution of artificial sweeteners for sucrose in mixed alcoholic beverages may have a marked effect on the rate of gastric emptying and the plasma alcohol response.

6.2 INTRODUCTION

Pre-mixed (“ready-to-drink”) alcoholic beverages have become increasingly popular amongst the young, and particularly women (Hughes et al. 1997). This group is especially likely to be following restricted energy or “low carb” diets.

While pre-mixed drinks were initially formulated with sugar as the sweetener, "diet" versions containing artificial sweeteners are now becoming available. Furthermore, many drinkers mix their own alcoholic drinks using "diet" beverages.

It is now well established that the rate of gastric emptying is a major determinant of alcohol absorption (Nimmo 1976; Holt et al. 1980; Holt 1981; Horowitz et al. 1989b). Peak blood ethanol concentrations are less when gastric emptying of an alcohol-containing drink is slowed by propantheline (Gibbons and Lant 1975), cigarette smoking (Johnson et al. 1991), or ingestion of a meal (Horowitz et al. 1989b), and greater when emptying is accelerated by metoclopramide (Gibbons and Lant 1975).

Gastric emptying is closely regulated to approximately 1 - 3 kcal per minute by feedback arising from interaction of nutrients with the small intestine (Brener et al. 1983a; Hunt et al. 1985a), with some variations related to meal volume and energy density (Hunt et al. 1985a). Hence, the addition of sucrose to an alcoholic beverage would be expected to slow gastric emptying when compared to a non-nutritive sweetener. However, the potential for sugar-free drinks to empty more rapidly from the stomach, and thereby increase the rate of alcohol absorption, when compared with the original formulations, has apparently not been recognised. This issue is particularly important when individuals judge their fitness to drive a motor vehicle by the number of "standard" measures of alcohol consumed.

The aim of this study was to evaluate both plasma alcohol concentrations, and the rate of gastric emptying, in healthy volunteers following ingestion of alcoholic drinks made with either sucrose-containing or artificially-sweetened mixers. The author hypothesised that the "diet" beverage would empty from the stomach more quickly than the "regular" drink, resulting in higher plasma alcohol concentrations.

6.3 METHODS

6.3.1 Subjects

Eight healthy males (median age 24 years, range 20 - 32; body mass index 23.8 kg/m^2, range 20.2 - 25) were studied. None used regular medication, was a smoker, or consumed more than 20 g alcohol daily.

6.3.2 Protocol

Each subject was studied twice, after fasting overnight and abstaining from alcohol for 48 hours. Studies were separated by at least 3 days, and were randomised and double-blinded.

At t = -5 min, subjects were given an orange-flavoured vodka drink, which they consumed within 5 min. On one day, a "regular" drink was given, consisting of 75 ml vodka (Wodka Wyborowa®, Turew, Poland) mixed with 525 ml Fanta Orange™ (Coca Cola Amatil, Sydney, Australia), which contains sucrose (total volume 600 mL, 30g alcohol, 65 g carbohydrate, 478 kcal, pH 3.4). On the other day, a "diet" drink was provided, containing 75 mL vodka mixed with 525 mL

Fanta Lite Orange ™ (Coca Cola Amatil, Sydney, Australia), which is sweetened with aspartame and acesulphame potassium (total volume 600 mL, 30 g alcohol, 2.6 g carbohydrate, 225 kcal, pH 3.3). Both drinks were served at 22 degrees, after dissipation of gas bubbles.

Blood was sampled via a cannula at t = -5, 15, 30, 60, 90, 120, and 180 min, and serum stored at -70 ºC until analysis of alcohol concentrations. A meal was provided after the study, and subjects observed until breath analysis indicated a plasma alcohol concentration less than 0.05 g%.

6.3.3 Measurements

Gastric emptying

Measurements of antral area were performed using an Aloka SSD-650 CL ultrasound machine (Aloka, Tokyo, Japan) with a 3.5-MHz annular array probe. Subjects were seated leaning slightly backward, and the transducer was positioned vertically to visualise the antrum in cross section, with the superior mesenteric vein and abdominal aorta in a longitudinal section (Bolondi et al. 1985; Hveem et al. 1996). Antral area was measured, using the built-in caliper and calculation software, at t = -5, and at 10 minute intervals from t = 0 to 120 min. Gastric half-emptying time was defined as the time when antral area decreased to half the maximum increase above baseline (Hveem et al. 1994). This method has been shown to correlates closely with scintigraphy for measurement of liquid half-emptying time (Hveem et al. 1996).

Plasma alcohol

Plasma alcohol concentrations were determined by gas chromatography (Cooper 1971).

6.3.4 Statistical analysis

Antral area and plasma alcohol concentrations were compared using repeated measures analysis of variance. Peak plasma alcohol concentrations, half-emptying values, baseline and maximum antral areas, and areas under the plasma alcohol concentration curves, calculated using the trapezoidal rule, were compared using Student's paired t-tests. Linear regression analysis was used to examine relationships between half-emptying time and plasma alcohol concentrations (Statview 5.0, SAS Institute, Cary, North Carolina, USA). Data are mean ± standard deviation. P-values < 0.05 were considered significant.

6.4 RESULTS

All subjects tolerated the study well, and none found either drink unpleasant.

6.4.1 Antral area and gastric emptying

Neither baseline nor maximum antral area differed between the two days. Antral area declined more rapidly with the "diet" drink ($P < 0.001$), and the gastric half-emptying time was less than for the "regular" drink (diet 21.1 ± 9.5 min vs regular 36.3 ± 15.3 min, $P < 0.01$) (Figure 6.1).

6.4.2 Plasma alcohol concentrations

Plasma alcohol concentrations were greater after the “diet” than the “regular” drink ($P < 0.001$). Both the peak plasma alcohol concentration (0.053 ± 0.006 vs 0.034 ± 0.008 g%, $P < 0.001$) and the area under the plasma alcohol concentration curve (5.2 ± 0.7 vs 3.2 ± 0.7 units, $P < 0.001$) were greater after the “diet” drink (Figure 6.2).

There was no significant relationship between the peak plasma alcohol concentration or the area under the curve, with the gastric half-emptying time for either beverage (data not shown).

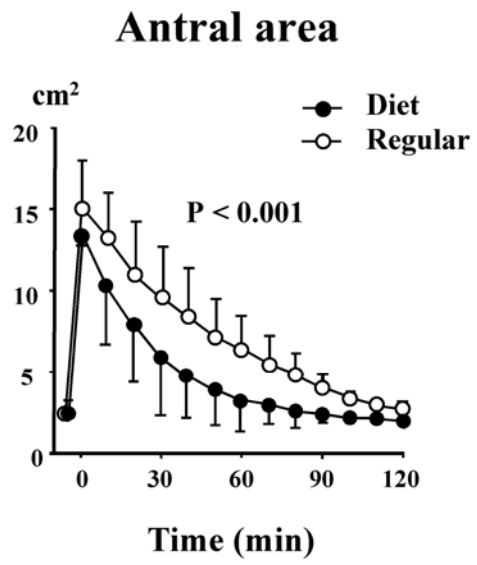

Figure 6.1

Antral area (mean ± standard deviation) after consumption of "regular" (open circles) or "diet" (filled circles) mixed alcoholic drinks in healthy subjects. The antral area at baseline is plotted as the first data point. The decline in antral area is more rapid after the "diet" drink, indicative of faster gastric emptying (P < 0.001).

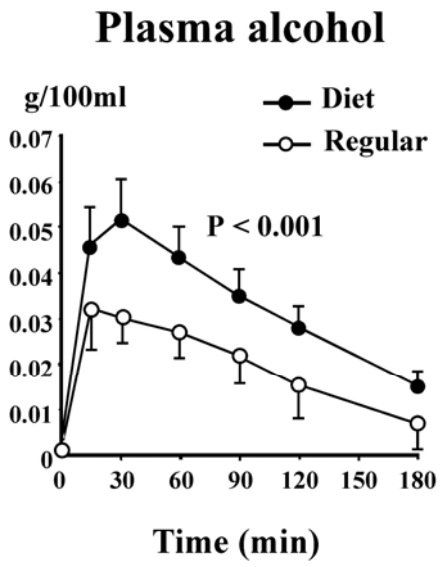

Figure 6.2

Plasma alcohol concentration (mean ± standard deviation) after consumption of "regular" (open circles) and "diet" (filled circles) mixed alcoholic drinks in healthy subjects. Both peak alcohol concentration and the area under the curve are greater ($P < 0.001$ for each) after the "diet" drink.

6.5 DISCUSSION

The study establishes that substitution of a "diet" mixer for a "regular" one containing sucrose, in a mixed alcoholic beverage, has a major impact on the rate of gastric emptying and alcohol absorption in healthy adults. While the author anticipated these effects from previous studies indicating that gastric emptying is regulated at a relatively constant caloric rate (Brener et al. 1983a) and that alcohol absorption is dependent on gastric emptying (Horowitz et al. 1989b), the magnitude of the difference in blood alcohol concentrations was striking. Indeed, the mean peak blood ethanol concentration (i.e. > 0.05 g%) after the "diet" drink would preclude subjects from legally driving a motor vehicle in many jurisdictions, while this act would be legal after consuming the "regular" drink.

These observations are consistent with those of Hey et al. (2004), who reported that the area under the curve for blood ethanol concentration over 180 min was about 30% less after a sucrose-containing vodka-based drink, when compared to drinking vodka alone (Hey et al. 2004). Although differences in gastric emptying were thought likely to account for this difference, it was not measured. Furthermore, the lower pH of the mixed drink (3.2) may have contributed, since the exposure of the small intestine to acid inhibits gastric emptying (Lin et al. 1993). Moreover, the volumes of the drinks were not matched. In the current study, the "diet" and "regular" beverages were matched for volume and pH, and gastric emptying was measured. The osmolarity of "diet" mixers is considerably less than that of "regular" soft drinks (about 30 versus 700 mOsm/kg (Feldman and Barnett 1995))

which may have contributed to more rapid emptying, although differences in caloric load are, almost certainly more important (Lin et al. 1993).

That the rate of gastric emptying is a major determinant of alcohol absorption (Gibbons and Lant 1975) is probably mainly due to the greater surface area for absorption provided by the small intestine compared to the stomach; metabolism of alcohol within the stomach by gastric alcohol dehydrogenase is almost certainly minor (Oneta et al. 1998). In addition to the substantially higher plasma alcohol concentrations after the "diet" compared to the "regular" drink, the area under the concentration curve was markedly greater (with no subsequent crossover of the curves), consistent with the concept of more rapid absorption due to accelerated gastric emptying, as well as proportionally less first-pass metabolism (Kechagias et al. 1999). Hepatic metabolism of alcohol is saturated at relatively low doses, so that disproportionate increments in plasma alcohol concentrations result from modest increases in the rate of gastric emptying (Levitt et al. 1997; Oneta et al. 1998; Kechagias et al. 1999). Given these multiple sources of variation in plasma alcohol concentrations, together with the relatively small number of subjects studied, it is not surprising that the study failed to demonstrate a linear relationship between plasma alcohol concentrations with the rate of gastric emptying for either beverage. Only lean males were studied, but the influence of gastric emptying on alcohol absorption has also been demonstrated in females (Horowitz et al. 1989b; Oneta et al. 1998), and emptying of nutrient liquids does not vary substantially by gender or body mass index (Wright et al. 1983). Therefore, it is likely the findings can be generalised. As neither drink was unpalatable (Wicks et al. 2005), it is

unlikely that minor taste differences between them would influence gastric emptying.

The study highlights the need for community awareness that factors other than just the alcohol content of a beverage need to be taken into account in considering safe levels of consumption and the potential for intoxication. In particular, the substitution of artificial sweeteners for sugars in mixed alcoholic drinks has potential to have a profound impact on blood alcohol concentrations, despite the drinks being similar in all other aspects.

CHAPTER 7:

CONCURRENT DUODENAL MANOMETRIC AND IMPEDANCE RECORDING TO EVALUATE THE EFFECTS OF HYOSCINE ON MOTILITY AND FLOW EVENTS, GLUCOSE ABSORPTION AND INCRETIN HORMONE RELEASE IN HEALTHY SUBJECTS

7.1 SUMMARY

Upper gastrointestinal motor function and incretin hormone (GLP-1 and GIP) secretion are major determinants of postprandial glycaemia and insulinaemia. However, the impact of small intestinal flow events on glucose absorption and incretin release is poorly defined. Intraluminal impedance monitoring is a novel technique that allows flow events to be quantified. Eight healthy volunteers were studied twice, in randomised order. After an overnight fast, a catheter incorporating 6 pairs of electrodes at 3 cm intervals, and 6 corresponding manometry sideholes, was positioned in the duodenum. Hyoscine butylbromide 20 mg, or saline, was given as an intravenous bolus, followed by a continuous intravenous infusion of either hyoscine (20 mg/h) or saline, over 60 min. Concurrently, glucose and 3-O-methylglucose (3-OMG) were infused into the proximal duodenum (3 kcal/min), with frequent blood sampling to measure glucose, 3-OMG, insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependant insulinotropic polypeptide (GIP). The frequency of duodenal pressure waves and propagated pressure wave sequences was reduced by hyoscine in the first 10 min ($P<0.01$ for both), but not after that time. In contrast, there were markedly fewer duodenal flow events throughout 60 min with hyoscine ($P < 0.005$). Overall blood glucose ($P < 0.01$) and plasma 3-

OMG concentrations ($P < 0.05$) were lower during hyoscine than saline, while plasma insulin, GLP-1, and GIP concentrations were initially ($t = 20$ min) lower during hyoscine ($P < 0.05$). In conclusion, intraluminal impedance measurement may be more sensitive than manometry in demonstrating alterations in duodenal motor function. A reduction in the frequency of duodenal flow events is associated with a decreased rate of glucose absorption and incretin release in healthy subjects.

7.2 INTRODUCTION

Glycaemic control is a major determinant of the development and progression of microvascular, and probably macrovascular, complications of type 1 (DCCT 1993; Nathan et al. 2005) and type 2 (UKPDS and Group 1998) diabetes, and the importance of transient postprandial hyperglycaemia to overall glycaemic control is now recognised (Del Prato 2002) (chapter 2). While it has been established that the rate of gastric emptying is a major determinant of the postprandial increase in blood glucose in healthy subjects (Horowitz et al. 1993a) and patients with type 1 (Merio et al. 1997) and type 2 (Jones et al. 1996b) diabetes, there is much less information about how much of the variation in the postprandial glycaemic response is determined by flow patterns of chyme in the upper small intestine. Several studies animal models (Sarr et al. 1980; Fioramonti et al. 1982; Defilippi and Gomez 1995) and in humans (Sjovall et al. 1990; Cherbut et al. 1994; Rayner et al. 2002; Schwartz et al. 2002) indicate that different patterns of motor activity in the small intestine influence the absorption of glucose from the lumen. We have reported a relationship between absorption of the glucose analogue, 3-OMG and the number of duodenal waves, and particularly antegrade propagated wave

sequences, in healthy humans (Schwartz et al. 2002) and patients with type 1 diabetes mellitus (Rayner et al. 2002). Furthermore, pharmacological inhibition of small intestinal motor activity has the potential to affect glucose absorption (Samsom et al. 1999).

While manometric recordings from closely spaced sideholes within the gut provide detailed information about the organisation of lumen-occluding contractions in space and time, they cannot measure contractions that are not lumen-occlusive, and at best allow incomplete inferences regarding the flow of luminal contents. Intraluminal impedance recording has the capacity to determine flow events by monitoring changes in electrical impedance between pairs of electrodes positioned within the gut lumen (Nguyen et al. 1999). The passage of a fluid bolus results in a fall, while an air bolus increases, impedance; the transit of the bolus along a gut segment can be monitored by recordings from sequential electrode pairs. Intraluminal impedance recording is now well established and validated in the oesophagus (Nguyen et al. 1999). The technique has also been applied in the duodenum (Nguyen et al. 1995), and has been used to characterise antropyloroduodenal flow events in healthy humans (Savoye et al. 2003; Savoye-Collet et al. 2003), and abnormalities of duodenal chyme transport in patients with diabetic gastroparesis (Nguyen et al. 1997). Concurrent duodenal impedance and manometry recordings have recently been compared with the “gold standard” of videofluoroscopy for the detection of flow events in the human duodenum (Imam et al. 2004). The outcome of this study indicates that impedance has a greater sensitivity for detecting flow than manometry, and, accordingly, is likely to

represent the most suitable technique for prolonged evaluation of intestinal flow patterns, where videofluoroscopy would entail excessive radiation exposure.

The presence of glucose in the small intestine stimulates the release of several peptides, including insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (Schirra et al. 1996b), which play a key role in determining the postprandial glycaemic response (chapter 2). As with glucose absorption, there is little information as to how variations in flow patterns in the upper small intestine may influence their release.

The aims of this study were to compare duodenal manometry and impedance recordings, and to evaluate the impact on glucose absorption and incretin hormone release, when duodenal motor function was suppressed pharmacologically by the anticholinergic drug, hyoscine butylbromide.

7.3 METHODS

7.3.1 Subjects

Eight healthy males (age 27.9 ± 2.3 years, body mass index 26.8 ± 0.7 kg/m^2) were recruited by advertisement. No subject was taking medication known to affect gastrointestinal function. The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital, and each subject provided written, informed consent.

7.3.2 Protocol

Each subject underwent paired studies, separated by an interval of 4–7 days, in single blind, randomised order. Subjects attended the laboratory at 0900h following an overnight fast (14 hours for solids and 12 hours for liquids). At that time, a combined manometry and impedance assembly was introduced into the stomach through an anaesthetised nostril, and allowed to pass into the duodenum by peristalsis. The assembly incorporated a multilumen silicone manometry catheter (external diameter 4 mm) (Dentsleeve, Adelaide, Australia) with 6 duodenal sideholes spaced at 3 cm intervals, and an additional sidehole for intraduodenal infusion located between the two most proximal manometric sideholes, in parallel with an impedance catheter with 6 electrode pairs spaced at 3 cm intervals (external diameter 2 mm) (Sandhill Scientific Inc, Highlands Ranch, Colorado, USA). The location of the manometric sideholes corresponded to the midpoint of each electrode pair (Figure 7.1). The manometric sideholes were perfused with degassed water, and the position of the assembly was monitored continuously by measurement of the transmucosal potential difference (TMPD) from two additional saline-perfused sideholes, one in the duodenum (1.5 cm proximal to those used for pressure measurement) and the other in the antrum (3 cm more proximal again), using established criteria (i.e. antral TMPD < -20mV, duodenal TMPD > -15mV, difference > 15mV) (Heddle et al. 1988c). This required the insertion of a 20G saline-filled cannula subcutaneously in the forearm, as a reference. When the catheter was positioned correctly, an intravenous cannula was inserted in each forearm, one for blood sampling and the other for infusion of hyoscine or saline.

Fasting duodenal motility was observed until five min after the onset of duodenal phase II. At this time (t = -5 min), 20 mg hyoscine butylbromide (Buscopan, Boehringer Ingelheim, Australia) made up to 10 mL in normal saline, or 10 mL of normal saline alone as a control, was infused intravenously over 2 min. At t = 0 min, an intravenous infusion of hyoscine butylbromide (20 mg made up to 60 mL with normal saline), or saline alone, was given over 60 min (i.e. 0.033 mg/min). This rate of hyoscine infusion was chosen to match the elimination rate of hyoscine in a 70 kg human (Herxheimer and Haefeli 1966). Concurrently (i.e. t = 0 - 60 min), an intraduodenal infusion of 45 g glucose together with 5 g 3-OMG, dissolved in water to a total volume of 200 mL, was given over 60 min (≡ 3 kcal/min) via the duodenal infusion channel. At t = 60 min both the hyoscine butylbromide and intraduodenal glucose infusions ceased.

Venous blood was sampled every 5 min from t = -5 to t = 60, and then at T = 70, 80, 90 105, 120, 150, and 180 min for measurement of blood glucose, every 10 min from t = 0 to t = 90 and then at t = 120 and 180 min for measurement of plasma 3-OMG, and at t = 0, 10, 20, 30, 40 and 60 min, for measurement of plasma insulin, GIP, and GLP-1 concentrations. Heart rate was recorded every 10 min for the first 90 min then every 15 min until 120 min and every 30 min until 180 min.

7.3.3 Measurements

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

Manometric analysis

Manometric data were analysed in an automated fashion, using previously described software (Samsom et al. 1998c). The frequency of duodenal waves ≥ 10 mmHg in amplitude (total number in all duodenal channels per 10 min) and propagated sequences of duodenal waves was analysed, assuming a propagation velocity between 0.9 cm/s and 16 cm/s (Rayner et al. 2002).

Impedance analysis

Impedance recordings were analysed by two independent observers (of whom one 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) (Figure 7.2). Flow events were classified as either antegrade or retrograde (Nguyen et al. 1995; Imam et al. 2004). The detection of flow events was compared between observers, and consensus was reached over discrepant observations.

Blood glucose, 3-OMG, plasma insulin, GLP-1, and GIP concentrations

Blood samples for determination of plasma insulin, GLP-1, and GIP were collected in ice-chilled tubes containing EDTA and 400 kIU aprotonin (Trasylol; Bayer Australia Ltd., Pymple, Australia) per litre blood. Plasma was separated by centrifugation and stored at -70 ºC for subsequent analysis. Blood glucose concentrations were determined immediately using a portable glucose meter (Medisense Precision QID, Abbott Laboratories, Bedford, Massachusetts, USA). 3-OMG concentrations were measured by gas liquid chromatography (Rayner et al. 2002). Plasma insulin concentrations were measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc., Webster, Texas, USA) (Horowitz et al. 1996a), and plasma GLP-1 and GIP concentrations by radioimmunoassay (RIA) (O'Donovan et al. 2004b).

7.3.4 Statistical Analysis

Data were evaluated using repeated measures ANOVA with treatment and time as factors, with post-hoc comparisons in the event of significant treatment x time interactions. Student's paired t-test was used to compare baseline blood glucose and plasma peptide concentrations, and areas under the curves for blood glucose and 3-OMG, calculated using the trapezoidal rule. A statistical software package (Statview 5, SAS Institute, Cary, North Carolina, USA) was used for all analyses. Statistical significance was accepted at $P < 0.05$, and data are presented as mean values ± standard error.

7.4 RESULTS

All subjects tolerated the study well, and the anticholinergic effect of hyoscine (increase in heart rate versus saline infusion) was maintained at a stable level throughout 60 min of hyoscine infusion (mean heart rate during hyoscine 85 ± 5, and during saline 67 ± 4 beats per min, $P < 0.005$), and remained higher with hyoscine until t = 70 min. Subjects could not discriminate between the two study conditions, and none noticed a dry mouth or blurred vision during hyoscine infusion.

7.4.1 Duodenal pressure waves and duodenal flow events

Duodenal pressure waves

There were fewer duodenal pressure waves with hyoscine compared to saline during the first 10 min ($P < 0.005$, treatment x time interaction). However, over 60 min, there was no overall difference (i.e. treatment effect) in the frequency of duodenal pressure waves (Figure 7.3a). Similarly, there were fewer propagated pressure wave sequences with hyoscine compared to saline during the first 10 min ($P < 0.01$, treatment x time interaction), but again, over 60 min, there was no overall difference in the frequency of propagated pressure wave sequences (Figure 7.3b). The mean amplitude of duodenal waves did not differ between study days, when compared over 60 min (27.7 ± 2.4 mmHg during hyoscine vs 25.1 ± 1.7 mmHg during saline infusion), or at 10 minute intervals (data not shown).

Duodenal flow events

The majority of duodenal flow events were antegrade (96 ± 2 % for hyoscine, 94 ± 3 % for saline). There were markedly fewer duodenal flow events with hyoscine when compared to saline throughout 60 min ($P < 0.005$) (Figure 3c).

7.4.2 Blood glucose, and plasma 3-OMG, insulin, GLP-1, and GIP concentrations

There was no difference in baseline blood glucose or plasma insulin, GIP, and GLP-1 concentrations between the study days.

