Investigations into the gastrointestinal factors involved in the regulation of appetite and energy intake

A thesis submitted by
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Chapter 4: Common methodologies

Thesis summary

The research presented within this thesis has focused on the complex and interrelated gastrointestinal mechanisms involved in the regulation of appetite and energy intake. The suppression of appetite and energy intake is mediated, at least in part, by a number of gastrointestinal factors, including gastric distension, the modulation of gastric emptying, gastrointestinal motility and gastrointestinal peptides, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY) and ghrelin. An understanding of these mechanisms is important to determine the pathophysiology of obesity and to allow the identification of targets for the treatment of obesity.

The effects of the fat on gastrointestinal function and appetite are dependent upon the digestion of fat to free fatty acids. Animal studies indicate that the effects of free fatty acids on energy intake are more potent than those of triglycerides. The comparative effects of a free fatty acid and a triglyceride on gastric emptying, appetite and energy intake were assessed in healthy lean male subjects. Free fatty acids slowed gastric emptying, stimulated the secretion of CCK, suppressed hunger, increased fullness and suppressed energy intake more potently than triglyceride (Chapter 5). These observations suggest that small amounts of free fatty acids in the small intestine potently modulate gastrointestinal function and energy intake.

We had previously demonstrated that intraduodenal infusion of the free fatty acid, lauric acid (C12) (at 0.375 kcal/min, 106 mM), stimulates isolated pyloric pressure waves (IPPWs), inhibits antral and duodenal pressure waves (PWs),

stimulates the release of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), and suppresses energy intake, and that these effects are much greater than those seen in response to isocaloric decanoic acid (C10) infusion. However, C12 was associated with nausea, confounding interpretation of these results. In order to determine whether the effects we had observed were physiological, or related to nausea, we assessed the effects of a range of doses of C12 (0.1 – 0.4 kcal/min) on the above parameters. Intraduodenal infusion of very small amounts of C12, potently modulate gastrointestinal motility, gut hormone secretion and suppresses energy intake at a subsequent meal in a dose-dependent fashion, in the absence of nausea (Chapter 6). However, as both the load and the concentration of the infusions varied, it was unclear whether these effects were load-, or concentration-, dependent. We, therefore, examined the independent effects of load, and concentration, of C12 on these variables, and demonstrated that the effects of C12 on gastrointestinal motility, gut hormone release and energy intake are dependent upon the load, but not the concentration of C12 administered to the small intestine in humans (Chapter 7).

Animal studies have indicated that the effects of nutrients on gastrointestinal function and energy intake are dependent upon the length of small intestine exposed to nutrient. In humans, we demonstrated that the modulation of gastrointestinal motility and gut hormone secretion by small intestinal glucose is dependent upon the length of small intestine exposed to nutrient, specifically, the suppression of antral motility, the release of GLP-1 and the suppression of plasma ghrelin concentrations is dependent upon greater than 60 cm of the small intestine being exposed to glucose (Chapter 8).

The inhibitory action of glucagon-like peptide-1 (GLP-1) on gastric emptying GE is likely to be important in mediating its effects on glycaemia, appetite and upper gastrointestinal symptoms. In healthy subjects (i) the slowing of solid and liquid gastric emptying by exogenous GLP-1 is associated with increased retention of both solid and liquid in the distal stomach and, even when administered in a "low" dose can induce "gastroparesis" and (ii) the effects of GLP-1 on postprandial glycaemic and insulinaemic responses are predictable on the basis of its effect on gastric emptying, supporting the concept that gastric emptying is a major target mechanism for the clinical use of incretin mimetics (Chapter 9). The feeding inhibitory effects of GLP-1 are likely to relate to the increased antral meal retention, as a close relationship has previously been demonstrated between antral area (and content) with the perception of fullness and subsequent energy intake.

An understanding of the physiological adaptations that occur in obesity is essential to enable the development of successful therapies for this condition. There is increasing evidence that consumption of a high-fat diet is associated with the development of obesity. The precise mechanisms by which this occurs are unclear, however, studies in animals suggest that adaptations in the gastrointestinal mechanisms involved in the regulation of appetite and energy intake occur, and may, therefore, predispose to obesity. In particular, studies have demonstrated that the acute effects of exogenous CCK, a hormone that potently suppresses energy intake, are attenuated following exposure to a high-fat diet in rats. In our study, healthy lean male volunteers were exposed to a high-fat

diet for a period of 3 weeks, following which the effects of an intravenous infusion of CCK on gastrointestinal motility and energy intake were evaluated. Fasting concentrations of CCK were greater following the high-fat diet, however, we did not demonstrate any differences in the antropyloroduodenal motility or energy intake response to exogenous CCK following ingestion of either diet, suggesting, that at least in the short-term, in healthy lean male subjects consumption of a high-fat diet does not alter the sensitivity to the effects of CCK on antropyloroduodenal motility and energy intake (Chapter 10).

The studies reported in this thesis have provided new insights into the mechanisms by which nutrients present within the gastrointestinal tract modulate gastrointestinal function and energy intake. Future studies in obese subjects will be required to determine whether sensitivity of the gastrointestinal tract to nutrients is modulated in the obese state.

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.

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Signed: _____

Tanya J. Little

Dedication

I dedicate this thesis to my parents, Janet and Brian Little. For your love, support and encouragement throughout these years, I will be forever grateful.

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The studies reported in this thesis were conducted in the Discipline of Medicine, the Gastrointestinal Investigation Unit and the Department of Nuclear Medicine, PET and Bone Densitometry at the Royal Adelaide Hospital. Whilst conducting the research reported in this thesis I was supported by a University of Adelaide Faculty of Health Science Divisional Scholarship.

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Publications arising from this thesis

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

Little TJ, Feltrin KL, Horowitz M, Smout AJPM, Rades T, Meyer JH, Pilichiewicz AN, Wishart J, Feinle-Bisset C. Dose-related effects of lauric acid on antropyloroduodenal motility, gastrointestinal hormone release, appetite and energy intake in healthy men. Am J Physiol Regul Integr Comp Physiol 289 (4): R1090 – R1098 (2005).

Little TJ, Horowitz M, Feinle-Bisset C. Role of cholecystokinin in appetite control and body weight regulation. Obesity Reviews 6: 297 - 306 (2005).

Little TJ, Pilichiewicz AN, Russo A, Phillips L, Jones KL, Nauck MA, Wishart J, Horowitz M, Feinle-Bisset C. Effects of intravenous GLP-1 on gastric emptying and intragastric distribution in healthy subjects – relationships with postprandial glycemic and insulinemic responses. J Clin Endocrinol Metab 91: 1916 - 1923 (2006).

Little TJ, Doran S, Meyer JH, Smout AJPM, O'Donovan DG, Wu KL, Jones KL, Wishart J., Rayner CK, Horowitz M, Feinle-Bisset C. The release of GLP-1 and ghrelin, but not GIP and CCK, by glucose is dependent on the length of small intestine exposed. Am J Physiol Endocrinol Metab 291: E647-E655 (2006).

Chapter 1

Gastrointestinal factors involved in the regulation of appetite, energy intake and postprandial glycaemia

1.1. Introduction

The incidence of obesity, defined as a body mass index equal to, or greater, than 30 kg/m^2, is increasing rapidly worldwide. Recent data from the Australian Diabetes, Obesity and Lifestyle study (AusDiab) indicate that 52 % of women, and 67 % of men are overweight or obese (Cameron *et al.*, 2003). This rise in overweight and obesity is the major factor accounting for the dramatic increase in the incidence of type 2 diabetes. Obesity is also associated with an increased risk of a number of other diseases, including cardiovascular disease, hypertension, cholelithiasis and infertility. Hence, obesity represents a major social and economic burden, with current estimates suggesting that obesity costs the Australian government between $680 – 1239 million in direct health costs per year (http://www.asso.org.au/home/obesityinfo). While the precise aetiology of obesity is poorly defined, it is clear that a number of factors, including genetic, environmental (e.g. an increased availability of foods high in fat and energy density, coupled with a decrease in physical energy expenditure), physiological and psychological (e.g. stress), contribute. The studies presented in this thesis focus on the gastrointestinal factors involved in the regulation of appetite and energy intake.

It is well established that the presence of nutrients, particularly fat, in the small intestinal lumen is associated with decreased perceptions of hunger, increased fullness and decreased energy intake at a subsequent meal (Chapman *et al.*, 1999; Cook *et al.*, 1997; Lavin *et al.*, 1996; MacIntosh *et al.*, 1999). These acute, inhibitory effects of small intestinal nutrients on appetite and energy intake are probably mediated by a number of inter-related factors, including the modulation of gastric emptying (Sepple and Read, 1989), gastrointestinal motility (Azpiroz and Malagelada, 1985; Brener *et al.*, 1983; Feinle *et al.*, 1996; Heddle *et al.*, 1988a; Heddle *et al.*, 1988b) and the stimulation of a number of gastrointestinal hormones, including cholecystokinin (CCK) (Cook *et al.*, 1997; Lilja *et al.*, 1984), glucagon-like peptide-1 (GLP-1) (Herrmann *et al.*, 1995; Lavin *et al.*, 1996; Lavin *et al.*, 1998), peptide YY (PYY) (MacIntosh *et al.*, 1999; Onaga *et al.*, 2002), and the suppression of ghrelin (Overduin *et al.*, 2005) (**Figure 1.1**). The signals released from the gastrointestinal tract play an important role in the short-term regulation of energy intake by modulating meal size (i.e. when feeding at an individual meal is terminated), intermeal interval and/or energy intake at a subsequent meal. Signals released in proportion to the amount of stored adipose tissue (which are not discussed in detail) play an important role in the longer-term regulation of energy balance and body weight.

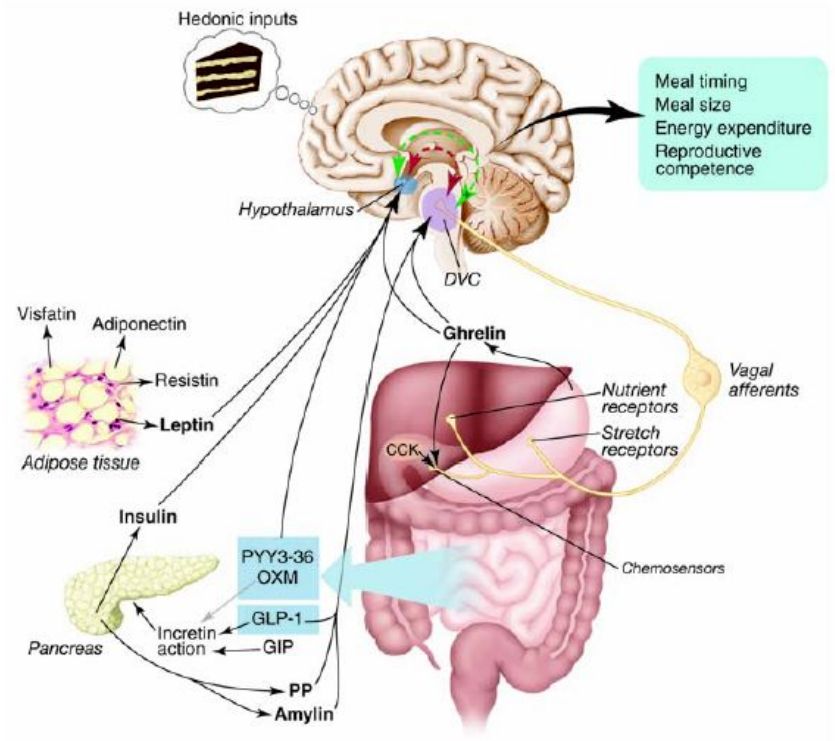

Figure 1.1: Overview of the peripheral hormonal factors involved in the regulation of gastrointestinal motor function, appetite and energy intake (from Badman and Flier, 2005).

This chapter provides an overview of the gastrointestinal factors involved in the regulation of appetite, energy intake and postprandial glycaemia, the major focus of the research presented in this thesis, followed by a brief discussion of the role of leptin and insulin in the long-term regulation of energy balance, and a summary of the central regulation of energy intake.

1.2. Peripheral regulation of appetite and energy intake

1.2.1. Stomach

The stomach is a J-shaped organ, which can be anatomically divided into the fundus, corpus, antrum and pylorus. Functionally, the stomach can be divided into three regions; the proximal compartment including the fundus and proximal

corpus (which acts as a reservoir for ingested food), the distal compartment, consisting of the antrum (which is responsible for the mixing and grinding of solid foods), and the pylorus, which modulates the emptying of chyme into the small intestine.

The mechanical function of the stomach is determined by the underlying electrical activity of the smooth muscle myocytes. Gastric myoelectrical activity can be divided into tonic and phasic components. The tonic component consists of the gastric slow wave, generated by the gastric pacemaker, which occurs at regular intervals (normally ~ 3 waves/min), originating in the proximal stomach and propagating distally towards the pylorus. Superimposed on the gastric slow wave, spike potentials, in the depolarised state, are responsible for phasic contractions.

1.2.1.1. Gastric emptying

Gastric emptying is regulated by coordinated activity of the proximal and distal regions of the stomach, which results in the delivery of chyme into the small intestine at a rate allowing for the optimal digestion and absorption of ingested nutrients. Two motor responses occur in the proximal stomach following meal ingestion. The first, termed “receptive relaxation”, is initiated by swallowing and associated with a decrease in intragastric pressure. Following this, “adaptive relaxation” of the proximal stomach occurs so that the meal can be accommodated, i.e. the increase in intragastric volume is not usually associated with a substantial increase in intragastric pressure (Azpiroz and Malagelada, 1987). The distal stomach, or the antrum, is involved in the grinding of solid

foods into small particles, and propels chyme into the duodenum. Phasic and tonic pyloric contractions are considered to play a major role in the regulation of gastric emptying by acting as a brake; emptying can only occur when the pylorus is open (Horowitz *et al.*, 1994). When quantified on a second-by-second basis, transpyloric flow is predominantly a pulsatile, rather than continuous, phenomenon – there is substantial variation in the individual characteristics of individual flow pulses and both antegrade and retrograde flow occur (Anvari *et al.*, 1995). Patterns of transpyloric flow are, thus, regulated by the integration of motor activity in the proximal stomach, the antrum and the pylorus (Horowitz *et al.*, 1994). The interaction of nutrients with the small intestine slows gastric emptying by relaxing the proximal stomach, inhibiting antral contractions, and stimulating tonic and phasic pyloric pressures (**Figure 1.2**)

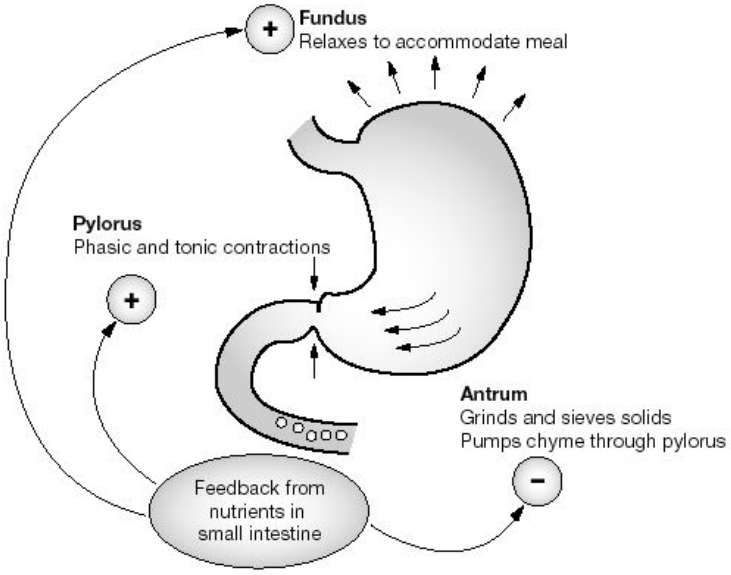

Figure 1.2: Motor patterns associated with normal gastric emptying (from Rayner and Horowitz, 2005).

Pattern(s) of gastric emptying are critically dependent upon the nature (i.e. liquid or solid) and the macronutrient composition of the ingested meal. Nutrient-containing liquids and liquefied solids empty from the stomach in a linear fashion. The emptying of solids is characterised by an initial lag phase during which food is processed into fine particles by mixing and retropulsion of antral contractions. In contrast, non-nutrient liquids empty relatively rapidly from the stomach in a mono-exponential fashion (Horowitz and Dent, 1991).

1.2.1.2. Gastric distension

While gastric distension has been implicated in the regulation of appetite and energy intake, the relationships between energy intake with the rate of gastric emptying, gastric distension, intragastric volume and intragastric meal distribution are poorly defined. The demonstrated relationship between increasing hunger and gastric emptying has been postulated to suggest that the return of hunger following a meal may be mediated, at least in part, by a decrease in gastric distension (Sepple and Read, 1989). The relationship between perceptions of appetite with gastric emptying is, however, relatively weak (Hveem *et al.*, 1996). Gastric distension, with a water-filled balloon, reduces energy intake in both healthy and obese subjects when the balloon volume is greater than, or equal to, 400 ml (Geliebter, 1988; Geliebter *et al.*, 1988), however, the site of gastric distension was not precisely defined in these studies. It is known that the mechanical properties and neural innervation vary in different regions of the stomach (Grundy, 1988; Grundy and Scratcherd, 1989) hence, the site of gastric distension is likely to be important in mediating appetite sensations. A role for the proximal stomach in the control of appetite is suggested by studies

in which distension of the proximal stomach, using an air-filled bag, increased the perception of fullness (Feinle *et al.*, 1997). However, this sensation of fullness was only induced when nutrients were concurrently infused into the duodenum; during duodenal saline infusion, the distension was perceived as discomfort or pain (Feinle *et al.*, 1997), suggesting that feedback signals arising from the small intestine may be relatively more important.

Recent studies (Hveem *et al.*, 1996; Santangelo *et al.*, 1998; Jones *et al.*, 1997; Sturm *et al.*, 2004), in both healthy young, and older, subjects indicate that the perception of postprandial fullness (Hveem *et al.*, 1996; Jones *et al.*, 1997; Santangelo *et al.*, 1998) and energy intake at a subsequent meal (Sturm *et al.*, 2004) are closely related to the content of the distal stomach. For example, following ingestion of a 350 ml glucose drink, the perception of fullness was closely related to antral area (and content) in healthy subjects, while there was no significant relationship between fullness and the content of either the total or proximal stomach (Hveem *et al.*, 2001; Jones *et al.*, 1997). In addition, energy intake after a "yoghurt preload" was inversely related to antral area in healthy young and older subjects, such that a larger antral area was associated with decreased energy intake (Sturm *et al.*, 2004) (**Figure 1.3**). Whilst these studies suggest that "antral distension" may be the dominant "intragastric" mechanism in the regulation of appetite and energy intake, the relationship of appetite with gastric emptying and intragastric meal distribution requires further investigation (Chapter 10).

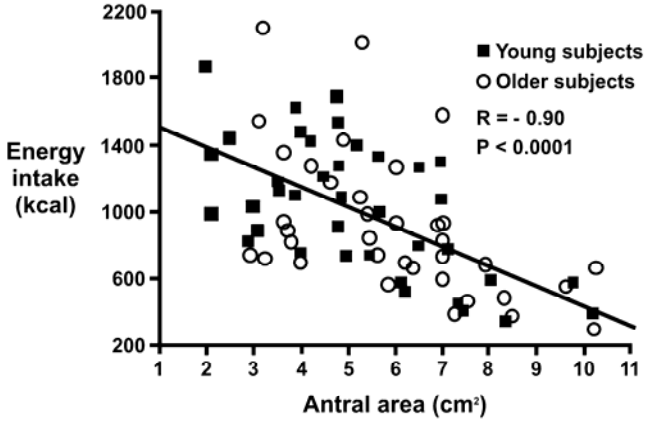

Figure 1.3: The relationship between energy intake at a buffet-meal with antral area (cm^2) immediately before meal ingestion in subjects who received "preloads" of water, 250 kcal or 750 kcal 70 min before the meal. R = -0.90, P < 0.0001 (from Sturm *et al.*, 2004).

1.2.2. Small intestine

The small intestine is a muscular tube, approximately 5 metres in length, which can be divided into three regions - the most proximal region is the duodenum (~ 25 cm), the jejunum (~ 2 metres), and the most distal, the ileum (~ 3 metres). During fasting the gastrointestinal tract exhibits a distinct cyclic pattern of motility, termed the migrating motor complex (MMC) (Code and Marlett, 1975). The MMC consists of three phases with a cycle time of ~ 120 minutes: phase I (a period of motor quiescence), phase II (irregular phasic contractions) and phase III. Phase III, which lasts for ~ 5 – 10 minutes, is characterised by powerful coordinated contractions, which occur at the maximal frequency, which in the stomach is 3, and in the duodenum is 12, contractions per minute. This ensures that any undigested food remaining in the stomach or small intestine is propelled distally (Sarna and Otterson, 1988).

1.2.2.1. Effects of small intestinal nutrients on gastrointestinal motor function

In the process of digestion, nutrients are broken down, absorbed and transported along the gut; thus exposure of intestinal receptors to nutrients can occur for a number of hours after a meal. The interaction of nutrients with receptors in the small intestine in turn results in a feedback inhibition of gastric emptying (Cooke, 1977; Heddle *et al.*, 1989; Hunt, 1963; Hunt and Knox, 1968), thereby prolonging gastric distension (Read *et al.*, 1994).

The major factor regulating gastric emptying is the feedback inhibition triggered by receptors distributed throughout the small intestine (Lin *et al.*, 1989; Lin *et al.*, 1990b). The presence of nutrients in the small intestine interrupts the MMC and results in relaxation of the proximal stomach (Azpiroz and Malagelada, 1985; Feinle *et al.*, 1996), suppression of antral and duodenal contractility (Heddle *et al.*, 1988a) and the stimulation of tonic and phasic pyloric pressures (Heddle *et al.*, 1988b). This process ensures that in healthy subjects chyme is delivered from the stomach to the small intestine at a rate of approximately 2-3 kcal/minute (Brener *et al.*, 1983), after an initial phase that may be more rapid (Horowitz *et al.*, 1993). These changes in gastrointestinal motility reflect, primarily, the interaction of nutrients with the small intestine. However, acute changes in blood glucose concentrations, even within the normal postprandial range, slow gastric emptying (Rayner *et al.*, 2001). The different macronutrients, carbohydrate, protein and fat, may vary in their effects on gastrointestinal motility, possibly dependent on the type of fat and carbohydrate administered. Long-chain triglycerides have been reported to induce proximal gastric relaxation (Azpiroz and Malagelada, 1985), slow gastric emptying (Kumar *et al.*, 1987), and

stimulate phasic and tonic pyloric motility (Cook *et al.*, 1997) to a greater extent than carbohydrate.

1.2.2.2. Effects of small intestinal nutrients on appetite and energy intake

The presence of nutrients in the small intestinal lumen decreases perceptions of hunger, increases fullness and decreases subsequent energy intake (Chapman *et al.*, 1999; Cook *et al.*, 1997; Lavin *et al.*, 1996; MacIntosh *et al.*, 1999). In contrast, intravenous administration of nutrients has little, if any, effect on energy intake (Lavin *et al.*, 1996; Welch *et al.*, 1985). Fat, carbohydrate and protein have all been shown to reduce subsequent energy intake in animals, and humans, when infused directly into the small intestine, and fat may be the most potent, increasing fullness and decreasing hunger in humans to a greater extent than isocaloric carbohydrate and protein loads (Andrews *et al.*, 1998; Burton-Freeman *et al.*, 1997; Chapman *et al.*, 1999; Cook *et al.*, 1997).

1.2.2.3. Influence of length and region of small intestine exposed to nutrient on gastric emptying, appetite and energy intake

Animal studies have established that the effects of nutrients on gastric emptying are dependent upon the length of small intestine exposed to nutrient (Cooke, 1977; Lin *et al.*, 1990a; Lin *et al.*, 1989; Lin *et al.*, 1990b). For example, studies in dogs implanted with small-intestinal fistula have demonstrated that infusion of glucose into the duodeno-jejunal junction inhibits gastric emptying, but has no effect when infused into only the proximal 5 cm of the duodenum (Cooke, 1977). Another study in dogs established that whilst both the proximal and distal small intestine are capable of participating in the inhibition of gastric emptying, the

length of small intestine exposed is critical. - Gastric emptying was only inhibited when >15 cm of the small intestine was exposed to nutrient, and maximal inhibition was achieved after exposure of >150 cm, irrespective of the concentration of nutrient (in this case, glucose); furthermore this inhibition of gastric emptying was the same whether 150 cm of the proximal, or distal, small intestine was exposed to nutrient, suggesting that the length, and not the region, of intestine exposed was the critical determinant of gastric emptying (Lin *et al.*, 1989). The inhibitory effect of fat (sodium oleate) on gastric emptying is also dependent on the length of small intestine exposed (**Figure 1.4**), however, with fat, feedback arising from the proximal small intestine may be more potent than that arising from the distal small intestine (Lin *et al.*, 1990b). However, studies in rats have demonstrated that ileal infusion of lipids and free fatty acids potently delays the transit of a meal through the small intestine (Brown *et al.*, 1990). These observations strongly indicate that feedback signals arising from the distal small intestine are critical for the regulation of gastric emptying, i.e. as more small intestinal receptors are activated along a greater length of small intestine, a greater feedback inhibition of gastric emptying will occur. These observations have important implications for the regulation of appetite and energy intake, and indeed, in animal studies the suppression of energy intake by small intestinal nutrients has been reported to be dependent on the length of small intestine exposed to nutrient. For example, in rats, when lactose or oleate were confined to a 35 cm region of the jejunum, they did not suppress energy intake; in contrast, when allowed access to the entire length of small intestine, they potently suppressed energy intake (Meyer *et al.*, 1998). The effects of exposing different

lengths of the small intestine to nutrient on gastrointestinal function in humans have, hitherto, not been investigated (Chapter 8).

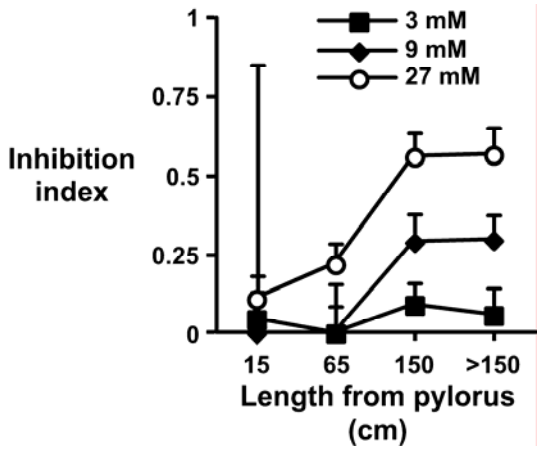

Figure 1.4: The inhibition index (= 1 – $AUC_{oleate}/AUC_{buffer}$) for gastric emptying (1 = maximal inhibition, 0 = minimal inhibition) of 3, 9, and 27 mM loads of sodium oleate in carbonate buffer expressed as a function of length of small intestine exposed to nutrient in dogs implanted with small-intestinal fistula (from Lin *et al*., 1990b).

1.2.3. Gastrointestinal peptides involved in the regulation of gastrointestinal motility, appetite and energy intake

A number of gastrointestinal hormones, including cholecystokinin (CCK) from the proximal small intestine (Lilja *et al.*, 1984; Matzinger *et al.*, 2000), and glucagon-like peptide-1 (GLP-1) (Flint *et al.*, 1998; Gutzwiller *et al.*, 1999b) and peptide tyrosine tyrosine (PYY) (Batterham *et al.*, 2003), from the distal small intestine are released in response to enteral nutrients, while the release of ghrelin, which is predominantly synthesised in the stomach (Kojima *et al.*, 1999), is suppressed (Cummings *et al.*, 2001; Wren *et al.*, 2001a). CCK, GLP-1, PYY and ghrelin are all likely to be important in mediating the suppressive effects of nutrients on appetite and subsequent energy intake (Batterham *et al.*, 2002; Flint *et al.*, 1998; MacIntosh *et al.*, 2001; Wren *et al.*, 2001a).

1.2.3.1. Cholecystokinin (CCK)

In the gastrointestinal tract, CCK is located mainly in the "I" cells of the duodenal and jejunal mucosa and is released in response to the presence of nutrients, particularly the digestion products of fat and protein (Larsson and Rehfeld, 1978; Liddle *et al.*, 1985; Lieverse *et al.*, 1994a), but also by glucose (Parker *et al.*, 2005). CCK is also present in enteric vagal afferent neurons (Larsson and Rehfeld, 1979); in the central nervous system, CCK is present in high concentrations in the thalamus, hypothalamus, basal ganglia and dorsal hindbrain (Moran and Kinzig, 2004). – In both the enteric and central nervous systems, CCK functions as a neurotransmitter (Crawley and Corwin, 1994). CCK has a large number of biological actions within the gastrointestinal tract, including the stimulation of gall bladder contraction, bile (Liddle *et al.*, 1985) and pancreatic secretion (Harper and Raper, 1943), inhibition of gastric acid secretion (Chen *et al.*, 2004), slowing of gastric emptying (Liddle *et al.*, 1986), modulation of small intestinal and colonic motility (Meyer *et al.*, 1989) and suppression of energy intake (Kissileff *et al.*, 1981). These physiological actions have been established by studies using the specific CCK_1 receptor antagonist, dexloxiglumide (Beglinger *et al.*, 2001; Fried *et al.*, 1991; Lal *et al.*, 2004; Meyer *et al.*, 1989).

<u>Effects on gastric and small intestinal motor function</u>

Exogenous administration of CCK slows gastric emptying (Liddle *et al.*, 1986; Muurahainen *et al.*, 1988; Yamagishi and Debas, 1978), which is associated with proximal gastric relaxation (Ludtke *et al.*, 1988), suppression of antral and

duodenal pressure waves and stimulation of tonic and phasic pyloric pressures (Brennan *et al.*, 2005; Fraser *et al.*, 1993; Rayner *et al.*, 2000), stimulation of jejunal, and suppression of colonic, motility (Kellow *et al.*, 1987). The inhibitory effect of fat on gastric emptying is attenuated by administration of the CCK_1 receptor antagonist, loxiglumide (Fried *et al.*, 1991), indicating that the inhibitory effects of fat on gastric emptying are mediated, at least in part, by CCK. These effects of CCK on gastric emptying and antral, pyloric and duodenal motility may be relevant to its appetite-suppressant properties (Brennan *et al.*, 2005).

Effects on appetite and energy intake

It is well established that acute exogenous administration of CCK suppresses energy intake. In their historic study, Gibbs and colleagues reported that intraperitoneal (i.p.) administration of the biologically active, sulphated octapeptide of CCK (CCK-8S) dose-dependently suppressed energy intake (Gibbs *et al.*, 1973a), as well as sham feeding (Gibbs *et al.*, 1973b) in rats. In humans, intravenous infusion of CCK-8 and CCK-33 increases the perception of fullness, decreases hunger and reduces subsequent energy intake (Kissileff *et al.*, 1981; Lieverse *et al.*, 1994b; MacIntosh *et al.*, 2001; Muurahainen *et al.*, 1988).

Most studies have investigated the inhibitory effects of exogenous CCK on energy intake at a single meal, defined as "satiation" (Blundell *et al.*, 1988). Whilst CCK appears to be involved in the induction of satiation, a "within-meal" process, its role in satiety, which can be defined as the inhibition of hunger and further eating as a consequence of food consumption, i.e. a "between-meal" process (Blundell *et al.*, 1988), is less clear. The use of the terms satiety and

satiation is often confused in the literature; only one study has, hitherto, evaluated the effects of exogenous CCK on satiety; in rats, administration of CCK-8 near the end of each free-feeding meal decreased meal size and, thus, energy intake (West *et al.*, 1987). However, infusion of CCK-8 during the intermeal interval was associated with a decrease, rather than an increase, in the intermeal interval (West *et al.*, 1987), i.e. the decrease in food intake associated with acute administration of CCK was counterbalanced over time by an increase in meal frequency, so that by day 6, total daily energy intake had returned to baseline levels (West *et al.*, 1987). The effects of CCK on satiety have not been investigated in humans.

In order to establish a physiological role for CCK in the regulation of appetite and energy intake, studies using antagonists specific for the CCK_1 receptor are required. Only a relatively small number of studies have evaluated the role of endogenous CCK in the regulation of energy intake using CCK_1 receptor antagonists (Beglinger *et al.*, 2001; Lieverse *et al.*, 1994a; Lieverse *et al.*, 1995; Matzinger *et al.*, 2000; Matzinger *et al.*, 1999). Infusion of the CCK_1 receptor antagonist, loxiglumide, attenuated the inhibitory effects of an intraduodenal lipid infusion on subsequent energy intake by approximately 10 % (Lieverse *et al.*, 1994a; Matzinger *et al.*, 1999). Furthermore, in a group of 40 healthy subjects, intravenous infusion of loxiglumide for one hour prior to, and during ingestion of, a mixed nutrient meal increased energy intake by about 10 % at that meal compared with the control infusion (Beglinger *et al.*, 2001) (**Figure 1.5**). In contrast, Lieverse *et al*. found, in both lean and obese subjects, that intravenous infusion of a lower dose of loxiglumide failed to affect intake of a carbohydrate

rich meal in a group of 14 subjects (Lieverse *et al.*, 1995), however, these observations may reflect a type 2 error due to the low subject number. Hence, while intravenous infusion of CCK decreases energy intake by perhaps 20 %, the outcome of studies using CCK_1 receptor blockade are inconsistent and suggest that the acute effects of endogenous CCK are more modest. It remains to be determined whether CCK_1 receptor antagonism increases energy intake of meals high in fat or protein, as the studies by Beglinger *et al*. (2001), and Lieverse *et al*. (1995), employed meals relatively low in, or containing no, fat and protein, respectively. However, given the moderate effects of loxiglumide on energy intake after duodenal lipid infusion (Lieverse *et al.*, 1994a; Matzinger *et al.*, 1999), this appears unlikely.

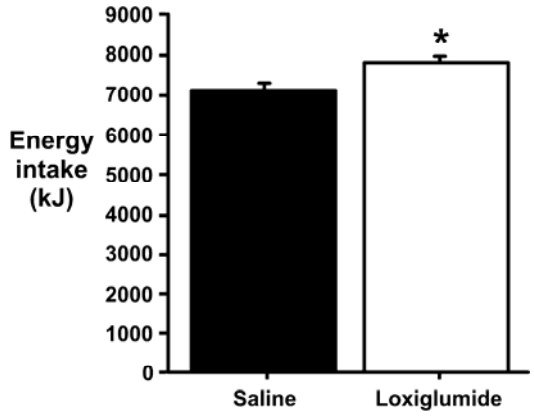

Figure 1.5: Effect of intravenous infusion of the CCK_1 receptor antagonist, loxiglumide (22 µmol/kg/h) and control (saline) on energy intake (kJ) in 40 healthy male subjects. Loxiglumide increased energy intake by about 10 % compared with control, * $P < 0.004$. Data are means ± SEM. (Data derived from Beglinger *et al.*, 2001, with permission of the authors).

It has been postulated, based on the observed acute effects of CCK, that chronic elevation of plasma CCK concentrations may suppress energy intake in the longer term. However, the outcome of animal studies indicates that this is not the

case (Covasa *et al.*, 2001; Crawley and Beinfeld, 1983; Lukaszewski and Praissman, 1988). For example, in rats, chronic intraperitoneal administration of CCK-8 via an implanted minipump for 14 (Crawley and Beinfeld, 1983) or 28 (Covasa *et al.*, 2001) days was ineffective in inhibiting food intake after the first day of administration, and had no effect on body weight. Similarly, chronic intravenous CCK-8 infusion over a 7-day period had no effect on food intake or body weight in rats (Lukaszewski and Praissman, 1988). Chronic elevation of plasma CCK concentrations, associated with the consumption of a high-fat diet (Covasa *et al.*, 2001; Spannagel *et al.*, 1996) has also been shown in rats to decrease the response to an acute dose of CCK-8 (0.5 μg/kg) administered directly before a meal (Covasa *et al.*, 2001). The effects of chronic CCK administration on energy intake in humans have not been evaluated. It appears that whilst exogenous CCK suppresses energy intake at a single meal, chronic elevation of plasma CCK concentrations is associated with decreased sensitivity to the effects of CCK on food intake, and a prompt attenuation of the response. This hypothesis will be evaluated by examining the effects of exposure to a high-fat diet on the gastrointestinal and energy intake responses to exogenous CCK (Chapter 10).

1.2.3.2. Glucagon-like peptide-1 (GLP-1)

Glucagon-like peptide-1 is a 33-amino acid peptide hormone product of the glucagon gene. The glucagon gene is expressed in the pancreas and in the small intestinal mucosal endocrine cells, where its primary translational product, proglucagon, is cleaved to release two peptides with a high sequence homology to glucagon, glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2

(GLP-2) (Flint *et al.*, 1998). GLP-1 is secreted from L cells, predominantly located in the distal small intestinal mucosa in response to carbohydrate (Näslund *et al.*, 1998), protein (Herrmann *et al.*, 1995) and fat (Feinle *et al.*, 2003). GLP-1 is rapidly degraded into a biologically inactive form in human serum by the enzyme dipeptidyl peptidase IV (DPP-IV) (Mentlein *et al.*, 1993).

Effects on gastric and intestinal motor function

Exogenous administration of glucagon-like peptide-1 (GLP-1) has diverse effects on gastrointestinal function in humans (Meier *et al.*, 2003; Nauck *et al.*, 1997; Schirra and Goke, 2005; Verdich *et al.*, 2001). Intravenous GLP-1 slows gastric emptying (Delgado-Aros *et al.*, 2002; Flint *et al.*, 2001; Nauck *et al.*, 1997), and this is associated with relaxation of the proximal stomach (Delgado-Aros *et al.*, 2002; Schirra *et al.*, 2002), inhibition of antral and duodenal motility, and stimulation of tonic and phasic pyloric pressure waves (Schirra *et al.*, 2000). A recent study using the specific GLP-1 antagonist, exendin (9-39), indicates that GLP-1 is a physiological modulator of gastroduodenal motility (Schirra *et al.*, 2006). The inhibitory effect of exogenous GLP-1 on gastric emptying has been demonstrated in healthy (Delgado-Aros *et al.*, 2002; Nauck *et al.*, 1997), type 2 diabetic (Meier *et al.*, 2003) and obese (Flint *et al.*, 2001; Näslund *et al.*, 1998) subjects, and has major implications for an understanding of its potential therapeutic efficacy, as well as reported adverse effects such as nausea, bloating and vomiting when GLP-1 agonists, such as NN2211 are administered (Agerso *et al.*, 2002; Elbrond *et al.*, 2002). There are, however, a number of limitations to current knowledge. It is not known whether GLP-1 has the capacity to markedly slow gastric emptying, i.e. induce “gastroparesis”, when administered at

physiological doses. This is an important issue, particularly as gastric emptying is delayed in a large number of type 2 diabetic patients (Horowitz *et al.*, 2002). Exogenous administration of GLP-1 at 0.3 pmol/kg/min has been shown to result in "physiological", while infusion of GLP-1 at 0.9 pmol/kg/min results in "supraphysiological", plasma concentrations (Schirra *et al.*, 2002). In type 2 diabetic patients, infusion of GLP-1 in a supraphysiological dose of 1.2 pmol/kg/min profoundly delays gastric emptying of a liquid drink (Willms *et al.*, 1996). While lower doses of GLP-1 slow gastric emptying in healthy subjects (Nauck *et al.*, 1997) and patients with type 2 diabetes (Meier *et al.*, 2003), the magnitude of this effect is uncertain, particularly as results were not compared to a control range.

Scintigraphy is the "gold standard" method for evaluating gastric emptying and allows concurrent assessment of solid and liquid emptying, as well as quantification of intragastric meal distribution (Collins *et al.*, 1983; Horowitz and Dent, 1991; Jones *et al.*, 2002). The majority of studies relating to the effects of GLP-1 on gastric emptying have employed less than optimal techniques, including dye dilution (Nauck *et al.*, 1997) and measurement of paracetamol absorption (Flint *et al.*, 2001; Näslund *et al.*, 1999); the effects of exogenous GLP-1 on gastric emptying of discrete solid and liquid meal components and intragastric meal distribution have not been evaluated (Chapter 9).

Effects on appetite and energy intake

Intravenous administration of GLP-1 has also been reported to suppress appetite and energy intake in some (Verdich *et al.*, 2001; Gutzwiller *et al.*, 1999a;

Näslund *et al.*, 1999), albeit not all (Brennan *et al.*, 2005; Long *et al.*, 1999), studies. In rats, the inhibitory effects of GLP-1 on both gastric emptying (Tolessa *et al.*, 1998) and food intake (Turton *et al.*, 1996) are blocked by the specific GLP-1 receptor antagonist, exendin-(9-39). Exendin (9-39) administered alone had no effect on feeding following a fast, but more than doubled food intake in fed rats, and augmented the feeding response to the appetite stimulant, neuropeptide Y (Turton *et al.*, 1996). These results strongly support the role of GLP-1 as a physiological mediator of satiety; however, as studies using specific GLP-1 receptor antagonists to evaluate the effects of GLP-1 on energy intake have not been performed in humans, the role of GLP-1 in the regulation of energy intake in humans remains uncertain.

The effects of GLP-1 on gastric emptying and intragastric meal distribution have potential implications for an understanding of its capacity to induce gastrointestinal symptoms, such as nausea and bloating (Agerso *et al.*, 2002) (particularly if the magnitude of the slowing of gastric emptying is sufficient to induce gastroparesis), and suppress appetite and energy intake (Verdich *et al.*, 2001). The relationships between the effects of GLP-1 on gastrointestinal symptoms and perceptions of appetite with those on gastric emptying and intragastric meal distribution have, hitherto, not been investigated (Chapter 9).

1.2.3.3. Peptide YY

PYY is a 36 amino acid peptide of the pancreatic polypeptide family synthesised by endocrine cells predominantly located in the ileum and large intestine, and released in response to nutrients, particularly long-chain fatty acids (Pappas *et al.*,

1986). In dogs, the secretion of PYY has been shown to occur in response to neurohumoral signals originating from the proximal gut, including the secretion of CCK (Lin and Chey, 2003; Lin *et al.*, 2000). PYY is secreted as PYY_{1-36}, and is rapidly degraded to PYY_{3-36} by dipeptidyl peptidase IV (Grandt *et al.*, 1994).

Effects on gastric and intestinal motor function

PYY_{3-36} inhibits meal-stimulated gastric and pancreatic secretion, and intravenous infusion of PYY_{3-36} dose-dependently slows gastric emptying and intestinal transit in healthy volunteers (Savage *et al.*, 1987).

Effects on appetite and energy intake

PYY_{1-36} is an agonist at the Y1 and Y2 receptors (of the NPY receptor family) and is a potent orexigen (Corp *et al.*, 1990), PYY_{3-36} is an agonist at the Y2 receptor and has been reported to have anorexigenic activity (Batterham *et al.*, 2002). The role of PYY_{3-36} in the regulation of energy intake is, however, controversial. It was originally reported that peripheral injection of PYY inhibits food intake and attenuates weight gain in rats (Batterham *et al.*, 2002), however, attempts to replicate these results have been largely unsuccessful (Tschop *et al.*, 2004). This issue has been comprehensively reviewed (Boggiano *et al.*, 2005; Tschop *et al.*, 2004). Recently, it was reported that pre-treatment of the arcuate nucleus with an antagonist specific for the Y2 receptor, BIIE0246, attenuated the inhibitory effect of an intraperitoneal dose of PYY_{3-36} on food intake in rats (Abbott *et al.*, 2005). Furthermore, when BIIE0246 was administered into the arcuate nucleus alone, food intake was increased (Abbott *et al.*, 2005). Hence, this study provides support for a role of endogenous PYY in the regulation of

energy intake. In humans, i.v. infusion of apparently "physiological" concentrations of PYY has been reported to inhibit energy intake for up to 12 hours, in both lean and obese subjects, suggesting that PYY may potentially play a role in the longer-term regulation of appetite (Batterham *et al.*, 2003; Batterham *et al.*, 2002). A later study, however, suggested that PYY_{3-36} only inhibits energy intake when infused at pharmacological plasma concentrations, and at such high doses nausea was a common side-effect (Degen *et al.*, 2005). Studies using a specific receptor antagonist to evaluate the effects of endogenous PYY on energy intake have not been performed in humans, thus the role of PYY in the regulation of human appetite and energy intake remains uncertain.

1.2.3.4. Ghrelin

Ghrelin is a 28-amino acid peptide with an acyl side-chain, n-octanoic acid, which is essential for its actions on appetite (Kojima *et al.*, 1999). Ghrelin is an endogenous ligand of the growth-hormone secretagogue receptor, primarily synthesised by oxyntic cells of the fundic mucosa (Kojima *et al.*, 1999) and unlike, other gut peptides, is suppressed rather than stimulated by nutrient ingestion (and also by infusion of PYY_{3-36} (Batterham *et al.*, 2003)) (Cummings *et al.*, 2001; Wren *et al.*, 2001a), supporting the concept that ghrelin plays a role in meal initiation. The suppression of ghrelin occurs in response to administration of nutrients in the stomach, duodenum or jejunum, and carbohydrate and protein appear to be more potent suppressors of ghrelin than fat (Overduin *et al.*, 2005). Animal studies have demonstrated that the inhibitory effects of intraduodenal nutrients on ghrelin secretion are not discretely dependent upon exposure of the stomach or duodenum to nutrient, and have

suggested that exposure of the small intestine distal to the duodenum may be involved in the regulation of ghrelin secretion (Overduin *et al.*, 2005). In humans, the relatively prolonged time required for the suppression of plasma ghrelin by enterally administered glucose (Parker *et al.*, 2005) and fat (Feinle-Bisset *et al.*, 2005), as well as a marked suppression of ghrelin observed following Roux-en-Y gastric bypass (Cummings *et al.*, 2004; Korner *et al.*, 2005), which shunts nutrients to the distal bowel, suggest that exposure of the distal intestine may be important. It must, however, be noted that ghrelin secretion is also suppressed during intravenous infusion of glucose (Shiiya *et al.*, 2002). Hyperinsulinaemia has also been shown to suppress plasma ghrelin under eu-, hypo- and hyperglycaemic conditions in humans (Flanagan *et al.*, 2003), suggesting that insulin may mediate ghrelin suppression independently of glucose. Other studies have, however, failed to demonstrate an inhibitory effect of intravenous insulin on circulating ghrelin concentrations, except at supraphysiological concentrations (Caixas *et al.*, 2002; Schaller *et al.*, 2003). Ghrelin suppression has also been observed in response to intravenous administration of CCK (Brennan *et al.*, 2006) and PYY (Batterham *et al.*, 2003), albeit at relatively supraphysiological concentrations. The relative importance of distal small intestinal exposure to nutrients for the suppression of ghrelin has not been investigated in humans. The postprandial suppression of ghrelin may be dependent upon the length, or region, of small intestine exposed to nutrient (Chapter 8).

<u>Effects on gastric and intestinal motor function</u>

Exogenous ghrelin exerts a gastrokinetic effect in rats (Dornonville de la Cour *et al.*, 2004), and in both healthy humans (Levin *et al.*, 2006) and patients with diabetic gastroparesis (Murray *et al.*, 2005). Intravenous ghrelin has also been reported to induce phase III of the fasting MMC, and increase proximal gastric tone in healthy subjects (Tack *et al.*, 2006). The physiological relevance of these effects of ghrelin on gastrointestinal motor function are, however, unclear as following meal ingestion, when gastric emptying is slowed, ghrelin is suppressed.

Effects on appetite and energy intake

Exogenous administration of ghrelin stimulates food intake in both rats and humans (Wren *et al.*, 2001a; Wren *et al.*, 2001b); in rats, chronic administration of ghrelin for 7 days induces weight gain and adiposity (Wren *et al.*, 2001b). No studies, have, hitherto, investigated the role of endogenous ghrelin on energy intake, or gastrointestinal function, i.e. by using an antagonist specific for the growth hormone secretagogue receptor, hence a physiological role of ghrelin in feeding behaviour has not been established.

1.2.4. Peripheral adiposity signals

The signals released from the gastrointestinal tract play an important role in the acute regulation of appetite and energy intake, largely by determining meal size. The long-term regulation of energy balance is, however, probably predominantly determined by signals that circulate in proportion to the amount of fat stored in the body's adipose tissue, particularly leptin and insulin. A comprehensive discussion of the role of leptin and insulin in appetite regulation is beyond the scope of this thesis; this topic has been recently reviewed (Cancello *et al.*, 2004).

Leptin, a 16 kDa circulating protein secreted predominantly from white adipose tissue in proportion to adipose mass, acts both centrally, and peripherally, to increase satiety (Zhang *et al.*, 1994). Leptin is also synthesised by endocrine cells in the fundic mucosa and is released in response to nutrients present within the gut (Bado *et al.*, 1998) and CCK (Bado *et al.*, 1998). Insulin is secreted from pancreatic β-cells in response to increased plasma glucose concentrations. The amount of insulin in the plasma, both under basal and postprandial conditions is in direct proportion to total body adiposity. Insulin enters the brain via a receptor-mediated process (Woods *et al.*, 2003), and, when administered intracerebroventricularly to baboons, decreases energy intake and body weight (Woods *et al.*, 1979).

1.3. Central regulation of appetite and energy intake

The signals arising from the peripheral organs must ultimately be integrated in the central nervous system, most importantly in the hypothalamus. Many of the peripheral signals, including the gastrointestinal peptides, have direct access to the arcuate nucleus of the hypothalamus. There are two populations of neurons located in the arcuate nucleus that are involved in the regulation of appetite and energy intake. One population expresses proopiomelanocortin (POMC)/cocaine and amphetamine related transcript (CART) and is involved in the suppression of energy intake, whereas neurons expressing neuropeptide Y (NPY)/agouti-related protein (AgRP), are involved in the stimulation of energy intake. Leptin and insulin exert their inhibitory effects on food intake by interacting with leptin receptors on these neurons (Cowley *et al.*, 2001). Leptin stimulates the POMC-containing neurons, while inhibiting the NPY/AgRP neurons (Williams *et al.*,

1999). Leptin and insulin have been shown to interact with peripheral signals to enhance their inhibitory effects on energy intake. For example, in rats the anorexic effects of intraperitoneal. CCK-8 were enhanced by intracerebroventricular injection of insulin 1 hour prior to a meal (Riedy *et al.*, 1995), and, daily administration of a combination of intraperitoneal CCK-8 and intracerebroventricular leptin resulted in greater weight loss than leptin alone (Matson *et al.*, 2000), in the absence of a change in food intake (Matson *et al.*, 2002).

1.4. Gastrointestinal factors involved in the regulation of postprandial glycaemia

Postprandial glycaemia is influenced by a number of factors, including the rate of delivery of nutrients to the small intestine, small intestinal absorption and the hepatic metabolism of glucose. The gastrointestinal tract plays an important role in the regulation of postprandial glycaemia by regulating gastric emptying, and releasing the two "incretin" hormones, glucose-dependent insulinotropic peptide (GIP) and GLP-1, which augment insulin secretion.

1.4.1. Gastric emptying

The rate of entry of carbohydrate into the small intestine is a critical determinant of postprandial glycaemia (Horowitz *et al.*, 1993; Jones *et al.*, 1996; O'Donovan *et al.*, 2004; Rayner *et al.*, 2001). Gastric emptying accounts for ~ 35 % of the variance in peak blood glucose concentrations after ingestion of a 75 g glucose load, in both healthy subjects (Horowitz *et al.*, 1993), and patients with type 2 diabetes (Jones *et al.*, 1996).

1.4.2. Incretin hormones

1.4.2.1. Effect of GIP

GIP is a 42 amino acid peptide, secreted from K cells in the proximal small intestine in response to nutrients, particularly carbohydrate (Ebert and Creutzfeldt, 1980). Intravenous administration of GIP, when administered in combination with glucose, has been demonstrated to stimulate insulin secretion (Dupre *et al.*, 1973); thus the insulinotropic action of GIP is dependent upon hyperglycaemia. Intravenous infusion of GIP has been demonstrated to have no effect on gastric emptying in healthy subjects (Meier *et al.*, 2004).

1.4.2.2. Effect of GLP-1

GLP-1 also plays an important role in the regulation of postprandial blood glucose levels. Exogenous GLP-1 stimulates insulin, and suppresses glucagon secretion, these effects being dependent on blood glucose concentrations (Kreymann *et al.*, 1987; Nauck *et al.*, 1993). These actions of GLP-1 have stimulated substantial interest in the potential for its use (Schirra *et al.*, 1998), and that of specific GLP-1 analogues and agonists (Nauck, 1998), to improve glycaemic control in type 2 diabetes (Meier *et al.*, 2003; Nauck *et al.*, 1998), particularly, as GIP apparently does not maintain its insulin secretory properties in type 2 diabetes (Nauck *et al.*, 1993), nor does it slow gastric emptying (Meier *et al.*, 2004). While there is increasing information that this is effective, the precise mechanisms by which GLP-1 (and GLP-1 agonists/analogues) improve glycaemia (Schirra *et al.*, 2000) remain poorly defined. For example, while exogenous GLP-1 stimulates insulin secretion in the fasted state (Kreymann *et*

al., 1987), when GLP-1 is administered with a meal there is an, apparently paradoxical, reduction in postprandial insulin levels (Nauck *et al.*, 1997). Observations that postprandial insulin secretion is reduced, rather than increased, by exogenous GLP-1 in healthy subjects (Nauck *et al.*, 1997) and type 2 diabetics (Meier *et al.*, 2003), suggest that the dominant mechanism by which exogenous GLP-1 improves postprandial glycaemia relates to the slowing of gastric emptying, and that GLP-1 may not be a "physiological" incretin hormone (Nauck *et al.*, 1997). This concept is supported by a recent study in which "reversal" of the inhibitory effect of exogenous GLP-1 on gastric emptying, by the gastrokinetic drug, erythromycin, was associated with a substantial attenuation of its glucose-lowering effect, despite augmentation of the insulin and GIP responses (Meier *et al.*, 2005). It is not known whether there is a direct relationship between postprandial blood glucose and insulin concentrations and the magnitude of the slowing of gastric emptying by GLP-1, i.e. whether the effect of GLP-1 on the glycaemic response to a carbohydrate-containing meal can be predicted in an individual by its effects on gastric emptying (Chapter 9).

1.5. Conclusions

This chapter has reviewed the peripheral, and (briefly) the central, factors involved in the regulation of appetite and energy intake, and the gastrointestinal mechanisms involved in the regulation of postprandial glycaemia. The studies described in subsequent chapters of this thesis address the following hypotheses:

(1) The effects of nutrients on gastric emptying and gastrointestinal hormone release are dependent on the length of small intestine exposed (Chapter 8).

(2) The magnitude of the slowing of gastric emptying by a “physiological” dose of exogenous GLP-1 would be sufficient to induce “gastroparesis” and be associated with increased meal retention in the distal stomach (Chapter 9).

(3) The suppression of postprandial glycaemia and insulinaemia by GLP-1 are closely related to the slowing of GE of carbohydrate (Chapter 9).

(4) Postprandial appetite perceptions and gastrointestinal symptoms induced by GLP-1 will be related to changes in intragastric meal distribution (Chapter 9).

(5) Chronic elevation of endogenous CCK induced by ingestion of a high-fat diet will attenuate the effects of exogenous CCK on gastrointestinal motility and energy intake (Chapter 10).

Chapter 2

Fat digestion and free fatty acids – effects on gastrointestinal function, appetite and energy intake

2.1. Introduction

There is a close relationship between the intake of dietary fat with total energy intake and body weight (Golay and Bobbioni, 1997); consequently, an understanding of the specific mechanisms, by which fat in the gastrointestinal tract modulates gastrointestinal function, appetite and energy intake, may enable the development of dietary strategies that maximise energy intake suppression. While dietary fat contributes to overeating (Golay and Bobbioni, 1997), fat does also have effects in the gastrointestinal tract that favour suppression of hunger and energy intake. For example, small intestinal infusion of lipid potently slows gastric emptying (Valenzuela and Defilippi, 1981), stimulates the release of gastrointestinal hormones, including CCK, PYY, GLP-1, and suppresses ghrelin (Feinle *et al.*, 2003; Feinle-Bisset *et al.*, 2005; Pilichiewicz *et al.*, 2006), and reduces subsequent energy intake (MacIntosh *et al.*, 2001). There is increasing evidence that the digestive products of fat, free fatty acids, are responsible for the gastrointestinal effects of fat (Feinle-Bisset *et al.*, 2005; O'Donovan *et al.*, 2003). Furthermore, the effects of free fatty acids on gastrointestinal function and appetite appear to be dependent upon their acyl chain length (Feltrin *et al.*, 2004; Hunt and Knox, 1968; McLaughlin *et al.*, 1999).

2.2. The importance of fat digestion for the effects of fat on gastrointestinal function, appetite and energy intake

The digestion of fat is accomplished by gastric and pancreatic lipases; the development of specific inhibitors of these lipases has demonstrated the importance of fat digestion, and consequently the release of free fatty acids for the effects of fat on gastrointestinal function and energy intake.

2.2.1. Fat emulsification

Dietary fats are ingested in the form of triglycerides, consisting of one glycerol and three fatty acid molecules. Fats entering the small intestine are emulsified by bile, which is secreted from the gallbladder. Emulsification generates a lipid-water interface, which facilitates the action of water-soluble lipases on the hydrophobic lipid molecules (Armand *et al.*, 1994; Armand *et al.*, 1996). The properties of this lipid-water interface influence fat digestion, and are dependent upon the physicochemical properties of the ingested lipid, including fat droplet size (Armand *et al.*, 1999). For example, studies in humans have revealed that duodenal lipolysis of a lipid emulsion containing fine particles (0.7 μm in diameter) was ~ 30 % greater than that of a lipid emulsion containing coarse particles (10 μm in diameter), despite the emulsions having an identical composition (Armand *et al.*, 1999). The fine emulsion emptied from the stomach at a slower rate than the coarse emulsion (Armand *et al.*, 1999), an observation that is likely to be explained by the comparatively greater feedback inhibition of gastric emptying arising from increased fat digestion, and increased liberation of free fatty acids in the small intestinal lumen.

2.2.2. The importance of fat digestion for the effects of fat on gastrointestinal function and energy intake

The ability to block fat digestion with pharmacological agents, such as tetrahydrolipstatin (THL), the active component of the anti-obesity drug, Xenical® (Hoffman-Roche, Basle, Switzerland), has provided evidence that the digestion of fat, and consequently the release of free fatty acids into the small intestine, is essential for the effects of fat on gastric emptying, antropyloroduodenal motility, gastrointestinal hormone secretion, appetite and energy intake (Borovicka *et al.*, 2000; Feinle *et al.*, 2003; Feinle *et al.*, 2001; Matzinger *et al.*, 2000; O'Donovan *et al.*, 2003; Pilichiewicz *et al.*, 2003; Schwizer *et al.*, 1997).

2.2.2.1. The importance of fat digestion for the effects fat on gastrointestinal function

Administration of THL with a mixed nutrient meal accelerates gastric emptying of both the solid and lipid phases of the meal (Borovicka *et al.*, 2000). Furthermore, THL attenuates the effects of intraduodenal fat on proximal gastric relaxation (Feinle *et al.*, 2001), antropyloroduodenal motility (Feinle *et al.*, 2003) (**Figure 2.1**) and the secretion of CCK, GLP-1 (Feinle *et al.*, 2003; Feinle *et al.*, 2001), pancreatic polypeptide (PP) and PYY, and the suppression of ghrelin (Feinle-Bisset *et al.*, 2005). Thus, the effects of fat on gastrointestinal function are dependent upon the presence of free fatty acids in the small intestinal lumen.

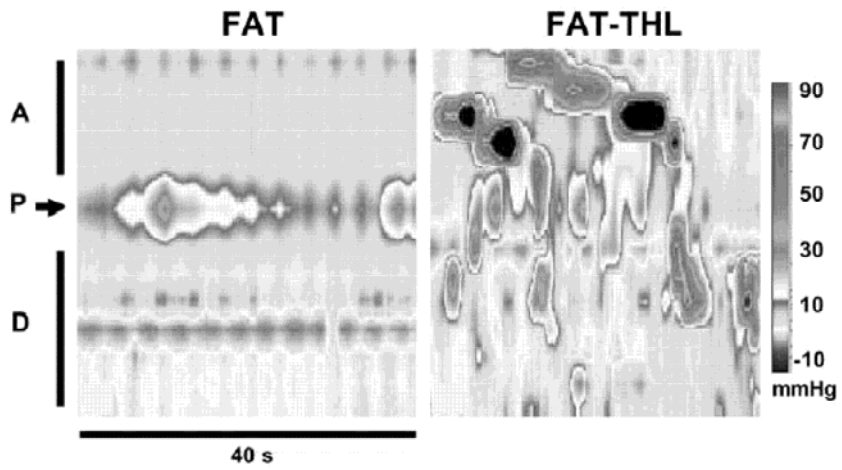

Figure 2.1: Example of antropyloroduodenal pressure patterns during duodenal infusion of a triglyceride emulsion without (FAT; left) and with (FAT-THL; right) 120 mg of the lipase inhibitor, THL. Left: infusion of FAT resulted in a "fed" motor pattern, characterised by stimulation of isolated pyloric pressure waves (P) and inhibition of antral (A) and duodenal (D) pressure waves. Right: In contrast, when THL was co-administered with fat (FAT-THL), there was pronounced propulsive antropyloroduodenal pressure activity (from Feinle *et al*., 2003).

2.2.2.2. The importance of fat digestion for the effects fat on appetite and energy intake

In healthy lean male subjects, the inhibitory effect of an oral fat load (70 % fat) on subsequent energy intake is attenuated following administration of 120 mg orlistat (O'Donovan *et al.*, 2003), with the increase in total daily energy intake approximating the amount of energy lost due to fat malabsorption. Furthermore, when orlistat is added to a long chain triacylglyceride emulsion, the inhibitory effects of the emulsion on appetite perceptions and energy intake are attenuated (Feinle *et al.*, 2003) (**Figure 2.2**). The increase in energy intake is likely to be related to the impaired gastrointestinal motility and gastrointestinal hormone responses to fat following lipase inhibition, and supports the important role of free fatty acids in the small intestinal lumen in the regulation of energy intake following ingestion of a fatty meal.

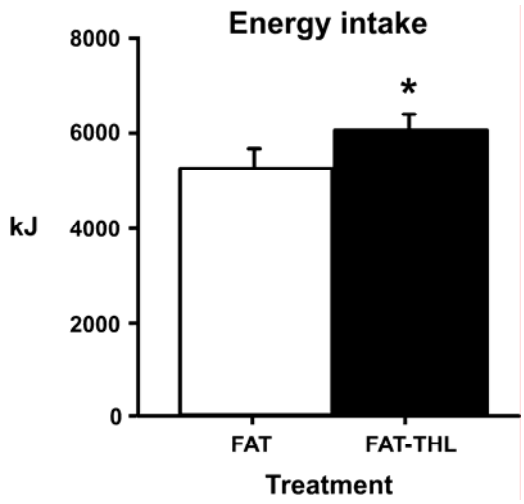

Figure 2.2: Energy intake from a buffet-meal presented immediately following duodenal infusion of a triglyceride emulsion without (FAT) and with (FAT-THL) 120 mg of the lipase inhibitor THL. * vs. FAT, $P < 0.05$ (from Feinle *et al.*, 2003).

The above findings potentially explain why Xenical® is less effective than expected, i.e. while the prescribed dose of 120 mg three times daily has been reported to decrease fat absorption by ~ 30 % (Zhi *et al.*, 1994), the weight loss observed in obese patients tends to be less than that predicted by the degree of fat malabsorption (Hill *et al.*, 1999). The observation that energy intake is, in fact, increased when fat absorption is inhibited suggests that a compensatory increase in energy intake may decrease the efficacy of this treatment for weight loss.