Blood glucose

On both study days, blood glucose increased to a plateau at about 40 min, then declined as soon as the intraduodenal glucose infusion ceased (t = 60 min) and returned to baseline by about 90 min (Figure 7.4 a). Blood glucose concentrations were lower during hyoscine infusion when compared to saline (t = 0 - 60 min) ($P < 0.01$). There was also a trend for the peak blood glucose concentration to be lower after hyoscine (9.6 ± 0.4 vs 10.3 ± 0.5 mmol/L, $P = 0.08$).

Plasma 3-OMG

3-OMG concentrations increased during intraduodenal glucose/3-OMG infusion, and continued to rise after the end of the intraduodenal infusion, with a later peak on the hyoscine day, but similar, and declining, concentrations at 180 min (Figure 7.4 b). However, 3-OMG concentrations were lower during hyoscine infusion

compared to saline (t = 0 - 60 min, $P < 0.05$), and the area under the 3-OMG curve over 180 min was also less on the hyoscine day (43 ± 4 vs 51 ± 4 units, $P < 0.05$).

Plasma insulin

There was a progressive rise in plasma insulin during intraduodenal glucose infusion (t = 0 - 60 min), from 10 min during saline and from 20 min during hyoscine, so that plasma insulin was less with hyoscine compared to saline at t = 20 min ($P < 0.05$ treatment x time interaction) (Figure 7.5 a).

Plasma GLP-1

GLP-1 increased from 20 min during saline and from 30 min during hyoscine infusion (Figure 7.5 b). While GLP-1 concentrations did not differ significantly over t = 0 - 60 min, GLP-1 was less at t = 20 min during hyoscine than saline ($P < 0.05$).

Plasma GIP

GIP concentrations increased from 10 min during saline infusion and from 20 min during hyoscine infusion, with lower concentrations during hyoscine compared to saline at t = 10 and t = 20 min ($P < 0.0005$, treatment x time interaction) (Figure 7.5 c).

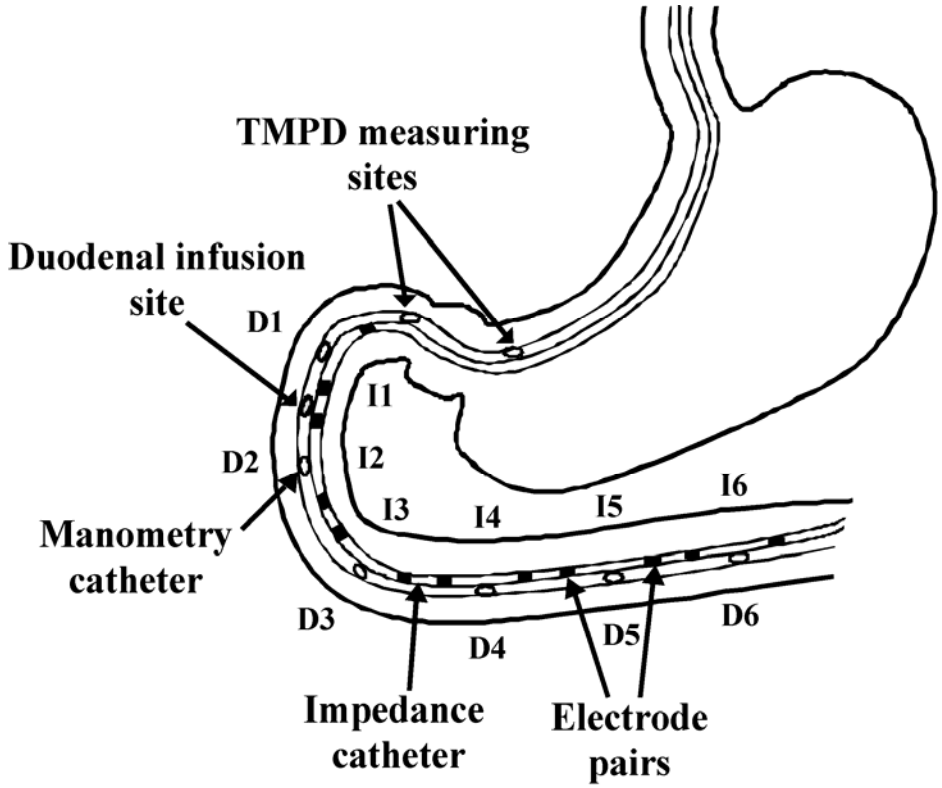

Figure 7.1:

Configuration of the manometry and impedance catheters used to evaluate duodenal pressures and flow events.

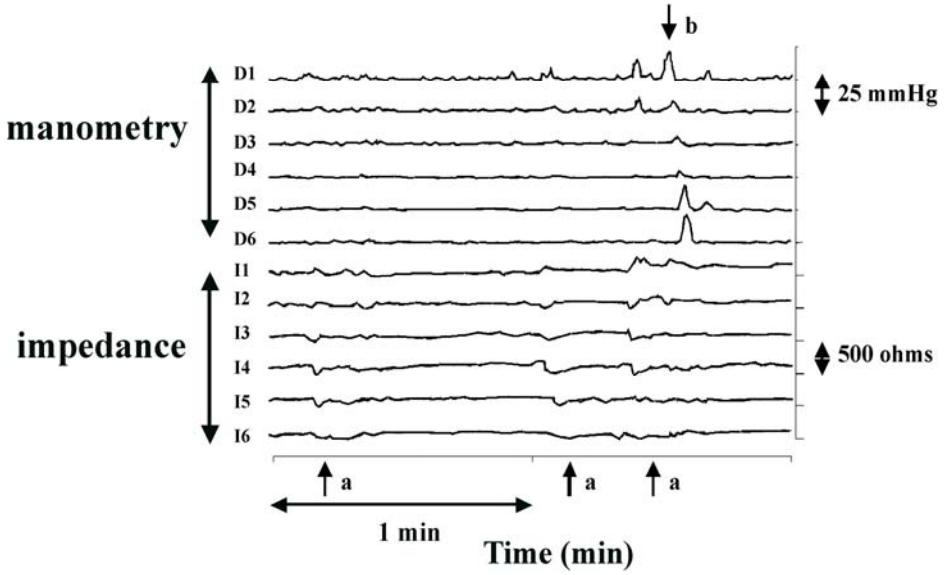

Figure 7.2:

Example of concurrent recording of intraluminal manometry (top 6 channels, D1-D6) and impedance monitoring (bottom 6 channels, I1-I6), demonstrating three flow events (a), of which one is associated with a propagated duodenal pressure wave sequence (b).

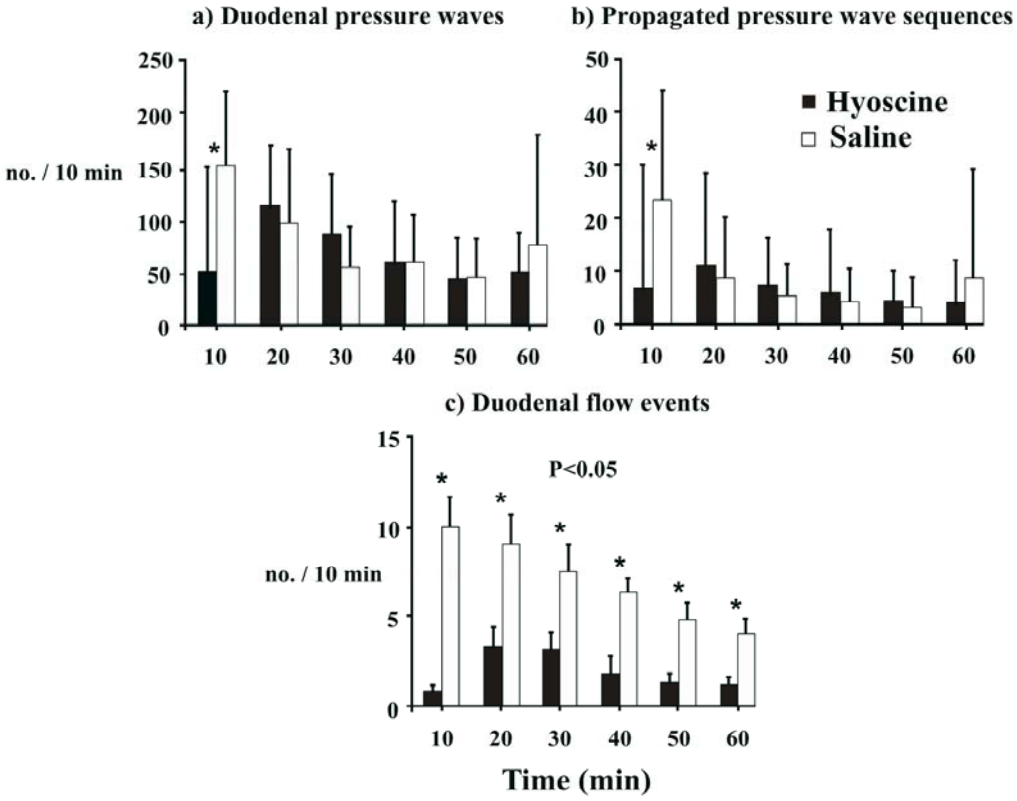

Figure 7.3:

*Effect of intravenous hyoscine on (a) duodenal pressure waves, (b) propagated pressure waves sequences and (c) duodenal flow events during intraduodenal glucose infusion (hyoscine - filled bars, saline - open bars, *P < 0.05 for post-hoc comparison of individual time points).*

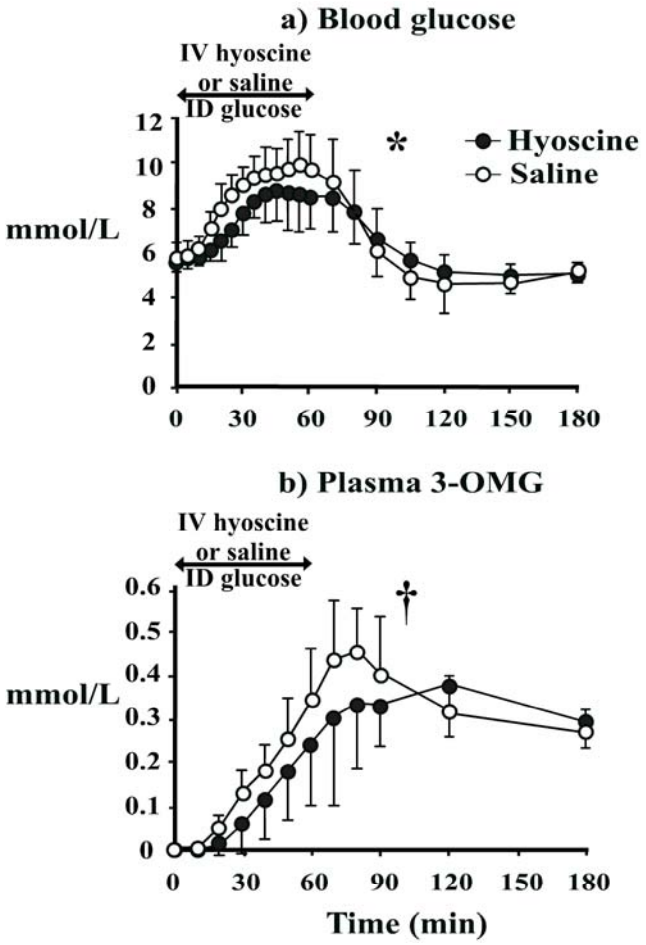

Figure 7.4:

*Effect of intravenous hyoscine on (a) blood glucose concentrations and (b) 3-OMG concentrations, during intraduodenal glucose infusion (hyoscine - filled circles, saline - open circles; *P < 0.05 for the period of IV drug administration (t = 0 - 60 min). †P < 0.05 for area under the 3-OMG curve, t = 0 - 180 min.*

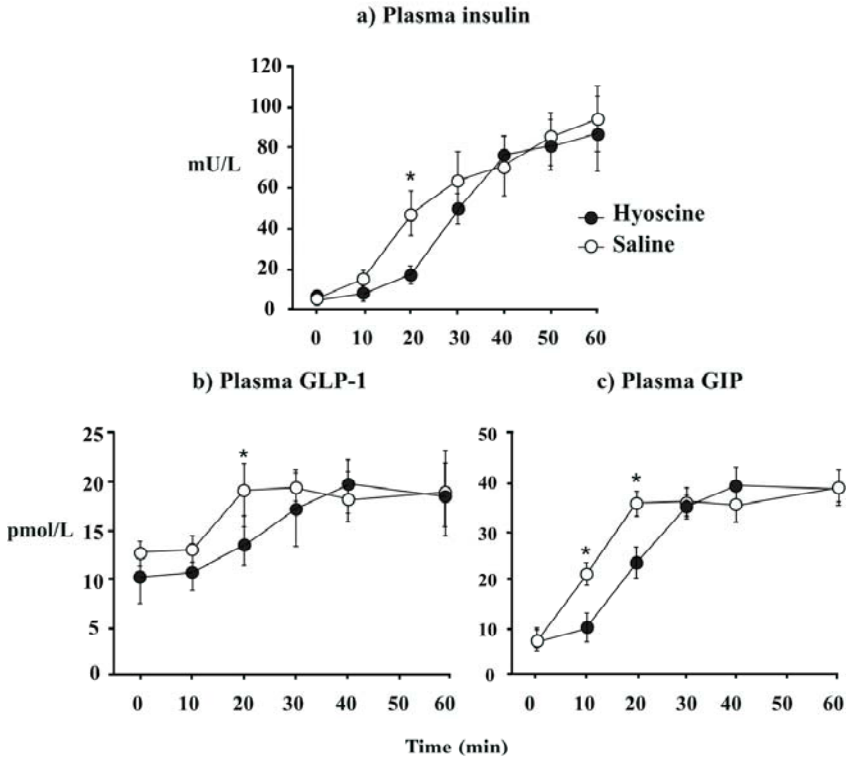

Figure 7.5:

*Effect of intravenous hyoscine on (a) plasma insulin concentrations, (b) plasma GLP-1 concentrations and (c) plasma GIP concentrations, during intraduodenal glucose infusion (hyoscine - filled circles, saline - open circles; *P < 0.05 for comparison of individual time points).*

7.5 DISCUSSION

While the rate of gastric emptying has been established as a major determinant of postprandial glycemia (Horowitz et al. 1993a; Jones et al. 1996b; Merio et al. 1997), the impact of small intestinal motor function in this regard has hitherto received little attention, and is poorly defined. In this study, intraduodenal pressure and impedance signals were recorded simultaneously while glucose was infused into the duodenum in healthy humans, in the presence and absence of the anticholinergic drug hyoscine butylbromide. The frequency of duodenal flow events (evaluated by impedance) was apparently suppressed by hyoscine much more markedly than that of duodenal pressure waves or propagated pressure wave sequences (evaluated by manometry). The disparity between impedance measurements and manometry in detecting alterations in flow during hyoscine infusion was marked, and emphasizes the utility of small intestinal impedance monitoring to evaluate alterations in gastrointestinal transit in various disease states. Manometry detects phasic changes in pressure that result from lumen-occluding contractions, whereas impedance measurement allows inferences to be made regarding transit of boluses of electroconductive fluid between pairs of electrodes. Since luminal flow is affected by variations in intestinal tone and diameter (Cooper et al. 1968), as well as by propagation of lumen-occluding contractions, it is not surprising there may be disparities between manometry and impedance recordings, as illustrated here in Figure 7.3.

By infusing glucose directly into the duodenum, our study design allowed the specific evaluation of the effect of variations in small intestinal motor function on

glucose absorption and incretin release, independent of the known effects of hyoscine on gastric emptying (Stacher et al. 1984). Loperamide has been used to suppress small intestinal motor activity in a previous study of glucose absorption (Samsom et al. 1999), but in rodent models, this drug has the potential to inhibit the intestinal glucose transporter SGLT1 (Klaren et al. 2000). Therefore we chose the anticholinergic drug, hyoscine butylbromide, which is widely used to inhibit small intestinal motor activity in radiologic and endoscopic procedures (Aschoff et al. 1999; Elson et al. 2000), has a rapid onset of action (20 - 30 min), and has little central anticholinergic action. The rate of hyoscine infusion was selected to maintain a stable anticholinergic effect over 60 min (Herxheimer and Haefeli 1966), and the observed heart rate response suggests that this was indeed achieved. Use of the glucose analogue, 3-OMG, which is absorbed in the same way as glucose, but not metabolised (Fordtran et al. 1962), allowed us to differentiate diminished glucose absorption from increased glucose utilisation.

The suppression of flow events that what we observed was associated with attenuation of the rise in blood glucose and a diminished rate of small intestinal glucose absorption, as indicated by plasma 3-OMG (Figure 7.4), as well as a delayed release of both the incretin hormones (GLP-1 and GIP) and insulin (Figure 7.5). We believe that the slowing of intestinal transit by hyoscine diminished the rate of absorption of glucose, delayed the spreading of glucose over the full length of GIP-bearing mucosa in the duodenum and proximal jejunum, and postponed the arrival of glucose into the GLP-1 bearing mucosa in the more distal jejunum and ileum. Both the smaller area under the 3-OMG curve, and the initial delay in

insulin release during the hyoscine day, are consistent with delayed absorption, rather than increased disposal of glucose, as the explanation for the difference in the blood glucose curves. Previous reports have demonstrated a strong relation between small intestinal motility and glucose absorption. Two studies in which glucose (Rayner 1991) or xylose (Fioramonti et al. 1982) were infused into the small intestine reported increased absorption of carbohydrate when the infusion was given during periods of motor activity (phase II or III of the migrating motor complex) compared to motor quiescence (phase I). Manometric observations in humans have also supported a relationship between the absorption of 3-OMG and the frequency of small intestinal pressure waves and propagated pressure sequences (Rayner et al. 2002; Schwartz et al. 2002). Suppression of flow events by hyoscine could temporarily slow glucose absorption by initially restricting the area of the absorptive surface. Spreading of glucose by intestinal transit over longer lengths of gut would allow the recruitment of progressively more glucose transporters, so that the overall rate of glucose absorption into the systemic circulation is no longer restricted to a localised maximum of about 0.5 g per minute over 30 cm of upper jejunum (Holdsworth and Dawson 1964; Modigliani and Bernier 1971; Duchman et al. 1997). In addition, the thickness of the unstirred water layer over the mucosal surface is affected by intraluminal flow rates, with luminal perfusion at higher rates resulting in a thinner unstirred water layer, and thereby, enhanced glucose absorption (Lewis and Fordtran 1975). It could be suggested that the suppression by hyoscine of pressure waves in the first 10 min might be at least as important as the reduction in flow events, in its impact on glucose absorption. However, the 3-OMG concentration curves, which reflect the systemic appearance of glucose

absorbed from the lumen, continued to diverge for up to 80 min, ie. well after the period during which manometric events were suppressed, which argues in favor of the importance of flow events in determining glucose absorption. While there appears to be some disparity between the profound inhibition of flow events by hyoscine, and the more modest reduction in glucose absorption, it should be recognised that, even if long distance flow events were completely abolished, glucose absorption would continue within the limits of the small intestinal surface area exposed to glucose. Moreover, the impedance technique lacks the sensitivity required to identify flow events occurring over short distances, which have a role in facilitating glucose absorption.

Hyoscine attenuated the initial rises in GLP-1, GIP and insulin. This could potentially be accounted for by inhibition of vagally mediated GLP-1 and GIP secretion, but the evidence for vagal control of incretin hormone release is limited (Deacon 2005). In rats, electrical vagal stimulation does not release GIP (Berthoud and Jeanrenaud 1982), while in dogs, neither vagotomy (Ohneda et al. 1985) nor electrical vagal stimulation (Greenberg and Pokol-Daniel 1994) affect GIP secretion in response to glucose. Rodent intestinal L cells, which secrete GLP-1, have muscarinic receptors, and atropine inhibits GLP-1 secretion *in vivo* in rats (Anini et al. 2002); atropine is said not to delay small intestinal transit in this species. GLP-1 secretion from a human L cell line in culture is also reportedly suppressed by muscarinic M_1 and M_2 receptor blockade (Anini and Brubaker 2003) although, as these L cells were derived from a colonic adenocarcinoma line, the relevance of the observations to upper small intestinal L cells *in vivo* is unclear.

The few reports regarding the effect of muscarinic blockade in humans indicate that atropine inhibits both GIP (Ahren and Holst 2001) and GLP-1 (Balks et al. 1997; Ahren and Holst 2001) secretion after a meal, but this may reflect the delay in gastric emptying induced by atropine, rather than any direct inhibition of secretion, since incretin hormone release is critically dependant on the rate of entry of nutrients into the small intestine (O'Donovan et al. 2004b; Chaikomin et al. 2005). There is good evidence, on the other hand, that first phase insulin secretion is under autonomic control (Ahren and Holst 2001) and, therefore could be inhibited by hyoscine. An alternative, and more plausible, explanation for the delay of GLP-1 and GIP secretion in our study may be that the hyoscine slowed the flow of glucose into more distal segments of the small intestine. This may be particularly relevant for GLP-1, given that the density of L-cells increases more distally in the gut (Eissele et al. 1992), where GLP-1 is released by local contact of glucose (Little et al. 2006a). Delayed secretion of GLP-1 and GIP, as opposed to decreased glucose absorption *per se*, also seems likely to account for the plasma insulin profile during hyoscine infusion, which was also attenuated initially, but did not differ from the saline day at 60 min, despite both blood glucose and plasma 3-OMG still being lower at this point. Conversely, suppression of vagally mediated GLP-1, GIP, and insulin secretion alone by hyoscine cannot explain the blood glucose profile, which would then be expected to be higher, rather than lower, than on the saline day, in the absence of any difference in glucose absorption. Studies are indicated to define more clearly the impact of intestinal flow on incretin action.

In summary, this study has established the utility of intraluminal impedance monitoring in detecting pharmacologically-induced alterations in duodenal motor function, and provides evidence that flow patterns of glucose within the small intestine impact on both the rate of glucose absorption as well as incretin hormone and insulin release. In view of our observations, the relevance of changes in impedance patterns to nutrient absorption in the small intestine in disease states should be explored in future studies. A particular priority is to determine the degree to which variations in small intestinal flow patterns influence postprandial glycaemia in patients with diabetes.

CHAPTER 8:

EFFECTS OF INTRALUMINAL LOCAL ANAESTHETIC ON DUODENAL GLUCOSE SENSING IN HUMANS

8.1 SUMMARY

Enteral administration of glucose modifies gut sensation, diminishes hunger, and slows gastric emptying by suppressing antral motility and stimulating pyloric pressures. The mechanism of small intestinal glucose sensing is unclear. We studied 8 healthy males on two days each, in random order. After an overnight fast, a catheter was positioned with a sleeve sensor across the pylorus, and sideholes in the antrum and duodenum. Benzocaine, or vehicle alone, was given into the proximal duodenum as an initial bolus, followed by a continuous infusion for 105 min (t = -15 to 90 min). Glucose was also infused into the proximal duodenum at 3 kcal/min for 90 min (t = 0 to 90 min). Sensations of hunger, bloating, and nausea were assessed at frequent intervals with visual analogue scales. Antral, duodenal, and isolated pyloric pressure waves (IPPWs) > 10 mmHg were counted, together with pyloric tone and propagated sequences of duodenal waves. Data are mean ± SEM. Sensations of bloating and nausea were markedly less with benzocaine when compared to vehicle ($P < 0.05$ for each), with no difference in hunger. In contrast, the suppression of antral waves and stimulation of IPPWs, pyloric tone, duodenal waves, and propagated duodenal wave sequences during 90 min of intraduodenal glucose infusion did not differ between the two days. There was no difference in blood glucose, plasma insulin or GLP-1 profile between benzocaine and control, but GIP concentrations were higher with benzocaine compared to control ($P <$

0.05). We conclude that nerves in the duodenal mucosa predominate over hormonal signals in mediating unpleasant sensations induced by enteral glucose, while feedback on appetite and gastroduodenal motility is conveyed by alternative mechanisms, such as gut peptides.

8.2 INTRODUCTION

Nutrients interact with the small intestine to modulate gastroduodenal motility, sensation, energy intake and glycaemia. Infusion of nutrients into the small intestine slows gastric emptying, associated with suppression of antral contractions, stimulation of pyloric pressures (Heddle et al. 1988c), and induction of irregular duodenal contractions (Husebye 1999) and as a result of these phenomena, nutrients empty from the stomach at about 3 kcal/min (Brener et al. 1983a). Small intestinal glucose decreases hunger and subsequent energy intake (Lavin et al. 1996b) and its effects of suppressing appetite, and stimulating insulin secretion, are much greater than when an equivalent glucose load is infused intravenously (Lavin et al. 1996b; Lavin et al. 1998b).