2.2.2.3. Comparative effects of free fatty acids versus triglycerides on gastrointestinal function, appetite and energy intake

There are limited data regarding the direct comparative effects of free fatty acids versus triglycerides, however, the effect of free fatty acids on gastrointestinal function and appetite appears to be greater than that of triglycerides. For example, in rats, intraduodenal infusion of oleic acid (0.064 kcal/min for 2 hours; total load 7.68 kcal) suppressed food intake by approximately 28 % more than an

isocaloric infusion of its respective triacylglyceride, triolein (Woltman *et al.*, 1995). Similarly, jejunal infusion of oleic acid (0.2 ml/h for 7 h; total load: 11.3 kcal) suppressed food intake more potently than an isocaloric infusion of corn oil (Cox *et al.*, 2004). In humans with pancreatic exocrine insufficiency, the effects of free fatty acids (oleic acid) on CCK secretion were greater (integrated response: 91 ± 11 pM.min vs. 49 ± 21 pM.min) and occurred faster (15 min vs. 30 min) when compared with triglyceride (20 % Intralipid®) (Guimbaud *et al.*, 1997). Indirect evidence from recent studies in healthy human subjects suggests that the effects of free fatty acids on gastrointestinal function and energy intake are more potent than triglycerides. For example, while peak plasma concentrations of CCK were ~ 7 pmol/l during intraduodenal infusion of a long-chain triacylglyceride emulsion (2.8 kcal/min; total energy delivered: ~ 1053 kJ), plasma concentrations of CCK reached ~ 12 pmol/l during infusion of lauric acid, a fatty acid with 12 carbon atoms ("C12") (0.375 kcal/min; total energy delivered: ~ 140 kJ). In addition, while the stimulation of pyloric pressure waves and the release of gastrointestinal hormones were maximal within ~ 30 - 45 min of the start of the triglyceride infusions (Feinle *et al.*, 2003; Heddle *et al.*, 1989; Heddle *et al.*, 1988), similar effects were evident during infusion of C12 within ~ 15 min. Furthermore, while energy intake was suppressed by ~ 900 kJ following triglyceride infusion (Feinle *et al.*, 2003), C12 suppressed energy intake by ~ 2800 kJ (Feltrin *et al.*, 2004), suggesting that the effects of free fatty acids on gastrointestinal function and energy intake are more potent than those of triglycerides. The direct comparative effects of free fatty acids and triglycerides on gastric emptying and energy intake have, hitherto, not been investigated in humans (Chapter 5).

2.2.3. The role of free fatty acids in the regulation of gastrointestinal function, appetite and energy intake

The mechanisms by which free fatty acids present within the gastrointestinal tract modulate gastrointestinal function and energy intake are unclear. There are, however, a number of factors, including fatty acid chain length, dose (energy load) and concentration that influence the actions of free fatty acids on gastrointestinal function and energy intake.

2.2.3.1. The role of the acyl chain length of fatty acids on gastrointestinal function, appetite and energy intake

Studies in both animals and humans indicate that the effects of free fatty acids on gastrointestinal motility, gastrointestinal hormone release, appetite and energy intake are dependent upon their acyl chain length (Feltrin *et al.*, 2004; Hunt and Knox, 1968; McLaughlin *et al.*, 1999; Meyer *et al.*, 1998b). In pioneering studies Hunt and Knox (1968) demonstrated that intragastric infusion of fatty acids with an acyl chain length ≥ 12 carbon atoms inhibit gastric emptying, while those with ≤ 10 carbon atoms are ineffective (Hunt and Knox, 1968). Although both decanoic acid (C10) and lauric acid (C12) appear to have the ability to relax the proximal stomach when administered intragastrically, fatty acids with ≥ 12 carbon atoms have a greater effect (McLaughlin *et al.*, 1999). Fatty acids with ≥ 12 carbon atoms also reduce the amplitude of antral contractions, as measured ultrasonographically, more than fatty acids with ≤ 10 carbon atoms (McLaughlin *et al.*, 1999). Moreover, intraduodenal administration of C12 has been shown to stimulate isolated pyloric pressure waves and basal pyloric pressure, and suppress

antral and duodenal pressure waves, when compared with control (water) and C10, whilst C10 had no effect on antral, and stimulated pyloric and duodenal, motility (Feltrin *et al.*, 2004).

The release of gastrointestinal hormones by free fatty acids is also dependent on their chain length. In humans, intraduodenal infusion of C12 resulted in a marked increase in plasma CCK concentrations when compared with C10 and control, both of which stimulated CCK secretion, although to a lesser extent than C12 (McLaughlin *et al.*, 1999). However, the control solution (containing the non-ionic surfactant, Tween 80) also raised plasma CCK concentrations, confounding interpretation of these observations. Similarly, intraduodenal infusion of C18:1, but not C8, stimulated CCK secretion (Matzinger *et al.*, 2000). Recent observations from our group have, however, reported that C10 does stimulate CCK release (peak concentration: 8.5 pmol/l) when compared with control (water) (peak concentration: 3 pmol/l), but the magnitude of the stimulation was far less than that induced by C12 (peak concentration 13.4 pmol/l) (Feltrin *et al.*, 2004). Thus, while C10 stimulates CCK to a degree, the effects of fatty acids with acyl chain length $\geq$ 12 carbon atoms are more potent. C12 also caused a significant increase in plasma GLP-1 (Feltrin *et al.*, 2004), PP and PYY concentrations, and suppressed ghrelin, whilst C10 did not (Feltrin *et al.*, 2006). Therefore, in contrast to the release of CCK, the secretion of GLP-1, PP and PYY, and the suppression of ghrelin, is dependent upon an acyl chain length of $\geq$ 12 carbon atoms.

Given the important role of fatty acid chain length in mediating the effect of free fatty acids on gastrointestinal motility and gut hormone secretion, it is likely that the effects of free fatty acids on appetite and energy intake are also dependent upon their acyl chain length. In rats, intraduodenal infusion of C12 or oleic acid (C18:1) significantly suppresses food intake, whilst fatty acids with ≤ 10 carbon atoms are ineffective (Meyer *et al.*, 1998b). In healthy male subjects, a 90-minute intraduodenal infusion of C18:1 (~ 288 kJ), but not C8 (~ 163 kJ), has been shown to suppress food intake (Matzinger *et al.*, 2000), however, as the infusions were not isocaloric, the data are difficult to interpret. Nonetheless, recent observations from our laboratory have confirmed a marked inhibitory effect of intraduodenal infusion of C12, when compared with an isocaloric infusion of C10, on energy intake (Feltrin *et al.*, 2004). Infusion of C12 (~ 140 kJ) was found to potently attenuate appetite-related sensations, significantly reducing hunger and desire to eat, and suppress energy intake at a buffet meal by ~ 2857 kJ, when compared with control (Feltrin *et al.*, 2004) (**Figure 2.3**). Therefore, the effects of fatty acids on energy intake are dependent upon an acyl chain-length ≥ 12 carbon atoms. However, in some subjects infusion of C12 was associated with nausea, confounding interpretation of the data. Further studies were thus required to consolidate and extend these observations to establish whether C12 would suppress energy intake in the absence of nausea (Chapters 6, 7).

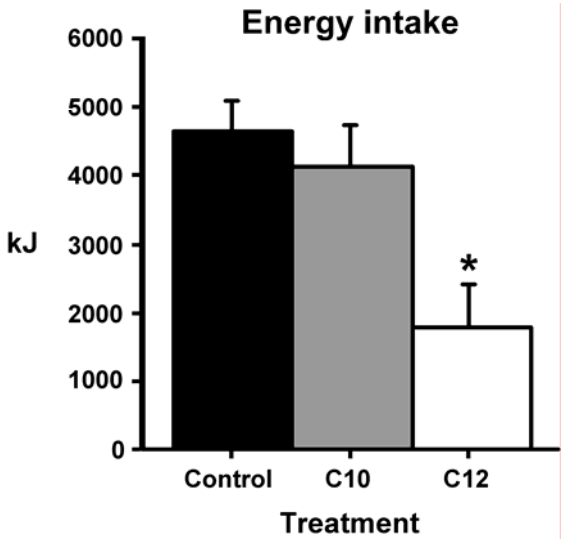

Figure 2.3: Energy intake at a buffet meal immediately following 90 min duodenal infusions of lauric acid (C12), decanoic acid (C10) and control (water). * vs. control/C10: $P < 0.001$ (from Feltrin *et al.*, 2004).

2.2.3.2. Mechanisms mediating the effects of fatty acids

The mechanisms by which carbon chain length mediates the gastrointestinal effects of fatty acids are unclear, however, differences in their post-absorptive processing are likely to be important. Fatty acids with a chain length of ≤ 11 carbon atoms are predominantly absorbed from the gut directly into the portal vein (Kiyasu *et al.*, 1952), while fatty acids with a chain length ≥ 12 carbon atoms are predominantly re-esterified and packaged into chylomicrons which pass through the lymph to the circulation (McDonald and Weidman, 1987). Chylomicron formation is essential for the actions of fatty acids with greater than 12 carbon atoms. In rats, inhibiting chylomicron formation, using the "detergent" Pluronic L-81, abolishes the effects of lipid on gastric emptying (Raybould *et al.*, 1998), the release of CCK and food intake (Meyer *et al.*, 1998a), and the activation of vagal afferents (Randich *et al.*, 2000). However, it must be noted

that a small percentage of fatty acids with ≥ 12 carbon atoms are transported via the portal vein, while a small percentage of fatty acids with ≤ 10 carbon atoms are transported via the lymphatic pathway (McDonald and Weidman, 1987), which may potentially explain the observed effects of C10 (Feltrin *et al.*, 2004). Thus, signalling arising from chylomicron formation may explain why fatty acids with an acyl chain length ≥ 12 carbon atoms have more potent effects on gastrointestinal function and energy intake than fatty acids with ≤ 11 carbon atoms.

The effects of fatty acids on gastrointestinal function and energy intake are mediated, at least in part, by the activation of vagal afferents. For example, in rats, the suppression of food intake by intraduodenal infusion of oleic acid is abolished by celiac vagotomy (Walls *et al.*, 1995). The activation of vagal afferents is likely to be chain-length dependent. Early studies identified two groups of vagal afferent receptors in the cat intestine, those responsive to oleic acid (C18) (i.e. to long-chain fatty acids), and those responsive to sodium caprylate (C8) (i.e. to short-chain fatty acids) and also glycerol (Melone, 1986), however, this study did not evaluate the mechanism by which fatty acids activate these fibres. More recently, studies in rats using the specific CCK-A receptor antagonist, devazepide, have demonstrated that fatty acids with ≥ 12 carbon atoms activate vagal afferent fibres via a CCK-dependent mechanism, whereas fatty acids with 11 or less carbon atoms act directly on vagal afferents (Lal *et al.*, 2001). There is some evidence that the effects of C12 are dependent on the release of CCK (Lal *et al.*, 2004), for example, the inhibitory effects of C12 on gastric emptying and the perception of intragastric volume (maximum volume

tolerated) were attenuated by the CCK_1 receptor antagonist, loxiglumide (Lal *et al.*, 2004). Furthermore, the inhibitory effects of C18:1 on energy intake were abolished by loxiglumide (Matzinger *et al.*, 2000). Therefore, CCK is likely to contribute to the effects of C12 on energy intake, although the role of CCK in the suppression of energy intake by C12 has not been assessed directly, i.e. by infusing loxiglumide. The effects of C12 on energy intake may also be mediated by GLP-1 (Feltrin *et al.*, 2004), PYY and ghrelin (Feltrin *et al.*, 2006)**,** as these hormones play an important role in the regulation of energy intake (Batterham *et al.*, 2002; Verdich *et al.*, 2001; Wren *et al.*, 2001). Therefore, the more potent effects of fatty acids with ≥ 12 carbon atoms on gastric emptying, antropyloroduodenal motility and appetite may reflect, at least in part, the different pathways of absorption, patterns of gastrointestinal hormone secretion and the mechanisms by which signals are transmitted to the feeding centres in the hypothalamus by vagal afferent fibres.

2.2.3.3. Load- and concentration-related effects of C12 on gastrointestinal function, appetite and energy intake

In animals, the effects of small intestinal fatty acids on gastrointestinal function and energy intake may be influenced by the concentration and/or the energy load administered (Lin *et al.*, 1990; Meyer *et al.*, 1998b). For example, the effects of free fatty acids on pancreatic enzyme secretion in dogs (Meyer and Jones, 1974) and energy intake in rats (Meyer *et al.*, 1998b) have been reported to be load-dependent, but concentration-independent. In dogs, intraluminal infusion of 500 ml of a solution containing 27 or 9 mM, but not 3 mM, of oleate (C18) inhibits gastric emptying (Lin *et al.*, 1990), however, as both the energy load and

concentration of the solutions varied, the relative contribution of these factors is uncertain. In rats plasma CCK concentrations are released in a load-, and concentration-dependent, fashion (Li *et al.*, 1990). Thus, studies in animals suggest that the effects of free fatty acids on gastric emptying and gastrointestinal hormone secretion may be dependent upon both the load, and concentration, administered, while the effects on energy intake are likely to be dependent upon the energy load administered.

In humans, gastric emptying of free fatty acids has been suggested to be dependent upon the concentration administered, such that as the concentration of fatty acid increased, gastric emptying was more potently delayed; however, in this experiment, both the concentration and the energy load varied in parallel (Hunt and Knox, 1968). The relative importance of load versus concentration for the effects of free fatty acids on gastrointestinal function and energy intake have not been directly investigated in humans. Postprandially fatty acids are present within the small intestine at concentrations ranging from approximately 20 – 80 mM (Ament *et al.*, 1972; Borgstrom *et al.*, 1957; Porter *et al.*, 1971), and calculations based on the data of Hunt and Knox (1968) suggest that C12 empties from the stomach at rates ranging from approximately 0.1 to 0.4 kcal/min (Hunt and Knox, 1968). Infusion of C12 at concentrations and energy loads closely resembling postprandial conditions is important to determine (i) whether C12 has physiological effects on gastrointestinal function and energy intake, in the absence of nausea (Chapter 6) and (ii) the relative effect of energy load versus concentration (Chapter 7).

2.3. Conclusions

This chapter has reviewed factors that mediate the effects of fat on gastrointestinal function and energy intake. Specifically, the effects of fat on gastrointestinal function and energy intake are dependent upon fat digestion and the subsequent release of free fatty acids in the small intestinal lumen. The comparative effects of free fatty acids and triglycerides on gastrointestinal function and energy intake have received relatively little attention, however, the effects of fatty acids appear to be greater than those of triglycerides, underlining the importance of fat digestion. The study presented in Chapter 5 was designed to evaluate the comparative effects of a free fatty acid and a triglyceride on gastric emptying, gastrointestinal hormone release and energy intake. Furthermore, the effects of free fatty acids are dependent upon their chain length. Acute administration of a very small amount of the free fatty acid, lauric acid (C12), but not decanoic acid (C10), markedly modulates gastrointestinal function and suppresses appetite and energy intake, however, at the load, or concentration, administered, C12 induced nausea. The studies, presented in Chapters 6 and 7, were therefore designed to determine: (i) a dose of C12 that would modulate gastrointestinal function, and suppress energy intake without inducing nausea, and (ii) whether the effects of small intestinal C12 were dependent upon the load or concentration (or both) administered.

Chapter 3

The modulation of gastrointestinal function, appetite and energy intake by exposure to a high-fat diet

3.1. Introduction

Obesity can, in the broadest sense, be considered to be the result of an energy intake which exceeds energy expenditure. As discussed in Chapter 1, signals arising from the gastrointestinal tract play a fundamental role in the regulation of appetite and energy intake, and there is evidence that the gastrointestinal, and hormonal, mechanisms involved in the regulation of appetite and energy intake are compromised in obesity (Baranowska *et al.*, 2000; Tschop *et al.*, 2001). Hence, obesity may, at least in part, reflect a decreased sensitivity to the gastrointestinal effects of nutrients, particularly in the face of excessive caloric intake. Studies in animals, and to a limited extent humans, indicate that consumption of a high-fat diet has the capacity to modulate the gastrointestinal responses to ingested fat, and thereby lead to impairments in appetite regulation and body weight. A number of factors potentially involved in the regulation of appetite and energy intake, including gastric emptying (Covasa and Ritter, 2000; Cunningham *et al.*, 1991a; Cunningham *et al.*, 1991b), gastrointestinal transit (Brown *et al.*, 1994), antropyloroduodenal motility (Boyd *et al.*, 2003), the secretion, and/or action, of gastrointestinal peptides, including ghrelin, CCK, GLP-1 and PYY, as well as the adiposity signals, leptin and insulin (Ainslie *et al.*, 2000; French *et al.*, 1995; Lee *et al.*, 2002; Spannagel *et al.*, 1996; Woods *et al.*, 2003), and central satiety signalling (Challis *et al.*, 2004) are altered by a high-fat diet. These studies support the concept that the development of obesity

may be related to an attenuation of the feedback inhibition of energy intake arising from fat present in the gastrointestinal tract. This chapter summarises current knowledge about the adaptive changes that occur in response to an excessive (high-fat) energy intake.

3.2. Role of a high dietary fat intake in the development of overweight and obesity

While the causes of obesity are heterogeneous, it is widely accepted that one of the salient environmental factors contributing to the epidemic is the increased availability, and over-consumption, of high-fat, energy-dense foods. Epidemiological studies have revealed a strong positive relationship between the incidence of overweight and obesity with dietary fat consumption (Golay and Bobbioni, 1997; Rolls, 1995). For example, in countries in which the incidence of obesity is rising, approximately 40 - 45 % of the daily energy intake is provided by fat (Golay and Bobbioni, 1997). Furthermore, there is evidence that obese subjects display an increased preference for the consumption of fatty foods (Mela and Sacchetti, 1991) and consume a higher proportion of dietary fat than lean individuals (Miller *et al.*, 1990). The consumption of a diet high in fat has also consistently been demonstrated to promote an increase in energy intake (Lissner *et al.*, 1987; Tremblay *et al.*, 1989), sometimes termed “high-fat diet hyperphagia”. It has been purported that the increased availability of nutritionally unbalanced diets promotes an increase in energy intake. – The so-called “protein leverage hypothesis” suggests that the intake of protein is tightly regulated, and in order to maintain protein consumption at a constant level while consuming high-fat, high-carbohydrate, low-protein diets, fat and carbohydrate

must be over-consumed (Simpson and Raubenheimer, 2005). In animal studies, it has been well established that *ad libitum* access to a high-fat diet promotes hyperphagia and obesity, and is associated with leptin and insulin resistance (Woods *et al.*, 2003). Furthermore, when rats are exposed to an energy-restricted high-fat diet they maintain a similar body weight to pair-fed animals consuming a low-fat diet, but gain more adipose mass (Woods *et al.*, 2003), suggesting that the fat content of the diet *per se* influences fat deposition. Thus, there is considerable evidence to implicate the consumption of a high-fat diet in the promotion of increased energy intake and body weight.

3.3. The effects of a high-fat diet on gastrointestinal function, appetite and energy intake – implications for the pathogenesis of obesity

The consumption of a high-fat diet has been demonstrated in animal, and in a small number of human, studies, to modify small intestinal morphology and pancreatic secretion (Spannagel *et al.*, 1996) and attenuate the effects of fat on gastric emptying (Covasa and Ritter, 2000; Cunningham *et al.*, 1991a; Cunningham *et al.*, 1991b), gastrointestinal transit (Brown *et al.*, 1994), antropyloroduodenal motility (Boyd *et al.*, 2003), the secretion, and/or action of gastrointestinal peptides, including ghrelin, CCK, GLP-1 and PYY, as well as the adiposity signals leptin and insulin (Ainslie *et al.*, 2000; French *et al.*, 1995; Lee *et al.*, 2002; Spannagel *et al.*, 1996; Woods *et al.*, 2003). These observations provide important insights into the pathogenesis of obesity, i.e. exposing animals to a high-fat diet provides a model in which the mechanisms regulating appetite and energy intake are attenuated, therefore, enabling the identification of factors that promote increased energy intake and body weight. There are, however, some

limitations to these studies. For example, the effects may relate to the high level of fat supplementation employed in most of the studies in rats as, typically, rats consume a diet containing ~ 5 % energy as fat. Furthermore, all studies conducted in humans have compared the effects of a high-energy, high-fat diet with either a pre-diet condition (i.e. the subjects habitual diet) or a low-energy, low-fat diet. Thus, whether the observed changes in gastrointestinal function are due to an increase in the fat content of the diet *per se*, or due to the increased carbohydrate or caloric intake is unclear. This section will review the effects of exposure to a high-fat diet on gastrointestinal function and energy intake first in animals, and then in humans.

3.3.1. Animal studies

3.3.1.1. Effect of a high-fat diet on small intestinal morphology

In rats, small intestinal morphology (Balint *et al.*, 1980; Singh *et al.*, 1972) and pancreatic lipase secretion (Spannagel *et al.*, 1996) are modified following a high-fat diet. For example, increasing the amount of fat consumed from the diet (fat intake: 45 % of daily energy) for four weeks increased small intestinal cell proliferation and increased the uptake of oleic acid from the small intestine (Balint *et al.*, 1980; Sagher *et al.*, 1991; Singh *et al.*, 1972). Furthermore, the secretion of pancreatic lipase, amylase and proteases changes in proportion to the dietary content of fat, carbohydrate and protein, respectively (Sabb *et al.*, 1986; Spannagel *et al.*, 1996); in rats fed a high-fat diet containing greater than, or equal to, 20 % of the daily energy intake as fat, the secretion of pancreatic lipase is increased (Brannon, 1990; Sabb *et al.*, 1986; Spannagel *et al.*, 1996). The hypertrophy of the intestinal mucosa, and increased capacity for fat digestion,

would be expected to enhance the absorption of fat from the proximal small intestine and result in a decreased length of small intestinal exposure.

3.3.1.2. Effects of a high-fat diet on gastrointestinal motor function

Studies in animals provide evidence that the gastrointestinal motor response to fat is attenuated following short-term exposure (i.e. 2 – 4 weeks) to a diet containing high amounts of fat. For example, following exposure to a diet providing 54 % of the total daily energy as fat, for two weeks, the inhibitory effect of a small intestinal oleate, but not maltotriose, infusion on gastric emptying of 5 ml saline was attenuated, when compared with rats consuming an isocaloric diet providing 5 % energy as fat (Covasa and Ritter, 2000). Increased exposure of the small intestine to fat has also been demonstrated to accelerate intestinal transit (Brown *et al.*, 1994). – In rats, infusion of palm oil into the ileum for three hours per day, three days per week for four weeks attenuated the lipid-induced slowing of small intestinal transit (Brown *et al.*, 1994). This relative acceleration of fat transit was still evident four weeks after the cessation of fat, suggesting that the "adaptation" of gastrointestinal function may occur more rapidly than the reversal of the changes (Brown *et al.*, 1994); longer-term studies are, therefore, indicated to evaluate the time course required for reversal of these changes.

3.3.1.3. Effects of a high-fat diet on the secretion of, and sensitivity to, gastrointestinal and adiposity hormones

The release of CCK, GLP-1, PYY and ghrelin has been reported to be modulated following a high-fat diet (le Roux *et al.*, 2006; Spannagel *et al.*, 1996; van Citters *et al.*, 2002). Changes in the secretion of, and sensitivity to these hormones, may

have important implications for the regulation of gastrointestinal function and energy intake. The following will discuss current knowledge regarding the effects of exposure to a high-fat diet on the secretion of, and sensitivity to, these hormones.

3.3.1.3.1. Cholecystokinin

There are a number of studies to indicate that both the secretion, and sensitivity to the actions, of CCK are modulated by a high-fat diet. For example, in rats, exposure to a high-fat diet (20 % energy as fat) for 14 days increased the CCK response to an intraduodenal triglyceride infusion by approximately 1.7-fold (Spannagel *et al.*, 1996). In rats fed a high-fat diet the sensitivity to exogenous CCK also appears to be attenuated. For example, the inhibitory effect of an i.p. injection of CCK on gastric emptying (Covasa and Ritter, 2000) and food intake (Covasa *et al.*, 2001; Covasa and Ritter, 1998), was reduced following exposure to a high-fat diet (34 or 54 % energy as fat) for two weeks, when compared with exposure to an isocaloric low-fat diet (5 % energy as fat), in the absence of any change in body weight or adiposity, suggesting that chronically increased plasma CCK concentrations, induced by increased consumption of dietary fat, mediate the change in sensitivity to the inhibitory effects of CCK on food intake. There is, however, some inconsistency in the available information. For example, the suppressive effect of i.p. CCK-8 on energy intake has been reported to be maintained following consumption of a 34 % fat diet for two weeks in rats (Torregrossa and Smith, 2003), and in rats consuming a 60 % fat diet for two weeks, sensitivity to the satiating effects of CCK has been reported to be increased, rather than decreased, when compared with rats maintained on a low-fat diet (Torregrossa and Smith, 2003). The reason(s) for the discrepant

observations are unclear, however, it should be noted that in these studies the rats consuming the 60 % fat diet gained more weight and adipose mass when compared with rats on the 5 or 34 % diets; it is possible that the effects of CCK on gastrointestinal function and energy intake are dependent on body weight.

3.3.1.3.2. GLP-1

There is limited information about the effect of a high-fat diet on plasma GLP-1. In one study plasma GLP-1 concentrations were approximately 2.5 times greater in the fasting state and 3.4 fold greater in the postprandial state in dogs, who had increased their body weight and adipose tissue mass after being provided with a hypercaloric, high-fat diet (~ 40 % energy from fat) for 12 weeks, when compared with a control low-fat diet (van Citters *et al.*, 2002). No studies have evaluated the effects of exogenous GLP-1 on appetite and energy intake and gastrointestinal function following exposure to a high-fat diet, hence, it is unknown whether sensitivity to the effects of GLP-1 is attenuated, as appears to be the case with CCK.

3.3.1.3.3. PYY

The secretion of PYY is modulated by a high-fat diet, however, PYY appears to maintain its inhibitory effect on energy intake. For example, in mice who have become obese in response to a high-fat diet (60 % energy as fat) for 16 weeks, plasma concentrations of PYY were lower in both the fasting and postprandial state when compared with rats maintained on a low-fat diet (2.6 % energy as fat) (le Roux *et al.*, 2006). The sensitivity to the inhibitory effects of exogenous PYY on energy intake, however, appears to be maintained. For example, in wild type

mice exposure to a high-fat diet (45 % energy as fat) did not attenuate the inhibitory effects of an i.p. injection of PYY on food intake (Challis *et al.*, 2004).

3.3.1.3.4. Ghrelin

The secretion of ghrelin has been reported to be modulated following ingestion of a high-fat diet. In Long-Evans rats, who gained weight when fed a hypercaloric, high-fat diet (70 % energy as fat) for a period of 14 weeks, fasting plasma ghrelin concentrations were decreased by approximately 30 % when compared with rats fed a control (chow) diet (Beck *et al.*, 2002). Likewise, in Sprague-Dawley rats exposed to a high-fat diet (48 % energy as fat) for 30 days, ghrelin mRNA levels and ghrelin secretion were decreased (Lee *et al.*, 2002). The suppression of ghrelin by a high-fat diet may reflect an increased substrate-(fat-)induced inhibition of ghrelin secretion. This decrease in plasma ghrelin may be an appropriate response to the increased caloric intake associated with high-fat diet consumption and serve to reduce the drive for further intake. The mechanism(s) by which ghrelin suppression occurs following high-fat diet exposure is unclear, however, as discussed, ghrelin has been shown to be decreased during hyperglycaemia (Shiiya *et al.*, 2002) and hyperinsulinaemia (Flanagan *et al.*, 2003), two common features of increased body weight associated with obesity.

3.3.1.3.5. Leptin

In rats exposure to a high-fat diet (36 % energy as fat) for a period of four weeks decreased plasma leptin, with concentrations being some 24 % lower in rats consuming the high-fat diet than in rats consuming a control diet (6 % energy as fat) (Ainslie *et al.*, 2000). Contrary to what would be expected, this decrease in

plasma leptin is associated with an increase, rather than a decrease, in abdominal adipose mass. Furthermore, in rats exposed to a high-fat diet (20 % energy as fat) for 32 weeks, the inhibitory effects of centrally administered leptin on energy intake were attenuated (Tulipano *et al.*, 2004). In the marsupial, *Sminthopsis crassicaudata*, consumption of a high energy, high-fat diet (30 % energy as fat) induced an increase in adiposity, and this was associated with resistance to the actions of i.p. leptin on both energy intake and body weight (Hope *et al.*, 1999). Impaired leptin action may potentially account for changes in sensitivity to gastrointestinal hormones, including CCK (Matson *et al.*, 2000; Matson *et al.*, 2002).

3.3.1.3.6. Insulin

There is evidence that exposure to a high-fat diet impairs the central effects of insulin on energy intake. For example, in rats fed a high-energy, high-fat diet (20 % energy as fat) for 70 days, the inhibitory effect of i.c.v. insulin was attenuated when compared with rats maintained on chow or a low-energy, low-fat diet (Woods *et al.*, 2004). It should, however, be noted that these rats were also substantially heavier than the low-fat and chow-fed controls, and, hence, the observed effects may potentially represent a response to increased body fat. Subsequent reports demonstrating that in rats fed a high-fat diet the effects of i.c.v. insulin on energy intake were abolished when compared with low-fat fed rats, in the absence of a substantial change in body weight (Arase *et al.*, 1988; Chavez *et al.*, 1996), however, argue against this possibility. The observed change in sensitivity to insulin following exposure to a high-fat diet may relate to

reduced transport of insulin across the blood brain barrier following a high-fat, when compared with a low-fat, meal (Gerozissis *et al.*, 1997).

3.3.1.4. Effects of a high-fat diet on appetite and energy intake

In rodent studies, consumption of a high-fat diet has long been associated with the intake of an excessive amount of calories and weight gain (Golay and Bobbioni, 1997; Woods *et al.*, 2003), termed "high-fat diet hyperphagia", and it is well established that feeding a high-fat diet (20 % energy fat) to susceptible rats, leads to diet-induced obesity (Woods *et al.*, 2003). For example, in rats fed *ad libitum* with either an isocaloric (2.3 kcal/ml) high-fat (60 % energy fat) or high-carbohydrate (76 % carbohydrate) liquid diet for 16 days, the daily energy intake is substantially greater in rats consuming the high-fat (106 kcal/day), when compared with the high-carbohydrate (97.2 kcal/day), diet (Warwick *et al.*, 2002). It has been suggested that the increased energy intake associated with high-fat diets is due to increased palatability of these diets, however, it appears that comparable effects of fat are apparent after intragastric and small intestinal administration of nutrients, which bypasses any orosensory and hedonic inputs. For example, in rats fitted with intragastric pumps that allowed them to self-infuse either a high-fat or a high-carbohydrate liquid diet, the rats receiving the high-fat liquid infused a greater amount of calories than those receiving the high-carbohydrate liquid (Warwick and Weingarten, 1995).

3.3.2. Human studies

The available evidence from studies in animals suggests that exposure to a high-fat diet modulates the gastrointestinal response to fat. For example, the effects of fat on gastric emptying, CCK, GLP-1, PYY, ghrelin, leptin and insulin secretion

have been reported to be attenuated. These observations have important implications for the regulation of energy intake and body weight, and indeed, in animals consumption of a high-fat diet results in hyperphagia and weight gain, which may relate to the attenuation of these gastrointestinal factors. Data relating to the effects of exposure to a high-fat diet on gastrointestinal function and energy intake in humans are much less conclusive, perhaps due to the methodological limitations incurred by the need to provide subjects with normal food items. Furthermore, in the studies conducted to date in humans, only small numbers of lean subjects have been studied, the diets were of a relatively short duration and consumption of the high-fat diets was not associated with a substantial change in body weight and adiposity. Furthermore, the magnitude of fat supplementation is far less than in the studies conducted in animals, and thus, the magnitude of the adaptive changes may be smaller.

3.3.2.1. Effects of a high-fat diet on gastrointestinal motor function

Changes in gastric emptying have been demonstrated following exposure to a high-fat, high-energy diet. - In healthy male subjects consumption of a high-fat diet (2340 kJ of fat, 19.3 MJ daily) for a period of 14 days resulted in acceleration of the gastric emptying and mouth-to-caecum transit of a high-fat test meal, when compared with a low-fat diet (105 kJ fat, 9.1 MJ energy daily) (Cunningham *et al.*, 1991a), consistent with the animal data. Ingestion of a high-fat diet has also been shown to modulate antropyloroduodenal motility. In healthy male subjects, exposure to a high-fat, high-energy diet (40 % energy from fat, 20,123 kJ per day) for a period of 14 days attenuated the effects of an intraduodenal lipid infusion on antropyloroduodenal motility, when compared with a low-fat diet (11 % energy from fat, 11,191 kJ per day) (Boyd *et al.*, 2003).

Therefore, it appears that increasing the caloric content of the diet accelerates gastric emptying. However, because of the marked difference in the total energy content of the diets, it is impossible to determine whether the observed changes in gastric emptying and small intestinal transit were related to the fat content *per se*, or to the high-energy content of the high-fat diet. In contrast, in another study there was no significant difference in the gastric emptying of a high-fat test meal following exposure to a high-fat diet (55 % energy from fat) for 14 days (Castiglione *et al.*, 2002). However, the high-fat diet was compared with a pre-diet condition in which the fat and energy content were not controlled (i.e. the subjects habitual diet contained 30 – 40 % of energy as fat). Therefore, due to methodological limitations in the above studies it remains to be determined what the true effects of exposure to a high-fat diet on gastric emptying are in humans. Studies utilising isocaloric low- and high-fat diets are required to determine the precise role of increased dietary fat, as opposed to increased caloric intake, on gastric emptying and gastrointestinal motor function.

3.3.2.2. Effects of a high-fat diet on the secretion of, and sensitivity to, gastrointestinal, and adiposity, hormones

In human studies, the effects of high-fat diets on gastrointestinal hormone secretion are far less clear, and no studies have evaluated the effects of exogenous administration of these hormones on either gastrointestinal function or energy intake, following exposure to a high-fat diet.

3.3.2.2.1. Cholecystokinin

While increased postprandial CCK concentrations have been reported following exposure to a high-fat diet, when compared with a pre-diet condition (integrated

plasma CCK, high-fat: 1285 ± 153 pM/min; pre-diet: 897 ± 78 pM/min; $P < 0.01$) (French *et al.*, 1995), the CCK response to intraduodenal infusion of lipid (2.8 kcal/min), which bypasses the influence of gastric emptying, was not affected by exposure to a high-fat diet (Boyd *et al.*, 2003). It is, therefore, likely that, in humans, the increased postprandial plasma CCK response observed following consumption of a high-fat diet (French *et al.*, 1995) is primarily reflective of more rapid gastric emptying (Cunningham *et al.*, 1991a). There is indirect evidence that the sensitivity to the actions of CCK may be attenuated in humans. For example, in the study by Boyd et al. (Boyd *et al.*, 2003), demonstrating that the antropyloroduodenal response to an intraduodenal lipid infusion is attenuated following exposure to a high-fat diet, the plasma CCK response to lipid was not modified. Whether consumption of a high-fat diet attenuates the effects of exogenous CCK on gastrointestinal function and energy intake is, hitherto, unknown (Chapter 10).

3.3.2.2.2. GLP-1

The secretion of GLP-1 in response to an intraduodenal lipid infusion is not affected by a high-fat diet when compared with a low-fat, low-energy diet (Boyd *et al.*, 2003). It has not been determined whether exposure to a high-fat diet modulates the actions of GLP-1 on gastrointestinal function or energy intake.

3.3.2.2.3. Ghrelin

Plasma ghrelin concentrations have been reported to be decreased following exposure to a high-fat diet, although the results are inconsistent. Healthy males exposed to a high-fat, cafeteria-style diet *ad libitum* for a period of 16 weeks had unchanged fasting plasma ghrelin concentrations when compared with a high-

protein or high-carbohydrate diet (Paul *et al.*, 2005). Another study reported that following 3-weeks of supplementation of the habitual diet with high-fat products, the suppression of plasma ghrelin following an oral fat load was greater than at baseline, despite only a 3 % increase in body weight (Robertson *et al.*, 2004).

3.3.2.2.4. Leptin

In healthy human subjects, ingestion of a high-fat diet (60 % energy as fat) for a period of 7 days had no effect on fasting plasma leptin concentrations (Schrauwen *et al.*, 1997). However, the short time frame of this study probably limits the relevance of these observations, as an increase in adipose tissue mass is unlikely to have occurred during the 7 day diet period.

No studies have directly assessed the effects of exposing human subjects to a high-fat diet on plasma concentrations of insulin, or the sensitivity to the actions of exogenous insulin.

3.3.2.3. Effects of a high-fat diet on appetite and energy intake

The changes in gastrointestinal function and energy intake would be expected to promote increased energy intake. Studies in humans have shown that exposure to a high-fat diet increases energy intake. For example, in healthy female subjects, covert manipulation of the dietary fat content resulted in a 15.4 % increase in total daily energy intake when consuming a high-fat diet (45 - 50 % fat), when compared with a medium-fat diet (30 – 35 % fat) for 2 weeks, which was associated with weight gain (Lissner *et al.*, 1987). Furthermore, this increase in energy intake occurred in the absence of any change in perceptions of palatability

of the diets. Similarly, in healthy male subjects Tremblay and colleagues (1989) demonstrated a marked increase in acute energy intake following a very short-term (2 day) increase in the fat content of the diet (Tremblay *et al.*, 1989). French and colleagues (1995) reported that appetite perceptions were modified following exposure of to a high-fat diet (58 % energy as fat) for a period of two weeks, with hunger increasing, and fullness decreasing, in healthy male subjects who had gained ~ 2 kg of weight (French *et al.*, 1995). They also reported a modest increase in food intake from a pre-selected meal, and an increase in average daily food consumption, as measured by food diaries, was maintained for the two week dietary intervention (French *et al.*, 1995), and again, consumption of the high-fat diet was associated with an increase in body weight.

3.4. Conclusions

The evidence summarised above provides a rationale for the hypothesis that consumption of a high-fat diet promotes increased energy intake and the development of obesity. In general, animal studies suggest that the consumption of a high-fat diet attenuates the effects of fat on gastrointestinal motor function and gastrointestinal hormone secretion, particularly when the animals become obese. This “adaptation” of gastrointestinal function may potentially result in an attenuation of the signals involved in the feedback inhibition of appetite and energy intake, suggesting that changes in the sensitivity to intestinal fat is likely to contribute to the increased food intake and obesity manifested by high-fat diets. It is conceivable that the resistance to leptin and insulin, which is a common feature of obesity, may attenuate the effects of gastrointestinal hormones, including CCK (Matson *et al.*, 2002), and result in chronically

increased food intake, and consequently weight gain, in individuals exposed to a high-fat diet. In humans, gastric emptying of fat appears to be accelerated, and the secretion of gastrointestinal hormones modulated, however, no studies have investigated whether the sensitivity to the actions of these hormones on gastrointestinal function and energy intake are attenuated. In conclusion, the consumption of a high-fat diet appears to attenuate the effects of fat on gastrointestinal function and energy intake, and this may potentially promote increased body weight. Current studies in humans are, however, limited by the fact that they have compared high-fat, high-energy diets with low-fat, low-energy diets, thus, whether these adaptations occur in response to the fat content of the diet, or the increased caloric intake, is an issue which requires further investigation. In particular, evidence from animal studies strongly indicates that exposure to a high-fat diet attenuates the effects of CCK on gastrointestinal function and energy intake, when compared with an isocaloric low-fat diet, a hypothesis which has, hitherto, not been investigated in humans (Chapter 10).

Chapter 4

Common methodologies

This chapter describes the techniques that were used in the studies presented within this thesis. All of the techniques are well established within our laboratory and considered to be the "gold standard" for the assessment of gastrointestinal motor function, gastric emptying, gastrointestinal hormone concentrations, appetite and energy intake.

4.1. Subjects

For all studies, healthy young male subjects (aged 18 – 50 years) and of normal body weight for their height (BMI 19 – 25 kg/m^2) were enrolled. Subjects were recruited by advertisements placed in the local universities (University of Adelaide, University of South Australia, Flinders University) and at the Royal Adelaide Hospital. Each subject was screened prior to inclusion in the study to exclude:

- Restrained eaters (score > 12 on the eating restraint component (Factor 1) of the Three Factor Eating Questionnaire) (Stunkard and Messick, 1985)
- Significant gastrointestinal symptoms, disease or surgery
- Current use of medications which may affect gastrointestinal function including gastric emptying, antropyloroduodenal motility, body weight, appetite or energy intake (e.g. cisapride, metoclopramide, erythromycin, buscopan, orlistat, antidepressants)
- Current gallbladder or pancreatic disease

- Diabetes mellitus
- Epilepsy
- Cardiovascular or respiratory diseases
- Any other underlying medical conditions
- Weight change (either increase or decrease) of > 7.5 % of total body weight in the three months prior to their enrolment in the study
- Intake of > 20 g of alcohol per day
- Smoking > 10 cigarettes per day
- Exposure to ionising radiation (from X-ray machines or radioactive substances) as part of a research study in the previous twelve months (Chapters 5 and 9)
- For the study involving intravenous infusion of GLP-1 (Chapter 9), subjects were also required to undergo a blood screen to ensure that liver function tests and creatinine clearance were inside the following limits:
  - Alanine aminotransferase ≤ 35 IU/l
  - Alkaline phosphatase ≤ 300 IU/l
  - Aspartate transaminase ≤ 35 IU/l
  - Bilirubin ≤ 17 μmol/l (1.5 mg / 100ml)
  - Creatinine clearance ≥ 50 ml/min

All subjects were required to provide written, informed consent prior to commencement of the study and were informed of their right to withdraw from the study at any time. Subjects were reimbursed for their time spent at the hospital by way of an honorarium ($18 per hour).

4.2. Ethics approval

All study protocols were approved by the Royal Adelaide Hospital Research Ethics Committee. For studies utilising investigational drugs (i.e. CCK and GLP-1, Chapters 9 and 10), protocols were approved by the Royal Adelaide Hospital Investigational Drugs Subcommittee, prior to assessment by the Research Ethics Committee.

4.3. Assessment of antropyloroduodenal pressures

Pressures occurring within the antropyloroduodenal region were measured by high-resolution water-perfusion manometry, using a multi-lumen catheter with closely spaced side-holes linked to transducers. This technique enables the concurrent assessment of pressure waves in the antrum, pylorus and duodenum, basal pyloric tone and the antegrade and retrograde propagation of pressures (Horowitz and Dent, 1991). The reliable measurement of pressures in the pylorus using perfused side-holes is difficult due to the narrowness of the region of maximal sphincter pressure, and the mobility of the sphincter relative to the luminal recording assembly; the Dentsleeve, a perfused channel made of silicon rubber has, therefore, been developed for this purpose. The sleeve is long enough (4.5 cm) to span the normal range of sphincter movement (Dent, 1976). When the sleeve is positioned in the pylorus, the squeeze of the sphincter compresses the silicon rubber membrane against the sleeve bed, increasing the resistance of the sleeve to the passage of perfusate; this increased resistance is detected by an external pressure transducer (Dent, 1976).

The manometric channels were perfused with degassed distilled water at 0.15 ml/min (Heddle *et al.*, 1988a). The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by continuously measuring the transmucosal potential difference (TMPD) between the stomach and small intestine via the side-holes located either side of the sleeve sensor (i.e. the most distal antral channel and the most proximal duodenal channel) (Heddle *et al.*, 1988a). These channels were perfused with degassed 0.9 % saline, at 0.15 ml/min (Heddle *et al.*, 1988a). Potassium chloride-filled electrodes (Dri-Ref^{TM}, World Precision Instruments, Florida, USA) were connected to each of these side-holes and, together with a common reference electrode (a sterile plastic saline-filled 21-G cannula placed subcutaneously in the left forearm), were connected to voltage transducers to create an electrical circuit. The secretions of the stomach (hydrochloric acid) produce an electrically negative voltage (~ - 40 mV) whereas the secretions of the proximal duodenum (bicarbonate) produce a neutral (~ 0 mV) electrical voltage, when compared to a reference point (plasma). Therefore, the continuous measurement of these charges allows monitoring of the positioning of the catheter.

4.3.1. Catheter designs

The manometric catheter utilised in chapters 6, 7 and 10 consisted of sixteen side-holes (0.1 mm diameter) separated by 1.5 cm intervals, for the recording of luminal pressures (outer diameter: 3.5 mm. Dentsleeve, Adelaide, Australia). Six side-holes (channels 1 - 6) were positioned in the antrum, a 4.5 cm sleeve sensor (channel 7), with 2 channels present on the back of the sleeve (channels 8 and 9), was positioned across the pylorus, and 7 channels (channels 10 – 16) were

positioned in the duodenum (**Figure 4.1**). The catheter also incorporated an infusion port (1 mm diameter) located 14.5 cm distal to the pylorus, for the administration of intraduodenal infusions.

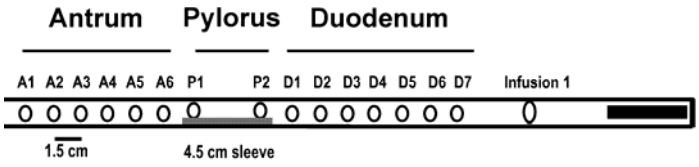

Figure 4.1: Schematic drawing of the manometric assembly used for the assessment of antropyloroduodenal pressures in Chapters 6, 7 and 10 (from Feinle *et al*., 2003).

The manometric assembly utilised for the study presented in chapter 8 (outer diameter: 4.5 mm, Dentsleeve, Adelaide, South Australia) included 5 manometric channels located at 1.5 cm intervals, with 3 channels positioned in the antrum, a 4.5 cm pyloric sleeve sensor (Dent, 1976), with two pyloric channels located on the back of the sleeve, and 1 duodenal channel, 2 small intestinal infusion ports, an aspiration channel at 60 cm distal to the pylorus, and a balloon port containing 3 air channels (**Figure 4.2**). An inflatable polypropylene balloon (5 cm in length, 4 cm in diameter, with a maximum volume of 40 ml) was attached to the balloon port (von Richter *et al.*, 2001) and, when inflated with air, created an "isolated" 60 cm segment of the proximal small intestine. The first of the two infusion ports was located 2 cm distal to the sleeve and used to infuse glucose into the isolated 60 cm segment of the proximal small intestine, while the second, located 75 cm distal to the sleeve, was used to infuse saline, or glucose, distal to the occluding balloon. The aspiration port with an air channel (to facilitate suction) was located 1 cm proximal to the balloon to enable aspiration from the isolated 60 cm segment of the proximal small intestine.

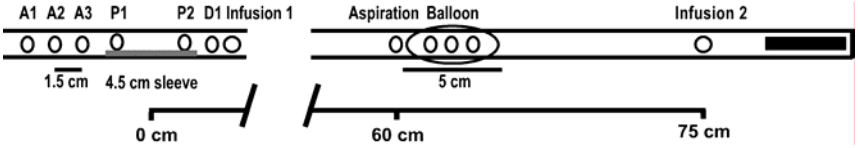

Figure 4.2: Schematic drawing of the manometric assembly used for the assessment of antropyloroduodenal pressures in Chapter 8.

4.3.2. Protocol

For the studies presented in Chapters 6, 7, 8 and 10, subjects attended the Department of Medicine at 0830 h after an overnight fast from 2200 h, and were intubated with a manometric catheter via an anaesthetised nostril (Co-phenylcaine forte, ENT Technologies, West Perth, Australia). The catheter was allowed to pass through the stomach and into the duodenum by peristalsis (Heddle *et al.*, 1988a). Correct positioning of the catheter took between 30 and 210 minutes. If the tube was not correctly positioned by 12 pm, the study was aborted and the subjects were required to come back on another occasion. Following correct positioning of the catheter, with the sleeve sensor straddling the pylorus, fasting motility was observed until the occurrence of a phase III of the interdigestive migrating motor complex (MMC) (Heddle *et al.*, 1988a). Following a baseline recording during a subsequent phase of motor quiescence (phase I), antropyloroduodenal pressures in response to experimental treatments were recorded.

4.3.3. Data acquisition and analysis

Manometric pressures were digitised and recorded on a computer-based system (PowerMac 7100/75; Apple Computer, Cupertino, CA, USA) running

commercially available software (HAD, Associate Professor GS Hebbard, Melbourne, Australia), written in Labview 3.1.1 (National Instruments), and stored for subsequent analysis. APD pressures were able to be analysed for: (i) number and amplitude of antral pressure waves (PWs), (ii) basal pyloric pressure ("tone"), (iii) number and amplitude of isolated pyloric pressure waves (IPPWs), (iv) number and amplitude of duodenal PWs, and (v) number and length of pressure wave sequences involving the antrum, pylorus and duodenum, using custom-written software (Gastrointestinal Motility Unit, University Hospital Utrecht, Utrecht, The Netherlands (Samsom *et al.*, 1998)), tailored to our requirements. The analyses performed for each individual study are described in detail in the relevant chapter. Phasic PWs in the antrum were defined by pressure increases which lasted 1 to 20 s and had an amplitude of > 10 mmHg, with a minimum interval of 15 s between peaks. Isolated pyloric pressure waves (IPPWs) were defined as pressure increases > 10 mmHg recorded by the sleeve with, or without, a concurrent PW in one side-hole along the sleeve, and without antral or duodenal PWs occurring within 5 sec of the onset of the IPPWs. Phasic PWs in the duodenum were defined as those having an amplitude of > 10 mmHg, with a minimal interval of 3 s between peaks. APD pressure wave sequences (APD PWSs) were defined as two or more temporally related PWs with onsets within ± 5 s (in the antrum), or ± 3 s (in the duodenum) of each other (Samsom *et al.*, 1998). Basal pyloric pressure was determined by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side-hole (2.25 cm from the centre of the sleeve) from the mean basal pressure recorded at the sleeve, using custom-written software (MAD, Professor Charles Malbert,

Institut National de la Recherche Agronomique (INRA), Rennes, France) (Heddle *et al.*, 1988b).

4.4. Assessment of gastric emptying

Gastric emptying was measured using scintigraphy, which is the "gold standard" method for the assessment of both solid and liquid emptying (Collins *et al.*, 1983; Collins *et al.*, 1991; Horowitz and Dent, 1991). Scintigraphy is a technique whereby the emptying of a radiolabelled meal from the stomach to the small intestine can be imaged continuously. The concurrent assessment of solid and liquid emptying can be conducted by using radiolabels specific for each phase of the meal.

4.4.1. Test meals

The meal used for the study presented in Chapter 5 consisted of an emulsion containing either (i) 40 g of oleic acid (Sigma Aldrich, Milwaukee, WI, USA) or (ii) 40 g of macadamia oil (as 600 ml oil-in-water emulsions stabilised with 4 % (w/v) powdered milk protein, or (iii) 600 ml aqueous solution of 4 % (w/v) powdered milk protein (24 g skim milk powder in 600 ml water). In study conditions (i) and (ii) the oleic acid (either as a free fatty acid, or in the macadamia oil) was labelled with 15 MBq of gamma-emitting ^{123}Iodine (^{123}I). The labelling was achieved by conjugating elemental ^{123}I to 0.5 g of oleic acid, or macadamia oil (Meyer *et al.*, 1994; Meyer *et al.*, 1996). In this previously validated method, the ^{123}I binds covalently to the unsaturated double bond of the oleic acid molecule. Briefly, 0.5 g of oleic acid or macadamia oil was dissolved in ethanol and added to a mixture of Na^{123}I (500 MBq, ARI), hydrogen peroxide

and hydrochloric acid. After 90 minutes, the ^{123}I-labelled product was extracted from the reaction mixture using petroleum spirit (40 - 60°C), which was subsequently evaporated. After the evaporation of the petroleum spirit, the radioactive residue was diluted with either cold oleic acid (3 x 2 ml), in the case of ^{123}I-oleic acid, or macadamia oil (3 x 2 ml), for the ^{123}I-macadamia oil. The radiochemical purity of the radio-iodinated product was determined to be > 98 %. Patient doses (15 MBq) were drawn up in volumes of 0.2 – 2 ml. On the control day (study condition iii), the liquid meal was labelled with 15 MBq of ^{99m}Technetium-sulphur colloid.

The meal used for the study presented in Chapter 9 consisted of a 100 g minced beef patty (270 kcal, 25 g protein, 21 g fat) labelled with 15 MBq ^{99m}Technetium-sulphur colloid chicken liver, followed immediately by 150 ml of 10 % dextrose (15 g dextrose in 150 ml water, 63 kJ) labelled with 4 MBq ^{67}Gallium-EDTA (Jones *et al.*, 2002).

4.4.2. Protocol

Subjects were either standing (Chapter 5) or were seated (Chapter 9) with their back against the gamma camera. The time of completion of the meal was defined as t = 0 min. Gastric emptying and intragastric distribution of the meal were then measured for 2 hours (Chapter 9) or 4 hours (Chapter 5). The time points at which radioisotopic data were acquired is described in the relevant chapters.

4.4.3. Data analysis

Data were corrected for subject movement, radionuclide decay and gamma-ray attenuation (Collins *et al.*, 1983). Regions-of-interest were drawn for total, proximal and distal gastric regions, and gastric emptying curves, expressed as percent retention over time, derived (Collins *et al.*, 1983; Jones *et al.*, 1995). The amount remaining in the total, proximal and distal stomach between t = 0 – 120, or t = 0 – 240 min was derived. The lag phase for solid and liquid was determined as the time period between meal completion and the appearance of radioactivity in the proximal small intestine (Collins *et al.*, 1983). The amount of solid remaining in the stomach at t = 100 min and the time for 50 % of the liquid to empty (T50) were calculated (Collins *et al.*, 1983). GE was classified as delayed when the solid % retention at t = 100 min was > 61 % and/or the liquid T50 was > 31 min, based on an established normal range (Jones *et al.*, 2002).

4.5. Blood sampling

Cannulae for repeated blood sampling were inserted into an antecubital vein. Blood samples were immediately transferred into tubes containing dipotassium EDTA and the protease inhibitor, Trasylol (Bayer Australia Ltd, Pymble, Australia, 400 kIU aprotinin per ml blood). Plasma was separated by centrifugation at 3200 rpm for 15 min at 4°C within 30 min of collection, and stored at -70°C until assayed.

4.6. Blood glucose and plasma hormone concentrations

4.6.1. Blood glucose

Venous blood glucose concentrations (mmol/l) were determined immediately by the glucose oxidase method using a portable glucose meter (Medisense Precision QLD, Abbott Laboratories, Bedford, MA). The accuracy of this method has been confirmed in our laboratory using the hexokinase technique (Horowitz *et al.*, 1991).

4.6.2. Cholecystokinin (CCK)

Plasma CCK concentrations (pmol/l) were determined following ethanol extraction using an established radioimmunoassay (MacIntosh *et al.*, 2001). A commercially available antibody raised in rabbits against synthetic sulphated CCK-8 was employed (C258, Lot 105H4852, Sigma Chemical, St Louis, MO, USA). This antibody binds to all CCK analogues with the sulphated tyrosine residue in position 7, has a cross-reactivity of 26 % with unsulphated CCK-8, less than 2 % cross-reactivity with human gastrin and does not bind to structurally unrelated peptides. The intra-assay coefficient of variation (CV) was 9 % and the inter-assay CV was 27 %. The assay has a sensitivity of 2.5 pmol/l.

4.6.3. Glucagon-like peptide-1 (GLP-1)

Plasma GLP-1 concentrations (pmol/l) were measured by radioimmunoassay (Wishart *et al.*, 1998). The antibody, supplied by Professor SR Bloom (Hammersmith Hospital, London), has been shown using chromatography to measure intact GLP-$1_{(7\text{-}36)}$ amide, and it is likely that this antibody also binds the degraded form of GLP-$1_{(9\text{-}36)}$ amide. The antibody did not cross-react with

glucagon, GIP or any other gut or pancreatic peptides. The intra-assay CV was 17 % and the inter-assay CV was 18 %. The assay has a sensitivity of 1.5 pmol/l.

4.6.4. Peptide YY (PYY)

Plasma PYY concentrations (pmol/l) were measured by radioimmunoassay using an antiserum (kindly donated by Dr B Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) raised in rabbits against human $PYY_{(1-36)}$ (Sigma-Aldrich, St Louis, MO, USA) (Pilichiewicz *et al.*, 2006). This antiserum showed < 0.001 % cross-reactivity with human pancreatic polypeptide and sulphated CCK-8 and 0.0025 % cross-reactivity with human neuropeptide Y. The assay has a sensitivity of 1.5 pmol/l, the inter-assay CV was 16.6 % and the intra-assay CV was 12.3 %.

4.6.5. Ghrelin

Total ghrelin (ng/l) was measured by radioimmunoassay using a commercial antiserum (RAST-4745, Bachem, CA, USA) (Parker *et al.*, 2005) that does not cross-react with human secretin, orexin, motilin, galanin or vasoactive intestinal peptide. The intra-assay CV was 17 % and the inter-assay CV was 23 %, with a sensitivity of 40 ng/l.

4.6.6. Glucose-dependent insulinotropic peptide (GIP)

Plasma GIP concentrations (pmol/l) were measured by immunoassay (Wishart *et al.*, 1992). A commercially available antiserum was used (Peninsula Laboratories (Bachem), CA, USA), which shows 100 % cross-reactivity with GIP (human)

and GIP (porcine). The assay has a sensitivity of 2 pmol/l, and both the intra-assay and inter-assay CVs were 15 %.

4.6.7. Insulin

Plasma insulin concentrations (mU/l) were measured by ELISA (Diagnostics Systems Laboratories Inc., Webster, TX). The sensitivity of the assay was 0.26 mU/l, the intra-assay and inter-assay CVs were 2.6 and 6.2 %, respectively (Horowitz *et al.*, 1996).

4.6.8. Glucagon

Glucagon was assayed by the Central Sydney Laboratory Service at the Royal Prince Alfred Hospital using a specific radioimmunoassay (Diagnostic Product Corporation Pty Ltd., Los Angeles, California, USA). The assay has a sensitivity of 13 pg/ml and both the intra-assay and inter-assay CVs were 15.7 %.

4.7. Assessment of appetite perceptions, gastrointestinal symptoms and energy intake

4.7.1. Assessment of appetite perceptions

Ratings of appetite, including hunger, fullness, desire to eat and prospective consumption ("how much food do you think you could eat right now?") were measured using validated visual analogue scale questionnaires (VAS) (Parker *et al.*, 2004) (**Appendix 1**). Nausea and bloating were also assessed. Each VAS evaluated a sensation on a 100 mm horizontal line, where 0 mm represented 'sensation not felt at all' and 100 mm 'sensation felt the greatest'. Subjects were asked to indicate how they were feeling at that particular time by placing a

vertical mark on the 100 mm line. Other perceptions, such as anxiety and drowsiness, were also assessed to distract from the main purpose of the questionnaire, but were not evaluated.

4.7.2. Assessment of habitual energy intake

Prior to enrolment in the study described in Chapter 10, subjects were instructed to complete a food diary over 5 consecutive days (3 weekdays and a weekend) to assess their habitual energy intake. Detailed instructions and examples were provided so that subjects understood the need for reporting of all food and drink consumed over this period. Subjects were required to weigh foods, or to provide standardised measures (i.e. cup or teaspoon quantities), and were instructed to provide recipes and methods of cooking for meals prepared. The average daily energy (kJ), amount (g) and macronutrient composition (%) of the food consumed was determined using commercially available software (Foodworks 3.01; Xyris Software, Highgate Hill, Queensland, Australia)

4.7.3. Assessment of energy intake following experimental treatments

Energy intake was assessed by quantifying the amount of energy consumed by a subject at an *ad libitum* cold, buffet-style meal (Feltrin *et al.*, 2004). The amount of food offered was in excess of what the subject would normally be expected to eat (Lavin *et al.*, 1996). The total energy content of the food was ~11,800 kJ. The meal consisted of 125 g white bread, 125 g wholemeal bread, 100 g sliced ham, 100 g sliced chicken, 85 g cheese slices, 100 g sliced tomato, 100 g sliced cucumber, 100 g lettuce, 200 g strawberry yoghurt, 140 g fruit salad, 150 g chocolate custard, 1 apple, 1 banana, 500 g unsweetened orange juice, 600 g iced

coffee, 600 g water, 20 g of margarine and 20 g mayonnaise (Feltrin *et al.*, 2004). The amount (g), energy (kJ) and macronutrient composition (%) of the individual food items is outlined in **Appendix 2**. Subjects were instructed to eat for up to 30 minutes until they felt "comfortably" full.

Energy intake (kJ), the total amount of food consumed (g) and the macronutrient distribution (% of energy from carbohydrate, fat and protein) was analysed using commercially available software (Foodworks 3.01; Xyris Software, Highgate Hill, Queensland, Australia) (Feltrin *et al.*, 2004).

4.8. Statistical analysis

Data were analysed using the commercially available statistical software packages Statview Version 5.0 (SAS Institute Inc., North Carolina, USA) and SuperANOVA Version 1.1 (Abacus Concepts Inc., Berkeley, CA, USA). The statistical analyses for each study are described in detail in the relevant chapter. Statistical significance was accepted at $P < 0.05$. For all studies data are displayed as means ± SEM.

Chapter 5

The effects of free fatty acids on gastric emptying, plasma CCK, appetite perceptions and energy intake are more potent than those of triglycerides

5.1. Abstract

The effects of fat on gastric emptying, gastrointestinal hormone release and energy intake are dependent on the digestion of fat to free fatty acids. There is little information about the comparative effects of free fatty acids and triglycerides on these factors. For example, animal studies suggest that oleic acid inhibits energy intake more potently than an isocaloric infusion of its respective triglyceride, triolein. We hypothesised that free fatty acids administered intragastrically would slow gastric emptying, stimulate CCK secretion and suppress appetite and energy intake more potently than triglyceride. Nine healthy males were studied on three occasions to evaluate the effects of intragastric administration of (i) 40 g of oleic acid (free fatty acid, "FFA") (1830 kJ) or (ii) 40 g of macadamia oil (triglyceride, "TG") (1856 kJ), both as 600 ml oil-in-water emulsions stabilised with 4 % (w/v) powdered milk protein, or (iii) 600 ml aqueous solution of 4 % (w/v) powdered milk protein ("control", 24 g skim milk powder in 600 ml water, 352 kJ), on gastric emptying, plasma CCK concentrations, appetite and energy intake. Gastric emptying of FFA was much slower than of TG ($P < 0.001$), with greater retention of FFA, than TG, in the proximal stomach ($P < 0.001$). Hunger was less ($P < 0.05$), and fullness greater ($P < 0.05$), with FFA when compared with control and TG. Plasma

concentrations of CCK were greater in response to FFA when compared with TG and control. Energy intake was lower in response to FFA, and higher in response to TG, when compared with control. In conclusion, this study has demonstrated that free fatty acids empty from the stomach more slowly, stimulate CCK release and suppress appetite more potently, when compared with triglycerides.