The so-called “incretin” peptides, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are key mediators of the effects of small intestinal glucose on gastric emptying, appetite, and insulin release (Kreymann et al. 1987; Wettergren et al. 1993; Lavin et al. 1998b; Schirra et al. 2006). GLP-1 is released from L-cells whose density is greatest in the distal jejunum, with fewer cells in proximal jejunum, ileum, and colon (Eissele et al. 1992), whereas GIP is released from duodenal K cells, and possibly also from a sub-group of L cells in the mid-

small intestine (Mortensen et al. 2003). The stimulus for GLP-1 release is unclear, since plasma levels rise rapidly after ingestion of a meal, before nutrients would be expected to have reached the distal small bowel (Herrmann et al. 1995; O'Donovan et al. 2005a). A so-called duodeno - jejuno/ileal "loop" has been postulated, but its neural or humoral mediators remain poorly defined (Holst 1994). There is a possibility that 'local' neural mechanisms might play a role in the regulation of GLP-1 secretion, and these could be blocked by local anaesthetic.

Administration of the local anaesthetic benzocaine into the duodenum has been shown to attenuate both the release of cholecystokinin (CCK), and the perceptions of fullness, discomfort, and nausea induced by gastric distension, during intraduodenal lipid infusion in healthy subjects (Feinle et al. 2001a). This implies that local neural mechanisms regulate CCK release in response to intraduodenal fat. Glucose is a weaker stimulus for CCK release than either fat or protein (Liddle et al. 1985), but is a potent stimulus for incretin hormone release. Whether similar mechanisms mediate the release of the incretin hormones, as well as the effects of glucose on gut sensation and gastroduodenal motility, is unknown.

The aim of the current study is to evaluate the effects of intraduodenal administration of benzocaine on (i) gut sensations and energy intake, (ii) antral, pyloric, and duodenal motility, and (iii) the release of GLP-1, GIP, insulin, and glycaemia, in response to intraduodenal glucose.

8.3 METHODS

8.3.1 Subjects

Eight healthy males (age 26.1 ± 2.4 years, body mass index 27.6 ± 1.0 kg/m^2) were recruited by advertisement.

8.3.2 Protocol

Each subject underwent paired studies, separated by an interval of 4 - 7 days, in randomised order. Following an overnight fast (14 hours for solids and 12 hours for liquids), the subject attended the laboratory at 0900h. A silicone rubber manometry catheter was introduced into the stomach through an anaesthetised nostril, and allowed to pass into the duodenum by peristalsis. The catheter incorporated multiple lumens within an external diameter of about 4 mm, with 4 sideholes spaced at 1 cm intervals to measure pressures in the antrum and 6 in the duodenum spaced at 1.5 cm intervals, together with a sleeve sensor positioned across the pylorus (Heddle et al. 1988b). The catheter also incorporated a channel for infusion of benzocaine and glucose into the proximal duodenum, located between the two most proximal duodenal manometry sideholes. The manometry channels were perfused with water, and the distal antral and proximal duodenal sideholes with saline, so that the correct position of the catheter could be monitored continuously by measurement of the transmucosal potential difference (TMPD) on either side of the pylorus (antral TMPD < -20mV, duodenal TMPD > -15mV, difference > 15mV) (Heddle et al. 1988c). This required the insertion of a 20G saline-filled cannula subcutaneously in the forearm as a reference. When the

catheter was positioned correctly based on TMPD criteria, a cannula was then inserted into a forearm vein to allow repeated blood sampling.

Following phase III of the duodenal migratory motor complex, Subcutin N (benzocaine 0.75%, polyoxyl-40-hydrogenated castor oil 9.25%, propylene glycol 20%, distilled water 70%) or vehicle alone (polyoxyl-40-hydrogenated castor oil 10%, propylene glycol 20%, distilled water 70%) (both a gift from Dr Stefan Ritsert, Dr E Ritsert & Company, Ritsert Inc, Eberbach, Germany) was infused into the duodenum with an initial 10 mL bolus, followed by 2 mL/min for 105 min (T= -15 to 90 min). Fifteen min after the beginning of the benzocaine infusion (ie T = 0 min), 270 mL hypertonic glucose (25%) was infused into the duodenum at 3 mL per minute (i.e. 3 kcal/min), and continued for 90 min (T = 0 - 90 min). Venous blood was sampled at T = -15, -5, 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 min for measurement of blood glucose and at T = 0, 10, 20, 30, 45, 60 and 90 min for plasma peptide concentrations. Subjects completed visual analogue questionnaires at T = -15, -5, 0, 15, 30, 45, 60, 75 and 90 min to rate sensations of hunger, desire to eat, fullness, abdominal discomfort, bloating, and nausea. Heart rate and blood pressure were recorded at regular intervals, and oxygen saturation was monitored continuously by pulse oximetry, in view of the theoretical risk of methaemoglobinaemia if benzocaine were absorbed (Moore et al. 2004).

At T = 90 min, the manometry catheter was removed, and subjects was presented with a buffet meal from which were allowed to eat *ad libitum* for 30 min, and from which food intake was calculated (Sturm et al. 2004).

8.3.3 Measurements

Sensations and energy intake

Sensations were rated with the use of 100 mm visual analogue scale as described previously (Sepple and Read 1989). Energy intake, as assessed by the amount of food consumed at the buffet meal, was quantified by using FOODWORKS software (version 3.1; Xyris Software Pty Ltd, Highgate Hill, Australia).

Manometric analysis

Manometric data were analysed in semi-automated fashion, using previously described software (Samsom et al. 1998c). The frequency of antral, duodenal and isolated pyloric pressure waves $\geq$ 10 mmHg was measured in 15 minute intervals. IPPWs were defined as waves recorded by the sleeve sensor in the absence of a pressure wave of onset within 5 seconds of the pyloric wave in the adjacent antral or duodenal sideholes (Heddle et al. 1988b). The frequency of propagated pressure wave sequences ($\geq$ 2 channels) was also recorded in 15 minute intervals (Rayner et al. 2002). Basal pyloric pressure ("tone") was calculated for each minute by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side hole from the mean basal pressure recorded at the sleeve (Heddle et al. 1988b), using custom-written software (MAD, C. H. Malbert, Institut National de la Recherché Agronomique, Rennes, France), and the mean basal pressure calculated in15 minute intervals.

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

Blood glucose concentrations were determined immediately using a portable glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, Massachusetts, USA). Blood samples for determination of plasma insulin, GLP-1, and GIP were collected in ice-chilled tubes containing EDTA and 400 kIU aprotonin (Trasylol; Bayer Australia Ltd., Pymple, Australia) per liter blood. Plasma was separated by centrifugation and stored at –70 ºC for subsequent analysis. Plasma insulin concentrations were measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc., Webster, Texas, USA) (Horowitz et al. 1996a), and plasma GLP-1 and GIP concentrations by radioimmunoassay (RIA) (O'Donovan et al. 2004b).

8.3.4 Statistical Analysis

Student's paired t-test (Statview 5, SAS Institute, Cary, North Carolina, USA) was used to compare sensation scores and pressure wave frequencies at baseline (T = -15 min) and at the onset of benzocaine or control infusion (T = 0 min), as well as energy intake. One way repeated measures ANOVA was used to examine changes in sensation scores, manometry data, blood glucose and plasma hormone concentrations over time, and two way repeated measures ANOVA was used to compare these data between studies, with treatment and time as factors (SuperANOVA version 1.11; Abacus Conceptes Inc, Berkeley, California, USA). Statistical significance was accepted at $P < 0.05$, and data are presented as mean values ± standard error.

8.4 RESULTS

All subjects tolerated the study well. Heart rate, blood pressure and oxygen saturation remained stable on all study days.

8.4.1 Gastrointestinal sensations and appetite (Figure 8.1)

There were no differences in any of the sensation scores between study days at baseline (T = -15 min), or at the onset of intraduodenal glucose infusion (T = 0 min), except that the perception of bloating was slightly greater for control than for benzocaine at T = 0 ($P < 0.05$), and had increased from baseline at this point on the control day ($P < 0.05$). Perceptions of fullness, abdominal discomfort, hunger, and desire to eat did not change during either study, and did not differ between the two studies. Nausea increased during intraduodenal glucose infusion (T = 0 to 90 min) on both study days (each $P < 0.05$), but the perception of nausea was markedly less with benzocaine when compared to control ($P < 0.05$). Bloating also increased during intraduodenal glucose infusion ($P < 0.005$ for benzocaine and $P=0.07$ for control), and was less with benzocaine than control ($P < 0.05$).

8.4.2 Energy intake

There was no difference in energy intake at the buffet meal after intraduodenal benzocaine (1014 ± 124 kcal) when compared to control (964 ± 93 kcal).

8.4.3 **Antropyloroduodenal motility** (Figure 8.2)

Antral Pressure waves

The frequency of antral pressure waves did not differ between the study days, either at baseline (T = -30 to -15 min) or during initiation of benzocaine or control infusion (T = -15 to 0 min). The frequency of antral waves was suppressed during intraduodenal glucose infusion (T = 0 to 90 min) on the control day ($P < 0.05$), and there was no difference in the frequency of antral waves during this period with benzocaine compared to control (Figure 8.2a).

Pyloric pressure waves

The frequency of IPPWs increased during infusion of either benzocaine ($P < 0.05$) or control ($P = 0.07$) into duodenum (T = -15 to 0 min versus baseline), and increased further with the onset of glucose infusion (T = 0 to 15 min, $P < 0.05$ on both days), followed by a decline until T = 90 min. There was no difference in the frequency of IPPWs between the two days (Figure 8.2b). Pyloric tone increased from baseline on both the control ($P < 0.05$) and benzocaine ($P = 0.08$) days, with the increase evident soon after the onset of intraduodenal glucose infusion, and a peak on each day between T = 15 and 30 min. There was no difference in the stimulation of pyloric tone between the two days (Figure 8.2c).

Duodenal Pressure Waves

There was a stimulation of duodenal pressure waves with the initiation of benzocaine or control infusion (T = -15 to 0 min, $P < 0.05$ for each study day). There was no difference in the frequency of duodenal pressure waves between two

study days (Figure 8.2d). A similar pattern was observed for the frequency of propagated duodenal wave sequences, with initial stimulation by benzocaine and control ($P < 0.05$ for each), and a later decline in frequency, with no difference between study days (Figure 8.2e).

8.4.4 **Blood glucose and plasma peptide concentrations** (Figure 8.3)

Blood glucose

Blood glucose increased soon after the onset of intraduodenal glucose infusion on both days ($P < 0.0005$), with a peak between 30 and 45 min, followed by a slight decline; there was no significant difference in blood glucose profile between benzocaine and control (Figure 8.3a).

Plasma insulin concentrations

Plasma insulin concentrations increased during intraduodenal glucose infusion on both days ($P < 0.0005$), reaching a plateau from about $T = 45$ min, and without a significant difference between benzocaine and control (Figure 8.3b).

Plasma GLP-1 concentrations

Plasma GLP-1 concentrations also increased during intraduodenal glucose infusion on both days ($P < 0.0005$), and rose progressively throughout, again with no difference between the study days (Figure 8.3c).

Plasma GIP concentrations

Plasma GIP concentrations increased during intraduodenal glucose infusion on both days, but tended to plateau from T = 45 min. In contrast to GLP-1, GIP concentrations were significantly higher with benzocaine compared to control ($P < 0.05$), however the magnitude of their difference was small (Figure 8.3d).

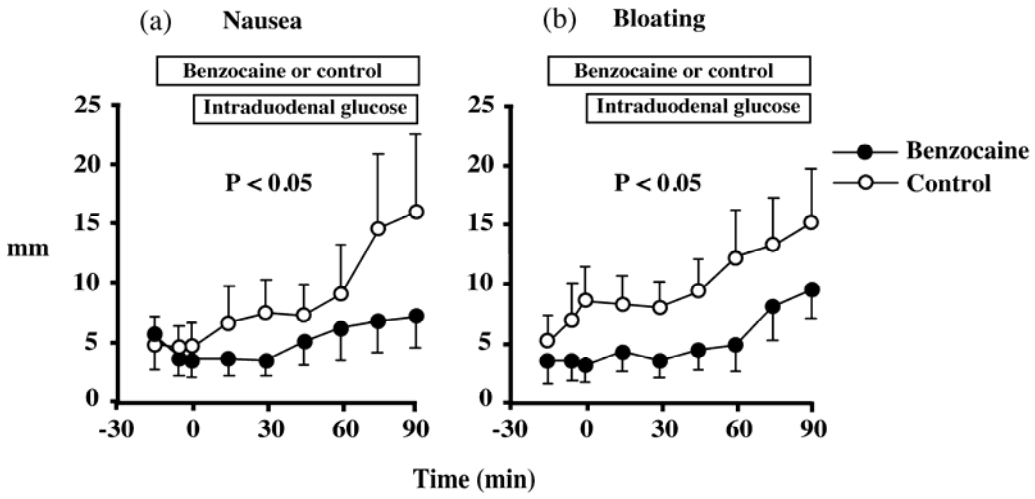

Figure 8.1

Perceptions of nausea and bloating in response to intraduodenal glucose with intraduodenal benzocaine (closed circles) or vehicle alone as a control (open circles). Nausea and bloating scores were lower for benzocaine than control, during intraduodenal glucose infusion (T = 0 to 90 min, P < 0.05).

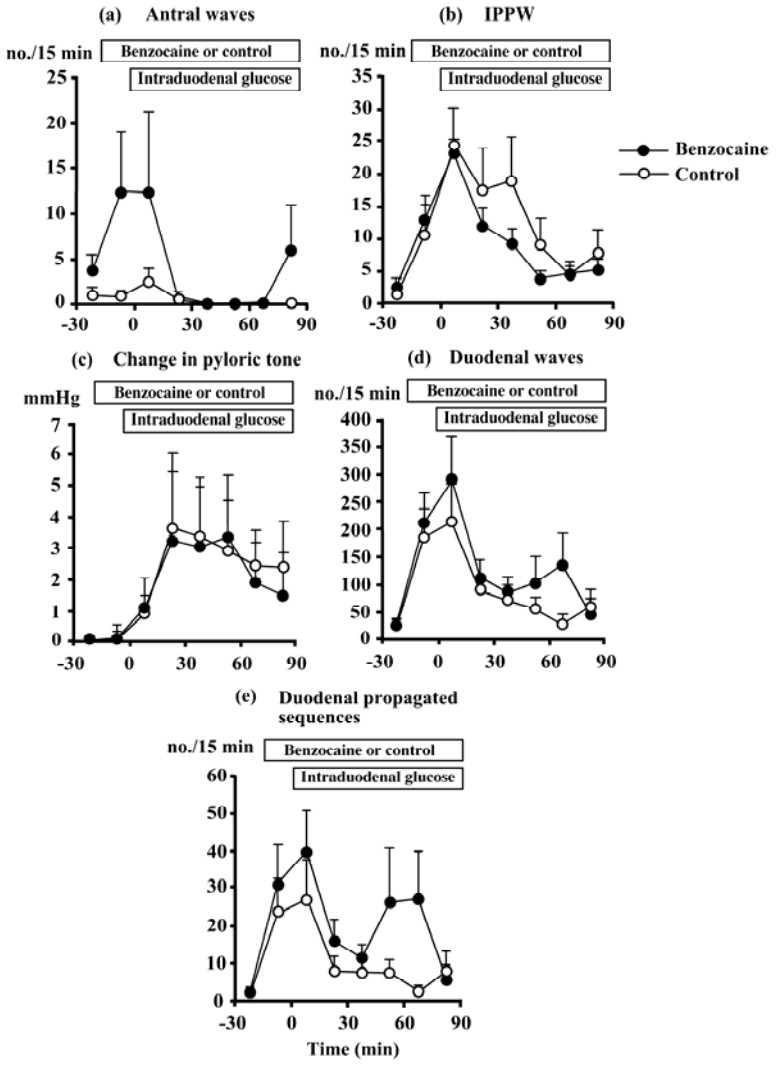

Figure 8.2

(a) Antral wave frequency (total in 4 antral channels), (b) isolated pyloric pressure wave (IPPW) frequency, (c) change in pyloric tone, (d) duodenal wave frequency (total in 6 duodenal channels), and (e) frequency of propagated duodenal wave sequences, with intraduodenal benzocaine (closed circles) or vehicle alone as a control (open circles), during intradudoenal glucose infusion (T = 0 to 90 min). All wave frequencies are shown as the number per 15 minute period.

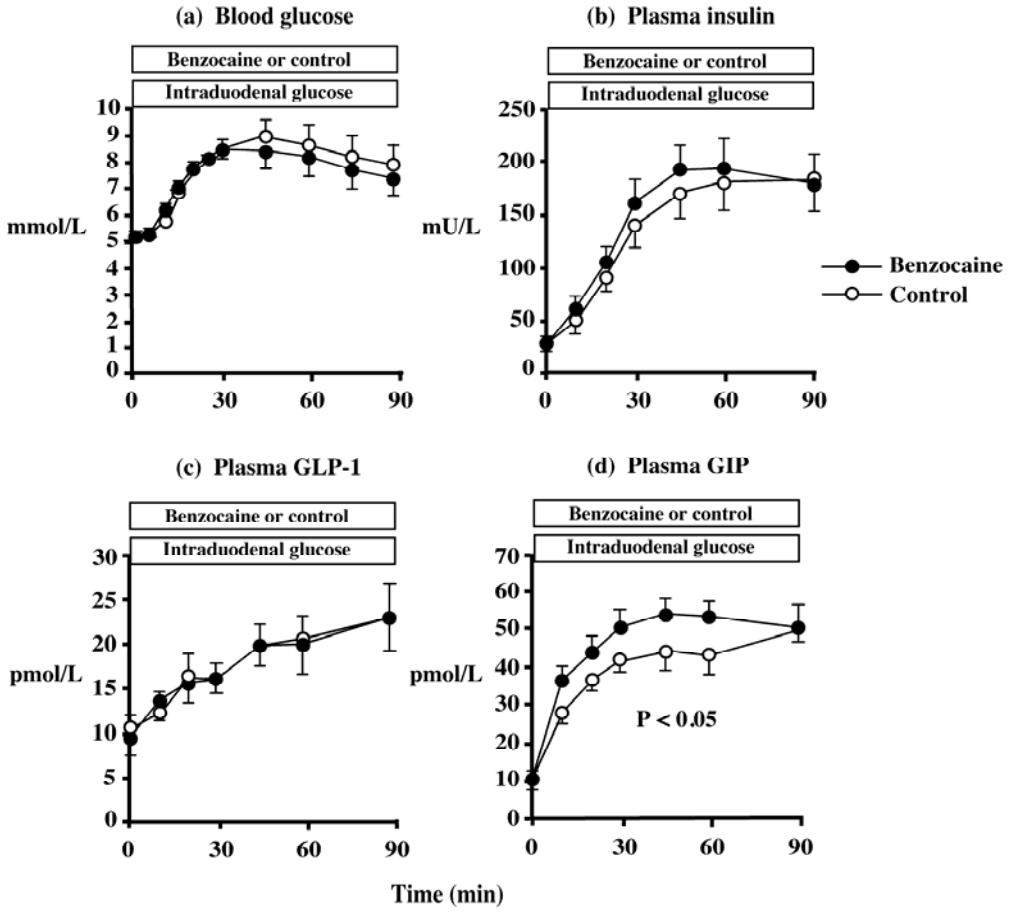

Figure 8.3

(a) Blood glucose, (b) plasma insulin, (c) plasma GLP-1, and (d) plasma GIP responses to intraduodenal glucose with intraduodenal benzocaine (closed circles) or vehicle alone as a control (open circles). Plasma GIP concentrations were higher with benzocaine than control ($P < 0.05$).

8.5 DISCUSSION

The mechanisms by which nutrients are sensed by the small intestinal mucosa in order to modulate gastric emptying, appetite, gut sensations, peptide hormone release, and glucose absorption, have been a major research focus in recent years (Furness et al. 1999). While important mediators have been determined in animal models (Lavin and Read 1995; Meyer et al. 1998), the number of invivo human studies is limited. However, this information is important in understanding gut function, and addressing disorders of its regulation.

We evaluated the role of duodenal mucosal nerves in mediating responses to intraluminal glucose by infusing the lipophilic local anaesthetic benzocaine directly into the duodenum, in a comparable dose to that used previously in humans (Feinle et al. 2001a). Benzocaine has been used to block vagal mucosal afferents in rats (Richards et al. 1996), and in contrast to other local anaesthetics, does not affect the mucosal absorption of monosaccharides (Strugala et al. 2000). Feinle et al. have reported previously that benzocaine attenuates both the release of CCK, and the perception of nausea during concurrent gastric distension, induced by intraduodenal lipid infusion (Feinle et al. 2001a). Enteral infusion of glucose suppresses appetite and subsequent energy intake much more than intravenous glucose, highlighting the importance of the interaction of nutrients with small intestinal receptors in the induction of satiety. However, the effects of benzocaine on small intestinal motor, sensory, and hormonal response to glucose had not been determined. We administered glucose intraduodenally at the same site as benzocaine, and at a rate (3 kcal/min), a rate known to generate antropyloric

feedback, suppress appetite, and stimulate incretin hormone release (Andrews et al. 1998; Lavin et al. 1998a), in order to determine whether mucosal anaesthesia could attenuate these effects. While the glucose infused was hypertonic (25%), and therefore also provided an osmotic stimulus (Houpt et al. 1983; Vist and Maughan 1995), provision of the same glucose load as an isotonic solution would have greatly increased the volume of infusion, with resultant dilution of the local anaesthetic and the possibility of distension itself inducing uncomfortable sensations.

The striking observation in our study was that benzocaine markedly suppressed the sensations of nausea and bloating associated with intraduodenal glucose administration. This suppression was not accounted for by a reduction in secretion of the incretin hormones, and in particular GLP-1, and therefore probably indicates that the sensory response to nutrient stimuli in the small intestine is mediated via mucosal afferent nerves. Although glucose is a much less potent stimulus for CCK secretion than lipid (Liddle et al. 1985), we did not measure plasma CCK in the current study, so we cannot discount the possibility that a reduction in CCK secretion (Feinle et al. 2001a) could also have contributed to the differences in sensation between the benzocaine and control days. Our data suggest that the vehicle itself may have also contributed to the sensation of bloating, and this too appeared to be suppressed by the presence of benzocaine.

Neither hunger nor energy intake at a subsequent buffet meal were affected by intraduodenal local anaesthetic. This may partly reflect the fact that intraduodenal

glucose is less potent than lipid in suppressing hunger (Andrews et al. 1998; MacIntosh et al. 2001a), but probably more importantly that the suppression of appetite by intraduodenal glucose is more dependent on the release of gut peptides including GLP-1 (Lavin et al. 1998a), than on the stimulation of mucosal afferent nerves. Benzocaine also had no effect on the suppression of antral pressures, stimulation of phasic and tonic pyloric motility, or transient stimulation of duodenal pressure waves or propagated pressure wave sequences induced by intraduodenal glucose infusion. This is consistent with the concept that gut peptides, including GLP-1, have a more dominant role than mucosal afferent nerves in mediating the upper gastrointestinal motor response to glucose. Indeed, a physiological role of GLP-1 in this regard has recently been established in healthy humans using the GLP-1 antagonist exendin(9-39)amide (Schirra et al. 2006). However, there may to be a role for intramural nerves in mediating pyloric responses to duodenal glucose at lower rates of glucose infusion, below the apparent threshold for GLP-1 release (Schirra et al. 1996a; O'Donovan et al. 2004b; Chaikomin et al. 2005); Treacy et al. (1992) reported in pigs that duodenal transection attenuated the pyloric motor response to low, but not high loads of glucose delivered into the duodenum (Treacy et al. 1992).