5.2. Introduction

It is well established that the presence of fat in the small intestine has a number of effects on gastrointestinal function, appetite and hormone release. For example, in healthy subjects, small intestinal infusion of lipid decreases hunger, increases sensations of fullness and decreases subsequent energy intake (Chapman *et al.*, 1999; Cook *et al.*, 1997; Lavin *et al.*, 1996; MacIntosh *et al.*, 1999). Small intestinal lipid also modulates gastrointestinal motility, including inhibition of fundic, antral and duodenal motility, stimulation of basal pyloric pressure and phasic pyloric pressure waves (Heddle *et al.*, 1988a), associated with a slowing of gastric emptying (Heddle *et al.*, 1989), stimulates the release of gastrointestinal hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) (MacIntosh *et al.*, 1999) and peptide YY (Feinle-Bisset *et al.*, 2005), and decreases ghrelin (Feinle-Bisset *et al.*, 2005). It has, however, become apparent that it is free fatty acids, and not triglyceride, that mediate these effects (Feinle *et al.*, 2003; Feinle-Bisset *et al.*, 2005; Feltrin *et al.*, 2006; O'Donovan *et al.*, 2003). It may, therefore, be expected that the putatively slow release of free fatty acids from triglycerides during lipolysis in the proximal small intestine may result in low luminal concentrations, and thus lower responses when compared with ingestion of free fatty acids. This may potentially explain why fat ingested in the diet, or administered directly into the stomach is relatively ineffective at suppressing energy intake (Blundell *et al.*, 1995), when compared with fat administered directly into the small intestine (Feinle *et al.*, 2003).

Fat digestion has been shown to be essential for the effects of fat on gastric emptying, gastrointestinal motility, gut hormone release and energy intake

(Feinle *et al.*, 2003; Feinle *et al.*, 2001; Matzinger *et al.*, 2000; O'Donovan *et al.*, 2003; Pilichiewicz *et al.*, 2003). The slowing of gastric emptying by lipid (oil) has been shown to be dependent on lipase activity in the duodenum of dogs equipped with chronic pancreatic fistulas (Meyer *et al.*, 1994). In humans, administration of the lipase inhibitor, tetrahydrolipstatin (THL) (Xenical®), which prevents duodenal hydrolysis of fat, accelerates gastric emptying of lipid (Pilichiewicz *et al.*, 2003; Schwizer *et al.*, 1997). Furthermore, administration of THL, attenuates the effects of duodenal fat infusion on proximal gastric relaxation (Feinle *et al.*, 2001), antropyloroduodenal motility (Feinle *et al.*, 2003), subjective ratings of fullness and energy intake (Feinle *et al.*, 2003; Feinle *et al.*, 2001; Matzinger *et al.*, 2000), gallbladder emptying and pancreatic secretion (Hildebrand *et al.*, 1998), and secretion of CCK, GLP-1, PYY and ghrelin (Feinle *et al.*, 2003; Hildebrand *et al.*, 1998).

These observations suggest that the effects of free fatty acids in the small intestine may be comparatively greater than the effects of triglycerides. There is, however, little information regarding the comparative effects of free fatty acids and triglycerides. In rats, intraduodenal infusion of oleic acid (0.064 kcal/min for 2 hours; total load 7.68 kcal) has been reported to suppress food intake by approximately 28 % more than an isocaloric infusion of triolein (Woltman *et al.*, 1995), and jejunal infusion of oleic acid (0.2 ml/h for 7 h; total load: 11.3 kcal) suppressed food intake more potently than isocaloric infusion of corn oil (Cox *et al.*, 2004). In humans with pancreatic exocrine insufficiency, the effects of free fatty acids (oleic acid) on CCK secretion were greater (integrated response: 91 ± 11 pM.min vs. 49 ± 21 pM.min) and occurred earlier (15 min vs. 30 min) when

compared with triglyceride (20 % Intralipid®) (Guimbaud *et al.*, 1997). Indirect evidence from recent studies in healthy human subjects suggests that free fatty acids have a more potent effect on gastrointestinal function and energy intake than triglycerides. For example, while the stimulation of pyloric pressure waves was maximal within ~ 30 - 45 min of the start of triglyceride infusion (2.8 kcal/min; total energy delivered: ~ 1053 kJ) (Feinle *et al.*, 2003; Heddle *et al.*, 1989; Heddle *et al.*, 1988b), similar effects were evident during infusion of lauric acid, a fatty acid with 12 carbon atoms (C12) (0.375 kcal/min, 56 mM; total energy delivered: ~ 140 kJ) within ~ 15 min. Furthermore, while peak plasma concentrations of CCK were ~ 7 pmol/l during intraduodenal infusion triglyceride, plasma concentrations of CCK reached ~ 12 pmol/l during infusion of C12, and while energy intake was suppressed by ~ 900 kJ following triglyceride infusion (Feinle *et al.*, 2003), C12 suppressed energy intake by ~ 2800 kJ (Feltrin *et al.*, 2004). Thus, the effects of free fatty acids on gastrointestinal function and energy intake appear to be more potent than the effects of triglycerides even though the latter are hydrolysed to free fatty acids in the small intestinal lumen.

Therefore, the “a priori” hypothesis of this study was that free fatty acids administered intragastrically would empty from the stomach more slowly, stimulate CCK secretion and suppress appetite and energy intake more potently than triglyceride. Oleic acid was compared with macadamia oil; this oil was used as it contains ~ 84 % monounsaturated free fatty acids, predominantly oleic acid. A control arm, a low-calorie protein solution, was used to determine that the protein in the emulsions did not markedly delay gastric emptying.

5.3. Subjects and methods

5.3.1. Subjects

Nine healthy lean male subjects were recruited along guidelines set by the Royal Adelaide Hospital Human Ethics Committee. Subjects had a mean age of 23.2 ± 1.8 years (range 21 - 26 years) and were of normal body weight for their height (mean BMI 22.1 ± 0.7 kg/m^2).

5.3.2. Study design

Subjects were studied on three occasions, separated by 3 – 10 days, in a single-blind, randomised, fashion to evaluate the effects of intragastric administration of (i) 40 g of oleic acid (free fatty acid, "FFA", Sigma Aldrich, Milwaukee, WI, USA, 1830 kJ) or (ii) 40 g of macadamia oil (triglyceride, "TG", Melrose Laboratories, Pty Ltd., Victoria, Australia, 1856 kJ), both as 600 ml oil-in-water emulsions stabilised with 4 % (w/v) powdered milk protein, or (iii) 600 ml aqueous solution of 4 % (w/v) powdered milk protein ("control" solution, 24 g skim milk powder in 600 ml water, Fonterra Brand Pty Ltd., Victoria, Australia, 352 kJ), on gastric emptying, plasma CCK concentrations, appetite and energy intake.

5.3.3. Protocol

Subjects attended the Department of Nuclear Medicine, PET and Bone Densitometry at the Royal Adelaide Hospital at either 0830 h or 1030 h on three occasions following an overnight fast from both solids and liquids from 2200 h. The reason for different start times was logistical; individuals attended at the same time on each visit. Immediately upon arrival, subjects ingested 120 g

potassium iodide to block the thyroid from uptake of the radiolabelled iodine. Subjects were then intubated with a nasogastric feeding tube (diameter 10 FR, Viasys Healthcare Medsystems, Warwick, UK) via an anaesthetised nostril. After collection of a baseline blood sample and completion of a VAS questionnaire to assess appetite perceptions (t = -10 min), the test emulsion/solution was instilled into the stomach over five minutes (t = -5 – 0 min) through the nasogastric tube. In study conditions (i) and (ii) the emulsions containing FFA or TG were labelled with 15 MBq of gamma-emitting ^{123}Iodine (^{123}I). The labelling was achieved by conjugating elemental ^{123}I to 0.5 g of oleic acid, or macadamia oil (Meyer *et al.*, 1994; Meyer *et al.*, 1996). In study condition (iii), the aqueous phase was labelled with 15 MBq of ^{99m}Technetium-sulphur colloid. Following instillation of the meal the nasogastric tube was removed (t = 0 min) and gastric emptying was measured for 240 min. Further blood samples were collected at t = 0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min, and VAS, to assess appetite perceptions were completed at t = 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min. At t = 240 min subjects were presented with a cold, buffet-style meal and invited to eat freely until they were comfortably full, for up to 30 min (Cook *et al.*, 1997). Immediately following the meal (i.e. at t = 270 min), a final blood sample was collected and another VAS completed; subjects were then free to leave the laboratory.

5.3.4. Measurement of gastric emptying

Gastric emptying was measured scintigraphically by obtaining 1 min anterior and 1 min posterior images at t = 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min. Gastric emptying curves were used to derive the % retention in the total,

proximal and distal stomach (Jones *et al.*, 1998). Data were corrected for subject movement, radionuclide decay and γ-ray attenuation, as described (Collins *et al.*, 1983). Correction factors for radioactive decay and attenuation of ^{123}I were determined in our laboratory using methods described for In^{113} and Ga^{67} (Collins *et al.*, 1983). The gastric emptying of the control arm was assessed to determine the gastric emptying of the aqueous phase.

5.3.5. Data and statistical analysis

We calculated that with 9 subjects we would observe a difference in gastric emptying and gastrointestinal hormone secretion at $\propto = 0.05$, with a power of 0.98. The amount of free fatty acids emptied from the stomach as part of the triglyceride was determined by subtracting the amount of glycerol from the total amount of triglyceride emptied (triglyceride, free fatty acid equivalent). For VAS scores and plasma CCK concentrations, baseline values (0) were calculated as the mean of values obtained at t = -10 and t = 0 min, and data were expressed as change from baseline values. The amount emptied (g) and % retention of the emulsions in the total, proximal and distal, stomach, and VAS data were analysed by repeated measures analysis of variance (ANOVA) with time and treatment as factors. Energy intake was assessed by one-way ANOVA. To investigate our "a priori" hypothesis, planned paired comparisons were performed to determine the differences between the TG and FFA. Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

5.4. Results

The study protocol was well tolerated. Intragastric administration of the two emulsions and the control solution was not associated with gastrointestinal symptoms or other adverse symptoms.

5.4.1. Gastric emptying

5.4.1.1. Total stomach

The emptying of the control solution approximated an overall mono-exponential fashion. The emptying of the emulsions occurred in an overall linear fashion. There was a treatment * time interaction for the amount of FFA and TG remaining in the total stomach ($P < 0.0001$). The intragastric retention of FFA was greater than that of TG between t = 90 – 240 min ($P < 0.001$) (**Figure 5.1A**). In contrast, the control solution completely emptied from the stomach over the 240 min (time effect: $P < 0.001$). The intragastric retention of FFA and TG was greater than that of the control solution between t = 10 – 240 min ($P < 0.001$).

When expressed as grams emptied, there was no significant difference in the amount of FFA and TG (free fatty acid equivalent) emptied over the first 30 min (**Figure 5.1B**), but between t = 45 – 240 min, the amount of TG emptied was greater than the amount of FFA ($P < 0.05$).

5.4.1.2. Intragastric meal distribution

Proximal stomach

The control solution emptied from the proximal stomach in a mono-exponential fashion. The emptying of the FFA and TG from the proximal stomach

approximated the same pattern as for the total stomach, i.e. was linear after an initial slower emptying phase. There was a treatment * time interaction for the amount remaining in the proximal stomach ($P < 0.0001$) (**Figure 5.2A**). At t = 45 and between t = 120 – 240 min, the amount of FFA in the proximal stomach was greater when compared with TG ($P < 0.05$, for all).

Distal stomach

There was a treatment * time interaction for the amount of the meal remaining in the distal stomach ($P < 0.01$) (**Figure 5.2B**). At t = 150 and t = 180 min, the amount of FFA in the distal stomach was greater when compared with TG.

5.4.2. Plasma cholecystokinin concentrations

Due to the small number of subjects analysed formal statistics have not been performed on this data. Preliminary data (n = 4) indicate that all three treatments stimulated the release of CCK, with peak values occurring at t = 10 min (**Figure 5.3**). The magnitude of this effect was greater for FFA, when compared with both TG and control solution, and was greater for the control solution when compared with TG. For all treatments, plasma CCK was elevated between t = 0 – 30 min, and then decreased over time. At t = 240 min, plasma CCK concentrations had returned to baseline for TG and control, but remained above baseline for FFA. Following the buffet-meal (t = 270 min), plasma concentrations of CCK increased following all treatments.

5.4.3. Appetite perceptions

There was a treatment * time interaction for scores of hunger ($P < 0.0001$) and fullness ($P < 0.05$) (**Figure 5.4A and B**). Hunger was significantly less in response to FFA, when compared with control between t = 10 – 150 min ($P < 0.05$), and compared with TG between t = 10 – 240 min. Hunger was significantly greater in response to TG when compared with control between t = 20 – 240 min ($P < 0.05$). Fullness increased from baseline immediately following intragastric administration of all three treatments (time effect: $P < 0.001$). Fullness was greater in response to FFA, when compared with both TG and control between t = 10 – 240 min ($P < 0.01$), with no difference between TG and control.

5.4.4. Energy intake

There was no effect of treatment on the amount (g), energy intake (kJ) or macronutrient composition (% fat, carbohydrate and protein) of food consumed from the buffet-meal ($P = 0.1$) (**Table 5.1**). Despite this, mean energy intake following FFA was reduced by ~ 555 kJ when compared with control. There was a significant difference between the FFA and TG; FFA reduced energy intake by ~ 1265 kJ when compared with TG ($P < 0.05$). In contrast, energy intake following TG was increased by ~ 710 kJ, when compared with control.

5.5. Discussion

This study has demonstrated for the first time in humans that the inhibitory effects of free fatty acids on gastric emptying and appetite are more potent than those of triglycerides, i.e. gastric emptying of free fatty acids was significantly slower than that of triglyceride; and was associated with greater retention in the proximal stomach. Furthermore, despite only a small amount of fatty acid emptying from the stomach, plasma CCK was increased, perceptions of hunger were decreased, and fullness was increased, more by free fatty acids than triglyceride. The most remarkable observation was that these differences were apparent after 10 min, indicating that the effects were not attributable to differences in intragastric content.

That the emptying of free fatty acids was much slower when compared with triglyceride is consistent with our hypothesis, which was based on previous studies demonstrating the importance of fat digestion, and consequently the release of free fatty acids in the small intestinal lumen, for the effects of fat on gastric emptying (Borovicka *et al.*, 2000; Meyer *et al.*, 1994; Meyer *et al.*, 1996; Schwizer *et al.*, 1997). It has been suggested that the presence of small amounts of lipolytic products in the small intestinal lumen profoundly inhibits gastric emptying of fat. For example, an early study by Cortot *et al.* suggested that fat emptying from the stomach at rates as low as 1.5 g/hour inhibited subsequent gastric emptying of fat by approximately 75 % (Cortot *et al.*, 1982). However, this study employed a duodenal marker perfusion-aspiration technique to measure gastric emptying, so that the radiolabels for the fat within the solid butter would have been aspirated more slowly than the perfused, emulsified markers,

potentially leading to inaccurate determination of the gastric emptying of fat. Our study, in which the radiolabel was tightly bound to the double-bond of the oleic acid molecule, has confirmed that free fatty acids empty very slowly from the stomach in a linear fashion, in support of the results by Hunt and Knox (1968) who reported that fatty acids empty from the stomach at rates between 0.1 – 0.4 kcal/min. The rate of gastric emptying was certainly far slower than the generally accepted rate of ~ 2 kcal/min at which nutrients, including fat, and carbohydrate have been reported to empty from the stomach (Brener *et al.*, 1983; Meyer *et al.*, 1996). This observation suggests that very small amounts of free fatty acids present in the small intestinal lumen potently inhibit gastric emptying.

The release of CCK by all three treatments occurred rapidly (i.e. peaked within 10 minutes), however, the magnitude of the effect was approximately two-fold greater for the free fatty acid when compared with the control solution, and four-fold greater than the triglyceride. At this time point no fatty acid was detectable in the small intestine; however, it is probable that a very small amount of fatty acid undetectable by the method used to assess gastric emptying, had emptied to stimulate the localised release of CCK from endocrine cells in the proximal small intestine. The release of CCK at t = 10 min was greater for the control solution than for the triglyceride. This is likely to relate to the fact that at this time a significantly greater amount of calories had emptied in the protein solution than for the triglyceride. The triglyceride released CCK only transiently, suggesting that the hydrolysis of triglycerides in the small intestinal lumen is relatively slow, resulting in lower responses.

Fullness was increased, and hunger decreased to a greater extent following free fatty acids, when compared with control and triglyceride. This difference was apparent at t = 10 min, at which time only a minute amount of free fatty acids had emptied from the stomach, suggesting that an "intragastric" mechanism sensitive to free fatty acids may mediate this effect. It is possible that the early release of CCK by the free fatty acid, served to enhance the effects of gastric distension. To support this concept, a study by Kissileff *et al.* demonstrated that the combination of intravenous infusion of CCK-8 (112 ng/ml) and gastric distension (300 ml water-filled balloon) reduced energy intake by ~ 25 %, compared with ~12 % and 4 % for CCK-8 and distension alone, i.e. the combined effect was greater than that of either stimulus alone (Kissileff *et al.*, 2003). In contrast, hunger increased to a greater extent following the triglyceride, than the protein. This suggests that free fatty acids may be released by duodenal lipolysis at concentrations that are insufficient to modulate appetite. This observation may also relate to the failure of triglyceride to stimulate a sufficient level of CCK.

The free fatty acids reduced energy intake when compared with triglyceride, but not control. Previous studies have reported that healthy male subjects most accurately compensate for the energy ingested in a preload (high-fat yoghurt) when the subsequent meal is offered 30 minutes later (Rolls *et al.*, 1991). In the current study, at t = 30 min plasma CCK concentrations were elevated, whereas by t = 240 min, plasma concentrations had decreased to levels similar to baseline for the control and triglyceride, but remained elevated for free fatty acids. Therefore, the long period of time between intragastric instillation of the emulsions and the presentation of the buffet-meal may have reduced the

magnitude of the difference in energy intake between the free fatty acid and the triglyceride. Furthermore, by 240 min, hunger did not differ between control and free fatty acids. Despite this, energy intake following free fatty acids was decreased by ~ 555 kJ when compared with control, and by ~ 1265 kJ when compared with triglyceride, suggesting that the effects of free fatty acids on energy intake are more potent than those of triglycerides. The mechanism mediating this difference is likely to result from greater feedback arising from the small intestine in response to the free fatty acid when compared with triglyceride, and is likely to involve CCK, since the effects of intraduodenal fat on energy intake are reduced when the CCK_1 receptor antagonist, loxiglumide, is administered (Lieverse *et al.*, 1994).

In conclusion, this study has demonstrated that free fatty acids empty from the stomach more slowly, stimulate the release of CCK and suppress appetite and energy intake more potently than triglyceride.

Table 5.1: Amount (g), energy intake (kJ) and macronutrient distribution of food consumed from the buffet meal presented 240 min after intragastric administration of control, triglyceride and free fatty acid.

Treatment	Energy intake (kJ)	Amount (g)	Macronutrient distribution (%)		
			Fat	Protein	CHO
Control	4754 ± 610	965 ± 102	34 ± 2	24 ± 2	41 ± 3
TG	5463 ± 662	1127 ± 138	36 ± 2	24 ± 2	40 ± 2
FFA	4199 ± 410*	958 ± 127	35 ± 2	25 ± 2	40 ± 3

Data are means ± SEM (n = 9). CHO; carbohydrate. TG = triglyceride, FFA = free fatty acid. * FFA vs. TG, $P < 0.05$.

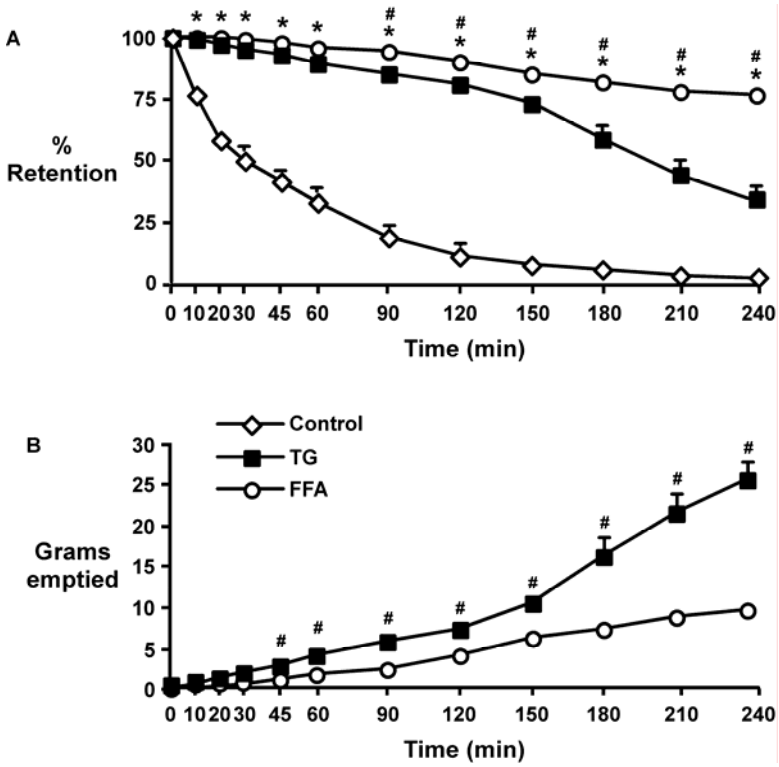

Figure 5.1: Gastric emptying (% retention) (A) and grams emptied (B) following intragastric administration of (i) 40 g oleic acid "FFA", (ii) 40 g macadamia oil "TG" as 600 ml oil-in-water emulsions, stabilised with 4 % (w/v) powered milk protein, or (iii) control solution (24 g skim milk powder in 600 ml water). Data are means ± SEM (n = 9). * TG and FFA vs. control solution, $P < 0.001$, # FFA vs. TG, $P < 0.001$.

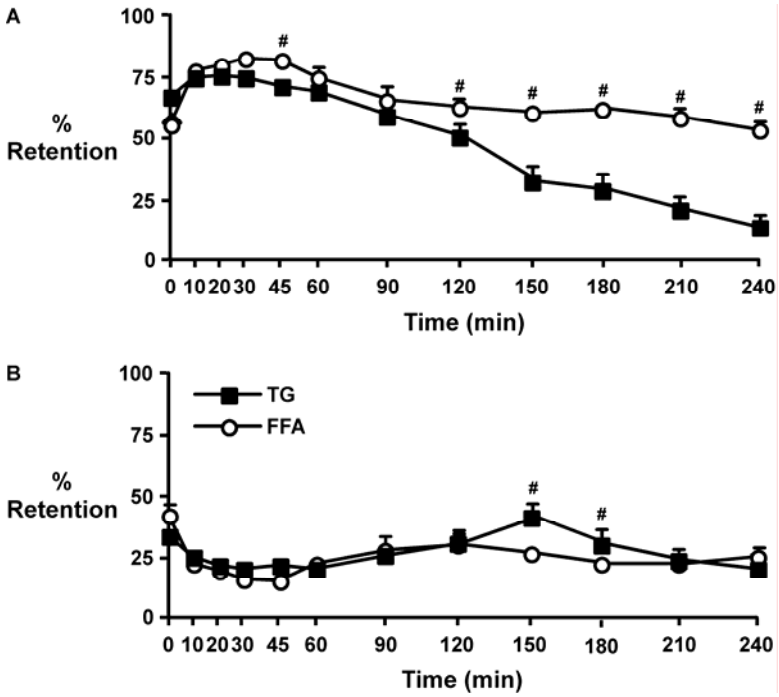

Figure 5.2: The retention of the meal in the proximal (A) and distal (B) stomach following intragastric administration of (i) 40 g oleic acid "FFA", (ii) 40 g macadamia oil "TG" as 600 ml oil-in-water emulsions, stabilised with 4 % (w/v) powered milk protein. Data are means ± SEM (n = 9). # FFA vs. TG, $P < 0.05$.

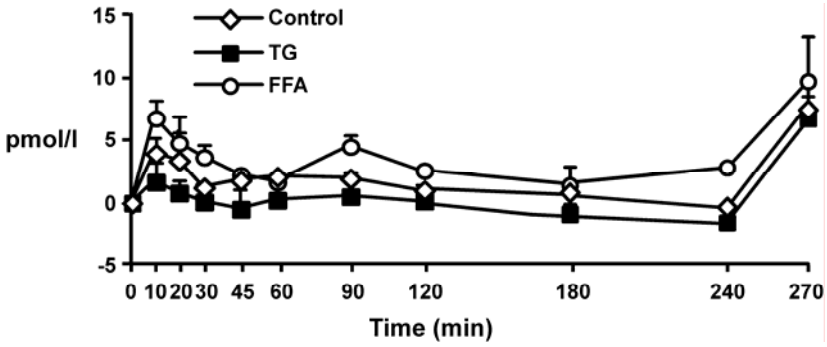

Figure 5.3: Plasma concentrations of CCK following intragastric administration of (i) 40 g oleic acid "FFA", (ii) 40 g macadamia oil "TG" as 600 ml oil-in-water emulsions, stabilised with 4 % (w/v) powered milk protein, or (iii) control solution (24 g skim milk powder in 600 ml water). Data are means ± SEM (n = 4).

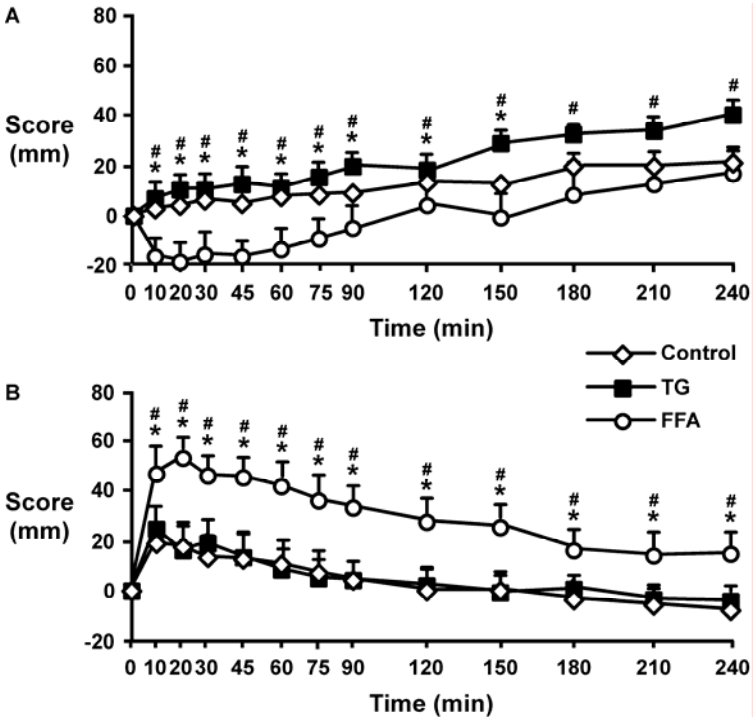

Figure 5.4: Hunger (A) and fullness (B) following intragastric administration of (i) 40 g oleic acid "FFA", (ii) 40 g macadamia oil "TG" as 600 ml oil-in-water emulsions, stabilised with 4 % (w/v) powered milk protein, or (iii) control solution (24 g skim milk powder in 600 ml water). Data are means ± SEM (n = 9). * FFA vs. control solution, $P < 0.05$, # FFA vs. TG, $P < 0.05$.

Chapter 6

Dose-related effects of lauric acid on antropyloroduodenal motility, gastrointestinal hormone release, appetite and energy intake in healthy lean men

6.1. Abstract

We have recently reported that intraduodenal infusion of lauric acid (C12) (at 0.375 kcal/min, 106 mM) stimulates isolated pyloric pressure waves (IPPWs), inhibits antral and duodenal pressure waves (PWs), stimulates the release of cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) and suppresses plasma ghrelin and energy intake, and that these effects are much greater than those seen in response to isocaloric decanoic acid (C10) infusion. Administration of C12 was, however, associated with nausea, confounding interpretation of the results. The aim of this study was to evaluate the effects of different intraduodenal doses of C12 on antropyloroduodenal (APD) motility, plasma CCK and GLP-1 concentrations, appetite and energy intake. 13 healthy males were studied on four days in double-blind, randomised, fashion. APD pressures, plasma CCK and GLP-1 concentrations and appetite perceptions were measured during 90 minute intraduodenal infusion of C12 at either (i) 0.1 (14 mM), (ii) 0.2 (28 mM) or (iii) 0.4 (56 mM) kcal/min, or (iv) saline (control) (rate: 4 ml/min). Energy intake was determined at a buffet meal immediately following the infusion. C12 dose-dependently stimulated IPPWs, decreased antral and duodenal motility, and stimulated secretion of CCK and GLP-1 ($r > 0.4$, $P < 0.05$ for all). C12 at 0.4 kcal/min suppressed energy intake compared with control,

C12 (0.1) and C12 (0.2) ($P < 0.05$). These effects were observed in the absence of nausea. In conclusion, intraduodenal C12 dose-dependently modulated APD motility and gastrointestinal hormone release in healthy male subjects, while effects on energy intake were only apparent with the highest dose infused (0.4 kcal/min), possibly because only at this dose modulation of antropyloroduodenal motility and gastrointestinal hormone secretion was sufficient for a suppressant effect on energy intake.

Am J Physiol Regul Integr Comp Physiol, 289(4): R1090-R1098 (2005).

6.2. Introduction

In a recent study from our laboratory intraduodenal administration of lauric acid, a fatty acid with 12 carbon atoms (C12), at a rate of 0.375 kcal/min and a concentration of 106 mM, was shown to stimulate pyloric motility and suppress antral and duodenal motility in healthy subjects much more than decanoic acid, a fatty acid with 10 carbon atoms (C10) (Feltrin *et al.*, 2004). Intraduodenal C12 also stimulated the release of cholecystokinin (CCK) (Feltrin *et al.*, 2004; McLaughlin *et al.*, 1999), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and suppressed ghrelin, whilst C10, in the dose evaluated, stimulated CCK, albeit to a lesser extent than C12, and had no effect on plasma concentrations of GLP-1, PYY and ghrelin (Feltrin *et al.*, 2004; Feltrin *et al.*, 2006). Intraduodenal infusion of C18, but not C8, has been shown to inhibit energy intake in humans (Matzinger *et al.*, 2000), and we have recently reported an inhibitory effect of intraduodenal infusion of C12, but not C10, on appetite and energy intake (Feltrin *et al.*, 2004), in healthy subjects. In this latter study, infusion of C12 potently attenuated ratings of hunger and desire to eat and suppressed energy intake at a subsequent meal (Feltrin *et al.*, 2004). However, in some subjects C12 also induced nausea, and the suppression of energy intake was greater in those subjects (3516 kJ) when compared with those that did not experience nausea (1801 kJ), confounding interpretation of the observations (Feltrin *et al.*, 2004). It is also possible, albeit less likely, that the observed effects on gastrointestinal motility and hormone release may also have been attributable to nausea. Therefore, it remains unclear whether the modulation of antropyloroduodenal (APD) motility, gastrointestinal hormone secretion, appetite, and energy intake

during intraduodenal infusion of C12 represents a physiological effect of lauric acid, or is secondary to the induction of nausea.

The mechanisms by which C12 inhibits subsequent energy intake are unclear. There is some evidence that the effects of C12 are dependent on the release of CCK (Lal *et al.*, 2004), for example, the inhibitory effects of C12 on gastric emptying and the perception of intragastric volume are attenuated by the CCK_1 receptor antagonist, loxiglumide (Lal *et al.*, 2004). The effects of fatty acids also appear to involve the activation of vagal afferents, either directly, or via CCK (Cox *et al.*, 2004; Lal *et al.*, 2001). The effects of C12 on energy intake may also be mediated through the actions of GLP-1 (Feltrin *et al.*, 2004) and possibly other peptides, and the changes in gastrointestinal motility, perhaps particularly the stimulation of pyloric motility (Xu *et al.*, 2005). In animals, the effects of small intestinal C12 on gastric emptying and energy intake may be influenced by both the concentration, and/or energy load (Lin *et al.*, 1990; Meyer *et al.*, 1998), although the energy load may be relatively more important (Lin *et al.*, 1990). The concentration of the C12 solution (106 mM) employed in our previous study was based upon that which had been infused intragastrically (100 mM) in humans without inducing nausea (McLaughlin *et al.*, 1999). The observation that infusion of C10 at a slightly higher (123 mM) concentration was not associated with adverse side effects also argues against the concept that the concentration of the C12 solution was responsible for the observed nausea. However, under physiological conditions, i.e. following ingestion of a meal, fatty acids are present within the small intestine at much lower concentrations, ranging from approximately 25 – 65 mM (Ament *et al.*, 1972; Borgstrom *et al.*, 1957; Porter *et*

al., 1971). Therefore, infusion of C12 at these concentrations may have more physiological effects on gastrointestinal function and energy intake. Likewise, it is possible that the energy load of C12 delivered to the small intestine may play a role in mediating the observed effects on APD motility, gastrointestinal hormone release, perceptions of appetite, and energy intake. The data of Hunt and Knox suggest that C12 empties from the stomach at rates ranging from approximately 0.1 to 0.4 kcal/min (Hunt and Knox, 1968), however, the load-dependency has not been investigated.

We have now evaluated the effects of increasing intraduodenal doses of C12, given over a range of concentrations and energy loads, on APD motility, plasma CCK and GLP-1 concentrations, appetite and energy intake, to test the hypothesis that C12 would dose-dependently stimulate phasic and tonic pyloric motility, suppress antral and duodenal pressures and stimulate the release of CCK and GLP-1, in the absence of nausea, and that these effects of C12 would be associated with a dose-dependent suppression of energy intake.

6.3. Subjects and methods

6.3.1. Subjects

13 healthy males were included in the study; the number of subjects was based on power calculations derived from a previous study (Chapman *et al.*, 1999); we calculated that with 13 subjects we would observe a 10 % decrease in energy intake at $\alpha = 0.05$, with a power of 80 %. Subjects had a mean age of 23.4 ± 1.7 years (range 19 - 30 years) and were of normal body weight for their height (mean BMI 23.6 ± 0.5 kg/m^2).

6.3.2. Study design

Each subject was studied on four occasions, separated by 3 – 10 days, in a double-blind, randomised fashion to evaluate the effects of 90 minute intraduodenal infusions of lauric acid (C12) at (i) 0.1 kcal/min, (ii) 0.2 kcal/min, (iii) 0.4 kcal/min, or (iv) control (isotonic saline), on APD pressures, appetite, energy intake and plasma CCK and GLP-1 concentrations.

6.3.3. Protocol

Subjects attended the laboratory at 0830 h after fasting from both solids and liquids from 10 pm the previous night and were intubated, via an anaesthetised nostril, with a 17-channel manometric catheter (Dentsleeve, Adelaide, Australia). Following correct positioning of the catheter, fasting motility was observed until the occurrence of a phase III of the interdigestive migrating motor complex (MMC) (Cook *et al.*, 1997). Immediately following the end of the phase III, during a period of motor quiescence (i.e. at t = -15 min), a baseline venous blood sample was taken, and the subject completed a visual analogue scale questionnaire (VAS) for the assessment of appetite-related sensations, as well as

nausea and bloating. At t = 0 min, intraduodenal infusion of C12 commenced at a rate of 4 ml/min for 90 min (i.e. t = 0 – 90 min). APD pressures were recorded throughout the infusion; blood samples were collected and VAS completed every 15 min. At t = 90 min, the infusion was terminated and the nasoduodenal catheter removed. The subjects were then offered a standardised, cold, buffet-style meal and allowed 30 min (t = 90 - 120 min) to consume as much food as they wished until they felt comfortably full. Further blood samples were collected and VAS completed by the subjects at t = 120 and t = 150 min, the intravenous cannula was then removed and the subjects were allowed to leave the laboratory.

6.3.4. Preparation and doses of C12 solutions

Fatty acid solutions were designed to deliver either: (i) 0.1 kcal/min, (C12 (0.1); concentration: 14 mmol; total energy in 90 min: 9 kcal (37.5 kJ)), (ii) 0.2 kcal/min (C12 (0.2); concentration: 28 mmol; total energy: 18 kcal (75 kJ)) or (iii) 0.4 kcal/min (C12 (0.4); concentration: 56 mmol; total energy: 36 kcal (150 kJ)). The 0.1 and 0.2 kcal/min loads were selected to encompass the range for gastric emptying of fatty acids reported in the study by Hunt and Knox (Hunt and Knox, 1968). The 0.4 kcal/min load, albeit at a lower concentration, was selected on the basis of our previous study in which C12 was administered intraduodenally at 0.375 kcal/min (106 mmol) to healthy subjects and shown to potently suppress energy intake (Feltrin *et al.*, 2004). The concentrations of the solutions were within the range of fatty acid concentrations observed in the small intestine after triglyceride digestion (Ament *et al.*, 1972; Borgstrom *et al.*, 1957; Porter *et al.*, 1971).

Solutions were prepared using the commercially available food grade saturated fatty acid, lauric acid (C12:0) (Sigma-Aldrich, Milwaukee, WI, USA). 1.13, 2.26 or 4.52 g of C12 were dissolved with 0.18, 0.36 or 0.75 g of sodium hydroxide (NaOH) (Sigma-Aldrich), respectively, in 0.9 % saline, to a total volume of 400 ml, with a resulting pH of 8.4. All solutions were infused at 37°C. The pH of the control solution (0.9 % saline) was adjusted to 8.4 by the addition of NaOH. All solutions were prepared on the morning of the study and were infused at a rate of 4 ml/min, so that the total volume infused in 90 min was 360 ml.

6.3.5. *Data and statistical analyses*

The number and amplitude of antral and duodenal PWs were used to calculate motility indices (MI) using the following equation: MI (mmHg * number) = natural logarithm [(sum of amplitudes x number of contractions (PWs)) + 1] (Camilleri and Malagelada, 1984). For the number, amplitude and motility indices of antral and duodenal PWs, number and amplitude of IPPWs, basal pyloric pressures and number of APD PWSs, baseline values (0) were calculated as the mean of values obtained between t = -15 to 0 min. For VAS and plasma CCK and GLP-1 concentrations, baseline values (0) were calculated as the mean of values obtained at t = -15 and t = 0 min. The number and amplitude of IPPWs and basal pyloric pressures were expressed as mean values over 15 min periods during the 90 min infusion period (i.e. 0 – 15, 15 – 30,…, 75 – 90 min), while the number, amplitude and motility indices of antral and duodenal PWs were expressed as mean values for the entire 90 min infusion period. APD PWSs were expressed as the total number of PWs travelling over 2 (i.e. 1.5 - < 3 cm), 3 (i.e. 3

- < 4.5 cm), …, 15 (i.e. 21 - < 22.5 cm) channels during the 90 minute infusion period. All data were expressed as changes from baseline values.

The number and amplitude of IPPWs, basal pyloric pressures, VAS scores and plasma hormone concentrations were analysed by repeated measures analysis of variance (ANOVA) with time (t = 0 – 15, 15 – 30,…, 75 – 90 min for IPPWs and basal pyloric pressures, and t = 0, 15, 30,…, 90 min for VAS scores and plasma hormone concentrations) and treatment as factors. The number of APD PWSs was analysed by repeated measures ANOVA with length of propagation (1.5 - < 3, 3 - < 4.5, …., 21 - < 22.5 cm) and treatment as factors. One-way ANOVA was used to analyse the effects of treatment on the number, amplitude and motility indices of antral and duodenal PWs, energy intake (kJ), macronutrient distribution, the total amount (g), and the amount of solid (g) and liquid (g), of food consumed at the buffet meal. Post-hoc paired comparisons, corrected for multiple comparisons by Bonferroni's correction, were performed if ANOVAs revealed significant effects. Dose-response relationships were determined using linear associations between the dose of C12 administered (i.e., 0, 0.1, 0.2 or 0.4 kcal/min) and the mean values over 90 minutes of the number, amplitude and motility indices of antral and duodenal PWs, the number of IPPWs, basal pyloric pressure, APD PWSs, energy intake, as well as the plasma CCK and GLP-1 concentrations at 90 min, by calculating correlation coefficients adjusted for repeated measures (Bland and Altman, 1995). Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

6.4. Results

All subjects completed the four randomised study days, and the study protocol was tolerated well by these subjects. The tube was correctly positioned and the infusion commenced within an average of 146 ± 45 (range 45 – 210) minutes.

6.4.1. Antropyloroduodenal pressures

6.4.1.1. Antral pressures

There was a trend for an effect of treatment on the number of antral PWs ($P = 0.08$). C12 (0.2) and C12 (0.4) appeared to decrease the number of antral PWs compared with control and C12 (0.1). There was an effect of treatment on the amplitude of antral PWs ($P < 0.01$). C12 (0.4) reduced the amplitude compared with control and C12 (0.1) ($P < 0.01$ for both). C12 (0.2) reduced the amplitude compared with control and C12 (0.1) ($P < 0.01$ for both). There was also an effect of treatment on the MI of antral PWs ($P < 0.001$) (**Figure 6.1**). C12 (0.4) decreased the MI compared with both control and C12 (0.1) ($P < 0.01$ for both). C12 (0.2) decreased the MI compared with control ($P < 0.01$). C12 (0.1) appeared to reduce the MI of antral PWs compared with control; however, this was not significant ($P = 0.2$).

There was an inverse relationship between the dose of C12 administered and the number, amplitude and motility index of antral PWs, such that the greater the dose of C12, the lower the number ($r = -0.3$, $P < 0.05$), amplitude ($r = -0.4$, $P < 0.01$) and motility index of antral PWs ($r = -0.5$, $P < 0.001$).

6.4.1.2. Pyloric pressures

Basal pyloric pressure (tone): There was no effect of treatment, or time, on basal pyloric pressure, although the mean values for C12 (0.4) were higher compared with control, C12 (0.1) and C12 (0.2) (**Figure 6.2A**). There was no relationship between the dose of C12 administered and basal pyloric pressure.

Phasic pressures: There was a treatment * time interaction for the number of IPPWs ($P < 0.01$) (**Figure 6.2B**). C12 (0.4) increased the number between t = 0 – 60 minutes compared with control and between t = 0 – 45 min compared with C12 (0.1), but decreased the number between t = 60 – 75 min compared with C12 (0.2) ($P < 0.05$ for all). C12 (0.2) increased the number between t = 0 – 90 minutes compared with control, and between t = 0 – 45 min and t = 75 – 90 min compared with C12 (0.1) ($P < 0.05$ for all). C12 (0.1) increased the number between t = 0 – 15 min compared with control ($P < 0.01$). There was a positive relationship between the dose of C12 administered and the total number of IPPWs over 90 min, such that the greater the dose of C12, the greater the number of IPPWs ($r = 0.4$, $P < 0.05$). There was no effect of treatment on, or a relationship between the dose of C12 administered and, the amplitude of IPPWs.

6.4.1.3. Duodenal pressures

There was an effect of treatment on the number of duodenal pressure waves ($P < 0.01$). Infusion of C12 (0.4) decreased the number compared with control, C12 (0.1) and C12 (0.2) ($P < 0.05$ for all). C12 (0.2) decreased the number compared with C12 (0.1) ($P < 0.05$). There was no effect of treatment on the amplitude of duodenal PWs. There was an effect of treatment on the MI of duodenal PWs ($P < 0.05$) (**Figure 6.3**). Infusion of C12 (0.4) decreased the MI compared with both

control and C12 (0.1) ($P < 0.05$ for both). There was no difference between C12 (0.1) or C12 (0.2) and control.

There was an inverse relationship between the number and MI, but not the amplitude, of duodenal PWs and the dose of C12 administered, such that the greater the dose of C12, the lower the number ($r = -0.5$, $P < 0.001$) and MI of duodenal PWs ($r = -0.4$, $P < 0.01$).

6.4.1.4. Antropyloroduodenal sequences

There was an effect of treatment on the number of pressure wave sequences travelling over 2 (i.e. 1.5 < 3 cm), 3 (i.e. 3 < 4.5 cm), 4 (i.e. 4.5 < 6 cm), 5 (i.e. 6 < 7.5 cm), 6 (i.e. 7.5 < 9 cm) and 7 (i.e. 9 < 10.5 cm) channels ($P < 0.001$) (**Figure 6.4**). Infusion of C12 (0.4) decreased the number of PWSs travelling over 2, 3, 4 and 5 channels compared with control, C12 (0.1) and C12 (0.2), the number of PWSs travelling over 6 channels compared with control, and the number of PWSs travelling over 7 channels compared with C12 (0.1) ($P < 0.05$ for all). Infusion of C12 (0.2) decreased the number of PWSs travelling over 2 channels compared with C12 (0.1) ($P < 0.001$). Infusion of C12 (0.1) increased the number of PWSs travelling over 2, 3 and 4 channels compared with control ($P < 0.01$). PWSs travelling over 8 and more (i.e. ≥ 10.5 cm) channels were not analysed statistically, as they were very infrequent (a total of 26 waves travelled over 8 – 15 channels, 9 during the control infusion, 14 during C12 (0.1), 2 during C12 (0.2) and < 1 during C12 (0.4)).

6.4.2. Plasma CCK and GLP-1 concentrations

Baseline plasma CCK concentrations did not differ between study days (Control: 4.0 ± 0.4 pmol/l, C12 (0.1): 3.9 ± 0.3 pmol/l, C12 (0.2): 3.8 ± 0.3 pmol/l and C12 (0.4): 3.9 ± 0.3 pmol/l). There was a treatment * time interaction for plasma CCK concentrations ($P < 0.001$) (**Figure 6.5A**). Plasma concentrations of CCK peaked at approximately 15 min. Infusion of C12 (0.4) increased plasma CCK concentrations between t = 15 – 90 minutes compared with control and C12 (0.1), and between t = 30 – 90 minutes compared with C12 (0.2) ($P < 0.01$ for all). Infusion of C12 (0.2) increased plasma CCK concentrations between t = 15 – 90 minutes compared with control, and at t = 15, 30, 60 and 90 min compared with C12 (0.1) ($P < 0.05$ for all). Infusion of C12 (0.1) increased plasma CCK concentrations at t = 15 and 45 - 90 minutes compared with control ($P < 0.05$ for all). There was a positive relationship between the dose of C12 administered and the plasma concentrations of CCK at t = 90 min, such that the greater the dose of C12, the greater the concentration of CCK at t = 90 min ($r = 0.7$, $P < 0.001$).

Baseline plasma GLP-1 levels were slightly variable over study days (Control: 13.9 ± 1.8 pmol/l, C12 (0.1): 17.6 ± 2.9 pmol/l, C12 (0.2): 16.7 ± 3 pmol/l, C12 (0.4): 14.9 ± 1.8 pmol/l). There was a treatment * time interaction for plasma GLP-1 concentrations ($P < 0.01$) (**Figure 6.5B)**. Infusion of C12 (0.4) increased plasma GLP-1 concentrations from t = 30 – 90 min compared with control, at t = 30 and 60 - 90 min compared with C12 (0.1), and at t = 45 and 75 min compared with C12 (0.2) ($P < 0.05$ for all). Infusion of C12 (0.2) increased plasma GLP-1 concentrations at t = 30, 60 and 90 min compared with control, and at t = 30 min compared with C12 (0.1) ($P < 0.05$ for all). Infusion of C12 (0.1) increased

plasma GLP-1 concentrations at t = 45 and 90 min compared with control ($P < 0.05$). There was a positive relationship between the amount of C12 administered and the plasma concentrations of GLP-1 at t = 90 min, such that the greater the dose of C12, the greater the concentration of GLP-1 at t = 90 min ($r = 0.5$, $P < 0.001$).

6.4.3. Appetite sensations and energy intake

There was no effect of treatment on ratings of appetite, i.e. hunger, desire to eat, fullness, prospective consumption, or gastrointestinal symptoms, i.e. bloating and nausea. There was an effect of treatment on energy intake ($P = 0.05$). C12 (0.4) decreased energy intake compared with control, C12 (0.1) and C12 (0.2) ($P < 0.05$ for all). There was, however, no effect on either the total amount (g), solid (g), liquid (g), or the macronutrient distribution, of food consumed at the buffet-meal (**Table 6.1**). There was no correlation between energy intake and the amount of C12 administered.

6.5. Discussion

This study establishes that intraduodenal administration of C12 modulates antropyloroduodenal motility and gastrointestinal hormone release in a dose-dependent fashion, such that the greater the dose of C12 administered, the greater the stimulation of isolated pyloric pressure waves, suppression of antral and duodenal pressure waves and APD PWSs, and stimulation of CCK and GLP-1. In contrast, at the doses used, appetite perceptions were not affected, and suppression of energy intake was only apparent with the 0.4 kcal/min dose, perhaps reflecting the greater effects of this dose on antropyloroduodenal motility and plasma CCK and GLP-1 secretion. The effect of C12 on motility, CCK, GLP-1 and energy intake occurred in the absence of nausea.

It has been established that the presence of C12 in the small intestinal lumen slows gastric emptying (Hunt and Knox, 1968; Lal *et al.*, 2004), stimulates isolated pyloric pressure waves (Feltrin *et al.*, 2004), increases proximal gastric relaxation (Lal *et al.*, 2004), suppresses antral (Feltrin *et al.*, 2004; McLaughlin *et al.*, 1999) and duodenal motility (Feltrin *et al.*, 2004), and stimulates the release of CCK (Feltrin *et al.*, 2004; McLaughlin *et al.*, 1999), GLP-1 (Feltrin *et al.*, 2004), PYY and PP and suppresses ghrelin (Feltrin *et al.*, 2006). The current study extends these observations by demonstrating that the responses are dependent upon the dose of C12 administered to the small intestine, and that even very low doses of C12 have potent effects. For example, infusion of C12 at doses as low as 0.1 and 0.2 kcal/min, resulting in a total energy delivery of only 9 and 14 kcal, respectively, over the 90 min infusion period, had substantial effects. In previous studies using intraduodenal lipid infusion at a rate of 2.8 kcal/min, the

stimulation of pyloric motility and plasma CCK concentrations were maximal at about ~ 30 – 45 min when approximately 84 – 126 kcal would have been delivered to the small intestine (Feinle *et al.*, 2003). This contrasts with the current study in which maximal effects of C12 infusion on pyloric motility and plasma hormone secretion were observed after 15 minutes, when only 1.5, 3 or 6 kcal had been delivered to the small intestine during infusion of C12 at 0.1, 0.2 and 0.4 kcal/min, respectively. This provides persuasive evidence that the effects of C12 on pyloric motility and plasma concentrations of CCK and GLP-1 are much more potent than those of long-chain triglycerides. The underlying reasons are currently unknown, but may perhaps reflect the fact that lauric acid probably accounts for only < 2 % of daily energy intake, so that, under normal conditions, exposure of the small intestine to lauric acid is likely to be limited.

The demonstrated dose-dependent effects of C12 on plasma concentrations of CCK and GLP-1 suggest that the release of CCK and GLP-1 in response to C12 is also dependent on the amount of C12 present in the small intestinal lumen, i.e. infusion of C12 at 0.4 kcal/min resulted in a greater secretion of CCK and GLP-1 than during infusion of C12 at 0.1 or 0.2 kcal/min. The secretion profiles varied between CCK and GLP-1; plasma CCK increased almost immediately after the start of the C12 infusions, with a plateau after 30 minutes, consistent with release of CCK from enteroendocrine cells in the proximal small intestine (Rehfeld, 1978). In contrast, there was a 30-minute delay before plasma GLP-1 concentrations increased from baseline, and this increase was progressive during the entire 90 minute infusion period. It is possible, that during infusion of C12, the absorption capacity of the proximal small intestine was exceeded, resulting in

progressively greater amounts of C12 reaching the distal small intestine, the primary site of GLP-1 release (Eissele *et al.*, 1992), thereby accounting for the gradual increase in GLP-1 secretion.

The effects of C12 on appetite and energy intake were not dose-dependent at the doses evaluated in this study. Infusion of C12 at 0.4 kcal/min, but not 0.1 and 0.2 kcal/min, decreased energy intake by 831 kJ compared with control, without inducing nausea. The reduction in energy intake was not associated with a change in the amount (weight) of food consumed, that is, subjects consumed a less energy-dense meal. While the highest dose suppressed energy intake, this occurred in the absence of changes in appetite perceptions. This suggests that perceptions of appetite may be regulated by mechanisms differing to those involved in the control of acute energy intake, and may perhaps require higher energy loads. The effects of C12 on energy intake were, however, much more marked in our previous study, in which C12 reduced ratings of hunger and suppressed subsequent energy intake by 2781 kJ when compared with control (Feltrin *et al.*, 2004). However, as discussed, the inhibition of hunger and energy intake following infusion of C12 (0.375 kcal/min, 106 mM) was associated with a marked increase in nausea (Feltrin *et al.*, 2004). Whilst the decrease in hunger and energy intake in our earlier study could not altogether be attributed to nausea, as the subjects who did not experience nausea still also decreased their food intake, these new observations provide further support for a physiological effect of C12 on the suppression of energy intake.

Whilst it has been suggested that the suppressive effects of nutrients on subsequent energy intake are mediated by changes in gastrointestinal motility and gastrointestinal hormone release (Lal *et al.*, 2004; Xu *et al.*, 2005), the current study suggests that the stimulation of IPPWs and secretion of CCK and GLP-1 have to reach a "threshold" to result in suppression of energy intake. The motor patterns associated with infusion of C12 at 0.2 and 0.4 kcal/min are known to be associated with the slowing of gastric emptying (Heddle *et al.*, 1989), which is thought to play a role in suppressing energy intake (Sepple and Read, 1989). Recent evidence suggests that electrical stimulation of the pylorus suppresses food intake in dogs (Xu *et al.*, 2005), thus implying an important role for the pylorus in the regulation of energy intake. It is, however, interesting to note that despite C12 at 0.2 kcal/min having a more prolonged stimulatory effect on IPPWs than C12 at 0.4 kcal/min, there was no effect on energy intake. Furthermore, at the time of the meal, the effects of C12 at 0.4 kcal/min on IPPWs had returned to baseline, yet energy intake was suppressed only following this infusion. Hence, factors other than the stimulation of isolated pyloric pressure waves are probably required to inhibit energy intake. The discrepant effects of C12 seen in our study may be attributable to the different patterns of secretion of the gastrointestinal hormones CCK and GLP-1, as intravenous infusion of both CCK and GLP-1 suppress energy intake in humans (Flint *et al.*, 1998; Kissileff *et al.*, 1981). C12 at 0.4 kcal/min stimulated the secretion of CCK and GLP-1 to a greater extent than C12 at 0.2 kcal/min. It is interesting to note that while C12 at 0.2 kcal/min stimulated the release of CCK to a similar extent to that previously observed in studies using intraduodenal infusion of a long-chain triglyceride emulsion in which there was a significant suppression of energy intake (Feinle *et*

al., 2003), it did not suppress energy intake. This may potentially reflect the number of subjects studied, i.e. a type II statistical error, however, it seems unlikely that increasing the number of subjects would show an effect of the lower doses on energy intake, as there was no trend at all towards decreased energy intake using these doses. Rather, it is likely that there are different threshold requirements for the effects observed on motility, gastrointestinal hormone release, appetite and energy intake, i.e. while the lower doses were sufficient to stimulate motility and hormone release, only the 0.4 kcal/min infusion suppressed energy intake, and this may have been due to appropriate modulation of motility and gastrointestinal hormones. Likewise, while none of the doses had an effect on perceptions of appetite, i.e. hunger, fullness, desire to eat and prospective consumption, C12 at 0.4 kcal/min suppressed subsequent energy intake.

While we have demonstrated a clear dose-responsive effect of C12 on antropyloroduodenal motility and gastrointestinal hormone release, it remains unclear whether the concentration, or the energy load, of C12 administered mediated these effects of C12, as in order to keep the volume of the infusion identical on all study days, the concentration of the solutions varied. Canine studies have suggested that inhibition of gastric emptying or stimulation of pancreatic enzyme secretion by intestinal oleate is length- and load-dependent at luminal concentrations near or above 20 mM, but that concentration becomes increasingly important for these responses, as it drops below 10 mM (Meyer and Jones, 1974). The effects of oleate and lauric acid on food intake, at concentrations ranging from 20 - 80 mM, were shown in rats to be load-, but not concentration-dependent (Meyer *et al.*, 1998). In our previous study, during

infusion of C12 at 0.375 kcal/min, we observed a more marked increase in the number of IPPWs, basal pyloric pressure, secretion of CCK and GLP-1, as well as decreased perceptions of hunger and desire to eat, and energy intake with a concentration of 106 mM (Feltrin *et al.*, 2004), compared with our current observations for C12 at 0.4 kcal/min and 56 mM, suggesting that concentration may be important. Increasing the load and/or concentration of C12 could suppress energy intake by increasing the effects of C12 on gastrointestinal motility and hormone secretion. This issue, therefore formed the basis for the study presented in Chapter 7.

In conclusion, our study has demonstrated a dose-dependent effect of acute intraduodenal C12 on antropyloroduodenal motility and gastrointestinal hormone secretion and, at the highest dose used, suppression of energy intake.

Table 6.1: Energy intake from the buffet meal, and macronutrient distribution, in response to 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control.

			% Energy		
Treatment	***Energy (kJ)***	***Amount eaten (g)***	**Fat**	**Protein**	**CHO**
Control	5932 ± 495	1369 ± 118	33 ± 1	22 ± 1	43 ± 2
C12 (0.1)	5902 ± 475	1367 ± 99	34 ± 2	22 ± 1	43 ± 2
C12 (0.2)	5815 ± 520	1399 ± 118	32 ± 2	22 ± 1	46 ± 2
C12 (0.4)	5101 ± 521 *	1305 ± 108	32 ± 1	21 ± 1	46 ± 2

Data are means ± SEM (n = 13). *C12 (0.4) vs. control, C12 (0.1) and C12 (0.2): $P < 0.05$. CHO = carbohydrate.

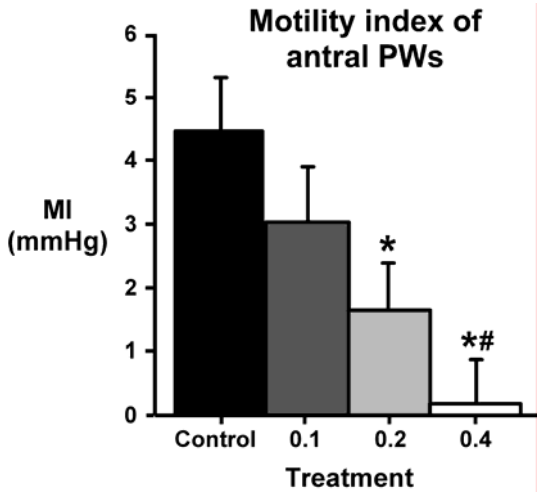

Figure 6.1: Motility index (MI) of antral pressure waves (PWs) during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. * C12 (0.4) and C12 (0.2) vs. control: $P < 0.01$, # C12 (0.4) vs. C12 (0.1): $P < 0.01$. Data are means ± SEM (n = 13).

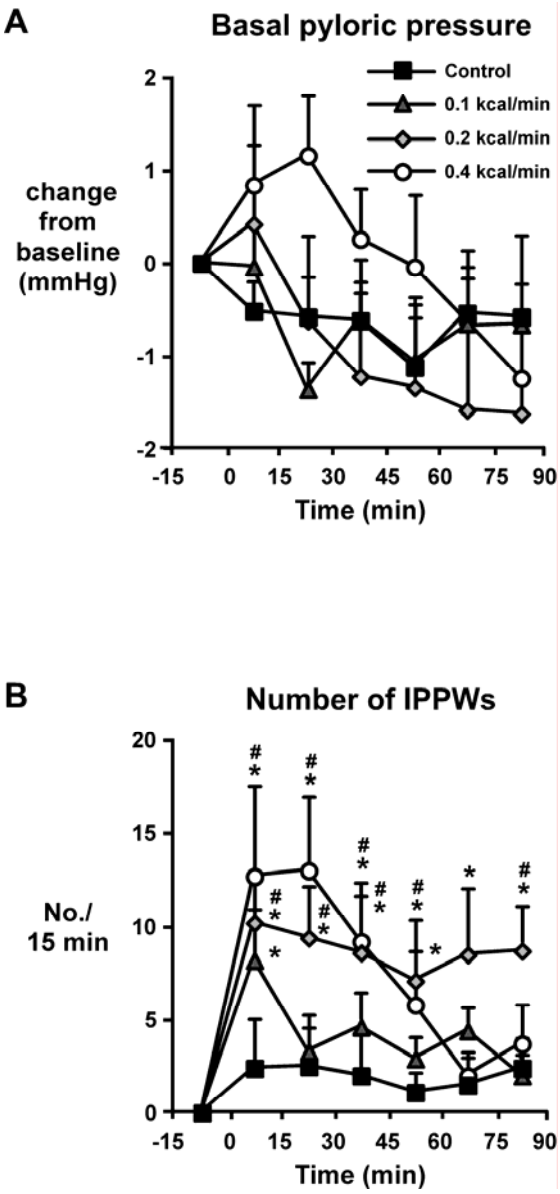

Figure 6.2: (A) Basal pyloric pressure (tone) and (B) number of isolated pyloric pressure waves (IPPWs) during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. * C12 (0.4), C12 (0.1) and C12 (0.2) vs. control: $P < 0.05$, # C12 (0.4) and C12 (0.2) vs. control: $P < 0.05$, α C12 (0.4) vs. C12 (0.2): $P < 0.01$. Data are means ± SEM (n = 13).

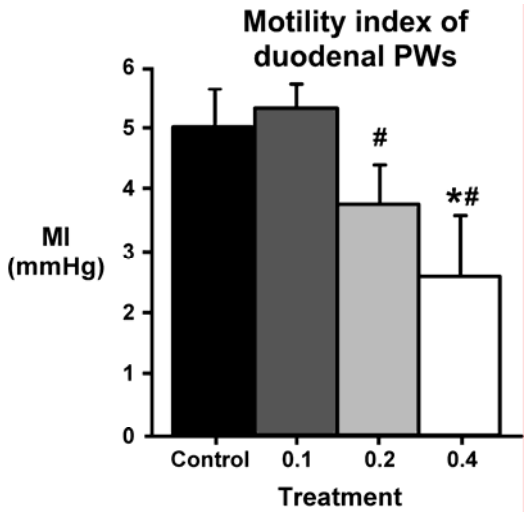

Figure 6.3: Motility index of duodenal pressure waves (PWs) during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. * C12 (0.4) vs. control: P = 0.01, # C12 (0.2) and C12 (0.4) vs. C12 (0.1): $P < 0.05$, α C12 (0.4) vs. C12 (0.2), $P < 0.05$. Data are means ± SEM (n = 13).

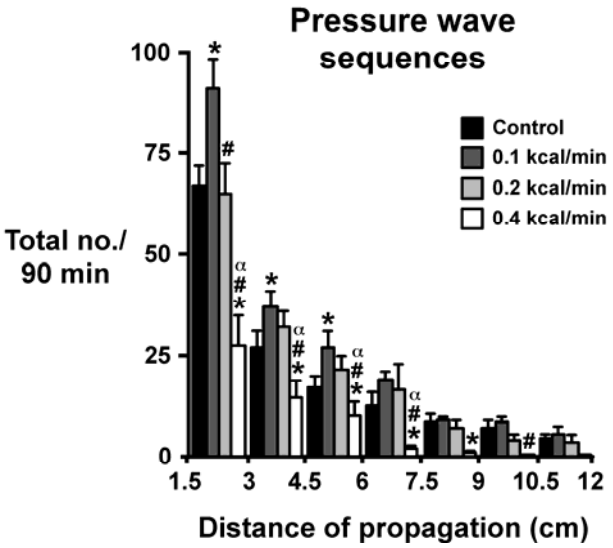

Figure 6.4: Antropyloroduodenal pressure wave sequences during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. * C12 (0.4), C12 (0.2) and C12 (0.1) vs. control: $P < 0.05$, # C12 (0.4) and C12 (0.2) vs. C12 (0.1): $P < 0.05$, α C12 (0.4) vs. C12 (0.2): $P < 0.05$. Data are means ± SEM (n = 13).