Despite the importance of GLP-1 and GIP on blood glucose homeostasis, it is remarkable how little information is available regarding the mechanisms that mediate their release. We did not observe any attenuation of the release of GLP-1 or GIP by benzocaine, in response to glucose. GLP-1 is secreted from L-cells, whose density is greatest in the distal small intestine, yet plasma levels rise within

~15 min after oral or intraduodenal glucose administration (Holst 1994; Schirra et al. 1996a; O'Donovan et al. 2004b; Chaikomin et al. 2005) - an observation confirmed in the current study. The presence of a neural or humoral "loop" linking the sensing of glucose in the duodenum with the release of GLP-1 from the distal small intestine has been postulated (Holst 1994). GIP, secreted from duodenal K cells, appears to fulfil this function in rodents but not humans (Meier et al. 2002a), and our current observations are against the stimulation of duodenal mucosal nerve endings mediating this "loop", since GLP-1 secretion was not inhibited by duodenal benzocaine. Alternative explanations for the early rise in GLP-1 include the recent discovery of a population of L cells located in the duodenum (Theodorakis et al. 2006), or that initial transit of glucose through the upper small intestine is initially rapid enough to allow contact with a sufficient density of jejunal L cells to elevate plasma GLP-1, and is then subsequently slower. The latter hypothesis is supported over the former by our recent report that GLP-1 was only released when intraluminal glucose was allowed exposure beyond the proximal 60 cm of the small intestine (Little et al. 2006a).

A novel and unexpected observation in the current study was that benzocaine increased the plasma concentration of GIP in response to glucose, when compared to infusion of vehicle alone. Since our assay was for total GIP rather than just the "intact" fraction, this is likely to reflect enhanced secretion of GIP, rather that inhibition of the enzyme responsible for its degradation (dipeptidyl peptidase IV) at the level of the duodenal mucosa (Gunnarsson et al. 2006). However, the increase in GIP was insufficient to stimulate significant additional insulin secretion, nor to

lower the blood glucose profile, when compared to the control day, and the observation further confirmation. Nevertheless, investigation of the mechanism involved in the stimulation of GIP release by benzocaine would be of interest, since GIP plays a major role in the postprandial stimulation of insulin in healthy humans (the "incretin effect"), although the response to GIP appears to be diminished in patients with type 2 diabetes (Holst and Gromada 2004). There is also recent evidence in a rodent model, that GIP may modulate small intestinal motility and, hence, glucose absorption (Eiichi et al. 2006).

In conclusion, this study indicates that nerves in the duodenal mucosa appear to predominate over humoral mechanisms in mediating unpleasant sensations induced by exposure of the small intestine to glucose. On the other hand, the release of peptide hormones, including GLP-1, appears more important in generating feedback on appetite and gastroduodenal motility.

CHAPTER 9:

EFFECTS OF MID-JEJUNAL, COMPARED TO DUODENAL, GLUCOSE INFUSION ON PEPTIDE HORMONE RELEASE AND APPETITE

9.1 SUMMARY

Small intestinal exposure to glucose releases hormones including cholecystokinin (CCK), and the "incretins" glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which modulate appetite and glycaemia. Enteroendocrine cells containing GIP and CCK predominate in the upper small intestine, while those containing GLP-1 are located more distally. The purpose of this study was to determine whether the hormonal, glycaemic and appetite responses to enteral glucose are dependent on the site of glucose delivery. Ten healthy males were each studied on two days, in random order. After an overnight fast, a multilumen catheter was positioned with one infusion channel terminating 15 cm beyond the pylorus ("duodenal"), and a second terminating 100 cm beyond ("mid-jejunal"). On one day, glucose (5%) was infused into the duodenum (1 kcal/min) while saline (0.9%) was infused into the mid-jejunum, both at 5 mL/min, for 90 min. On the other day, the sites of infusion were reversed. Blood was sampled for glucose, insulin, GLP-1, GIP, and CCK, and sensations of hunger were scored using visual analogue scales. At 90 min, the tube was removed and energy intake from a buffet meal was measured. Data are mean ± SEM. The stimulation of CCK (incremental area under the curve) and suppression of hunger were greater ($P < 0.05$ for each), and energy intake less (1252 ± 97 v 1413 ± 62 kcal, $P = 0.05$), with duodenal compared with mid-jejunal glucose infusion. There

were no differences in blood glucose, GIP, or insulin responses, and there was minimal GLP-1 increment on either day. In conclusion, there is substantial regional variation in CCK, but not incretin hormone release, in the upper small intestine, and modest differences in the intestinal site of glucose exposure affect appetite and energy intake.

9.2 INTRODUCTION

Nutrients interact with the small intestine to induce secretion of peptide hormones, which influence appetite and gut motility. Therefore, the suppression in hunger and subsequent energy intake (Welch et al. 1988b; Lavin et al. 1996b), and the stimulation of insulin secretion, are much greater when glucose is infused into the small intestine, than when the same glucose load is infused intravenously (Lavin et al. 1996b; Lavin et al. 1998b).

The “incretin” peptides, glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP), are key mediators of the effects of small intestinal glucose on gastric emptying, appetite, and insulin release (Kreymann et al. 1987; Wettergren et al. 1993; Lavin et al. 1998b). GLP-1 is released from L-cells whose density is greatest in the distal jejunum, with fewer cells in the proximal jejunum, ileum, and colon (Eissele et al. 1992). The stimulus for GLP-1 release is unclear, since plasma levels rise relatively rapidly after ingestion of a meal and before nutrients would be expected to have reached the distal small bowel (Herrmann et al. 1995). A duodeno - jejuno/ileal loop has been postulated, but its neural or humoral mediators have not been defined in humans (Holst 1994).

There is persuasive evidence that enhanced contact of carbohydrates with more distal gut regions stimulates GLP-1 release, and inhibits appetite. After jejuno-ileal bypass for the treatment of obesity, postprandial plasma GLP-1 concentrations are substantially higher than in obese controls (Mason 1999), while patients with extensive small bowel resection but an intact colon have greater GLP-1 responses to a meal than healthy subjects (Andrews and Irving 1992; Jeppesen et al. 2000b). Furthermore, when hydrolysis of sucrose is delayed by the α-glucosidase inhibitor acarbose, allowing contact of sucrose with the distal small bowel, GLP-1 release is prolonged when compared to sucrose ingested alone (Qualmann et al. 1995; Gentilcore et al. 2005). Our group recently reported that when an enteral glucose infusion is limited to the proximal 60 cm of small intestine by an occlusive intraluminal balloon, GLP-1 release is abolished (Little et al. 2006a), implying that GLP-1 secretion is dependent on exposure of either a minimum length of small intestine, or the distal small intestine, to glucose.

GIP is released from duodenal K cells, and possibly also from a sub-group of L cells in the mid-small intestine (Mortensen et al. 2003). Under physiological conditions, small loads of rapidly absorbable carbohydrate would preferentially release the proximal incretin hormone GIP, whereas ingestion of a larger meal containing complex carbohydrates would also release the distal incretin GLP-1 (Vilsboll et al. 2003). However, the effect of direct contact of glucose with different sites in the small intestine on the incretin response has not been evaluated in humans.

Cholecystokinin (CCK), another peptide hormone released by exposure of the small intestine to nutrients, is secreted by proximally-located I cells, which appear to be confined to the duodenum and jejunum (Polak et al. 1975; Buffa et al. 1976). Like GLP-1, CCK suppresses appetite and energy intake (Matzinger et al. 1999; Beglinger et al. 2001; Little et al. 2005), but unlike GLP-1 and GIP, is not an incretin hormone (Baum et al. 1992). In dogs, exposure of an isolated duodeno-jejunal loop to nutrient stimulates CCK release, while exposure of an ileal loop does not (Konturek et al. 1986). However, when the enteric nerves remain intact, even distal small intestinal perfusion with lipid stimulates CCK secretion, presumably via an intrinsic neural connection (Lin and Chey 2003). In humans, both proximal- (at the ligament of Treitz) and mid-jejunal mixed nutrient infusion (60 cm more distally) stimulated CCK release, with no difference between these sites (Vu et al. 1999).

Information regarding the effects of glucose on different gut regions may be important in designing nutritional strategies for patients with obesity or diabetes mellitus, or those requiring enteral nutrition. We compared the effects of glucose infused into the duodenum (15 cm post-pylorus) with an isocaloric infusion into the mid-jejunum (100 cm post-pylorus), on (i) the release of the peptides GLP-1, GIP, CCK and insulin, (ii) blood glucose concentrations, and (iii) appetite and food intake.

9.3 METHODS

9.3.1 Subjects

Ten healthy males (mean age 27.0 ± 1.8 years, body mass index 26.2 ± 0.8 kg/m^2) were studied on two days each, at least 2 days apart, in randomised single blinded order. The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital, and each subject gave written, informed consent.

9.3.2 Protocol

Following an overnight fast (14 hours for solids and 12 hours for liquids), subjects attended the laboratory at 0900h. A multilumen silicone rubber catheter (Dentsleeve, Wayville, Australia) was introduced into the stomach through an anaesthetised nostril, and allowed to pass into the duodenum by peristalsis. The catheter tip incorporated tungsten weights and a 10 mL balloon that was inflated after passage into the duodenum, to facilitate progression through the small intestine, and was deflated again when the catheter had reached the correct position (Fone et al. 1990b). The catheter incorporated two infusion channels, one terminating 15 cm beyond the pylorus and the other 100 cm beyond. There were also 2 channels terminating in the distal antrum & proximal duodenum, which were perfused with saline, so that the correct position of the catheter could be monitored continuously by measurement of the transmucosal potential difference (TMPD) (antral TMPD < -20mV, duodenal TMPD > -15mV, difference > 15mV) (Heddle et al. 1988c). A 20G saline-filled cannula was inserted subcutaneously in the forearm as a reference. When the catheter was positioned correctly on TMPD

criteria, an intravenous cannula was inserted into a forearm vein to allow repeated blood sampling.

450 mL isotonic glucose (5%) was infused into the small intestine through either the proximal (duodenal) or distal (mid-jejunal) infusion channel at 5 mL per minute (1 kcal/min), and continued for 90 min (t = 0-90 min). Saline (0.9%) was infused via the other infusion channel, also at 5 mL/min. Blood samples were taken at t = 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, and 90 min for measurement of blood glucose, and at t = 0, 10, 20, 30, 40, 60, and 90 min for GIP, GLP-1, CCK, and insulin. Subjects were asked to complete visual analogue questionnaires at t = 0, 15, 30, 60, and 90 min to score sensations of hunger, desire to eat, fullness, abdominal discomfort, bloating, and nausea. At t = 90 min, the catheter was removed, and subjects were allowed to eat ad libitum for 30 min to assess energy intake, using FOODWORKS software (version 3.1; Xyris Software Pty Ltd, Highgate Hill, Australia).

9.3.3 Measurements

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

Blood samples for determination of plasma insulin, GLP-1, and GIP were collected in ice-chilled tubes containing EDTA and 400 kIU/L aprotonin (Trasylol; Bayer Australia Ltd., Pymble, Australia). Plasma was separated by centrifugation and stored at −70 ºC for subsequent analysis. Blood glucose concentrations were determined immediately using a portable glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, Massachusetts, USA). Plasma insulin concentrations

were measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc., Webster, Texas, USA) (Horowitz et al. 1996a), and plasma GLP-1 and GIP concentrations by radioimmunoassay (RIA) (O'Donovan et al. 2004b). Plasma CCK was determined by RIA, after ethanol extraction (MacIntosh et al. 2001c).

9.3.4 Statistical Analysis

Student's paired t-test (Statview 5, SAS Institute, Cary, North Carolina, USA) was used to compare (i) blood glucose and plasma hormone concentrations, and visual analogue scores for each sensation, at baseline (t = 0), (ii) incremental areas under the blood glucose and plasma hormone curves, calculated using the trapezoidal rule, and (iii) energy intake at the buffet meal. One way repeated measures ANOVA was used to examine changes in blood glucose and plasma hormones over time, and two way repeated measures ANOVA was used to compare changes in fullness, nausea, discomfort, bloating, hunger, and desire to eat, with treatment and time as factors (SuperANOVA version 1.11; Abacus Concepts Inc, Berkeley, California, USA). Statistical significance was accepted at $P < 0.05$, and data are presented as mean values ± standard error.

9.4 RESULTS

All subjects tolerated the study well. There were no differences at baseline (t = 0) in blood glucose or plasma hormone concentrations between the study days. Baseline scores for hunger (38.5 ± 7.3 v 50.8 ± 7.3 mm, $P < 0.05$) and bloating (12.1 ± 3.3 v 25.4 ± 6.8 mm, $P < 0.05$) were lower on the mid-jejunal than the

duodenal infusion day. Scores for fullness, nausea, discomfort, and desire to eat did not differ at baseline.

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

Blood glucose (Figure 9.1 A)

Blood glucose increased during each infusion ($P < 0.0005$ for each), tending to plateau after 60 min, with no difference in the incremental area under the curve between the two study days (mid-jejunal infusion 147 ± 8 units, duodenal infusion 158 ± 12 units).

Plasma insulin (Figure 9.1 B)

Plasma insulin increased on both days ($P < 0.0005$ for each), and rose progressively throughout the study, with no difference in the incremental area under the curve (mid-jejunal infusion 1524 ± 275 units, duodenal infusion 1198 ± 292 units).

Plasma GLP-1 and GIP (Figure 9.1 C and Figure 9.1 D)

There was no significant increase in plasma GLP-1 over 90 min of glucose infusion on either day. However, a transient peak in GLP-1 at 10 min was apparent with the duodenal infusion ($P < 0.05$ compared to baseline). The incremental area under the GLP-1 curve did not differ between the two days (mid-jejunal infusion 217 ± 124 units, duodenal infusion 40 ± 115 units). Plasma GIP increased on both study days ($P < 0.0005$), with the rate of increase declining after the first 30 min and no

difference in the incremental area under the curve (mid-jejunal infusion 1076 ± 170 units, duodenal infusion 1055 ± 196 units).

Plasma CCK concentrations (Figure 9.1 E)

Plasma CCK increased from baseline during duodenal glucose infusion ($P < 0.005$) and was subsequently sustained at about the same levels throughout the study. During mid-jejunal infusion, plasma CCK did not change significantly over time, so that the incremental area under the CCK curve was less during mid-jejunal infusion (27 ± 25 units), than duodenal infusion (108 ± 23 units), ($P<0.05$).

9.4.2 Gastrointestinal sensations and appetite

Hunger and energy intake (Figure 9.2 and Figure 9.3)

Perceptions of fullness, nausea, discomfort, and bloating did not change significantly over time with glucose infusion at either site, nor was there any difference between the two days (data not shown). Hunger tended to increase with mid-jejunal glucose infusion and decrease with duodenal infusion, with a significant difference between the studies ($P < 0.05$). Desire to eat also increased with mid-jejunal glucose infusion ($P < 0.05$), but the difference from duodenal infusion was not significant. Subjects tended to consume more energy at the buffet meal after mid-jejunal, than duodenal, glucose infusion, with the difference at the borderline of statistical significance (1413 ± 62 versus 1252 ± 97 kcal, $P = 0.05$).

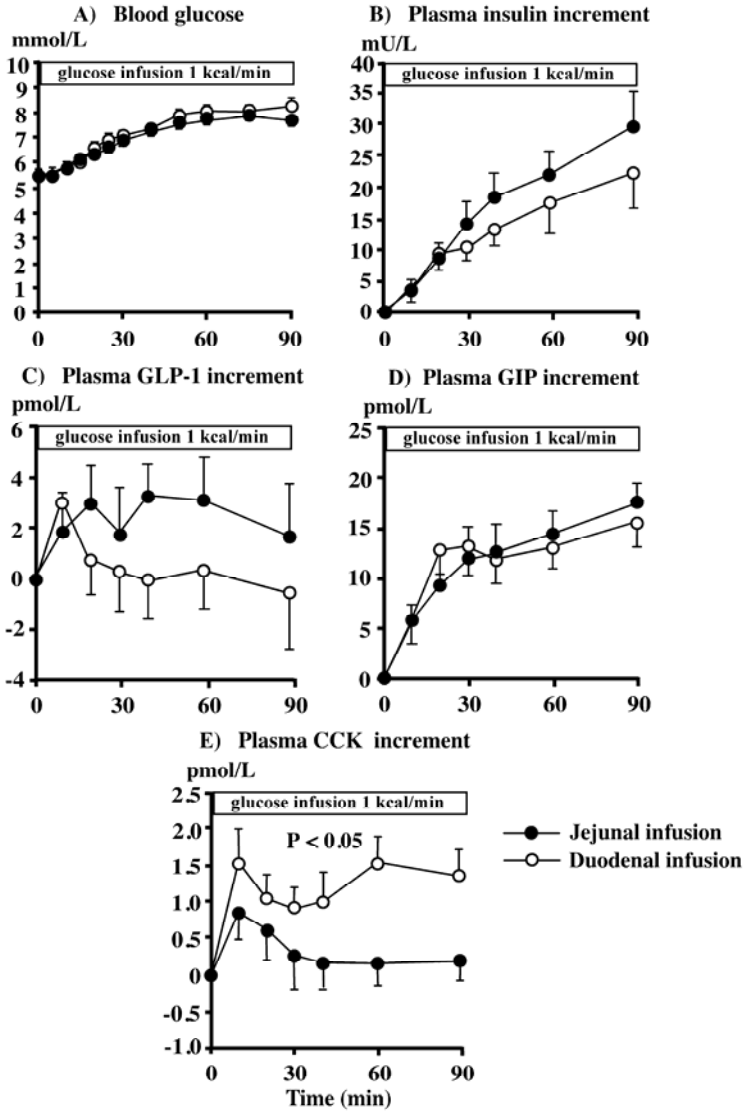

Figure 9.1

Blood glucose (A), change in plasma insulin (B), GLP-1 (C), GIP (D) and CCK (E) in response to intraduodenal glucose (open circles) or intrajejunal glucose (closed circles) in 10 healthy subjects. Data are mean ± SEM.

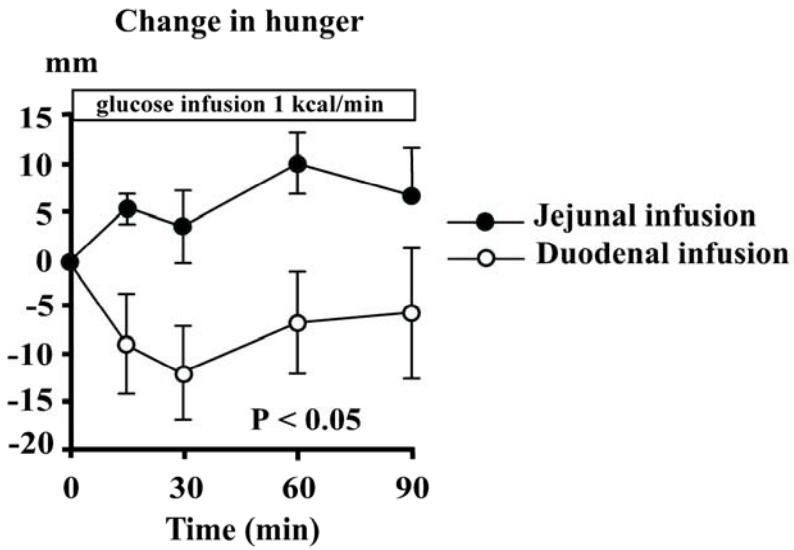

Figure 9.2

Change in perception of hunger during intraduodenal glucose (open circles) or intrajejunal glucose (closed circles) infusion in 10 healthy subjects. Data are mean ± SEM.

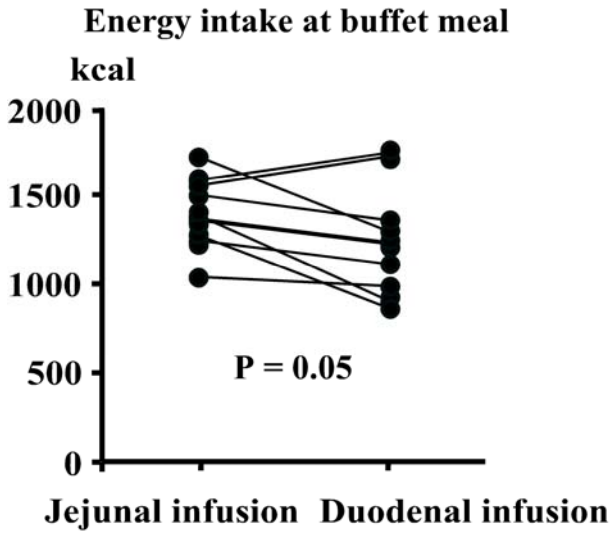

Figure 9.3

Energy intake at a buffet meal after intraduodenal glucose or intrajejunal glucose infusion in 10 healthy subjects. Data are mean ± SEM.

9.5 DISCUSSION

Given the importance of peptide hormones released from the small intestine on exposure to nutrients, in terms of appetite and glycaemic control, the lack of information regarding factors determining their release in humans is remarkable. We have shown that infusion of glucose into the mid-jejunum, when compared to an isocaloric duodenal glucose infusion, resulted in less stimulation of CCK and suppression of hunger, and was associated with a trend for greater energy intake at a subsequent buffet meal, without any difference in glycaemia, incretin hormone or insulin release. The study was designed to be as physiological as possible (ie. no balloon occlusion of gut segments). As the maximum glucose absorption rate in the small intestine is about 2 kcal/min/30cm (Holdsworth and Dawson 1964; Modigliani and Bernier 1971; Duchman et al. 1997), a glucose infusion of 1 kcal/min was chosen to ensure complete absorption in the segment being evaluated, without "spilling over" into more distal regions. We chose the mid-jejunum as the distal site of infusion because this was likely to be better tolerated than ileal administration of glucose, since the intubation is less demanding and there is a greater length of small bowel distal to the infusion site to absorb the glucose before it reaches the colon, which can cause abdominal discomfort due to bacterial fermentation (Spiller et al. 1988; Jain et al. 1989).

The enteral glucose infusion was associated with a rise in blood glucose concentration early in the study, to a plateau after 60 min of either duodenal or jejunal glucose infusion; this may be due to the progressive increase in insulin secretion, leading to an attenuation in the blood glucose rise late in the study. The

enteral glucose load also stimulated a rapid initial increase in plasma GIP during the first 30 min, with a more gradual increase thereafter, and no difference between the mid-jejunal and duodenal infusion sites. This is consistent with the notion that GIP release in response to glucose, even though load-dependent, does not display a threshold below which GIP secretion does not occur (Schirra et al. 1996a). Given that GIP-bearing K cells are localised to the duodenum (Polak et al. 1975; Buffa et al. 1976), it appeared remarkable that there was no difference in plasma GIP between duodenal and mid-jejunal glucose infusions. However, the recent report of a sub-group of L cells in the mid-small intestine that also release GIP may account for GIP secretion in response to jejunal glucose exposure (Mortensen et al. 2003).

The mechanism of GLP-1 release remains perplexing, given the rapid, load-dependent rise in plasma GLP-1 after oral or intraduodenal administration of glucose (Holst 1994; Schirra et al. 1996a), despite the predominance of GLP-1 bearing L-cells in the distal jejunum, ileum, and colon (Eissele et al. 1992). Moreover, a threshold load of glucose delivery to the small intestine has been suggested (~1.4 kcal/min), below which no GLP-1 is released (Schirra et al. 1996a). One explanation for these observations is that transit of glucose to the jejunum is initially more rapid, allowing direct contact of glucose with L cells, but only when the glucose load exceeds the maximum that can he absorbed within the duodenum (Schirra et al. 1996a; Chaikomin et al. 2005). We have observed an early, transient peak in GLP-1 with intraduodenal glucose infusions at 1 kcal/min (O'Donovan et al. 2004b; Chaikomin et al. 2005), which was also evident in the current study. Since there is evidence that exposure of the duodenum to glucose up-

regulates the rate of subsequent glucose absorption by insertion of GLUT2 transporters into the apical membrane of the enterocyte (Kellett and Brot-Laroche 2005), it is possible that glucose loads as low as 1 kcal/min could exceed the absorptive capacity of the duodenum in the initial, fasting state; studies of maximal small intestinal absorption rates of glucose have generally involved perfusion studies at steady state (Holdsworth and Dawson 1964; Modigliani and Bernier 1971; Duchman et al. 1997). Furthermore, the initial rapid transit of glucose may be slowed as soon as GLP-1 is released, since GLP-1 is reported to inhibit small bowel transit in the rat (Tolessa et al. 1998); therefore, ongoing transit of glucose to the GLP-1 bearing mucosa would be attenuated. An alternative explanation is that a "loop" exists, whereby duodenal glucose exposure stimulates neural or humoral signals that release GLP-1 indirectly from L cells in the distal small intestine and colon (Holst 1994). GIP appears to mediate such a loop in rats, but does not stimulate GLP-1 in humans (Meier et al. 2002a). However, we recently reported that limiting the spread of glucose to the proximal 60 cm of small intestine by an occlusive intraluminal balloon prevents GLP-1 release in healthy humans (Little 2006), which favors the requirement for jejunal exposure to glucose on order to stimulate GLP-1. Nevertheless, in the current study, there was no significant increment in GLP-1 during mid-jejunal glucose infusion. Possible explanations include the relatively low rate of glucose infusion (1 kcal/min), or that the jejunal infusion was not sufficiently distal to expose a significant density of L cells to glucose. Alternatively, there may be synergy between a duodenal signal and contact of glucose with the jejunal L cells; this could be examined in future studies by infusing glucose at both sites concurrently. In the only other study comparing

duodenal with mid-jejunal nutrient infusion in healthy humans, a mixed nutrient formula delivered at about 2 kcal/min stimulated GLP-1 release from the mid-jejunal, but not the duodenal, infusion site (Kaushik et al. 2005), although blood sampling for GLP-1 was infrequent (0, 120, and 360 min).

The observation that CCK was stimulated during duodenal, but not mid-jejunal, infusion of glucose, is novel and potentially important. While immunohistochemical studies indicate that CCK-bearing I cells are confined to the proximal small intestine (duodenum and jejunum) (Polak et al. 1975; Buffa et al. 1976), and nutrient exposure of an isolated loop of duodenum and jejunum stimulates CCK release, while exposure of an ileal loop does not (Konturek et al. 1986), the point of transition in the small intestine where the capacity to secrete CCK is lost has not been clearly demarcated. Moreover, Lin et al. reported that CCK was stimulated by lipid infusion in either the proximal or distal small bowel in dogs, and suggested that in the latter case, secretion was mediated by an intrinsic neural loop (Lin and Chey 2003). In humans, Vu et al found no difference in CCK release between proximal- and distal-jejunal nutrient infusion, although both gallbladder contraction and pancreatic exocrine secretion were less with infusion at the distal site (Vu et al. 1999). It is possible that CCK release stimulated by glucose occurs at a different site from that stimulated by lipid. Kaushik et al. reported that a mixed nutrient formula infused into the duodenum or mid-jejunum at about 2 kcal/min stimulated CCK at both sites in healthy humans, but the mid-jejunal infusion failed to induce pancreatic exocrine secretion, the major stimulus for which is CCK (Kaushik et al. 2005). Their data were insufficient to allow

direct comparison between CCK release at each infusion site, but taken together with our observations involving a lower nutrient load, a gradient between the duodenum and mid-jejunum in the capacity to secrete CCK could be postulated. The established, potent effects of CCK on appetite and food intake (Matzinger et al. 1999; Little et al. 2005) suggest that the difference we observed in CCK secretion was responsible for the effects on hunger and energy intake in our subjects. In contrast to GLP-1, the lack of contribution of the mid-jejunum to CCK release is supported by our previous observation that there is no augmentation of plasma CCK when glucose is allowed to contact the mid- to distal small intestine, when compared to the proximal 60 cm (Little et al. 2006a). One clinical application of our current observations is that enteral feeding delivered to the mid-jejunum is likely to safe in patients with acute pancreatitis, in whom the stimulation of pancreatic exocrine secretion is to be avoided (Kaushik et al. 2005).

In conclusion, both the load of nutrient (Edelbroek et al. 1992b; Rayner et al. 2000a) and the length of small intestine exposed (Lavin et al. 1998a; Chapman et al. 1999) are well established determinants of the degree of intestinal feedback generated. The current study has indicated that the site of small intestinal glucose exposure is also a determinant of CCK release and appetite.

CHAPTER 10:

EFFECTS OF PROTEIN ON GLYCAEMIC AND INCRETIN RESPONSES AND GASTRIC EMPTYING AFTER ORAL GLUCOSE IN HEALTHY SUBJECTS

10.1 SUMMARY

Dietary interventions represent a promising therapeutic strategy to optimise postprandial glycaemia in type 2 diabetes mellitus. The addition of protein to oral glucose has been reported to improve the glycaemic profile. This study evaluated the mechanisms by which protein supplementation lowers blood glucose concentrations after oral glucose. We studied 9 healthy males on three days each, in random order. After an overnight fast, subjects consumed 300 mL drinks containing either 50 g glucose ("glucose"), 30 g gelatin ("protein"), or 50 g glucose with 30 g gelatin ("glucose + protein") in water, labelled with 150 mg ^{13}C-acetate. Blood and breath samples were subsequently collected for 3 hours for blood glucose, plasma insulin, GLP-1, GIP and gastric emptying measurements. The blood glucose response was less after "glucose + protein" than "glucose" ($P < 0.005$), with no differences in plasma insulin or GLP-1, and lower GIP ($P < 0.005$). "Protein" alone stimulated insulin, GLP-1, and GIP ($P < 0.05$ for each), without elevating blood glucose. The gastric half-emptying time was greater after "glucose + protein" (51.2 ± 1.4 min) than "glucose" (45.6 ± 1.3 min) ($P < 0.05$), and tended to be greater for "glucose" than "protein" (42.1 ± 1.3 min) ($P = 0.06$). In conclusion, in healthy humans, addition of protein to oral glucose lowers postprandial blood glucose concentrations acutely, predominantly by slowing

gastric emptying, although protein also stimulates incretin hormone, and non-glucose-dependent insulin release.

10.2 INTRODUCTION

Glycaemic control is a major determinant of the development and progression of microvascular, and probably macrovascular, complications associated with diabetes (DCCT 1993; UKPDS and Group 1998; Nathan et al. 2005). Recent studies have highlighted the importance of postprandial hyperglycaemia to overall glycaemic control (Ohkubo et al. 1995; Ceriello et al. 2004). High protein diets have generated substantial, and increasing interest, particularly because of their potential impact on energy intake and body weight (Brinkworth et al. 2004; Simpson and Raubenheimer 2005). An additional potential benefit of protein supplementation, independent of weight loss, is a decrease in both postprandial blood glucose and glycated haemoglobin in type 2 diabetes (Gannon et al. 2003). The mechanisms responsible for the latter effects are, however, poorly defined.

The capacity for protein to enhance insulin secretion has been recognised for some years. In particular, the presence of raised blood amino acid concentrations appears to stimulate insulin release regardless of ambient blood glucose concentrations (Fieseler et al. 1995). Gannon et al reported that the addition of 25 g protein (from sources such as meat, fish, or gelatin) to a 50 g oral glucose load reduced the subsequent blood glucose response in type 2 patients (by about 30% for gelatin), while increasing plasma insulin (by about 2.5 times for gelatin) when compared to glucose alone (Gannon et al. 1988). The same group subsequently investigated the

contribution to this effect of various amino acids present in gelatin, in healthy subjects; both glycine (Gannon et al. 2002) and proline (Nuttall et al. 2004) reduced the blood glucose response to oral glucose, but without enhancing insulin secretion.

It is now established that the rate of gastric emptying is a major determinant of postprandial glycaemia, so that even modest changes may have a substantial impact on the magnitude and timing of the postprandial increase in blood glucose and insulin (Horowitz et al. 1993a; Rayner et al. 2001). Gastric emptying is normally closely regulated, predominantly by feedback arising from the small intestine, such that nutrients are delivered into the duodenum at a relatively constant rate of 2 – 3 kcal per minute (Brener et al. 1983b), with minor variation depending on meal volume and energy density (Hunt et al. 1985b). The addition of energy in the form of protein to an oral glucose load would be expected, therefore, to slow gastric emptying of glucose, and thereby reduce the glycaemic response. In this circumstance, it may be expected that there would be a reduction, rather than an increase, in the plasma insulin response. Previous studies evaluating the effects of protein supplementation on oral glucose tolerance (Gannon et al. 1988; Gannon et al. 2002; Nuttall et al. 2004) have not measured the rate of gastric emptying.

Oral glucose stimulates insulin secretion to a much greater degree (about two-fold) than an equivalent intravenous glucose load (Holst and Gromada 2004). This so-called “incretin effect” is mediated by the small intestinal peptides, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), and

the capacity for these peptides to stimulate insulin secretion is dependent on an elevated blood glucose concentration. GLP-1 probably has a physiological role in the slowing of gastric emptying induced by the presence of nutrients in the small intestine (Edwards et al. 1999; Schirra et al. 2006). Both carbohydrate and fat are potent stimuli for GLP-1 and GIP secretion (Elliott et al. 1993), but the response to protein is apparently less, and more variable. For example, while amino acids (Thomas et al. 1976; Miller et al. 1978; Thomas et al. 1978) and casein hydrolysates (Herrmann et al. 1995) stimulate GIP and GLP-1 with varying degrees of potency, the response to intact proteins appears to be less than to other macronutrients, at least in healthy humans (D'Alessio et al. 1993; Elliott et al. 1993). Strategies to enhance the incretin response are of particular interest in the management of patients with type 2 diabetes, in whom both the secretion of GLP-1 (Toft-Nielsen et al. 2001) and the beta cell response to GIP (Nauck et al. 1993b) appear to be impaired.

We have now examined the potential contributions of gastric emptying, incretin peptides, and insulin, in mediating the reduction in the glycaemic response to oral glucose by the addition of protein, in healthy humans.

10.3 METHODS

10.3.1 Subjects

Nine healthy male volunteers (median age 24 years, range 19 - 35; median body mass index 24.2 kg/m^2. range 21.6 - 27.5) were studied. Each gave written, informed consent, in accordance with the guidelines in the Declaration of Helsinki.

The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital. In each subject, measurements were performed on 3 days after an overnight fast, each separated by at least 3 days.

10.3.2 Protocol

The order of the studies was randomised and single-blinded. An intravenous cannula was placed in a forearm vein 30 min prior to each study to allow repeated blood sampling.

On each day, a 300 mL drink was consumed, which contained either (i) 50 g glucose ("glucose", 200 kcal), (ii) 30 g powdered gelatin (incorporating 25g protein) (Davis Gelatine, Gelita NZ Ltd, Christchurch, New Zealand) ("protein", 100 kcal), or both glucose and gelatin ("glucose + protein", 300 kcal), dissolved in water. Each drink also contained 150 mg ^{13}C-acetate and non-nutritive fruit flavouring, and was served at approximately 37°C and consumed within 3 min (t = 0 - 3 min).

Blood samples were collected at t = -5, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min for measurement of blood glucose and plasma insulin, GLP-1, and GIP concentrations. Breath samples were obtained at 5 minute intervals from t = 0 to 60 min, then at 15 minute intervals from t = 60 to 180 min, to calculate the rate of gastric emptying.

10.3.3 Measurements

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

Blood samples for determination of plasma insulin, GLP-1, and GIP were collected in ice-chilled tubes containing EDTA and 400 kIU aprotonin (Trasylol; Bayer Australia Ltd., Pymple, Australia) per liter blood. Plasma was separated by centrifugation and stored at -70 ºC for subsequent analysis. Blood glucose concentrations were determined immediately using a portable glucose meter (Medisense Precision QID, Abbott Laboratories, Bedford, Massachusetts, USA). Plasma insulin concentrations were measured by ELISA immunoassay (Diagnostics Systems Laboratories Inc., Webster, Texas, USA) (Horowitz et al. 1996a), and plasma GLP-1 and GIP concentrations by radioimmunoassay (RIA) (O'Donovan et al. 2004b).

Gastric emptying

The $^{13}CO_2$ enrichment in the breath samples was measured by mass spectroscopy (ABCA 20-20 mass spectrometer, Europa Scientific, Crewe, UK) to determine the percentage $^{13}CO_2$ recovery per hour and the cumulative percentage $^{13}CO_2$ recovery over 3 hr (Chew et al. 2003). The method of Ghoos et al. (Ghoos et al. 1993), which has been validated against scintigraphy (Choi et al. 1997; Choi et al. 1998), was applied to calculate the gastric half emptying time and gastric emptying coefficient (GEC).

10.3.4 Statistical Analysis

Data were evaluated using ANOVA for comparisons of areas under the blood glucose and plasma insulin curves, peak blood glucose levels, gastric half emptying time, and GEC. Repeated measures ANOVA was used to compare blood glucose and plasma peptide data, with post hoc comparisons in the event of a treatment x time interaction. For these data, the baseline was calculated as the mean of values at t = -5 and t = 0 min. Analyses were performed using a statistical software package (Statview 5, SAS Institute, Cary, North Carolina, USA). P-values less than 0.05 were deemed significant.

10.4 RESULTS

All subjects tolerated the study well. Subjects could distinguish the drinks containing gelatin because of their viscous texture.

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

Blood glucose concentrations (Figure 10.1 A)

Blood glucose concentrations increased from baseline after the "glucose" and "glucose + protein" drinks ($P < 0.05$ for each), but not after the "protein" drink, and the blood glucose profile differed between the three study days ($P < 0.005$). Blood glucose concentrations were lower after the "glucose + protein" compared to the "glucose" drink ($P < 0.05$), with a smaller area under the incremental blood glucose curve for "glucose + protein" (123 ± 59 units) versus "glucose" (214 ± 28 units), and a lower peak blood glucose value for "glucose + protein" (8.0 ± 0.4 vs

9.4 ± 0.3 mmol/L) (both $P < 0.05$). For the drinks containing glucose, blood glucose levels fell below baseline late in the study, to that blood glucose was lower for "glucose + protein" than "protein" at t = 120 and 150 min, and also for "glucose" than "protein" at t = 150 min (all $P < 0.05$).

Plasma insulin concentrations (Figure 10.1 B)

Plasma insulin increased from baseline after all three drinks ($P < 0.05$ for each). Insulin concentrations were less after "protein" than the other drinks ($P < 0.05$), but did not differ between "glucose" and "glucose + protein". The area under the incremental insulin curve did not differ between "glucose" (4261 ± 819 units) and "glucose + protein" (4936 ± 1505 units), but was greater for each than for the "protein" drink (1190 ± 386 units) ($P < 0.05$ for each). Insulin remained elevated above baseline levels after drinks containing glucose, even after blood glucose had returned to, or fallen below, baseline values.

The ratio of areas under the curves for insulin to glucose showed a non-significant trend to be greater after the "glucose + protein" drink (insulin:glucose area 40.0 ± 11.1) than the "glucose" drink (insulin:glucose area 21.2 ± 3.1) ($P = 0.097$).

Plasma GLP-1 concentrations (Figure 10.1 C)

Plasma GLP-1 increased in all three days ($P < 0.05$), with a relatively early peak followed by a steady decline, without any difference between the three drinks.

Plasma GIP concentrations (Figure 10.1 D)

Plasma GIP increased on each study day ($P < 0.05$ for each), and GIP concentrations differed between study days ($P < 0.005$). Plasma GIP concentrations were greater after "glucose" than each of the other drinks ($P < 0.05$ for each), and greater after "glucose + protein" than "protein" ($P < 0.05$). As for insulin, GIP remained elevated above baseline beyond the return of blood glucose to baseline.

10.4.2 Gastric emptying

Gastric emptying (Figure 10.2)

The half emptying time differed between the three study days ($P < 0.0005$) and was greater for "glucose + protein" (51.2 ± 1.4 min) than either the "glucose" (45.6 ± 1.3 min, $P < 0.05$) or "protein" (42.1 ± 1.3 min, $P < 0.005$) drinks. Half emptying time tended to be greater for "glucose" than "protein" ($P = 0.06$).

The GEC tended to differ between the three study days ($P = 0.05$), and direct comparison was greater for "glucose" (3.82 ± 0.07) than "glucose + protein" (3.61 ± 0.06) ($P < 0.05$), but did not differ significantly between "protein" (3.98 ± 0.16) and "glucose" ($P = 0.29$) or "protein" and "glucose + protein" ($P = 0.07$).

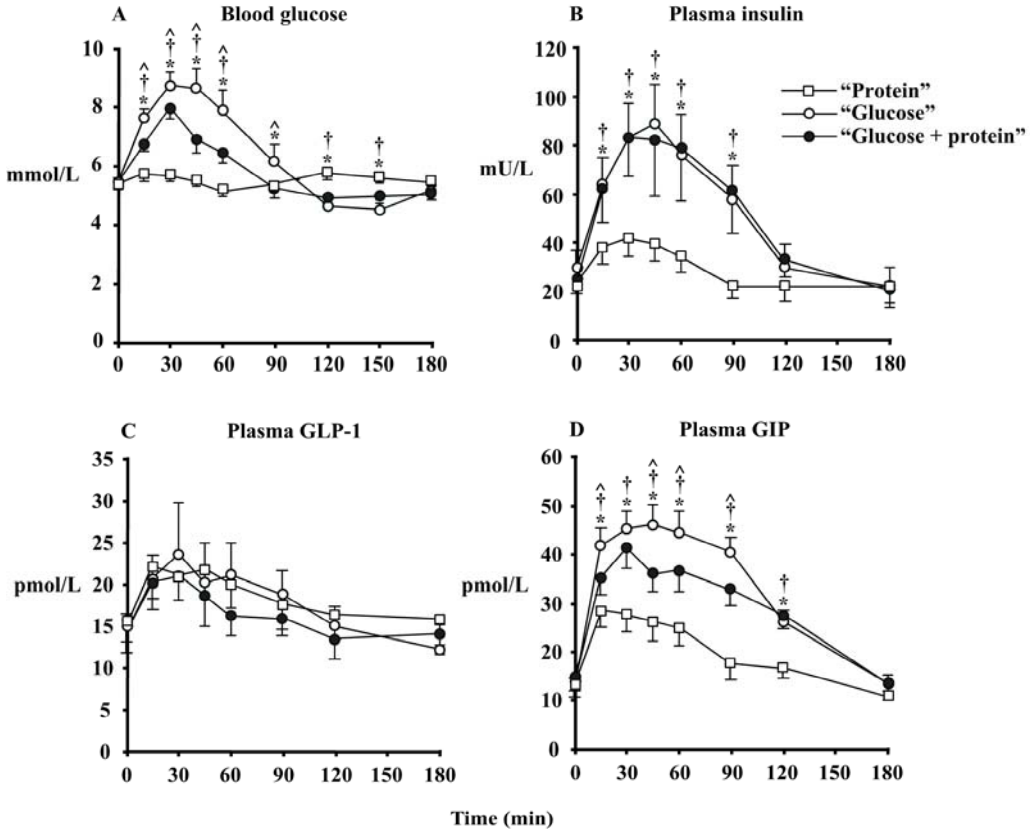

Figure 10.1

*A. Blood glucose, B. plasma insulin, C. plasma GLP-1, and D. plasma GIP concentrations following 50 g oral glucose ("glucose" drink, open circles), 25 g oral protein ("protein" drink, open squares), and 50 g oral glucose with 25 g oral protein ("glucose + protein" drink, filled circles). ^ $P < 0.05$ "glucose + protein" versus "glucose", * $P < 0.05$ "glucose" versus "protein", † $P < 0.05$ "glucose + protein" versus "protein".*

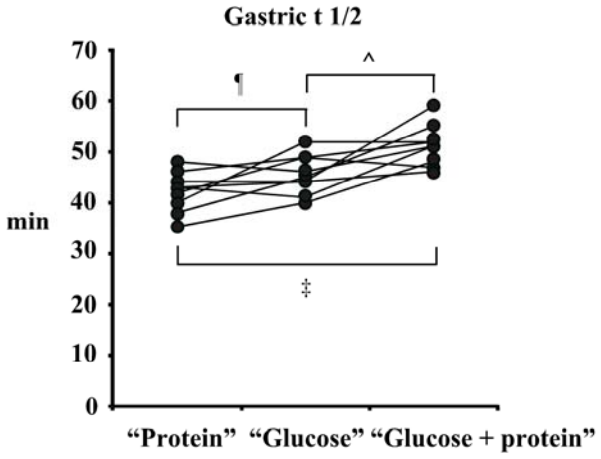

Figure 10.2

Gastric half-emptying time for the three drinks. Data for individual subjects are shown. ^ P < 0.05 "glucose + protein" versus "glucose", ¶ P = 0.06 "glucose" versus "protein", ‡ P < 0.005 "glucose + protein" versus "protein".

10.5 DISCUSSION

This is the first evaluation of both gastric emptying and incretin responses to the addition of protein to an oral glucose load in humans. We demonstrated that the addition of protein reduces the glycaemic response in healthy subjects, and that the dominant mechanism accounting for this effect is the slowing of gastric emptying. Protein did not stimulate additional GLP-1, GIP, or insulin release, and indeed plasma GIP concentrations were lower after "glucose + protein" than after the "glucose" drink alone. This is despite the fact that protein, when consumed alone, stimulated both incretin and insulin release. The increase in plasma insulin after the "protein" drink was not associated with any elevation in blood glucose, indicating that it was mediated by a mechanism other than GLP-1 and GIP (Holst and Gromada 2004).

The loads of glucose and protein administered in this study were chosen to match those evaluated previously in type 2 patients (Gannon et al. 1988), and the use of a ^{13}C breath test to measure gastric emptying is well validated in healthy subjects (Ghoos et al. 1993; Chew et al. 2003). The observed lowering of glycaemia by the addition of protein to the glucose drink is in accordance with the report of Gannon et al. (Gannon et al. 1988). In contrast to our findings, these authors observed a 2.5 fold stimulation of insulin secretion. However, the insulin response to the glucose drink alone in their type 2 patients was markedly less than in our healthy subjects. The same investigators then studied the affects of adding glycine (Gannon et al. 2002) or proline (Nuttall et al. 2004), both of which are found in gelatin, to oral glucose in healthy volunteers, and reported a reduction in the blood glucose, but no

increment in the insulin response. These observations are consistent with evidence that type 2 diabetes is associated with an impaired early phase insulin secretion to oral glucose, and a diminished glucose-sensing ability of the beta cell, while the insulin responses to other stimuli may be relatively intact (Polonsky et al. 1996), at least in the early stages of the disease.

Amino acids stimulate insulin secretion by different mechanisms than glucose, which raises the possibility of synergy between nutrient stimuli (Nuttall and Gannon 1991). However, it has been recognised for some time that plasma amino acid concentrations alone do not entirely account for the insulin response to ingested protein (Floyd et al. 1966), suggesting the contribution of another factor, such as the incretin peptides. Several groups have investigated the insulin response to ingested protein, and found that not only the presence of diabetes, but also the relative quantities of protein and carbohydrate, and the presence of free amino acids (as opposed to intact protein), are important variables. When ingested in isocaloric quantities, glucose and protein from lean beef have additive effects on insulin secretion in healthy subjects (Krezowski et al. 1986), whereas if the amount of protein is less than glucose, the increase in insulin is substantially less than additive (Westphal et al. 1990).

When an amino acid/protein hydrolysate mixture, chosen to optimise insulin secretion, was given to type 2 patients and healthy controls, the type 2 patients were found to have an impaired insulin response to oral glucose, but an intact response to the amino acid/protein mixture, ie. the glucose-independent stimulus to

insulin secretion seemed to be preserved (van Loon et al. 2003). Furthermore, there was a correlation between plasma insulin and plasma leucine and phenylalanine concentrations. The insulin response to intact protein is less than that observed with protein hydrolysates, presumably reflecting the more rapid increase in plasma amino acid concentrations with the latter. A mixture of leucine, phenylalanine, and wheat protein hydrolysate was found to be the most potent of a number of combinations (van Loon et al. 2000).