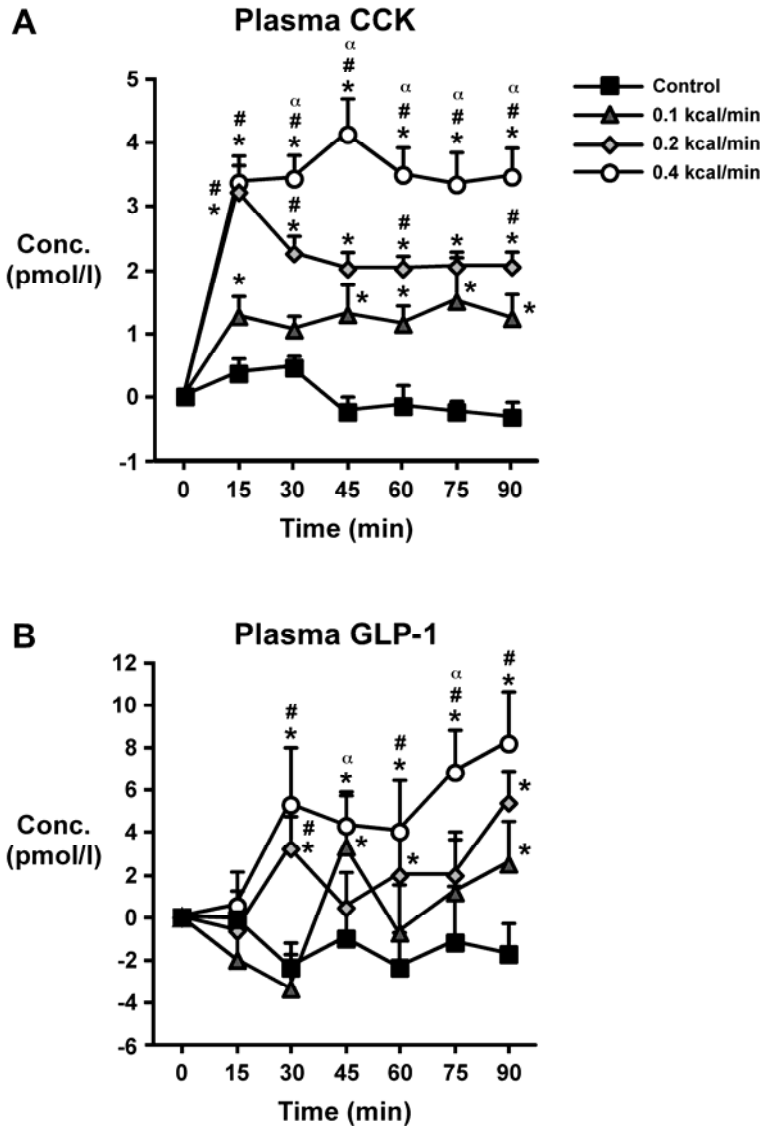

Figure 6.5: Plasma concentrations of (A) CCK and (B) GLP-1 during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. * C12 (0.4), C12 (0.2) and C12 (0.1) vs. control: $P < 0.05$, # C12 (0.4) and C12 (0.2) vs. C12 (0.1): $P < 0.05$, α C12 (0.4) vs. C12 (0.2): $P < 0.05$. Data are means ± SEM (n = 13).

Chapter 7

Effects of lauric acid on antropyloroduodenal motility, plasma CCK and PYY, appetite and energy intake are dependent on load, but not concentration

7.1. Abstract

Intraduodenal (ID) infusion of lauric acid (C12) dose-dependently inhibits antral and duodenal pressures, stimulates pyloric motility, modulates gut hormone release and reduces energy intake. Animal studies suggest that the effects of fatty acids on gastric emptying are dependent on both the load and concentration administered, while the effect of C12 on energy intake is load-dependent. Our recent studies suggest that, in humans, the suppression of energy intake by C12 may be concentration-dependent. At a constant load (0.4 kcal/min), ID infusion of C12 at 106 mM suppressed energy intake much more than infusion at 56 mM, although 106 mM is supraphysiological and induced nausea. It is, however, unclear whether the energy load, or the concentration, of C12 mediates these effects in humans. We hypothesised that the modulation of antropyloroduodenal (APD) pressure waves (PWs), the stimulation of CCK and PYY, and the suppression of energy intake by C12 is both load- and concentration-dependent in humans. 24 males (12 in each arm of the study) (age 18-36 yrs, BMI 19–25 kg/m^2) were studied on three occasions in double-blind, randomised fashion. APD PWs and plasma CCK and PYY concentrations were assessed during ID C12 infusion at (1) increasing loads (0.2, 0.3 or 0.4 kcal/min, all 56 mM) for 90 min or (2) increasing concentrations (40, 56 or 72 mM, all 0.4 kcal/min) for 60

min. Energy intake at a buffet-meal was quantified following the infusions. C12 stimulated plasma CCK ($r = 0.9$, $P < 0.0001$) and PYY ($r = 0.8$, $P < 0.0001$), and suppressed antral ($r = 0.6$, $P < 0.05$) and duodenal pressures ($r = 0.9$, $P < 0.001$) and energy intake ($r = 0.7$, $P < 0.001$) in a load-dependent manner. There were no effects of concentration on APD motility, plasma CCK and PYY, or energy intake. Nausea was not experienced during any of the infusions. In contrast to our hypothesis and animal data, the effects of intraduodenal C12 on APD motility and energy intake are load-dependent, and concentration-independent.

7.2. Introduction

In the study presented in Chapter 6 (Little *et al.*, 2005), intraduodenal infusions of lauric acid (C12) inhibited antral and duodenal contractions, stimulated pyloric motility, released cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), and suppressed food intake in a dose-related fashion when C12 was infused at loads of 0.1, 0.2, and 0.4 kcal/min, as 14, 28, and 56 mM solutions, respectively. A concentration as low as 14 mM (at 0.1 kcal/min) significantly stimulated pyloric pressure waves and the release of CCK, however, as the load and concentration of the solutions varied in parallel, we could not determine whether the load, or the concentration, administered mediated these responses. In an earlier study (Feltrin *et al.*, 2004), C12 infused at 106 mM (0.375 kcal/min) inhibited food intake twice as much, and released CCK three times as much, as the 0.4 kcal/min load of C12 at 56 mM (Little *et al.*, 2005). Because 106 mM is much greater than the postprandial concentrations of luminal fatty acids after a fatty meal (Borgstrom *et al.*, 1957; Porter *et al.*, 1971) and induced nausea, it is unclear whether we were observing physiological effects, and thus, whether the differences at 0.4 kcal/min between the 106 and 56 mM solutions reflected an effect of concentration, independent of load.

In animals, several stimuli in the small intestinal lumen have been reported to stimulate physiological responses in a load-dependent, but concentration-independent, fashion. For example, the stimulation of pancreatic bicarbonate secretion by acid (Meyer *et al.*, 1970a, 1970b); the stimulation of pancreatic enzyme secretion by oleic acid (Meyer and Jones, 1974); and the inhibition of food intake by luminal maltose, by oleic or lauric acids (Meyer *et al.*, 1998). In

contrast, other responses are concentration-dependent, but load-independent; for example, the stimulation of pancreatic bicarbonate secretion by oleic acid (Meyer and Jones, 1974). Furthermore, some responses show both load- and concentration-dependence; for example, the slowing of gastric emptying by oleic acid at 1.5 – 6 mM within a fixed segment of the proximal small intestine (Lin *et al.*, 1990).

We have, therefore, contrasted the effects of intraduodenal infusion of C12 at loads of 0.2, 0.3 or 0.4 kcal/min at a fixed concentration (56 mM) with the effects of C12 at concentrations of 40, 56 and 72 mM at a fixed energy load of 0.4 kcal/min) on antropyloroduodenal motility, plasma CCK and PYY concentrations, appetite perceptions and energy intake in healthy lean male subjects. We hypothesised that if fatty acid concentration (above 40 mM) was the dominant stimulus, we should see no response in the load study, but a definite concentration-dependent effect, whereas, if load dominated we would observe no response to concentration, but a definite load-dependent effect.

7.3. Methods

7.3.1. Subjects

A total of 24 healthy male subjects were studied, 12 subjects for each of the studies described in the protocols below; the number of subjects was based on power calculations derived from a previous study (Little *et al.*, 2005). We calculated that with 12 subjects we would observe a 15 % decrease in energy intake at $\alpha = 0.05$, with a power of 80 %. Subjects had a mean age of 23 ± 1 (range 18 - 36) years and were of normal body weight for their height (body mass index 22.3 ± 0.3 kg/m^2).

7.3.2. Study design

Each subject was studied on three occasions, separated by 3 - 10 days, to evaluate, in double-blind, randomised fashion the effects of intraduodenal infusion of C12 on APD motility, plasma CCK and PYY, appetite and energy intake. For this purpose, solutions were designed to deliver to the small intestine, (i) varying loads of C12 (0.2, 0.3 and 0.4 kcal/min) at a constant concentration (56 mM) (Study A: increasing loads of C12) or (ii) varying concentrations of C12 (40, 56 and 72 mM) at a constant load (0.4 kcal/min) (Study B: increasing concentrations of C12).

7.3.3. Preparation of C12 solutions

All solutions were prepared on the morning of the study; C12 was maintained in solution by heating it to 37ºC. The energy load of the solutions was based on calculations from a previous studies in humans, which showed that fatty acids empty from the stomach into the small intestine at ~ 0.2 – 0.4 kcal/min (Hunt and

Knox, 1968) and that intraduodenal loads of C12 at 0.1- 0.4 kcal/min for 90 min are well tolerated, in the absence of nausea (Little *et al.*, 2005). The concentrations of the solutions were selected to be within the range of fatty acid concentrations observed in the small intestine after triglyceride digestion, ~ 20 – 80 mM (Borgstrom et al, 1957). The pH of all solutions was 8.4.

7.3.3.1. Study A: Increasing loads of C12

C12 was delivered to the small intestine for 90 min at loads of (i) 0.2 kcal/min ("C12 (0.2)"; total energy: 18 kcal (75 kJ)), (ii) 0.3 kcal/min ("C12 (0.3)"; total energy: 27 kcal (112.5 kJ)) or (iii) 0.4 kcal/min ("C12 (0.4)"; total energy: 36 kcal (150 kJ)). All three solutions were prepared using 4.52 g of C12 (Sigma-Aldrich, Milwaukee, WI, USA) dissolved in 0.75 g of NaOH (Sigma-Aldrich, Louis, MO, USA) and 0.9 % saline, to a total volume of 400 ml. All solutions had a concentration of 56 mM and were infused at a rate of (i) 2 ml/min, (ii) 3ml/min or (iii) 4 ml/min, so that the total volume infused in 90 min was 180 ml, 270 ml and 360 ml, respectively.

7.3.3.2. Study B: Increasing concentrations of C12

C12 was delivered to the small intestine for 60 min at concentrations of (i) 40 mM ("C12 (40)"), (ii) 56 mM ("C12 (56)"); or (iii) 72 mM ("C12 (72)"). 4.8 g, 4.52 g and 3.6 g of C12 were dissolved in 0.67 g, 0.75 g and 0.45 g of NaOH, respectively, with 0.9 % saline, to a total volume of 600 ml, 400 ml and 250 ml respectively. Solutions were infused at a rate of (i) 5.7 ml/min (40 mM), (ii) 4.0 ml/min (56 mM) or (iii) 3.1 ml/min (72 mM), to deliver a load of 0.4 kcal/min of C12 into the small intestine, so that the total amount of C12 infused over 60 min

was 24 kcal (100 kJ) with all three solutions. We had originally planned to infuse these solutions for 90 min, in order to be directly comparable to the "C12 load" condition; however, infusion of the 40 mM C12 solution (5.7 ml/min) for 90 min, induced abdominal cramps or diarrhoea in some subjects and could only be tolerated for 60 min in the absence of these side-effects. This intolerability was only determined after completion of the "C12 load" condition, hence, the difference in the duration of the C12 infusions between the two study conditions.

7.3.4. Protocol

Subjects attended the laboratory at 0830 h after fasting from 2200 h the previous night from both solids and liquids. They were immediately intubated with a 16-channel manometric catheter. An intravenous cannula was also placed into a right forearm vein for blood sampling to determine plasma CCK and PYY concentrations. Once the catheter was positioned correctly, fasting motility was monitored until the occurrence of a phase III of the interdigestive migrating motor complex (MMC) (Cook *et al.*, 1997). Immediately after the cessation of phase III activity (at t = -15 min), a baseline venous blood sample was taken, and a visual analogue scale questionnaire (VAS) (Hill *et al.*, 1995; Parker *et al.*, 2004), assessing appetite-related sensations, nausea and bloating, was administered. At t = 0 min (i.e. during phase I of the MMC), the duodenal infusion of C12, at varying loads, but constant concentration (Study A), or varying concentrations, but constant loads (Study B), was commenced. APD pressures were monitored throughout the infusion period and blood samples were taken and VAS administered every 15 min from t = 0 – 90 min (Study A) or from t = 0 – 60 min (Study B). At t = 90 min (Study A), or t = 60 min (Study B) the

infusion was terminated, and the subject was immediately extubated and offered a cold buffet-style meal. The subject was given 30 min (i.e. t = 90 – 120 min (Study A), or t = 60 – 90 min (Study B) to consume the meal and instructed to eat until comfortably full. The intravenous cannula was then removed, and the subject was allowed to leave the laboratory.

7.3.5. Data and statistical analysis

Baseline ('0') values were calculated as the mean of values obtained at t = -15 and 0 min for VAS scores and plasma hormone concentrations, and between t = -15 to 0 min for basal pyloric pressures, number and amplitude of IPPWs, antral and duodenal PWs. The number and amplitude of antral and duodenal PWs were used to calculate motility indices (MI) using the following equation: MI (mmHg * number) = natural logarithm [(sum of amplitudes x number of contractions (PWs)) + 1] (Camilleri and Malagelada, 1984). Basal pyloric pressures and the number and amplitude of IPPWs were expressed as means over 15 min periods during the infusion. Numbers and amplitudes of antral and duodenal PWs were expressed as total number and mean values, respectively, during the infusion period. All data, except for amplitude of antral, pyloric and duodenal PWs and plasma CCK and PYY concentrations, were expressed as changes from baseline. The AUC of plasma CCK and PYY between t = 0 – 60 min was calculated using the trapezoidal rule. Total number of IPPWs, antral and duodenal PWs and the AUC of plasma CCK and PYY concentrations were calculated between t = 0 – 60 min for the 0.4 kcal/min infusion in Study A ("increasing loads of C12") to enable a comparison to be made with the total number of IPPWs and antral and duodenal PWs during the 56 mM infusion of Study B ("increasing concentrations

of C12") – as both infusions delivered the same load and concentration (0.4 kcal/min, 56 mM).

VAS scores, basal pyloric pressures, number and amplitude of IPPWs and plasma hormone concentrations were analysed by repeated measures analysis of variance (ANOVA), with time and treatment as within-subject factors. One-way ANOVA was used to assess the effect of treatment on energy intake, amount of food consumed, macronutrient distribution, total number, MI and mean amplitudes of antral and duodenal PWs. Post-hoc paired comparisons, corrected for multiple comparisons by Bonferroni's correction, were performed if ANOVA's revealed significance. To determine whether the effects of C12 were dependent upon the load, or the concentration administered, the slope of the linear regression of the sum of IPPWs, antral and duodenal MI, the AUC (t = 0 – 60 min) plasma CCK and PYY and energy intake, on the natural logarithm (ln) of the load, or concentration, for each individual was determined. T-tests were then performed to determine whether the average slope was greater than zero (Elashoff, 1981). Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

7.4. Results

All subjects completed the three randomised study days in both experimental conditions, and the study protocols were well tolerated.

7.4.1. Study A: Increasing loads of C12

7.4.1.1. Antropyloroduodenal pressures

7.4.1.1.1. Antral pressures

There was an effect of treatment on the number of antral PWs ($P < 0.01$). Both C12 (0.3) and C12 (0.4) reduced the number of antral PWs when compared with C12 (0.2) ($P < 0.01$), with no difference between C12 (0.3) and C12 (0.4) (**Table 7.1**). There was no difference in the amplitude of antral PWs between treatments. There was an effect of treatment on antral MI ($P < 0.01$). Both C12 (0.3) and C12 (0.4) decreased the MI when compared with C12 (0.2) ($P < 0.05$), with no differences between C12 (0.3) and C12 (0.4).

There was an inverse relationship between the MI of antral PWs with the load of C12 administered, such that the greater the load of C12, the lower the MI ($r = -0.66$, $P < 0.05$).

7.4.1.1.2. Pyloric pressures

Basal pyloric pressure (tone): There was no effect of treatment on basal pyloric pressure (data not shown). However, there was an effect of time ($P < 0.001$). C12 (0.2) increased basal pyloric pressure in the first 15 min when compared with baseline ($P < 0.05$), and both C12 (0.3) and C12 (0.4) increased basal pyloric pressure over the first 30 min of the infusion when compared with

baseline ($P < 0.05$). C12 (0.3) decreased basal pyloric pressure between t = 60 – 75 min when compared with baseline ($P < 0.05$).

There was no relationship between basal pyloric pressures with the load of C12 administered.

Phasic pressure: There was a treatment * time interaction for the number of IPPWs ($P < 0.01$). Both C12 (0.3) and C12 (0.4) increased the number of IPPWs when compared with C12 (0.2) between t = 0 – 30 min ($P < 0.01$), with no difference between C12 (0.3) and C12 (0.4) (**Figure 7.1A**). C12 (0.2) increased the number of IPPWs when compared with C12 (0.4) between t = 60 – 75 min (P 0.05), with no difference between C12 (0.2) and C12 (0.3). C12 (0.3) increased the number of IPPWs when compared with C12 (0.4) between t = 75 – 90 min (P < 0.01), with no differences between C12 (0.2) and (0.4). All C12 infusions increased the number of IPPWs throughout the 90 min infusion period compared with baseline ($P < 0.05$, for all). There was no effect of treatment on the amplitude of IPPWs, however, all treatments increased the amplitude of IPPWs throughout the 90 min infusion period when compared with baseline ($P < 0.05$).

There was a strong trend for a positive relationship between the total number of IPPWs with the load of C12 administered, such that the greater the load of C12, the greater the number of IPPWs ($r = 0.7$, $P = 0.06$).

7.4.1.1.3. Duodenal pressures

There was an effect of treatment on the number of duodenal PWs ($P < 0.001$). C12 (0.3) decreased the number of duodenal PWs when compared with C12 (0.2) ($P < 0.01$) (**Table 7.1**). C12 (0.4) decreased the number of duodenal PWs when compared with C12 (0.2) ($P < 0.01$) and C12 (0.3) ($P < 0.05$). C12 (0.3) decreased the number of duodenal PWs when compared with C12 (0.2) ($P < 0.01$). There was no effect of treatment on the amplitude of duodenal PWs. There was an effect of treatment on the MI of duodenal PWs ($P < 0.01$). Both C12 (0.3) and C12 (0.4) decreased duodenal MI when compared with C12 (0.2) ($P < 0.05$), with no differences between C12 (0.4) and C12 (0.3).

There was an inverse relationship between the MI of duodenal PWs with the load of C12 administered, such that the greater the load of C12, the lower the MI ($r = -0.7$, $P < 0.001$).

7.4.1.2. Plasma CCK and PYY concentrations

7.4.1.2.1. CCK

Baseline CCK concentrations did not differ among study days. There was a treatment * time interaction for plasma CCK concentrations ($P < 0.001$) (**Figure 7.2A**). Both C12 (0.3) and C12 (0.4) increased plasma CCK concentrations between t = 15 – 90 min when compared with C12 (0.2) ($P < 0.05$), and C12 (0.4) increased plasma CCK when compared with C12 (0.3) at t = 45 min ($P < 0.01$). There was also an effect of time for plasma CCK concentrations ($P < 0.001$). All treatments increased plasma CCK between t = 15 – 90 min when compared with baseline ($P < 0.001$).

There was a positive relationship between the AUC for plasma CCK concentrations with the load of C12 administered, such that the greater the load of C12, the greater the plasma CCK concentration ($r = 0.9$, $P < 0.0001$).

7.4.1.2.2. PYY

Baseline plasma PYY concentrations did not differ among study days. There was a treatment * time interaction for plasma PYY concentrations ($P < 0.001$) (**Figure 7.3A**). Both C12 (0.3) and C12 (0.4) increased plasma PYY concentrations when compared with C12 (0.2) between t = 30 – 90 min ($P < 0.01$), and C12 (0.4) increased plasma PYY when compared with C12 (0.3) at t = 30, 45 and 90 min ($P < 0.05$). There was also an effect of time for PYY concentrations ($P < 0.001$). C12 (0.2) increased plasma PYY concentrations between t = 45 – 90 min when compared with baseline ($P < 0.001$) and both C12 (0.3) and C12 (0.4) increased plasma PYY between t = 30 – 90 min, when compared with baseline ($P < 0.01$).

There was a positive relationship between the AUC for plasma PYY concentrations with the load of C12 administered, such that the greater the load of C12, the greater the plasma PYY concentration ($r = 0.8$, $P < 0.0001$).

7.4.1.3. VAS scores and energy intake

There was no effect of treatment on perceptions of hunger, fullness, nausea or bloating. There was an effect of treatment on energy intake (kJ) ($P < 0.01$) (**Table 7.2**). C12 (0.4) decreased energy intake when compared with C12 (0.3)

and C12 (0.2) ($P < 0.01$), with no differences between C12 (0.2) and C12 (0.3). There was no effect of treatment on the macronutrient composition of the food consumed, hence, the reduction in energy intake was due to an equal reduction in all three macronutrients.

There was an inverse relationship between energy intake with the load of C12 administered, such that the greater the load of C12, the lower the energy intake ($r = 0.7$, $P < 0.05$).

7.4.2. Study B: Increasing concentrations of C12

7.4.2.1. Antropyloroduodenal pressures

7.4.2.1.1. Antral pressures

There was no effect of treatment on the number, amplitude or MI of antral PWs (**Table 7.1**).

7.4.2.1.2. Pyloric pressures

Basal pyloric pressure (tone): There was no effect of treatment on basal pyloric pressure, however, there was an effect of time ($P < 0.001$) (data not shown). Both C12 (40) and C12 (56) increased basal pyloric pressure between t = 15 – 60 min, and C12 (72) between t = 15 – 45 min, when compared with baseline ($P < 0.05$).

Phasic pressure: There was no effect of treatment on the number, or amplitude, of IPPWs, however there was an effect of time ($P < 0.001$) (**Figure 7.1B**). All three

treatments increased the number and amplitude of IPPWs between t = 15 – 60 min, when compared with baseline (P < 0.001).

7.4.2.1.3. Duodenal pressures

There was no effect of treatment on the number, amplitude or MI of duodenal PWs (**Table 7.1**).

7.4.2.2. Plasma CCK and PYY concentrations

7.4.2.2.1. CCK

Baseline plasma CCK concentrations did not differ between study days. There was no effect of treatment of plasma CCK concentrations, however, there was an effect of time (P < 0.001) (**Figure 7.2B**). All three treatments increased plasma CCK between t = 15 – 60 min, when compared with baseline (P < 0.001).

7.4.2.2.2. PYY

Baseline plasma PYY concentrations did not differ between study days. There was no effect of treatment on plasma PYY, however, there was an effect of time (P < 0.001) (**Figure 7.3B**). C12 (56) increased plasma PYY between t = 30 – 60 min (P < 0.001), and both C12 (40) and C12 (72) between t = 45 – 60 min (P < 0.001), when compared with baseline.

7.4.2.3. Appetite perceptions and energy intake

There was no effect of treatment on scores for hunger, fullness nausea or bloating. There was an effect of time on scores for bloating (P < 0.05). C12 (40) increased scores for bloating between t = 15 – 30 min (P < 0.01) compared with

baseline, while C12 (56) and C12 (72) had no effect, i.e. the effect of C12 (40) may have been related to the larger volume administered (data not shown). There was no effect of treatment on energy intake (kJ) or the macronutrient composition of the food consumed (**Table 7.2**).

There were no relationships between antropyloroduodenal motility responses, plasma CCK and PYY, or energy intake, with concentration administered.

7.4.3. Comparison between conditions ("C12 load" vs. "C12 concentration")

There were no differences in the total number of IPPWs, antral and duodenal PWs (between t = 0 – 60 min) or plasma CCK and PYY concentrations (t = 60 min) between the 0.4 kcal/min infusion of the "C12 load" and the 56 mM infusion of the "C12 concentration" studies. There was no difference in energy intake following 0.4 kcal/min infusion of the "C12 load" and the 56 mM infusion of the "C12 concentration", even though the 0.4 kcal/min infusion of the "C12 load" study delivered an extra 12 kcal, when compared with the 56 mM infusion of the "C12 concentration" study.

7.5. Discussion

This study has demonstrated that load, but not concentration, mediates the effects of intraduodenal C12 on APD motility, plasma CCK and PYY concentrations and energy intake. Specifically, the greater the load of C12, the greater the (i) suppression of antral and duodenal pressure waves, (ii) the stimulation of isolated pyloric pressure waves, (iii) the stimulation of CCK and PYY secretion and (iv) the suppression of energy intake. In contrast, we observed no significant differences in these parameters when the load was fixed at 0.4 kcal/min, but the concentration was altered from 40 – 72 mM.

While this study was the first to directly compare the effects of variations in load and concentration of intraduodenal C12 in humans, previous studies in animals had reported that the effects of fatty acids on gastrointestinal function and energy intake are dependent on both the load and concentration of fatty acids administered (Li *et al.*, 1990; Lin *et al.*, 1990; Meyer *et al.*, 1998; Meyer and Jones, 1974). For example, the stimulation of pancreatic secretion by acid (Meyer *et al.*, 1970b) and oleic acid (Meyer and Jones, 1974), the inhibition of gastric emptying by glucose (Lin *et al.*, 1989), and the inhibition of energy intake by oleic or lauric acids (Meyer *et al.*, 1998) have been shown to be dependent on the load, but not the concentration administered. In each case, load-dependence was related to the length of small intestine exposed to nutrient. This is also likely to be the case for the current observations. Previously, Pilichiewicz *et al.* had demonstrated that low loads of lipid, which would be expected to be rapidly absorbed in the proximal small intestine, stimulate the release of CCK from the proximal small intestine, whereas, as the load of lipid increased, and presumably

a greater length of small intestine was exposed to nutrient, PYY was much more potently released by fat locally in the distal small intestine than in the jejunum (Pilichiewicz *et al.*, 2006). A similar pattern of CCK and PYY secretion was observed in the current study, suggesting that increasing loads of C12 may exceed the absorptive capacity of the proximal small intestine, resulting in more C12 reaching the distal small intestine to stimulate the localised release of PYY from the distal "L cells". The initial secretion of PYY in response to C12 may be attributable to the stimulation of CCK and the subsequent response to the exposure of the distal small intestine to lipid, as CCK has previously been implicated in the secretion of PYY in dogs (Lin *et al.*, 2000).

In humans, the average postprandial concentration of fatty acids in the small intestine ranges from ~ 29 – 67 mM following consumption of a high-fat meal. (Ament *et al.*, 1972; Borgstrom *et al.*, 1957; Porter *et al.*, 1971). Since these studies were performed, studies in animals (Meyer *et al.*, 1994) and humans (Meyer *et al.*, 1996) have demonstrated that the rate of gastric emptying of dietary fat, and thus the rate of release of free fatty acids, varies with the amount, as well as the physical properties, of the ingested fat. The above estimates of luminal fatty acid concentrations were, thus, likely dependent on the composition of the test-meals. We were unable to demonstrate any concentration-dependent effects of C12 when infused at these concentrations. This observation was unexpected, as previous studies in our laboratory had indirectly provided evidence that C12 may have concentration-dependent effects. – The effects of intraduodenal C12 administered at a constant rate of 0.4 kcal/min on antropyloroduodenal motility, plasma CCK and GLP-1 concentrations and

energy intake were far more marked when infused at 106 mM (Feltrin *et al.*, 2004), than at 56 mM (Little *et al.*, 2005) (Chapter 6). For example, while infusion of C12 at 106 mM decreased energy intake by ~2800 kJ (Feltrin *et al.*, 2004), C12 at 56 mM reduced energy intake by ~ 900 kJ (Little *et al.*, 2005). However, C12 at 106 mM induced nausea in some of the subjects, and is much greater than the physiological postprandial concentrations observed in the small intestinal lumen (Borgstrom *et al.*, 1957). While we were unable to demonstrate concentration-dependent effects of C12 in the current study, it is possible that the concentrations used were within too narrow a range to detect differences in gastrointestinal function and energy intake. Furthermore, it is possible that once C12 is infused at a threshold rate of 0.4 kcal/min, gastrointestinal and energy intake responses are maximal, thus eliminating the ability to observe concentration-dependent effects at this energy load. It is likely that the concentrations of C12 used in the current study are atypical of normal postprandial luminal concentrations. It should be noted that while lauric acid is contained in a variety of foods, including coconut milk (up to ~ 50 %), butter (~ 5 - 8 %) and beef, it, at least in most cases, does not represent a major component of our total energy intake. – On the basis of compositions of stored bodily fatty acids, it is estimated that C12 represents ≤ 6 % of dietary fatty acids. Breast milk, which contains up to 20 % of lauric acid, is only part of the human diet in early life. However, as previous studies reported that the effects of C12 and the most prevalent dietary fatty acid, oleic acid (C18), exerted similar load-dependent effects on food intake in rats (Meyer *et al.*, 1998), we speculate that the current observations can be generalised to all fatty acids with a chain-length ≥ 12 carbon atoms.

While we cannot directly compare the two studies, due to the differences in the duration of the infusions, the infusion of 56 mM (0.4 kcal/min) for 60 minutes in Study B ("increasing concentrations of C12") was sufficient to induce changes in gastrointestinal motility and plasma CCK and PYY concentrations of a similar magnitude as the 0.4 kcal/min load at 56 mM infused for 90 minutes in Study A ("increasing loads of C12"). When the total number of IPPWs and antral and duodenal PWs were determined in the first 60 min for the C12 (0.4) infusion and were compared with C12 (56) infusion there was no difference between the effects. Furthermore, mean energy intakes from the buffet meal following the C12 (0.4) and C12 (56) infusions were very similar, ~ 4510 kJ and ~ 4482, respectively, despite the C12 (0.4) infusion delivering ~ 30 % more energy (~ 12 kcal). These comparisons show that infusion of C12 at 0.4 kcal/min at 56 mM, in the different arms of the study, had comparable effects on APD motility, plasma CCK and PYY concentrations and energy intake. It is, therefore, doubtful that infusing for a further 30 minutes in the "C12 concentration" condition would have enabled us to demonstrate a concentration-dependent effect.

In conclusion, the current study has demonstrated that the effects of C12 on antropyloroduodenal motility, plasma CCK and PYY concentrations and energy intake are dependent upon the energy load, but not the concentration, administered.

Table 7.1: Total number, mean amplitude and motility index (MI) of antral and duodenal pressure waves (PWs) during intraduodenal infusion of C12 at *increasing loads* (0.2, 0.3 or 0.4 kcal/min, all 56 mM) for 90 min or *increasing concentrations* (40, 56 or 72 mM, all 0.4 kcal/min) for 60 min.

	C12 Load (kcal/min)			**C12 concentration (mM)**		
	0.2	**0.3**	**0.4**	**40**	**56**	*72*
Antral						
Number	60. 8 ± 20.3	11.1 ± 4.2*	11.4 ± 6.1*	1.1 ± 3.9	5.3 ± 3.2	1.1 ± 4.5
Amplitude	18.2 ± 3.9	12 ± 1.8	10.1 ± 3.6	14.4 ± 4	15 ± 3	15 ± 3.1
MI	5.5 ± 0.9	3.7 ± 0.7*	2.7 ± 0.9*	3.5 ± 0.6	4.1 ± 0.6	4.5 ± 0.9
Duodenal						
Number	733.9 ± 70.9	530.9 ± 77.5*	361.9 ± 47.7#	318.2 ± 44.4	301.6 ± 64.7	310.6 ± 66.7
Amplitude	25.1 ± 0.6	25.5 ± 1.4	22.9 ± 1.9	24.9 ± 1.4	23.8 ± 1.4	25.3 ± 1.5
MI	9.8 ± 0.1	9.2 ± 0.3*	8.9 ± 0.2*	8.9 ± 0.2	8.8 ± 0.3	8.8 ± 0.3

Data are mean ± SEM (n = 12). * vs. C12 (0.2), $P < 0.05$; # vs. C12 (0.2) and C12 (0.3), $P < 0.05$.