There is little information about the effect of supplementing carbohydrate with protein on the incretin response. Frid et al. reported that the addition of whey protein to a high glycaemic index carbohydrate meal in type 2 patients increased the insulin response and decreased the glycaemic profile when compared to the addition of lean ham (Frid et al. 2005). The GIP response was greater (area under the curve increased by 30 % over 120 min) with whey, but GLP-1 concentrations did not differ. The patients studied by Frid et al. had good glycaemic control and were managed by diet alone (mean glycated haemoglobin 5.4%), and may therefore not be representative of the majority of type 2 patients, who are reported to have a diminished or absent insulin response to GIP (Nauck et al. 1993b).

Gunnarsson et al. recently reported that in gastric gavage-fed mice, the insulin response to glucose (ie. area under the curve) was trebled by the addition of an equal weight of whey protein, and this was associated with a substantial decrease in plasma glucose (Gunnarsson et al. 2006). The total plasma GLP-1 concentration was increased, but GIP was not. However, analysis of intact (or active) incretins

indicated an increase in intact GIP, and the authors suggested that fragments of whey protein could inhibit dipeptidyl peptidase IV (DPP-IV), the enzyme that degrades the incretin peptides, in the small intestine. In this study, the incretin measurements were taken only at one time point, 15 min after the meal, due to limitations in blood sampling from the mice. As in our study, gastric emptying (assessed in this case by the less than optimal paracetamol absorption method) was slowed by the addition of whey protein to glucose. The fact that addition of protein in our study did not increase plasma GLP-1 and decreased GIP is likely to reflect the relatively smaller load and the nature of the protein used. Although we did not measure intact, as opposed to total, GIP, gelatin may be less easily digested to fragments that block DPP-IV when compared to whey protein.

We evaluated acute effects of modest protein supplementation, in healthy individuals. Medium- and long-term studies, incorporating more physiological meals, are now indicated in type 2 patients to evaluate the impact of protein supplementation on glycaemic control, and the load and type of protein could be optimised for a greater incretin response (van Loon et al. 2000). In a 5 week dietary crossover study in type 2 patients, 24 hour integrated area under the blood glucose curve was reported to be decreased by 40% with a high protein diet, although insulin secretion was not changed (Gannon et al. 2003). An alternative approach, of ingesting a protein “preload” at a given interval before meals, could be evaluated to determine whether the stimulation of incretins and insulin could achieve improvements in glycaemia after the subsequent meal (Gentilcore et al. 2006a). Potentially, an effect comparable to many emerging pharmacological

therapies such as GLP-1 analogues or DPP IV inhibitors could be achieved. Another advantage of protein "preloads" may be to increase satiety (Vandewater and Vickers 1996), and therefore reduce food intake at a subsequent meal. Possible adverse effects of protein supplementation also need to be considered. High protein diets have the potential to increase renal calcium excretion (Eisenstein et al. 2002), although medium term studies have not shown detrimental effects on bone turnover (Farnsworth et al. 2003). There is also an association between high intake of red and processed meat and colorectal cancer, although protein from poultry is not associated with increased risk, while fish is protective (Norat et al. 2005).

In summary, we have confirmed the capacity for protein supplementation to improve the glycaemic response to glucose in healthy humans, and demonstrated that this is accounted for predominantly by slowing of gastric emptying. Further studies aimed at optimising this effect are indicated in patients with type 2 diabetes, who may derive additional benefit, in terms of postprandial glycaemia, from the stimulation of non-glucose-dependent insulin secretion.

CHAPTER 11:

EFFECTS OF VARIATIONS OF DUODENAL GLUCOSE DELIVERY ON GLYCAEMIA, GASTROINTESTINAL HORMONE RELEASE, ANTROPYLORODUODENAL MOTILITY, APPETITE AND ENERGY INTAKE IN HEALTHY MEN

11.1 SUMMARY

As a result of small intestinal feedback glucose empties from the stomach at ~2 - 3 kcal/min. The rate of small intestinal glucose delivery is a major determinant of the effects of a meal on glycaemia, gastrointestinal hormone release and appetite. The aims of this study were to determine the effects of different intraduodenal (ID) glucose loads on glycaemia, incretin hormone and CCK release, antropyloroduodenal (APD) motility, appetite and energy intake, as well as the relationships between these parameters. 10 healthy males (age: 32 ± 4 yrs; BMI: 25.1 ± 0.4 kg/m^2) were studied on four separate occasions in double-blind, randomised fashion. APD motility, blood glucose, plasma GLP-1, GIP and CCK concentrations and appetite perceptions were measured during 120 min ID glucose infusions at (i) 1 ("G1"), (ii) 2 ("G2"), and (iii) 4 ("G4") kcal/min, or (iv) saline control ("C"). The concentration of all solutions was 1390 mOsmol/L. Immediately after completion of the infusions energy intake at a buffet meal was quantified. There was a rise in blood glucose in response to all glucose infusions ($P < 0.05$ vs. C), with the effect of G4 and G2 being greater than that of G1 ($P < 0.05$), but with no difference between G2 and G4. Blood glucose subsequently fell ($P < 0.01$) during G2 and G4, but not G1. There was a dose-related progressive rise in GLP-1

($P < 0.05$), and rapid rises in plasma GIP ($P < 0.001$) and CCK ($P < 0.01$), during all glucose infusions when compared with control. The stimulation of CCK by G4 was substantially greater ($P < 0.001$) than that of G2 and G1. All glucose infusions suppressed the number of antral pressure waves ($P < 0.05$), however, only G4 reduced duodenal pressure waves ($P < 0.01$), and stimulated basal pyloric pressure ($P < 0.01$); none of the glucose infusions had any effect on isolated pyloric pressure waves when compared with control. Only G4 decreased energy intake compared with C, G1 and G2 ($P < 0.05$). In conclusion, variations in the delivery of glucose loads into the small intestine have differential effects on the blood glucose, incretin and CCK responses, antropyroloduodenal motility, appetite and energy intake. These observations have implications for strategies to minimise postprandial glycaemic excursions in type 2 diabetes.

11.2 INTRODUCTION

Gastric emptying is a complex and coordinated process designed to deliver chyme into the small intestine at a rate that allows efficient nutrient digestion and absorption (chapter 1). The rate of gastric emptying of carbohydrates, particularly glucose, has fundamental implications for postprandial glycaemia (Horowitz et al. 1993a), appetite (Lavin et al. 1996b; Cook et al. 1997a) and energy intake (Lavin et al. 1998c) (chapter 1). In healthy subjects, glucose normally empties from the stomach in an overall linear pattern at a rate of ~2 - 3 kcal/min (Horowitz et al. 1996b). This regulation of gastric emptying results primarily from a length-dependent feedback arising from the small intestine (Lin et al. 1989), which in turn modulates antropyloroduodenal (APD) motility and stimulates the release of a

number of gastrointestinal hormones, including cholecystokinin (CKK) (Liddle et al. 1985) and the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (Schirra et al. 1996b; Lavin et al. 1998c; O'Donovan et al. 2004b). The relationships of APD motility, hormone release and appetite with the load of duodenal glucose are poorly defined, particularly the time-course of these effects.

The stimulation of the incretin hormones, GLP-1 and GIP, (Schirra et al. 1996b; Lavin et al. 1998c; O'Donovan et al. 2004b), accounts for ~ 50 % of the rise in plasma insulin after oral glucose (Nauck et al. 2004) as discussed (chapter 2). GLP-1 is secreted predominantly from L cells in the distal small intestine (Eissele et al. 1992), whereas GIP is secreted from duodenal K cells (Fehmann et al. 1995). It has been suggested that for GLP-1 a threshold of >1.8 kcal/min needs to be exceeded in order to stimulate its release (Schirra et al. 1996b). However, this observation is inconsistent with two recent studies, which have demonstrated that duodenal glucose infusions as low as 1 kcal/min were sufficient for the stimulation, albeit transient, of GLP-1 in healthy subjects (O'Donovan et al. 2004b; Chaikomin et al. 2005) and type 2 patients (O'Donovan et al. 2004b). In contrast, there is evidence that the release of GIP is load-dependent at relatively low duodenal glucose loads, but the ceiling for the maximum response is uncertain (O'Donovan et al. 2004b; Chaikomin et al. 2005). It is also recognised that the presence of glucose in the proximal small intestine stimulates the release of CCK (chapter 1), although the magnitude of this effect is not as marked as that observed in response

to protein or fat (Liddle et al. 1985). Whether the release of CCK by glucose is load-dependent remains to be determined.

The slowing of gastric emptying by the presence of glucose in the small intestine is associated with suppression of antral and duodenal pressure waves and the stimulation of phasic and tonic pressure localised to the pylorus (Heddle et al. 1988c; Edelbroek et al. 1992a). Studies in animals indicate that small intestinal feedback on gastric emptying is load-, but not concentration-dependent (Lin et al. 1989). In humans, intraduodenal infusion of glucose at 2.4 kcal/min and 4 kcal/min for 10 min has been shown to stimulate isolated pyloric pressure waves (IPPWs) and increase basal pyloric pressure, with a greater response to the 4 kcal/min infusion, indicative of load-dependence (Heddle et al. 1988c). In another study, duodenal infusion of glucose at 2.4 kcal/min for 120 min increased the number of IPPWs and basal pyloric pressure during the first 20 min, but after this time, there was a decrease with a return to baseline within 80 min (Edelbroek et al. 1992a), indicating that there may be "adaptation" in the pyloric motor response during more sustained small intestinal nutrient exposure. In contrast, in this study antral pressure waves remained suppressed during the entire infusion (Edelbroek et al. 1992a).

The effects of glucose on gut motility may also be mediated by the consequent rise in blood glucose - acute hyperglycaemia (blood glucose concentration 12 - 16 momol/L), is associated with an increase in proximal gastric compliance (Hebbard et al. 1996c), suppression of antral waves (Hasler et al. 1995), and stimulation of

pyloric contractions (Fraser et al. 1991c). Changes in blood glucose that are within the normal postprandial range also affect gastrointestinal motility (Hasler et al. 1995; Schvarcz et al. 1997) (chapter 2).

Hunger, fullness and subsequent energy intake are also suppressed by small intestinal infusion of glucose in young, obese and healthy older subjects (Cook et al. 1997a; Lavin et al. 1998c; Chapman et al. 1999). For example, an intraduodenal infusion of 20 % glucose for 90 min, at 3.2 kcal/min, decreased hunger and increased fullness during the last 30 min of the infusion and reduced subsequent energy intake when compared with the saline control (Lavin et al. 1998c). These effects may reflect the release of CCK (Kissileff et al. 1981) and GLP-1 (Verdich et al. 2001) both of which may reduce energy intake (chapter 2). It has been suggested in dogs that the stimulation of pyloric motility may, per se, reduce energy intake (Xu et al. 2005). It is not known whether there is a relationship between the effects of enteral glucose on APD motility with those on appetite / energy intake, or whether the suppression of energy intake is load-dependent in humans.

Therefore, the aims of this study were to evaluate the effects of different intraduodenal glucose loads on APD motility, glycaemia, incretin hormone, and CCK release, appetite and energy intake, as well as the relationships between these parameters. It was hypothesised that (i) the effects of intraduodenal infusion of glucose at loads lower (1 kcal/min), comparable to (2 kcal/min), and higher than (4 kcal/min) the rate of normal gastric emptying on APD motility, glycaemia, incretin

hormone, and CCK release, appetite and energy intake would be load-dependent and (ii) the effects of small intestinal glucose on appetite and energy intake would be related to those on APD motility.

11.3 METHODS

11.3.1 Subjects

10 healthy males (aged: 32 ± 4 yrs; body mass index: 25.1 ± 0.4 kg/m^2) participated in the study. None of subjects was a restrained eater (score ≤ 12 on the Eating Restraint component (Factor 1) of the Three Factor Eating Questionnaire (Stunkard and Messick 1985)), had a history of gastrointestinal disease, or was taking medication known to affect gastrointestinal motility or appetite. No subject was a smoker, or habitually consumed more than 20 g of alcohol per day. The number of subjects included was based on power calculations derived from previous work (MacIntosh et al. 1999; MacIntosh et al. 2001b).

11.3.2 Protocol

Each subject was studied on four occasions, each separated by 3 - 7 days, on which they received, in randomised, double-blind fashion, an intraduodenal infusion of a 25 % glucose solution, at (i) 1 kcal/min ("G1"), (ii) 2 kcal/min ("G2"), or (iii) 4 kcal/min ("G4"), or (iv) intraduodenal hypertonic (4.2 %) saline ("control") for 120 min. The intraduodenal glucose solutions were prepared by dissolving glucose powder (Glucodin, Boots Healthcare, North Ryde, NSW, Australia) in distilled water. The glucose solutions were diluted with hypertonic saline (4.2 %) to achieve

the specific loads. All infusions were administered at a concentration of 1390 mOsmol/L and at a rate of 4 ml/min, so that in all study conditions the total volume infused was 480 ml. The subject was blinded to the composition of the infusion on each study day and the infusions were prepared by one of the other investigators who was otherwise not involved in either the performance of the studies, or data analysis. During the studies the infusion apparatus was covered at all times.

Each subject attended the Discipline of Medicine at 0830 h after an overnight fast (14 hours for solids, 12 hours for liquids). A manometric catheter (diameter: 3.5 mm; Dentsleeve International Ltd. Mui Scientific, Ontario, Canada), used to measure pressures in the APD region, was inserted into the stomach via an anaesthetised nostril and then allowed to pass into the duodenum by peristalsis. The catheter included 16 manometric side-holes located at 1.5 cm intervals. Six side-holes (channels 1 – 6 each 1.5 cm apart) were positioned in the antrum, a 4.5 cm sleeve sensor (channel 7) with two side-holes on the side opposite the sleeve (channels 8 and 9), was positioned across the pylorus, and seven side holes (channels 10 – 16 each 1.5 cm apart) were positioned in the duodenum. An additional channel, used for intraduodenal infusion, was located 11.75 cm distal to the end of the sleeve sensor (i.e. ~ 14.5 cm from the pylorus). The correct positioning of the catheter, with the sleeve straddling the pylorus, was maintained by continuous measurement of the transmucosal potential difference (TMPD) at the most distal antral (channel 6) (~ -40 mV), and the most proximal duodenal (channel 10) (~ 0 mV), channel (Heddle et al. 1988a). For this purpose, a cannula filled with sterile saline was placed subcutaneously in the subject's forearm and

used as a reference electrode (Heddle et al. 1988a). All manometric channels were perfused at 0.15 ml/min with degassed, distilled water, except for the two TMPD channels, which were perfused with 0.9 % saline (Heddle et al. 1988a). An intravenous cannula was placed in a forearm vein for blood sampling.

Once the catheter was positioned correctly, fasting motility was monitored until the occurrence of a phase III of the interdigestive migrating motor complex (MMC) (Feltrin et al. 2004b). Immediately after the cessation of phase III activity (at t = -15 min), a baseline blood sample was taken and a visual analogue scale (VAS) questionnaire, assessing appetite-related sensations and gastrointestinal symptoms (Parker et al. 2004) was completed. At t = 0 min, during phase I of the MMC, the intraduodenal infusion commenced and was continued for 120 min. APD pressures were recorded during the 15 min baseline period (i.e. t = -15 - 0 min) and the infusion period (i.e. t = 0 - 120 min). Blood samples (~10 ml volume) were taken, and VAS completed, at 15 minute intervals between t = 0 - 60 min, and then at 30 minute intervals between 60 - 120 min. At t = 120 min, the infusion was terminated and the manometric catheter removed. Each subject was then presented with a standard, cold, buffet-style meal and allowed 30 min (i.e. t = 120 - 150 min) to eat freely until they were comfortably full (Feltrin et al. 2004b). Further blood samples were taken at t = 150 min and 180 min. At this time (t = 180 min) the intravenous cannula was removed and the subject allowed to leave the laboratory.

11.3.3 Measurements

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

Venous blood glucose concentrations (mmol/L) were determined by the glucose oxidase method using portable glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, Massachusetts, USA).

For measurement of plasma GLP-1, GIP and CCK, blood samples (~10 ml) were collected in ice-chilled EDTA-tubes containing 400 kIU aprotinin (Trasylol, Bayer Australia Ltd, Pymble Australia) per litre of blood. Plasma was separated by centrifugation (3200 rpm, 15 min, 4°C) within 30 min of collection and stored at -70°C until assayed.

Total plasma GLP-1 (pmol/L) was measured by radioimmunoassay (O'Donovan et al. 2004b). Plasma GIP (pmol/L) was measured by radioimmunoassay (O'Donovan et al. 2004b). Plasma CCK (pmol/L) was measured after ethanol extraction using an adaptation of a previously described radioimmunoassay (Santangelo et al. 1998a) (chapter 9).

Antropyloroduodenal pressures

APD pressures were recorded and digitised using a computer-based system running commercially available software (Flexisoft® Version 3, Assoc Prof GS Hebbard, Royal Melbourne Hospital Melbourne, Australia, written in Labview 3.1.1 (National Instruments)) and stored for subsequent analysis. APD pressures were analysed for (i) number and amplitude of antral and duodenal pressure waves

(PWs), (ii) number and amplitude of isolated pyloric pressure waves (IPPWs), (iii) basal pyloric pressure and (iv) number and length of pressure wave sequences (PWSs) (Feltrin et al. 2004b). PWs in the antrum, pylorus and duodenum were defined by an amplitude ≥10 mmHg, with a minimum interval of 15 s between peaks for antral and pyloric waves and 3 s for duodenal waves (Samsom et al. 1998b). Basal pyloric pressure was calculated by subtracting the mean basal pressure recorded at the most distal antral side-hole from the mean basal pyloric pressure recorded at the sleeve (Heddle et al. 1989b), using custom written software. PWs in the antrum, pylorus and duodenum were defined as PWSs, if their rate of travel between side-holes was between 9 - 160 mm/s (Samsom et al. 1998b). PWSs were characterised by their distance travelled, i.e. number of sideholes.

Appetite perceptions and energy intake

Appetite perceptions (hunger and fullness) were rated on validated visual analogue scales (VAS) (Parker et al. 2004). Nausea and bloating were also assessed.

Energy intake was assessed by measuring consumption at the buffet-style meal (Feltrin et al. 2004b) (chapter 3). The amount (g) and energy (kJ) consumed was evaluated using commercially available software (Foodworks Version 3.01; Xyris Software, Highgate Hill, QLD, Australia) (Feltrin et al. 2004b) (chapter 3).

11.3.4 Statistical Analysis

Baseline values ('0') were calculated as the mean values obtained between t = -15 - 0 min for the number and amplitude of IPPWs and antral and duodenal PWs, basal pyloric pressures and PWSs, and at t = -15 and 0 min for plasma hormone concentrations and VAS scores. Antral and duodenal motility indicies (MI) were derived using the equation: natural logarithm [(sum of amplitudes x number of phasic pressure waves) + 1] (Camilleri and Malagelada 1984b). Antral and duodenal PWs were expressed as total numbers, whereas amplitude and MI were expressed as mean values, over the 120 min infusion. Basal pyloric pressures and number and amplitude of IPPWs were expressed as the mean of 15 min periods over the 120 min infusion period. APD PWSs were expressed as the total number of PWs travelling over two (1.5 - 3 cm), three (3 - 4.5 cm), etc. up to fifteen (21 - 22.5 cm) channels during the 120 min infusion period. All motility and VAS data were expressed as changes from baseline, whilst blood glucose and plasma GLP-1, GIP, and CCK were expressed as absolute values. Blood glucose and plasma hormone data were evaluated for three time periods; 0 - 30 min (arbitrarily defined as the "early" response), 0 - 120 min (entire infusion period) and 120 - 180 min ("post-meal" period). Peak hormone concentrations and the times at which they occurred were calculated by determining the highest concentration and its timing, in each subject. The area under the curve (AUC) for hormone concentrations during the infusion period was determined using the trapezoidal rule.

The number, amplitude and MI of antral and duodenal PWs and energy intake were analysed by one-way ANOVA. Basal pyloric pressure, number and amplitude of

IPPWs, blood and plasma hormone concentrations and VAS scores were analysed by repeated-measures ANOVA, with time and treatment as factors. The total number of PWSs was analysed by repeated-measures ANOVA, with number and length (cm) as factors. Peak glucose and plasma hormone concentrations (and the time at which this occurred) and the AUCs were analysed using Student's t-test.

Correlations, corrected for repeated measures, were determined for (i) the total number and mean amplitude and MI of antral and duodenal PWs, AUC for number and amplitude of IPPWs, basal pyloric pressures, APD PWSs, blood glucose, plasma GLP-1, GIP, and CCK concentrations, and appetite perception scores between 0 - 120 min, and energy intake and amount eaten at the buffet meal with the ln (natural logarithm) - transformed loads of lipid administered (0.25, 1.5 and 4 kcal/min (ii) appetite perceptions and energy intake (and amount eaten) with APD motility, blood glucose and plasma GLP-1, GIP and CCK concentrations, using the method described by Bland and Altman (Bland and Altman 1995). Only R values > 0.5 were considered physiologically relevant. Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

11.4 RESULTS

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

11.4.1 Gastrointestinal hormone concentrations

Blood glucose concentrations (Figure 11.1A)

Early response: There was a progressive rise in blood glucose at t = 15 and 30 min with all glucose infusions, when compared with control ($P < 0.001$). Blood glucose concentrations during G2 and G4 were greater than during G1 between 15 - 30 min ($P < 0.01$ for both) and greater during G4 than G2 at t = 30 min ($P < 0.001$).

Entire infusion period: Blood glucose concentrations were higher during all glucose infusions, from t = 15 - 120 min, when compared with control ($P < 0.05$). The increase in blood glucose between t = 15 - 60 min was greater during G2 and G4, when compared with G1 ($P < 0.05$ for both). There was no difference between G2 and G4. Peak blood glucose concentrations were: control: 5.5 ± 0.3 mmol/L at 74 ± 14 min, G1: 7.5 ± 0.6 mol/L at 68 ± 9 min, G2: 8.7 ± 0.4 mmol/L at 48 ± 7 min, G4: 9.1 ± 0.4 mmol/L at 45 ± 4 min; $P < 0.001$, for concentration only. Peak glucose concentrations were higher during G2 and G4, when compared with G1 ($P < 0.01$), with no difference between G2 and G4. Blood glucose then progressively fell ($P < 0.01$) during G2 and G4, to concentrations close to baseline by t = 120 min ($P = 0.07$ for both). The AUC between 0 - 120 min, was greater for G1, G2 and G4 compared with control (control: -6 ± 9 mmol/L.min, G1: 147 ± 38 mmol/L.min, G2: 198 ± 36 mmol/L.min, G4: 226 ± 26 mmol/L.min; $P < 0.001$). There was a trend for blood glucose during G4 to be greater than G1 ($P = 0.06$). There was no

relationship between the AUC for blood glucose concentration with the load of glucose administered.

Post meal period: After consumption of the buffet meal, blood glucose concentrations decreased after all glucose infusions when compared with control and the level immediately before the meal (i.e. t = 120 min) ($P < 0.001$) - the magnitude of the fall was greater for G4 than for G1 and G2 ($P < 0.05$). In contrast, there was a postprandial increase in blood glucose ($P < 0.001$), after the control infusion.

Plasma GLP-1 (Figure 11.1B)

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

Entire infusion period: G2 and G4 increased plasma GLP-1 compared with control; G2 at t = 15 min and G4 between t = 15 - 120 min ($P < 0.05$). During G4 there was a progressive rise in plasma GLP-1 after t = 45 min ($P < 0.001$) and levels were higher compared with G1 between t = 15 - 120 min and with G2 between t = 30 - 120 min ($P < 0.05$ for both). There was no difference between

control, G1 and G2, except at t = 15 min. The AUC for plasma GLP-1 was greater for G4 compared with control, G1 and G2 (control: 591 ± 119 pmol/L.min, G1: 212 ± 301 pmol/L.min, G2: 447 ± 182 pmol/L.min, G4: 1805 ± 445 pmol/L.min; $P < 0.001$). There was a positive relationship between the AUC between t = 0 - 120 min for plasma GLP-1 concentrations with the load of glucose administered, such that the greater the load the higher the concentration ($r = 0.89$, $P < 0.01$).

Post meal period: After consumption of the buffet meal (i.e. t = 150 min), plasma GLP-1 declined after G4 and increased with control, and both these concentrations were higher compared with those for G1 and G2 ($P < 0.01$). At t = 180 min, there was no difference between control and any glucose infusion.

Plasma GIP (Figure 11.1C)

Early response: At t = 15 and 30 min, there was a marked rise in plasma GIP during all glucose infusions, when compared to control ($P < 0.001$ vs. control). The GIP responses to G2 between t = 0 - 30 min, and to G4 at t = 15 min, were greater when compared with G1. There was no difference between G2 and G4.

Infusion period: All treatments increased plasma GIP, between t = 15 - 120 min when compared with control ($P < 0.001$ for all). There was a rapid increase in GIP during all glucose treatments, after which concentrations remained relatively stable. G2 and G4 increased plasma GIP compared with G1 between t = 15 - 120 min, and t = 30 -120 min, respectively ($P < 0.05$ for all). G4 increased plasma GIP

compared with G2 between t = 30 - 90 min ($P < 0.05$). Peak plasma GIP concentrations were: control: 9 ± 2 pmol/L at 20 ± 5 min, G1: 32 ± 2 pmol/L at 20 ± 4 min, G2: 42 ± 4 pmol/L at 80 ± 17 min, G4: 52 ± 3 pmol/L at 99 ± 8 min; $P < 0.001$ for concentration only. Peak GIP concentrations were greater for G2 and G4 when compared with G1 ($P < 0.001$), and G4 when compared with G2 ($P < 0.05$). The AUC for plasma GIP was greater for all glucose treatments, when compared with control (control: 225 ± 106 pmol/L.min, G1: 1146 ± 486 pmol/L.min, G2: 2846 ± 306 pmol/L.min, G4: 3648 ± 329 pmol/L.min; $P < 0.001$). There was a positive relationship between the AUC for plasma GIP with the load of glucose administered, such that the greater the load the higher the concentration ($r = 0.91$, $P < 0.01$).

Post meal period: There were no changes in GIP concentrations for G2 and G4, however, there were postprandial increases after control and G1 (i.e. t = 150 min). GIP concentrations remained elevated for G2 and G4 compared with control and G1 after the meal ($P < 0.01$). At t = 180 min, there was no difference between control and any glucose infusion.

Plasma CCK (Figure 11.1D)

Early response: There was a marked prompt rise (i.e. within 15 min) in CCK concentrations during all glucose infusions, when compared with control ($P < 0.05$ vs. control), with no difference between G1 and G2. CCK concentrations during G4 were higher compared with G1 and G2 ($P < 0.001$). Between t = 15 - 30 min,

plasma CCK fell during G1 and G2, and there were no differences between control, G1, and G2l. At t = 30 min plasma CCK remained higher during G4 compared with control, G1 and G2 ($P < 0.01$).

Infusion period: There was a rapid increase in CCK during all glucose treatments, after which concentrations remained relatively stable. G1 and G2 increased plasma CCK when compared with control at t = 15 min ($P < 0.01$). CCK concentrations remained elevated compared to baseline for G1 and G2 between t = 15 - 120 min ($P < 0.01$). G4 increased plasma CCK when compared with control, G1 and G2 between t = 15 - 120 min ($P < 0.001$ for all). Peak plasma CCK concentrations were: control: 5 ± 0 pmol/L at 62 ± 1 min, G1: 6 ± 1 pmol/L at 53 ± 11 min, G2: 5 ± 0 pmol/L at 63 ± 13 min, G4: 7 ± 1 pmol/L at 30 ± 8 min; $P < 0.01$ for concentration only. Peak plasma CCK was greater for G4 when compared with control, G1 and G2 ($P < 0.01$). The AUC between t = 0 - 120 min for plasma CCK was greater for G4, when compared with control, G1 and G2 (control: 142 ± 28 pmol/L.min, G1: 146 ± 40 pmol/L.min, G2: 186 ± 23 pmol/L.min, G4: 344 ± 40 pmol/L.min; $P < 0.001$). There was a positive relationship between the AUC for plasma CCK with the load of glucose administered, such that the greater the load the higher the concentration ($r = 0.82$, $P < 0.01$).

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

11.4.2 Antropyloroduodenal pressures

Antral pressure waves

There was a treatment effect for the number, but not the amplitude, of antral PWs ($P < 0.01$) (Table 11.1). G1 ($P < 0.05$), G2 ($P < 0.05$) and G4 ($P < 0.001$) decreased the number of antral PWs when compared with control, with no significant difference between G1, G2 and G4, although the mean value was least for G4. There was also an effect of treatment on the MI of antral pressure waves ($P < 0.05$) (Table 1); G2 ($P < 0.01$) and G4 ($P < 0.05$) decreased the MI when compared with control. G2, but not G4, decreased the MI compared with G1 ($P < 0.05$), while there was no difference between G2 and G4. There was no significant relationship between the number, amplitude or MI of antral PWs and the load of glucose administered.

Pyloric pressures

Basal pressures (Figure 11.2A): There was a treatment*time interaction for basal pyloric pressures ($P < 0.001$). G1, G2 and G4 stimulated basal pyloric pressure when compared with control; G1 and G2 between t = 90 - 105 min ($P < 0.05$) and G4 between 15 - 120 min ($P < 0.01$). During G4 basal pyloric pressure progressively rose until t = 45 min ($P < 0.001$), after which pressure fell. G2 stimulated basal pyloric pressure between t = 15 - 45 min ($P < 0.05$) and G4 between t = 15 - 120 min ($P < 0.001$), when compared with G1. G4 simulated basal pyloric pressure between t = 30 - 120 min when compared G2 ($P < 0.01$). There was a trend for a positive relationship between the AUC for basal pyloric

pressure with the load of glucose administered, such that the greater the load the higher the pressure ($r = 0.89$, $P = 0.07$).

Phasic pressures: There was no effect of treatment on the number (Figure 11.2B) or amplitude of IPPWs (data not shown). All infusions initially stimulated the number of IPPWs (non-significant) followed by a progressive decline. There was, however, a transient increase in the number during G4 compared with control (between t = 15 - 45 min), G1 (between t = 0 - 45 min) and G2 (between t = 15 - 30 min) ($P < 0.05$ for all). There were no significant relationships between the number and amplitude of IPPWs with the load of glucose administered.

Duodenal pressures

There was a treatment effect for the number, but not the amplitude, of duodenal PWs ($P < 0.001$) (Table 11.1). G4 deceased the number of duodenal PWs compared with control ($P < 0.001$), G1 ($P < 0.001$) and G2 ($P < 0.01$) with no difference between G1, G2 and control. There was an effect of treatment on the MI of duodenal PWs ($P < 0.05$) (Table 11.1); G2 and G4 decreased the MI of duodenal PWs when compared with control ($P < 0.05$ for both). There was a trend for G4 to decrease the MI when compared with G1 ($P = 0.06$), with no significant differences between control and G1, G1 and G2, or G2 and G4. There was an inverse relationship between the number, but not amplitude or MI, of duodenal PWs with the load of glucose administered, such that the greater the load the lower the number ($r = - 0.75$, $P < 0.001$).

Pressure wave sequences (PWSs)

Only PWSs that travelled over 2 - 9 channels (1.5 - 13.5 cm) were analysed statistically, as PWSs travelling over 8 - 15 channels were infrequent (control: 3.4 ± 0.3; G1: 1.6 ± 0.2; G2: 2.3 ± 0.2; G4: 1.2 ± 0.1). There was a treatment*length interaction for the number of pressure wave sequences (PWSs) travelling along two (1.5 - 3 cm), three (3 - 4.5 cm), four (4.5 - 6 cm), five (6 - 7.5 cm), six (7.5 - 9 cm, seven (9 - 10.5 cm), eight (10.5 - 12 cm) and nine (12 - 13.5 cm) channels ($P < 0.05$) (data not shown). G2 decreased the number of PWSs that travelled over two channels ($P < 0.001$) and G4 over two to four channels ($P < 0.001$) when compared with control. G2 ($P < 0.05$) and G4 ($P < 0.01$) decreased the number of PWSs that travelled over two and three channels compared with G1. There were no differences between control and G1 or G2 and G4. There was an inverse relationship between the number of PWSs with the load of glucose administered, such that the greater the load the lower the number ($r = - 0.7$, $P < 0.05$).

11.4.3 Appetite perceptions and energy intake

Appetite perceptions and gastrointestinal symptoms

There was a treatment*time interaction for change in scores for hunger ($P < 0.001$) (Figure 11.3A). Change in scores for hunger were greater for G1 and G2 between t = 45 - 120 min and G4 between t = 30 - 45 min, when compared with control ($P < 0.05$ for all). Change in scores for hunger were greater during G1 between t = 60 - 120 min ($P < 0.05$) and G2 between t = 90 - 120 min ($P < 0.001$), when compared with G4. Change in scores for hunger were greater for G2 at t = 120 min, when

compared with G1 ($P < 0.001$). There was a treatment*time interaction for change in scores for fullness ($P < 0.001$) (Figure 3B). Change in scores for fullness were greater during G1 between t = 15 - 120 min and control and G4 between t = 30 - 120 min, when compared with G2, which remained at baseline values ($P < 0.05$ for all). There were no significant relationships between change in scores for hunger and fullness and the load of glucose administered.

There was a treatment*time interaction for change in scores for nausea ($P < 0.01$) (Figure 11.3 C). Although mean change in scores for nausea were low, they were greater during control, at t = 15 min compared with all glucose treatments and at t = 90 min when compared with G1 and G2 ($P < 0.05$ for all). Change in scores for nausea were also elevated during G4 between t = 90 - 120 min, when compared with control, G1 and G2 ($P < 0.05$). There was a positive relationship between change in nausea scores with the load of glucose administered, such that the greater the load the higher the score ($r = 0.76$, $P = 0.01$). Change in scores for bloating were elevated for control compared with G1, G2 and G4 with no difference between the glucose treatments (data not shown). There was no relationship between change in scores for bloating with the load of glucose administered.

Energy intake

There was an effect of treatment on the amount eaten (g) at the buffet meal ($P < 0.001$) (Table 11.2). G2 ($P < 0.05$) and G4 ($P < 0.001$) reduced the amount eaten when compared with control, and G4 when compared with G1 ($P < 0.001$) and G2 ($P < 0.01$). There was also an effect of treatment on the energy consumed at the

buffet meal ($P < 0.01$), so that G4 reduced energy intake when compared with G1 ($P < 0.001$) and G2 ($P < 0.01$), with a trend for a decrease compared with control ($P = 0.09$) (Table 11.2). There was no effect of treatment on the proportion of energy from fat, carbohydrate or protein (Table 11.2). There was an inverse relationship between the amount of food and energy consumed at the buffet meal with the load of glucose administered, such that the greater the load the lower the amount ($r = - 0.79$, $P < 0.001$) and energy ($r = -0.73$, $P < 0.01$).

11.4.4 Relations of APD motility and blood glucose and plasma GLP-1, GIP and CCK with appetite perceptions and energy intake.

Relation of antropyloroduodenal motility with blood glucose and plasma GLP-1, GIP and CCK

There was a weak inverse relationship between the number of antral PWs with the AUC for blood glucose concentrations ($R = -0.42$, $P < 0.01$). There was a positive relationship between the AUC for basal pyloric tone ($R = 0.63$, $P < 0.001$) and an inverse relationship between duodenal PWs ($R = -0.58$, $P < 0.001$) with the AUC for plasma GLP-1. There was a positive relationship between the AUC for basal pyloric tone ($R = 0.55$, $P < 0.001$) and a weak inverse relationship between the number of antral ($R = -0.39$, $P < 0.05$) and duodenal ($R = -0.47$, $P < 0.05$) PWs, with the AUC for plasma GIP. There was a positive relationship between the AUC for basal pyloric tone ($R = 0.66$, $P < 0.001$) and a negative relationship between the number of duodenal PWs ($R = -0.63$, $P < 0.001$) with the AUC for plasma CCK.

Relation of APD motility with appetite perceptions, energy intake and amount eaten

There was an inverse relationship between the AUC for IPPWs ($R = -0.58$, $P < 0.001$), and a weak positive relationship between the MI for duodenal PWs ($R = 0.44$, $P < 0.01$) with energy intake. There was also an inverse relationship between AUCs for basal pyloric pressure ($R = -0.42$, $P < 0.01$) and number of IPPWs ($R = -0.58$, $P < 0.001$) with the amount eaten (g) at the meal. There were no significant relationships between APD motility and scores for hunger, fullness, nausea or bloating.

Relation of plasma GLP-1, GIP and CCK concentrations with appetite perceptions, amount eaten and energy intake

There were no significant relationships between blood glucose and plasma GLP-1, GIP or CCK concentrations with appetite perceptions, the amount eaten or energy intake.

Relation of appetite perceptions and gastrointestinal symptoms with energy intake.

There were no significant relationships between hunger and fullness with the amount eaten and energy intake. There were inverse relationships between the AUC's for nausea ($R = -0.55$, $P < 0.001$) and bloating ($R = -0.51$, $P < 0.001$) scores and energy intake at the buffet meal.

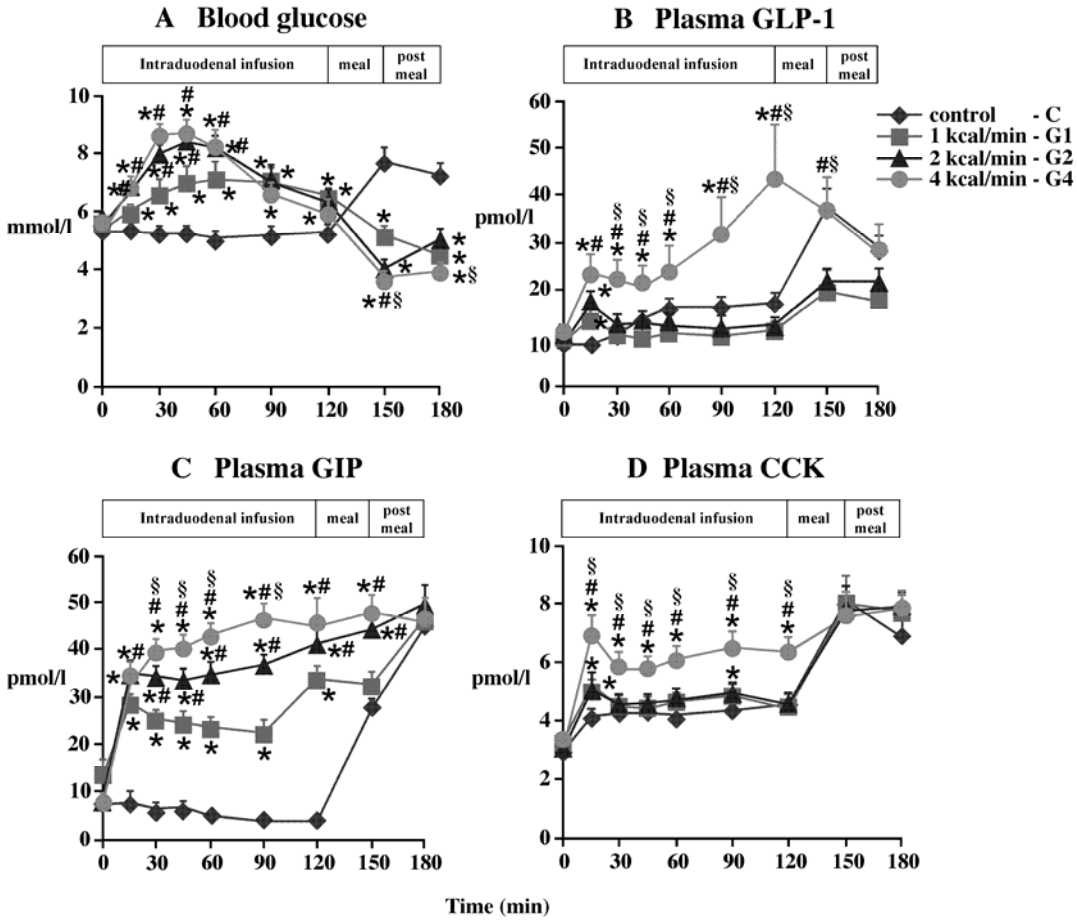

Figure 11.1

*Blood glucose (A) and plasma GLP-1 (B), GIP (C), and CCK (D) in response to intraduodenal glucose at 1 (■), 2 (▲), 4 (●) kcal/min and control (♦) in 10 healthy males. (A) # vs G1: P < 0.05, § vs. G2: P < 0.05. (B) * vs control: P < 0.05, # vs G1: P < 0.05, § vs G2: P < 0.05. (C) * vs control: P < 0.01, # vs G1: P < 0.05. (D) * vs control: P < 0.05, # vs G1: P < 0.01, § vs G2: P < 0.01. Data are means ± SEM.*

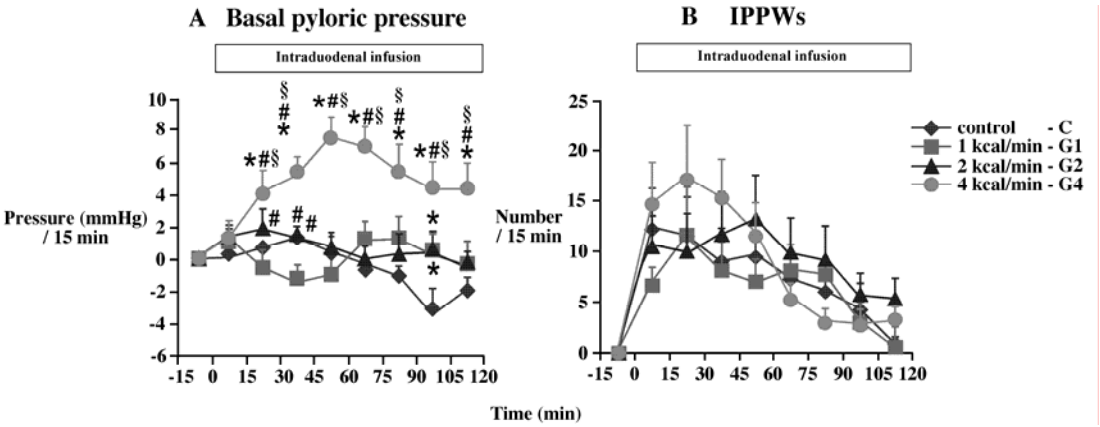

Figure 11.2

*Change in basal pyloric pressures (A) and isolated pyloric pressure waves (IPPWs) (B) in response to intraduodenal glucose at 1 (■), 2 (▲), 4 (●) kcal/min and control (♦) in 10 healthy males (A) G4 stimulated basal pyloric pressure between t = 30 - 120 min when compared with G2. * vs control: P < 0.05, # vs G1: P < 0.05, § vs G2: P < 0.01. (B) There was no effect of treatment on the number of isolated pyloric pressure waves. Data are means ± SEM (n = 10).*

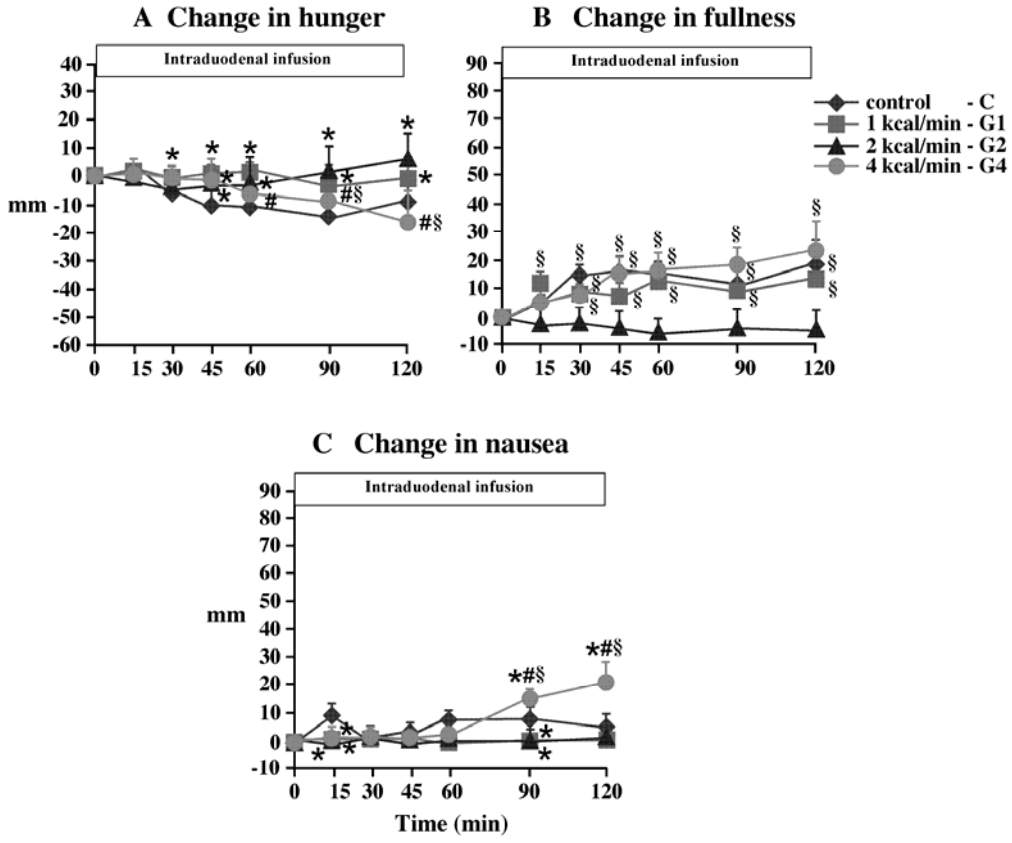

Figure 11.3

*Changes in hunger (A), fullness (B) and nausea (C) in response to intraduodenal glucose at 1 (■), 2 (▲), 4 (●) kcal/min and control (♦) in 10 healthy males (A) Hunger was greater during G1 between t = 60 - 120 min and G2 between t = 90 - 120 min when compared with G4. * vs control: P < 0.05, # vs G1: P < 0.001, § vs G2: P < 0.05. (B) There was a treatment*time interaction for fullness (P < 0.001). Fullness was greater during G1 between t = 15 - 120 min and control and G4 between t = 30 - 120 min, when compared with G2. § vs G2: P < 0.05. (C)* G1 and G2. * vs control: P < 0.05, # vs G1: P < 0.05, § vs G2: P < 0.05. Data are means ± SEM (n = 10).*

Table 11.1 Mean values for antral and duodenal PWs during intraduodenal infusions of 25 % glucose and 4.2 % saline between t = 0 - 120 min.

	Saline (control)	**Glucose G1**	**Glucose G2**	**Glucose G4**	***P* value ANOVA**
Antral PWs Number	70 ± 16	40 ± 10*	40 ± 12*	24 ± 7*	< 0.01
Amplitude (mmHg)	26 ± 6	27 ± 7	8 ± 3	22 ± 11	NS
MI (mmHg)	5 ± 1	4 ± 1	3 ± 1*#	3 ± 0*	< 0.05
Duodenal PWs Number	928 ± 78	1132 ± 125	865 ± 118	390 ± 93*#§	< 0.001
Amplitude (mmHg)	15 ± 3	11 ± 4	9 ± 4	7 ± 2	NS
MI (mmHg)	7 ± 1	6 ± 1	5 ± 1	4 ± 1	NS

Data are means ± SEM; n = 10. G1, 1 kcal/min; G2, 2 kcal/min; G4, 4 kcal/min; MI, motility index; NS, not significant; PWs, pressure waves. Significant differences: * from control, # from G1, § from G2.

Table 11.2 Amount eaten, energy intake and % macronutrient distribution at the buffet meal (i.e. between t = 120 - 150 min) following intraduodenal infusions of 25 % glucose and 4.2 % saline.

	Saline (control)	**Glucose G1**	**Glucose G2**	**Glucose G4**	***P* value ANOVA**
Amount (g)	1568 ± 142	461 ± 126	1395 ± 132*	1157 ± 166*#§	0.01
Energy (kJ)	4444 ± 492	4999 ± 255	5020 ± 364	3935 ± 480#§	0.001
% kJ from fat	28 ± 2	29 ± 2	30 ± 4	27 ± 2	NS
% kJ from CHO	53 ± 3	50 ± 3	0 ± 2	57 ± 4	NS
% kJ from protein	20 ± 2	20 ± 1	20 ± 1	19 ± 1	NS

Data are means ± SEM; n = 10. CHO, carbohydrate; G1, 1 kcal/min; G2, 2 kcal/min; G4, 4 kcal/min; MI, motility index; NS, not significant. Significant differences: * from control, # from G1, § from G2.

11.5 DISCUSSION

This study provides novel insights into the effects of increasing doses of glucose, at loads lower (1 kcal/min), comparable to (2 kcal/min), and higher (4 kcal/min) than the rate at which gastric emptying normally occurs, on blood glucose, incretin hormone and CCK concentrations, antropyloroduodenal motility, appetite and energy intake in healthy men. Of particular note are that: (i) while there was a rise in blood glucose in response to all the intraduodenal (ID) glucose infusions, there was no significant difference in the response to 2 kcal/min and 4 kcal/min, (ii) there was a transient, modest, rise in GLP-1, in response to ID glucose, but a sustained and progressive elevation was only evident with the 4 kcal/min from ~60 min, and a 'meal-related' rise occurred in response to the 1 and 2 kcal/min, but not the 4 kcal/min, infusions, (iii) there was a load-dependent stimulation of GIP and CCK with all infusions with a subsequent plateau and a 'meal-related' increase only after the 1 kcal/min for GIP and after the control, 1 and 2 kcal/min infusions for CCK, (iv) while antral pressures were suppressed during all glucose infusions, the stimulation of basal pyloric pressure and suppression of duodenal pressure waves only occurred during the 4 kcal/min infusion, (v) a reduction in energy intake was only observed after the 4 kcal/min infusion, and (vi) there were significant relationships between plasma GLP-1, GIP and CCK with basal pyloric pressure and duodenal pressure waves, and between pyloric pressures with energy intake.

The effects of intraduodenal glucose were specifically evaluated at loads that encompass rates below, similar to, and above the normal rate of gastric emptying.

A load-dependent stimulation of blood glucose and incretin concentrations was evident. Predictably, all loads increased blood glucose concentrations; previous studies have demonstrated that in both healthy subjects and patients with type 2 diabetes relatively minor differences in duodenal glucose delivery may have a major effect on the initial glycaemic response (O'Donovan et al. 2004b; Chaikomin et al. 2005). Another important observation was that the 2 kcal/min and 4 kcal/min glucose infusions resulted in comparable blood glucose concentration profiles, indicating that 2 kcal/min load was sufficient to cause a maximal blood glucose response. The maximal capacity of glucose absorption from the small intestine into the systemic circulation is probably ~0.5 g per minute or 2 kcal/min per 30 cm (Holdsworth and Dawson 1964; Duchman et al. 1997). That the loads of 2 and 4 kcal/min used in the present study are equal to, or exceed, maximal capacity for the segment of small intestine, the length of the duodenum, may explain why blood glucose responses are similar. The fall in blood glucose after 60 min is probably indicative of the release of insulin and incretin hormone response. - At the time of the submission of this thesis the results of plasma insulin measurements, which are fundamental to interpretation of the data, are not yet available. A prompt increase in plasma GIP occurred during all glucose infusions. As GIP is released from duodenal K cells (Fehmann et al. 1995), which are located near the site of infusion, it is not unexpected for glucose loads to result in a dose-dependent increase. On the otherhand, GLP-1 is released from L-cells whose concentrations is greatest in the distal small intestine (Eissele et al. 1992). A slow, progressive rise in GLP-1 concentrations may hypothetically be expected, however the peak concentrations during 1 and 2 kcal/min glucose infusions occurred within the first ~ 21 min of

infusion, approximately 10 min earlier than GIP. Schirra and colleagues suggested that GLP-1 secretion requires a threshold of caloric delivery of ~1.8 kcal/min to be exceeded (Schirra et al. 1996b). In the present, as well as two recently published (O'Donovan et al. 2004b; Chaikomin et al. 2005) studies, loads as low as 1 kcal/min were shown to have the capacity to cause an early transient, stimulation of plasma GLP-1. While the latter may reflect recently discovered GLP-1 releasing L cells located in the duodenum (Theodorakis et al. 2006), another recent study indicates that GLP-1 was only released during glucose infusions along the whole gut, and not when localised to the proximal 60 cm of the small intestine (Little et al. 2006b). Accordingly, the mechanism(s) underlying the initial increase in plasma GLP-1 still warrant(s) further investigation; presumably, it can be attributed to initially more rapid small intestinal transit and subsequent slowing.

CCK is released from I cells which are confined to the proximal small intestine (duodenum and jejunum) (Polak et al. 1975; Buffa et al. 1976), therefore, as expected, all glucose infusions increased plasma CCK concentrations in a dose-dependent fashion. In the study reported in chapter 9, only duodenal infusion of glucose, and not mid-jejunal infusion, stimulated the release of CCK, suggesting a gradient between the duodenum and mid-jejunum in the capacity to secrete CCK. This may explain why the increase was transient in response to the 1 and 2 kcal/min loads.

The regulation of glucose entry into the small intestine has important implications for postprandial glycaemia (Horowitz et al. 1993a; Rayner et al. 2001); the

stimulation of pyloric pressures has been shown to regulate gastric emptying (Heddle et al. 1988c; Houghton et al. 1988a). All glucose treatments, as well as the control, increased the number of IPPWs, during the first 30 min of infusion, followed by a decline. It should be recognised that the hypertonic saline given as a control would itself have an osmotic stimulus. While there was no overall difference between the treatments, the highest load (4 kcal/min) increased the number of IPPWs to a greater extent, up to 45 mins of the infusion. Similar to the present study, Edelbroek et al (1992) reported that 120 min glucose infusion at 2.4 kcal/min, in healthy men, resulted in an initial stimulation of IPPWs and basal pyloric tone, which was not sustained for the entire infusion period (Edelbroek et al. 1992a). These authors suggested that there may be adaptation of the pylorus, which could be specific for glucose, as previous studies using lipid have shown a continued stimulation of phasic and tonic pyloric activity throughout the infusion period (Fraser et al. 1992a; Feinle et al. 2003b). This adaptive change may reflect a decrease in the number, or affinity of small intestinal receptors for glucose, or a reduction in the area exposed to glucose as a result of enhanced absorption at more proximal duodenal sites (Edelbroek et al. 1992a). In the present study, however glucose loads of 4 kcal/min were able to stimulate basal pyloric tone throughout the entire infusion, whereas loads of 1 and 2 kcal/min had no effect. The current study also confirms previous data, showing a suppression of antral pressure waves during intraduodenal glucose infusions (Heddle et al. 1988c; Edelbroek et al. 1992a; Rayner et al. 2000b). In a recent study, the suppression of antral motility only occurred when glucose was infused into both the duodenum and distal small intestine (i.e. allowed access to the entire small intestine), and not when it was

confined to the proximal 60 cm (Little et al. 2006b). In the current study, the 1 kcal/min load was able to suppress antral pressure waves, which is surprising, as one would not expect this small load to reach the glucose sensors beyond 60 cm of the small intestine, but may reflect to the longer duration of glucose infusion in the present study.

There is a strong relationship between the rise in blood glucose after oral carbohydrate with gastric emptying (Horowitz et al. 1993a). Accordingly, interventions, which result in a slowing of gastric emptying, and are associated with a pattern of antropyloroduodenal motility which favour this, may reduce postprandial glycaemic excursions (Gentilcore et al. 2005; Gentilcore et al. 2006a). There was an association, albeit weak, between the release of GIP, but not GLP-1, and antral pressure waves. The magnitude of the rise in blood glucose was also associated with the magnitude of suppression of antral pressure waves. There is evidence that blood glucose concentrations as low as 7.7 mmol/L, can inhibit antral pressures (Barnett and Owyang 1988). In the current study even, the 1 kcal/min load resulted in glucose concentrations in this range. There was also a correlation between GLP-1, GIP and CCK and the stimulation of basal pyloric tone and suppression of duodenal pressure waves, which may account for slowing of gastric emptying.

Energy intake was only suppressed after the highest intraduodenal glucose infusion (4 kcal/min; 480 kcal), however the amount (in grams) of food consumed was also suppressed by 2 kcal/min. That a decrease in energy intake was only evident after

4 kcal/min is not surprising, as previous studies using glucose loads of 2 kcal/min for 90 min (180 kcal) (Rayner et al. 2000b) and 2.86 kcal/min for 120 min (343.2 kcal) (Chapman et al. 1999; MacIntosh et al. 2001b) demonstrated no significant reduction in energy intake in healthy young males. There was however, a reduction in appetite in these studies. The only study, to date, which has resulted in a reduction in energy intake during intraduodenal glucose infusions, compared with saline, occurred after a 3.2 kcal/min infusion of glucose for 90 min, which approximates to 288 kcal (Lavin et al. 1998c). Interestingly, this load (288 kcal) was lower than the 2.86 kcal for 120 min studies (Chapman et al. 1999; MacIntosh et al. 2001b), in which no reduction in energy intake was observed. This may indicate that the rate of glucose infusion, or similarly the length of small intestinal exposure to glucose, rather than the duration of infusion *per se*, are more important in the regulation of energy intake, as digestion and absorption are probably completed over a shorter length of intestine during slower infusions. The effect of exposing greater lengths of the small intestine (e.g. the mid jejunum), warrants further investigation. It is important to note that while there was an inverse relationship between mean nausea scores and energy intake, these scores were very low in absolute terms, and energy intake was also reduced in those individuals who did not experience nausea.

In conclusion, this study has established that variations in the delivery of glucose into the small intestine, have differential effects on glycaemia, incretin and CCK responses, gastric motility, appetite and energy intake in healthy subjects. These observations have implications for an understanding of the regulation of

postprandial glycaemia and energy intake in type 2 diabetes. As stated previously, the outcome of the plasma insulin measurements is important to the interpretation of the data and is awaited with interest

CHAPTER 12:

CONCLUSIONS

The studies presented in this thesis provide novel information about the interrelationships of upper gastrointestinal function, glycaemia and appetite in both health and type 2 diabetes. These insights have predictably led to a number of questions, as yet unanswered, which should now be addressed.

The study reported in chapter 4 represented a logical development of a previous study, which evaluated the effects of variations in the initial rate of small intestinal delivery of glucose on glycaemic, insulin and incretin hormone responses in healthy subjects and patients with type 2 diabetes (O'Donovan et al. 2004b). The underlying hypothesis in that study; that a rapid, and subsequently slower, rate of small intestinal glucose delivery would lead to a reduction in the overall glycaemic response, was not supported, but this may have reflected the relatively low rate of small intestinal glucose delivery with the variable infusion (3 kcal/min). In the study conducted by the author, the initial rate of small intestinal glucose delivery was, accordingly, increased to 6 kcal/min. While the magnitude of the initial rises in blood glucose, plasma insulin and plasma GLP-1, but not GIP, in response to the variable infusion, were substantially greater and blood glucose concentrations between 75 - 180 min were significantly less, when compared with those which resulted from the constant infusion, there was again no difference in the overall glycaemic response. Hence, these observations add to the rationale for the use of dietary and pharmacological strategies designed to reduce postprandial glycaemic

excursions in health and type 2 diabetes by slowing gastric emptying, rather than initially accelerating it. The study also confirmed that there is a modest, albeit-transient, stimulation of GLP-1 in response to a 1 kcal/min duodenal glucose infusion (O'Donovan et al. 2004b), which apparently conflicts with the report by Schirra et al (1996) suggesting that a threshold of duodenal glucose delivery in excess of ~1.4 kcal/min is required for GLP-1 release. It has been assumed that the stimulation of GLP-1 by enteral glucose is a load-, rather than concentration-dependent, phenomenon, but this has not been formally evaluated. Similarly, the relationship between the plasma GLP-1 response and the small intestinal glucose load is poorly defined (chapter 11). The cause (s) of the transient 'early' release of GLP-1 observed in response to both the 1 and 6 kcal/min infusions remains to be determined. In contrast to GLP-1, there was no difference in the initial GIP response between the variable infusions in the current and previous (O'Donovan et al. 2004b), study suggesting that stimulation by 3 kcal/min glucose may have been maximal (this issue is addressed in the study reported in chapter 11). A differential effect of small intestinal glucose on GIP and GLP-1 is not unexpected, given that they are released from different cell types which are located predominantly in differing regions (duodenum and jejunum respectively). While the observed lowering of blood glucose between 75 - 180 min by the variable infusion is likely to be attributable to the greater stimulation of plasma insulin which preceded it, it should be recognised that the rate of duodenal glucose delivery during this time was less with the variable, when compared with the constant, infusion. The study did not employ glucose tracer techniques, which would have been of interest to determine which of these phenomena made the greater contribution.

Fat is a potent inhibitor of gastric emptying; the study reported in chapter 5 establishes that, in type 2 patients managed by diet alone, ingestion of a relatively small amount of olive oil as a 'preload' 30 min before a carbohydrate meal, markedly slows gastric emptying, affects intragastric meal distribution, delays the postprandial rises in blood glucose, plasma insulin and GIP, and stimulates the secretion of GLP-1. In contrast, the effects of including the same amount of oil within an identical carbohydrate meal on gastric emptying, and glycaemic and incretin responses, were relatively modest. Oil should empty from the stomach with minimal delay when administered before a meal, so that both digested and non-digested fat would be expected to be present in the small intestine when the meal is consumed. As the 'incretin effect' is an important determinant of the postprandial insulin response (Nauck et al. 2004), and fat stimulates the secretion of GLP-1 and GIP (Herrmann et al. 1995; Feinle et al. 2003a), it was logical to anticipate that fat when given as a 'preload' also had the potential to attenuate the glycaemic response to carbohydrate by this mechanism. It should be recognised that there was only a trend for a reduction in the peak blood glucose level and that because blood glucose levels had not returned to baseline by 210 min, effects on the overall glycaemic (or insulinaemic) response could not be determined. The latter issue represents a priority for future studies. Furthermore, while these observations establish the capacity for the administration of a relatively small quantity of fat before a carbohydrate-containing meal to minimise glycaemic excursions and potentiate GLP-1 secretion in type 2 diabetes, further studies using more physiological 'preloads', and examining the effects of chronic administration of fat, would now be indicated. It would also be of interest to determine the effects

of chronic ingestion of fat 'preloads' on energy intake - a high fat preload may be expected to reduce energy intake at a subsequent meal and, therefore, have the potential to facilitate weight loss, rather than weight gain. If so, this would also benefit glycaemic control in type 2 diabetes. Accordingly, studies to evaluate the effects of fat 'preloads' on energy intake in type 2 patients would also be of interest.

The rate of gastric emptying is a major determinant of alcohol absorption (Holt 1981; Horowitz et al. 1989b); the energy content of a meal is, in turn, a major determinant of gastric emptying (Brener et al. 1983a). The study reported in chapter 6 demonstrated that the substitution of a lower calorie, artificially sweetened, ("diet") soft drink for a higher calorie, sucrose-containing ("regular") soft drink in a mixed alcoholic beverage, has a major impact on the rate of gastric emptying and alcohol absorption in healthy adults, as was to be expected.-The low calorie drink (with "diet" mixer) emptied from the stomach much more rapidly and resulted in higher blood alcohol concentrations when compared with the higher calorie alcoholic drink (with "regular mixer"). As well as the substantially higher peak plasma alcohol concentrations after the "diet" compared to the "regular" drink, the area under the blood alcohol concentration curve was markedly greater. These observations are consistent with the concept of more rapid absorption due to accelerated gastric emptying (Horowitz et al. 1989b), as well as proportionally less 'first-pass' alcohol metabolism (Kechagias et al. 1999) and highlight the need for community awareness that the caloric content of the non-alcoholic components of a beverage are a major determinant of the rate of alcohol absorption and the potential

for inebriation. Dietary and pharmacological strategies designed to optimise glycaemic control in type 2 diabetes by slowing gastric emptying would be expected to also diminish the blood alcohol response to alcohol-containing meals.

In the study reported in chapter 7, intraduodenal pressures and impedance signals were recorded simultaneously while glucose was infused into the duodenum in healthy humans, in the presence and absence of the anticholinergic drug, hyoscine butylbromide. The frequency of duodenal flow events (evaluated by impedance) was shown to be suppressed by hyoscine much more markedly than that of duodenal pressure waves and propagated pressure wave sequences (evaluated by manometry). The disparity between impedance measurements and manometry in detecting alterations in flow during hyoscine infusion was marked, and supports the potential utility of small intestinal impedance monitoring to evaluate alterations in gastrointestinal transit in various disease states. Hyoscine was also shown to reduce the rises in blood glucose and 3-OMG, indicative of slower glucose absorption. The suppression of flow events by hyoscine could account for the reduction in glucose absorption by initially restricting the area of the absorptive surface. The spreading of glucose by intestinal transit over longer lengths of gut would normally allow the recruitment of progressively more glucose transporters, so that the overall rate of glucose absorption into the systemic circulation would be no longer restricted to a localised maximum, suggested to be about 0.5 g per minute over 30 cm of upper jejunum (Holdsworth and Dawson 1964; Modigliani and Bernier 1971; Duchman et al. 1997). Hyoscine also attenuated the initial rises in GLP-1, GIP and insulin, which may potentially be accounted for by inhibition of

vagally mediated GLP-1 and GIP secretion, although evidence to suggest vagal control of incretin hormone release is limited (Deacon 2005). An alternative, and more plausible, explanation is that hyoscine delayed the flow of glucose into more distal segments of the small intestine - limitation of distal spread may be of particular relevance to GLP-1, given that the density of L-cells increases more distally (Eissele et al. 1992), where GLP-1 is released by local contact of glucose with L cells (Little et al. 2006a). Conversely, suppression of GLP-1, GIP, and insulin secretion by hyoscine per se cannot account for the blood glucose profile, which would then be expected to be higher, rather than lower, than on the saline day, in the absence of any difference in glucose absorption. Altogether the results of this study support the concept that proximal small intestinal motility/flow is a significant determinant of postprandial glycaemia, in addition to gastric emptying. Comparable studies in type 2 patients would be of interest.

Intraduodenal administration of the local anaesthetic, benzocaine, has been reported to attenuate both the release of cholecystokinin (CCK), and the perceptions of fullness, discomfort, and nausea induced by gastric distension, implying that local neural mechanisms may regulate CCK release in response to intraduodenal nutrients. In the study reported in chapter 8, intraduodenal administration of benzocaine was shown to attenuate the perceptions of abdominal discomfort and nausea, but had no effect on antropyloroduodenal motility, blood glucose concentrations, or the GLP-1 response, to intraduodenal glucose at a rate of 3 kcal/min in healthy subjects. These observations suggest that, at the relatively high duodenal glucose load used, mucosal afferent nerves mediate unpleasant gut

sensations, but other mechanisms such as GLP-1 release play the greater role in the feedback on gastric motility and appetite. - It is planned to measure CCK on the plasma samples; these assays had not been performed at the time of submission of this thesis.

As discussed, the “incretin” peptides, GLP-1 and GIP, are likely to be key mediators of the effects of small intestinal glucose on gastric emptying, appetite, and insulin release and that, GLP-1 is released from L-cells whose density is greatest in the distal jejunum, whereas GIP is released predominantly from duodenal K cells. Cholecystokinin (CCK) is secreted by proximally-located I cells, which appear confined to the duodenum and jejunum. In the study reported in chapter 9, the effects of glucose infused into the mid-jejunum on the release of GLP-1, GIP, CCK and insulin, blood glucose concentrations and energy intake were compared to those of an isocaloric duodenal infusion (1 kcal/min) in healthy subjects. While there was no difference in the incretin response between the two sites, the stimulation of plasma CCK and suppression of energy intake were greater with the duodenal, compared to the jejunal, infusion. Given the absence of any difference in incretin hormones, it is not surprising that there was no difference in either plasma insulin or blood glucose concentrations. These observations may reflect the relatively low duodenal glucose load (1 kcal/min) and/or that the site of the jejunal infusion was not distal enough to result in substantial stimulation of GLP-1. Given the potential importance of the site of small intestinal glucose exposure to postprandial glycaemia, these issues should be explored in future studies, in both healthy subjects and type 2 patients. It would also be of interest to

determine the effects of a specific CCK antagonist on the appetite responses to small intestinal glucose to clarify the suggested physiological role of CCK in mediating the observed effects of duodenal glucose on energy intake.

The study reported in chapter 10 represents the first evaluation of the effects of the addition of protein to an oral glucose load on both gastric emptying and incretin responses in humans. The addition of protein reduced the glycaemic response in healthy subjects, and the dominant mechanism accounting for this effect was the slowing of gastric emptying. Protein, when combined with glucose, did not stimulate additional GLP-1, GIP, or insulin release, and indeed plasma GIP concentrations were lower after the "glucose + protein" than after the "glucose" drink alone. This is despite the fact that protein, when consumed alone, stimulated both incretin and insulin release. Amino acids potentially stimulate insulin secretion by different mechanisms than glucose (Nuttall and Gannon 1991) and this may be particularly advantageous in patients with type 2 diabetes, in whom the beta cell response to glucose is impaired, but the response to other stimuli may be relatively intact. While the study confirmed the capacity for protein supplementation to improve the glycaemic response to glucose in healthy humans, and demonstrated that this is accounted for predominantly by slowing of gastric emptying, further studies aimed at optimising this effect are indicated in patients with type 2 diabetes, who may derive additional benefit, in terms of postprandial glycaemia, from the stimulation of non-glucose dependent insulin secretion. It is also likely, as appears to be the case with fat (chapter 5), that the beneficial effects of protein on glycaemia and slowing of gastric emptying may be more marked

when given as a ‘preload’. Furthermore, protein also has the potential to diminish energy intake. These issues should now be addressed.

The study reported in chapter 11, provides novel insights into the effects of increasing doses of glucose, at loads lower (1 kcal/min), comparable to (2 kcal/min), and higher than (4 kcal/min), the normal rate of gastric emptying, on glycaemia, incretin hormone release, and CCK concentrations, antropyloroduodenal motility, appetite and energy intake in healthy males. While there was a rise in blood glucose in response to all the intraduodenal (ID) glucose loads there was no significant difference in the response to infusions at 2 kcal/min and 4 kcal/min. There was also a transient, small, rise in GLP-1 in response to ID glucose (as noted in chapter 4), but a sustained and progressive elevation was only evident with the 4 kcal/min from ~60 min. In contrast to GLP-1, there was a load-dependent stimulation of GIP with all infusions with a subsequent plateau. The stimulation of CCK was much more marked in reported to the 4 kcal/min infusion. While antral pressures were suppressed during all glucose infusions, energy intake was only decreased by the 4 kcal/min glucose load. This may potentially reflect the greater stimulation of CCK, consistent with observations in chapter 9. Hence, this study establishes that there is a substantial discordance in the effects of small intestinal glucose on glycaemia, insulinaemia, incretin hormones, pyloric motility and appetite. These observations, by definition, can only be extrapolated to the glucose loads that were evaluated over the time interval over which they were given comparable studies in type 2 patients would be interest. All glucose treatments increased the number of isolated pyloric pressure waves IPPWs, during

the first 30 min of infusion; the highest load (4 kcal/min) increased the number of IPPWs to a greater extent up to 45 min, which was not sustained for the entire infusion period. This is consistent with the study by Edelbroek et al. (1992) who suggested that there is an adaptation of the pylorus to intraluminal glucose, which may be specific for glucose (Fraser et al. 1992b; Feinle et al. 2003a). That energy intake was only suppressed during the highest intraduodenal glucose infusion (4 kcal/min; 480 kcal) is not surprising, as previous studies using glucose loads of 2 kcal/min for 90 min (180 kcal) (Rayner et al. 2000a) and 2.86 kcal/min for 120 min (343.2 kcal) (Chapman et al. 1999) failed to demonstrate any effect on energy intake in healthy young males. This suggests that the rate of glucose infusion, or similarly the length of small intestinal exposure to glucose, rather than the duration of the infusion, may be more important in the regulation of energy intake, as digestion and absorption are probably completed over a shorter length of intestine during slower infusions. Accordingly, the effect of exposing greater lengths of the small intestine (i.e. the mid jejunum), warrants further investigation to assess effects on energy intake. It is planned to perform measurements of plasma insulin on the stored samples-these results were, unfortunately, not available at the time of the submission of this thesis and are critical to the overall interpretation of the data.

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