Table 7.2: Energy intake from the buffet meal, and macronutrient distribution, in response to intraduodenal infusion of C12 at *increasing loads* (0.2, 0.3 or 0.4 kcal/min, all 56 mM) for 90 min or *increasing concentrations* (40, 56 or 72 mM, all 0.4 kcal/min) for 60 min.

	C12 Load (kcal/min)			**C12 concentration (mM)**		
	0.2	**0.3**	**0.4**	**40**	**56**	**72**
Energy intake, kJ	5610 ± 545	5488 ± 271	4510 ± 470*	4946 ± 397	4483 ± 446	4398 ± 359
Energy (%)						
Fat	31 ± 1	33 ± 2	32 ± 1	33 ± 2	32 ± 2	33 ± 2
CHO	48 ±2	46 ± 2	47 ± 2	45 ± 2	46 ± 2	45 ± 3
Protein	22 ± 1	21 ± 1	21 ± 1	23 ± 1	22 ± 1	22 ± 1

Data are mean ± SEM (n = 12). * vs. C12 (0.3) and C12 (0.2), $P < 0.01$. CHO = carbohydrate.

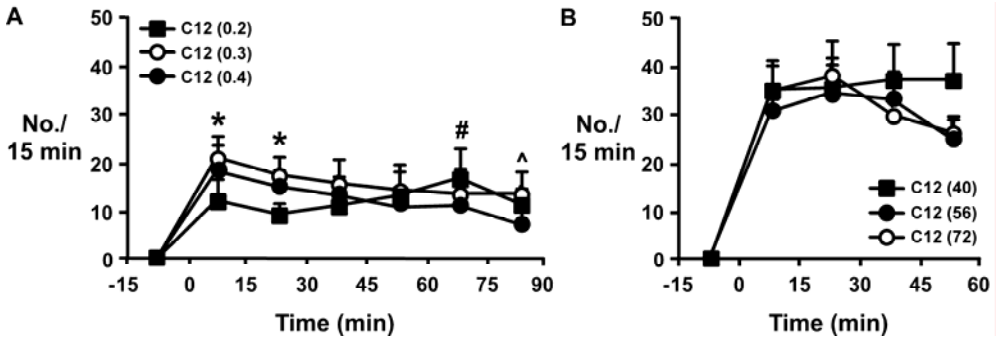

Figure 7.1: Number of isolated pyloric pressure waves during intraduodenal infusion of C12 at (A) *increasing loads* (0.2, 0.3 or 0.4 kcal/min, all 56 mM) for 90 min or (B) *increasing concentrations* (40, 56 or 72 mM, all 0.4 kcal/min) for 60 min. * C12 (0.4) and (0.3) vs. C12 (0.2), $P < 0.01$, ^ C12 (0.2) vs. C12 (0.4), $P < 0.05$, # C12 (0.3) vs. C12 (0.4), $P < 0.01$.

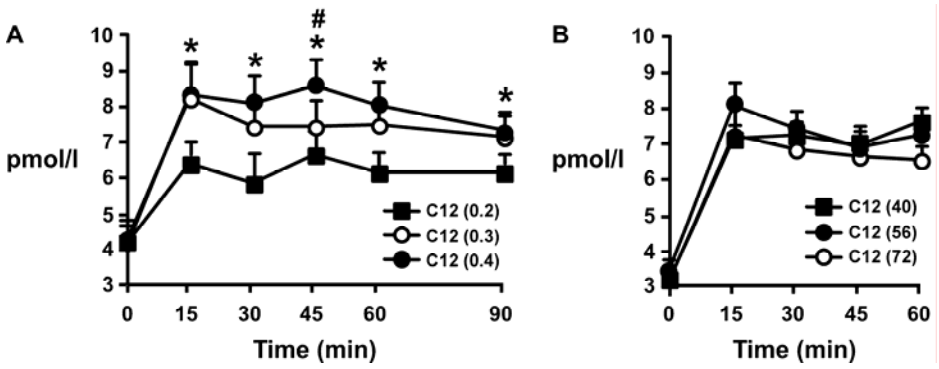

Figure 7.2: Plasma CCK concentrations during intraduodenal infusion of C12 at (A) *increasing loads* (0.2, 0.3 or 0.4 kcal/min, all 56 mM) for 90 min or (B) *increasing concentrations* (40, 56 or 72 mM, all 0.4 kcal/min) for 60 min. * C12 (0.4) and C12 (0.3) vs. C12 (0.2), $P < 0.05$, # C12 (0.4) vs. C12 (0.3), $P < 0.01$.

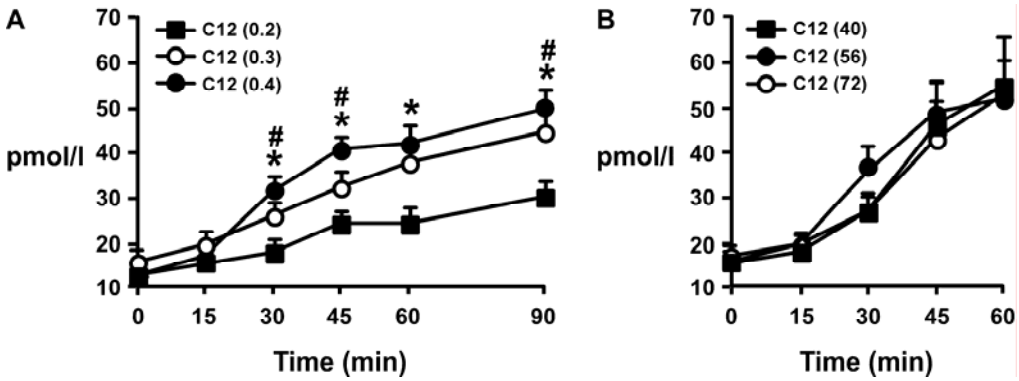

Figure 7.3: Plasma PYY concentrations during intraduodenal infusion of C12 at (A) *increasing loads* (0.2, 0.3 or 0.4 kcal/min, all 56 mM) for 90 min or (B) *increasing concentrations* (40, 56 or 72 mM, all 0.4 kcal/min) for 60 min. * C12 (0.4) and C12 (0.3) vs. C12 (0.2), $P < 0.01$, # C12 (0.4) vs. C12 (0.3), $P < 0.05$.

Chapter 8

The release of GLP-1 and ghrelin, but not GIP and CCK, by glucose is dependent upon the length of small intestine exposed

8.1. Abstract

Previous observations suggest that glucagon-like peptide-1 (GLP-1) is released into the bloodstream only when dietary carbohydrate enters the duodenum at rates that exceed the absorptive capacity of the proximal small intestine to contact GLP-1 bearing mucosa in more distal bowel. The aims of this study were to determine the effects of modifying the length of small intestine exposed to glucose on plasma concentrations of GLP-1, and also glucose-dependent insulinotropic peptide (GIP), insulin, cholecystokinin (CCK) and ghrelin, and antropyloric pressures. Glucose was infused at 3.5 kcal/min into the duodenum of eight healthy males (age: 18 - 59 years) over 60 min – on the first day into an isolated 60 cm segment of the proximal small intestine ("short segment infusion"); on the second day, the same amount of glucose was infused with access to the entire small intestine ("long segment infusion"). Plasma GLP-1 increased and ghrelin decreased ($P < 0.05$ for both) during the "long", but not the "short", segment infusion. By contrast, increases in plasma CCK and GIP did not differ between days. The rises in blood glucose and plasma insulin were greater during the "long", when compared with the "short", segment infusion ($P < 0.05$). During the "long", but not the "short", segment infusion antral PWs were suppressed ($P < 0.05$). Isolated pyloric pressure waves and basal pyloric

pressure were stimulated on both days. In conclusion, the release of GLP-1 and ghrelin, but not CCK and GIP, is dependent upon greater than 60 cm of the intestine being exposed to glucose.

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8.2. Introduction

The known regulatory gut peptides have differing distributions along the small intestine. For example, GIP is secreted from endocrine K cells (Fehmann *et al.*, 1995) and CCK by endocrine I cells (Rehfeld, 1978) in the proximal small intestine, whereas PYY and GLP-1 are secreted from endocrine L cells in the distal small intestine (Eissele *et al.*, 1992; Onaga *et al.*, 2002). Whether these peptides are released primarily, or exclusively, by direct local contact with specific nutrients in their regions of storage, or are released reflexively from the distal small intestine by signals arising from the proximal small intestine, or vice versa, is still debatable. For example, in rats, intravenous infusion of GIP has been shown to stimulate the secretion of GLP-1 (Roberge and Brubaker, 1993). In dogs, Lin *et al.* demonstrated that CCK was released equipotently whether fat contacted the proximal, or distal, small intestine (Lin and Chey, 2003) and that the release of PYY was dependent, at least in part, on the stimulation of CCK by fat confined to proximal small intestine (Lin *et al.*, 2000). On the other hand, Pilichiewicz et al. concluded recently that in humans CCK was released only by fat in the proximal small intestine and PYY was much more potently released by fat locally in the distal small intestine than in the jejunum (Pilichiewicz *et al.*, 2006). These last observations suggest that the nutrient-stimulated release of gut peptides in humans may differ substantially from the patterns observed in experimental animals.

Because the “incretin” peptides, GIP and GLP-1, play an important role in glucose homeostasis, both in health (Nauck *et al.*, 1997) and diabetes (Meier *et al.*, 2003), it is important to establish how the release of each peptide is

controlled by luminal glucose; specifically, whether in humans, GLP-1 is released only by local, distal jejunal, or ileal contact with glucose when dietary carbohydrates empty from the stomach at rates high enough to overcome the absorptive capacity of the proximal jejunum and thus to contact more distal small intestine. The observations of Schirra *et al.* (1996) strongly suggest that in humans, as opposed to rats, the release of GLP-1 is dependent on direct local contact of glucose with endocrine L cells. When human volunteers ingested 50 or 100 grams of glucose in 400 ml of water, rates of gastric emptying of glucose in the first post-cibal hour ranged, respectively, from 3.0 - 1.5 kcal/min and from 4.5 - 3.0 kcal/min. In that first post-cibal hour, plasma concentrations of GLP-1 were more than twice as high after the 100 g, when compared with the 50 g, glucose load, whereas plasma concentrations of GIP were similar after the two loads for most of that time (Schirra *et al.*, 1996). Thus, Schirra's observations are consistent with the idea that in humans, GLP-1 is released only by local contact of glucose with GLP-1 bearing L cells in the distal jejunum and ileum when the loads of glucose entering the duodenum exceed the absorptive capacities of the proximal gut. Yet, to date, this hypothesis has not been directly tested.

The major aim of the present study was to compare the effect of limiting glucose to the proximal 60 cm ("short segment infusion") of the small intestine during duodenal infusion with the effect of allowing access of this duodenal load to the jejunum beyond 60 cm ("long segment infusion"). Limiting glucose to the "short (0-60 cm) segment" was accomplished by inflating an occluding balloon 60 cm distal to the pylorus and draining glucose from the segment from an aspiration

port just proximal to the balloon. On the "long segment" day, the blocking balloon was inflated and glucose was infused beyond 60 cm at a rate determined by the rate of glucose recovery from the distal end of the 60 cm segment on the day of the "short segment infusion". On the basis of Schirra's observations, we postulated that release of GIP would be similar whether the "short", or "long", segments were perfused, whereas GLP-1 secretion would be observed only during the "long segment infusion".

The secretion of the orexigenic peptide ghrelin, by the oxyntic mucosa of the gastric fundus, is suppressed when nutrients are ingested (Overduin *et al.*, 2005; Parker *et al.*, 2005). In animals, this suppression is dependent upon exposure of the distal small intestine to nutrients (Overduin *et al.*, 2005). In humans, the relatively prolonged time required for the suppression of plasma ghrelin by enterally administered glucose (Parker *et al.*, 2005) and fat (Feinle-Bisset *et al.*, 2005), as well as a marked suppression of ghrelin observed following Roux-en-Y gastric bypass (Cummings and Shannon, 2003; Korner *et al.*, 2005), which shunts nutrients to the distal bowel, suggest that exposure of the distal intestine is required. Our "short" vs. "long" segment design afforded the opportunity to determine whether confining glucose to 60 cm of the proximal small bowel would blunt the suppression of ghrelin by glucose.

Since we aimed to identify regional effects of glucose based on the distribution of the two "incretin" peptides along the small intestine, the ideal experimental design for this purpose would have been to compare the effects of glucose confined to the proximal 0-60 cm, with glucose confined to 60-120 cm, of the

small intestine. However, as we found that we could not consistently, or easily, intubate the full 120 cm of the small intestine in the six, or so, hours that ambulatory volunteers were willing to give to this study on each day, we compromised to use only a 0-60 cm intubation. This comparison allowed us to look at the effect of extending the length of glucose contact with sensors along the small intestine on antral and pyloric motility. In dogs, glucose has been demonstrated to increasingly inhibit gastric emptying of liquid meals as the length of contact was extended from 15, to 60, to 150 cm of the small intestine (Lin *et al.*, 1989). This inhibition was maximal when 150 cm of the proximal small intestine was contacted; and maximal inhibition was not significantly different whether the proximal 150 cm (jejunum), or the distal 150 cm (ileum), were contacted (Lin *et al.*, 1989). Thus, the inhibition of gastric emptying of a liquid meal by intestinal glucose appeared to be length-, but not region-, dependent. While we did not measure gastric emptying in the present experiment, we were able to determine whether the effects of luminal glucose on antral and pyloric motility were maximal during the “short segment infusion” or were increased by lengthening glucose contact with the small intestine during the “long segment infusion”.

8.3. Subjects and methods

8.3.1. Subjects

Eight healthy males with a mean age of 30.4 ± 13.7 (range 18 - 59) years and with a mean body mass index of 26.2 ± 3.0 (range 22 – 32) kg/m^2 were studied.

8.3.2. Study design

Each subject was studied on two occasions separated by 3 – 10 days in single-blind, non-randomised fashion. This non-randomised study design was required in order to determine the rate at which glucose should be infused into the distal small intestine during the second, "long segment" study (a rate equal to the amount of glucose aspirated from the distal end of the proximal segment during the "short segment" study). On both days subjects received an infusion of glucose into the small intestine over 60 min – on the first day glucose was infused into an isolated 60 cm segment of the proximal small intestine ("short segment infusion") and on the other, the same amount of glucose was infused with access to the entire small intestine ("long segment infusion").

8.3.3. Protocol

Subjects attended the Department of Medicine at 0830 h following an overnight fast (14 hours for solids, 12 hours for liquids). On each day, a 7-channel manometric assembly (outer diameter: 4.5 mm, Dentsleeve, Adelaide, South Australia) was inserted into the stomach via an anesthetised nostril (or orally if nasoduodenal intubation was not tolerated [3 of 8 subjects]) and allowed to pass into the small intestine by peristalsis. An intravenous cannula was placed into a right antecubital vein for blood sampling.

Following correct positioning of the catheter, the balloon was slowly inflated with air to a volume of ~ 30 - 40 ml. In all cases balloon inflation ceased when subjects reported a sensation of pressure, without discomfort. Intra-balloon pressure (~ 20 mmHg) was monitored continuously throughout each study using a pressure gauge. A previous study had validated this technique by achieving complete recovery of the non-absorbable marker, polyethylene glycol 4000 (PEG 4000), from the isolated segment, i.e. total occlusion of the small intestine was achieved (von Richter *et al.*, 2001). "Baseline" blood samples were collected at t = - 5 and 0 min and baseline antropyloric pressures recorded between t = - 10 - 0 min. At t = 0 min, intraduodenal infusion of 1 M glucose (4.9 ml/min, 3.5 kcal/min) was commenced in a single-blind, non-randomised fashion. Thus, on the first study day ("short segment infusion") 1 M glucose was always infused into the isolated 60 cm segment of the proximal small intestine. During the infusion, the intestinal perfusate was continuously aspirated, by gentle suction using a hand-held syringe, from this segment via the aspiration port. The aspirate collected over each 10 min period was weighed and the glucose concentration determined. Between t = 10 - 60 min, the small intestine distal to the balloon was infused with 0.9 % saline at a rate corresponding to the weight of the aspirate collected from the proximal segment in the preceding 10 min interval, i.e. the volume infused into the distal small intestine approximated that removed from the proximal segment. On the second study day ("long segment infusion"), 1 M glucose was infused into the isolated proximal segment of the small intestine at the same rate as on the first day, while glucose was infused into the distal small intestine. The concentration of glucose for the distal infusion was calculated

from the mean glucose concentration of the aspirate recovered on the first study day, i.e. the amount of glucose not absorbed from the proximal segment, and was infused according to the weight of the aspirate collected in the preceding 10 min (i.e. at each successive 10 min period on the second, "long segment" day) (**Table 8.1**), as described for day 1. Venous blood samples were obtained at t = 5, 10, 15, 20, 30, 45 and 60 min for the determination of blood glucose and plasma insulin, GIP, GLP-1, CCK and ghrelin concentrations. At t = 60 min, the infusion was ceased, the subject extubated and the intravenous cannula removed. The subject was then free to leave the laboratory.

8.3.4. Preparation of glucose solutions

The 1 M glucose solution was prepared by dissolving 90 g of glucose in water, made up to a total volume of 500 ml. The glucose infusion rate of 210 kcal/h (3.5 kcal/min) is somewhat less than the initial rate of gastric emptying for the first 30 min following a glucose meal of moderate to high volume and energy density, but greater than the slower, steady rates of gastric emptying (Brener *et al.*, 1983; Hunt *et al.*, 1985).

8.3.5. Data and statistical analysis

Baseline values were calculated as the mean of values obtained at t = -5 and t = 0 min for blood glucose and plasma hormone concentrations. Blood glucose and plasma insulin, GIP, GLP-1 and CCK concentrations were expressed as raw data, while data for plasma ghrelin were expressed as changes from baseline due to the substantial variation in fasting concentrations (probably resulting from variable times for tube positioning). Baseline values for the number and amplitude of

antral pressure waves and isolated pyloric pressure waves (IPPWs) and basal pyloric pressure were calculated as the mean of values obtained between t = -10 - 0 min. Data were expressed as mean values over 10 min intervals during the 60 min infusion period, and analysed as changes from baseline.

Areas under the curve (AUC) were calculated, using the trapezoidal rule, for the magnitude of the increase in blood glucose, plasma insulin, GIP, GLP-1 and CCK and the decrease in plasma ghrelin between t = 0 - 60 min. Blood glucose, plasma insulin, GIP, GLP-1, CCK and ghrelin concentrations, antral PWs, basal pyloric pressures and IPPWs were analysed by repeated-measures analysis of variance (ANOVA), with time and treatment as factors. The AUCs for plasma variables were analysed by one-way ANOVA. Post-hoc paired comparisons, corrected for multiple comparisons by Bonferroni's correction, were performed if ANOVAs revealed significant effects. Correlations, corrected for repeated measures, were performed to determine the relationship between the amount of glucose (kcal/h) infused into the "distal segment" with the AUCs of plasma GLP-1 and ghrelin. Statistical significance was accepted at $P < 0.05$, and data are presented as mean values ± SEM.

8.4. Results

The study protocol was tolerated moderately well by all subjects. The average time taken from intubation to the commencement of the infusion was 3.5 ± 2.4 (range 1 - 8) hours. The amount of glucose infused on the two study days for each subject was variable due to the aspiration process, i.e. it was dependent on the amount of glucose recovered from the proximal segment on each day. **Figure 8.1** shows the amount of glucose (kcal) recovered per 10 min from the proximal 60 cm segment, **Table 8.1** shows the absolute amount of glucose infused into both the proximal and distal segments, during the "short", and "long", segment infusions, respectively. While glucose was infused at the average concentration of the aspirate recovered on the "short segment" day, the rate of the distal glucose infusion was determined by the volume of glucose recovered from the proximal segment on the "long segment" day. Hence, given that the volume of glucose absorbed from the proximal segment was greater on this day, only an average of 60 % of the glucose recovered from the proximal segment during the "short segment infusion" was infused distally during the "long segment infusion".

8.4.1. Blood glucose

There was a treatment * time interaction ($P < 0.05$) and an effect of time ($P < 0.001$), but no effect of treatment, for blood glucose (**Figure 8.2A**). While blood glucose increased progressively on both days between t = 15 - 60 min ($P < 0.05$), the magnitude of the rise was greater during the "long", when compared with the "short", segment infusion between t = 30 - 60 min ($P < 0.05$). There was no effect of treatment on the AUC for blood glucose.

8.4.2. Insulin

There was a treatment * time interaction ($P < 0.001$) and an effect of time ($P < 0.001$), but no effect of treatment, for plasma insulin concentrations (**Figure 8.2B**). While plasma insulin concentrations increased progressively from baseline during both treatments between t = 20 - 60 min ($P < 0.05$), the magnitude of the rise was greater during the "long", when compared with the "short", segment infusion, between t = 30 - 60 min ($P < 0.05$). There was a strong trend for the AUC for plasma insulin to be greater during the "long", when compared with the "short", segment infusion ($P = 0.08$).

8.4.3. Glucose-dependent insulinotropic peptide (GIP)

There was an effect of time ($P < 0.001$), but no effect of treatment and no treatment * time interaction, for plasma GIP concentrations (**Figure 8.2C**). During both conditions, plasma GIP increased rapidly from baseline at t = 10 min ($P < 0.001$) and plateaued at approximately 30 min. There was no effect of treatment on the AUC for plasma GIP.

8.4.4. Glucagon-like peptide-1 (GLP-1)

There was a treatment * time interaction ($P < 0.001$) and an effect of time ($P < 0.001$), but no effect of treatment, for plasma GLP-1 concentrations (**Figure 8.2D**). During the "short segment infusion" plasma GLP-1 did not change from baseline, while during the "long segment infusion", plasma levels of GLP-1 increased progressively from baseline between t = 30 - 60 min ($P < 0.01$). At t = 20 min and between t = 40 - 60 min, plasma GLP-1 was greater during the

"long", when compared with the "short", segment infusion ($P < 0.01$). There was a strong trend for the AUC for plasma GLP-1 to be greater during the "long", when compared with the "short", segment infusion ($P = 0.06$). There was no relationship between the amount of glucose (kcal/h) infused distal to the balloon and the AUC for plasma GLP-1 ($r = 0.38$, $P = 0.1$).

8.4.5. Cholecystokinin (CCK)

There was an effect of time ($P < 0.001$), but no effect of treatment and no treatment * time interaction, for plasma CCK concentrations (**Figure 8.3A**). On both days, plasma CCK increased rapidly from baseline at $t = 5$ min ($P < 0.001$) and over the first 20 min of infusion, and plateaued thereafter. There was no effect of treatment on the AUC for plasma CCK.

8.4.6. Ghrelin

There was a trend for an effect of treatment ($P = 0.06$), but no effect of time and no treatment * time interaction, for plasma ghrelin concentrations (**Figure 8.3B**). Plasma ghrelin tended to be lower during the "long", when compared with the "short", segment infusion. Moreover, there was an effect of treatment on the AUC for plasma ghrelin ($P < 0.05$). There was no relationship between the amount of glucose (kcal/h) infused distal to the balloon and the AUC for plasma ghrelin ($r = 0.42$, $P = 0.1$).

8.4.7. Antral and pyloric pressures

There was a treatment * time interaction ($P < 0.05$), but no effect of time or treatment, for the number of antral pressure waves (**Figure 8.4A**). The number

of antral pressure waves was greater during the “short” when compared with the “long”, segment infusion between t = 30 - 60 min ($P < 0.05$). There was no effect on the amplitude of antral PWs.

There was an effect of time ($P < 0.05$, for both), but no effect of treatment and no treatment * time interaction, for basal pyloric pressure (tone) and the number of IPPWs (**Figures 8.4B and C**). Basal pyloric pressure increased from baseline between t = 20 - 60 min during the “short” ($P < 0.05$), but not the “long” segment infusion. In contrast, the number of IPPWs increased from baseline between t = 20 - 60 min on both days ($P < 0.05$). There was no effect on the amplitude of IPPWs.

8.5. Discussion

This study has provided new insights into the small intestinal regulation of gastrointestinal hormone secretion and antropyloric motility in humans. Our observations reveal that exposure of greater than 60 cm of the small intestine to glucose is required for the secretion of GLP-1, and the suppression of ghrelin, but not for the secretion of GIP and CCK. Insulin secretion was also greater during the "long segment infusion", likely reflecting the higher blood glucose concentrations. Exposure of greater than 60 cm of the small intestine to glucose was also required to suppress antral pressure waves, while both infusions equally stimulated isolated pyloric pressure waves and increased basal pyloric pressure. Given that there was no significant relationship between the amount of glucose infused and either the AUC for plasma GLP-1 or ghrelin, the length of small intestine exposed to nutrient, rather than the amount of glucose infused, appears to be the important factor in determining GLP-1 and ghrelin release.

While previous studies have established that intraduodenal infusion of glucose stimulates the release of GIP, GLP-1, insulin and CCK, and suppresses ghrelin secretion (O'Donovan *et al.*, 2004; Parker *et al.*, 2005), it has, hitherto, been unknown whether the release of these hormones is dependent upon the length of small intestine exposed to glucose. In the current study, plasma GLP-1 concentrations increased promptly (i.e. within 20 min) and progressively only during exposure of greater than 60 cm of the small intestine to glucose. This observation is consistent with the localisation of endocrine L cells in the distal small intestine (Eissele *et al.*, 1992). In humans, GLP-1 is detectable in the duodenum (which extends 30 cm in length from the pylorus), has a moderate

concentration in the jejunum (a segment that spans approximately 30 – 80 cm from the pylorus), and high concentrations in the ileum (beyond 80 cm from the pylorus) (Theodorakis *et al.*, 2005). While it has been postulated that the early rise in plasma GLP-1 following a glucose load may be explained by the release of GLP-1 from duodenal L cells (Theodorakis *et al.*, 2005), our observations argue against this possibility. On both study days, most of the glucose infused in the proximal segment was absorbed during transit through the 60 cm duodeno-jejunal segment (Table 8.1); but on the second day, about 20 % of the infused glucose was given access to the jejunum beyond 60 cm distal to the pylorus. While we cannot say precisely how much jejunal length was exposed to glucose, contact surely extended to the GLP-1 bearing mucosa. For example, it has been demonstrated in human volunteers that glucose transport per cm of the proximal jejunum reaches a maximum at loads above inflows of 1.4 kcal/min, but that absorption is considerably slower at inflows of around 0.6 kcal/min, as was the case beyond the blocking balloon on day 2 (Table 8.1) (Holdsworth and Dawson, 1964; Modigliani and Bernier, 1971). Our observations are, therefore, consistent with the release of GLP-1 from the distal small intestine by direct, local contact with glucose. By contrast, the release of GIP and CCK, both distributed mainly in the duodenum and proximal jejunum (Fehmann *et al.*, 1995; Rehfeld, 1978), was similar on both days in which the duodeno-jejunal mucosa was contacted by glucose. Despite the fact that GIP was released similarly on both days, the failure of GLP-1 to rise significantly above basal concentration on day 1 suggests that in humans, unlike the rat (Roberge and Brubaker, 1993), the release of GLP-1 is not stimulated by GIP. This is supported by the observation that in humans intravenous infusion of GIP did not stimulate the secretion of GLP-1 (Nauck *et*

al., 1993). Despite the above considerations, our design does not allow us definitively to distinguish between (a) a length-dependent release of GLP-1 reflexively from L-cells (that is, some reflex mechanism, not involving GIP, that depends on an increasing number of glucose sensors contacted along a length of small intestine) and (b) a region-specific response that depends on local contact of luminal glucose with L cells in the distal small intestine, rather than the total length of small intestine contacted. Given the distribution of the L cells along the gut, we believe the second possibility is more likely.

Our conclusion that the release of the incretin hormones by glucose is dependent upon direct nutrient contact with GIP- or GLP-1-bearing mucosa in the duodenum versus mid jejunum, respectively, is consistent with the observations of Schirra *et al.* (1996). When human volunteers ingested 50 and 100 g of glucose in water, glucose emptied from the stomach initially (i.e. over the first 30 post-cibal minutes) at rates of 3.0 and 4.5 kcal/min, respectively. Plasma concentrations of GIP rose somewhat sooner than those of GLP-1 after both glucose drinks, but both hormones peaked within 30 min during higher rates of duodenal entry of glucose. However, the peak GIP concentration was ~ 800 pg/ml following both glucose loads, while the peak GLP-1 concentrations were significantly different at ~ 3.5 and 7.0 pmol/l, for the 50 and 100 g loads, respectively. After both drinks, gastric emptying of glucose at, or above, 3.0 kcal/min was sufficient to expose the proximal 60 cm of small intestine to glucose (as in our case with infusion of 3.5 kcal/min) to release GIP, whereas only after the larger glucose load and faster inflow rate was there sufficient, unabsorbed glucose in the proximal and distal jejunum to release GLP-1. It is

important to understand how gastrointestinal hormone release is regulated in this way by interactions between ingested load, gastric emptying rate, and length of small intestine contacted by nutrient as this has important implications for the regulation of gastric emptying (Lin *et al.*, 1989) and energy intake (Meyer *et al.*, 1998). The observations relating to GIP and GLP-1 also have major implications for the regulation of postprandial glycaemia and insulinaemia (Kreymann *et al.*, 1987).

Insulin secretion was also enhanced during exposure of greater than 60 cm of the small intestine to glucose when compared with infusion into the isolated 60 cm segment of the proximal small intestine; this observation is most likely to be predominantly attributable to the relative hyperglycaemia on this day. Both GIP and GLP-1 stimulate the release of insulin (Kreymann *et al.*, 1987; Nauck *et al.*, 1993). GLP-1 stimulates the secretion of insulin in a glucose-dependent manner (Kreymann *et al.*, 1987), hence, it is likely that the greater blood glucose, and GLP-1, response observed during exposure of greater than 60 cm of the small intestine interacted to enhance insulin secretion. These observations suggest that contact of glucose with the distal small intestine may be an important determinant of the postprandial insulin response.

Plasma ghrelin was also suppressed during the “long” when compared with the “short”, segment infusion. Animal studies have demonstrated that the inhibitory effects of intraduodenal nutrients on ghrelin secretion are not discretely dependent upon exposure of the stomach, or duodenum, to nutrient, and have suggested that exposure of the small intestine distal to the duodenum may play a

role (Overduin *et al.*, 2005). This is also supported by observations that Roux-en-Y gastric bypass in morbidly obese subjects, an operation which markedly increases exposure of the ileum to nutrients, rapidly decreases plasma ghrelin and increases PYY (Korner *et al.*, 2005). Again, however, our design does not allow us to distinguish a length-dependent, from a region-specific, process. One possible explanation for our observations is that ghrelin suppression requires the exposure of greater than 60 cm of the small intestine to glucose. It must, however, be noted that ghrelin secretion is also suppressed during intravenous infusion of glucose (Shiiya *et al.*, 2002), hence the increased blood glucose response during exposure of greater than 60 cm of the small intestine to glucose may have mediated the suppression of ghrelin. Hyperinsulinaemia has also been shown to suppress plasma ghrelin concentrations under eu-, hypo- and hyperglycaemic conditions in humans (Flanagan *et al.*, 2003), which suggests that insulin may mediate ghrelin suppression independently of glucose. Other studies have, however, failed to demonstrate an inhibitory effect of intravenous insulin on circulating ghrelin concentrations, except at supraphysiological concentrations (Caixas *et al.*, 2002; Schaller *et al.*, 2003). Our observations suggest that either elevated blood glucose or plasma insulin may, indeed, play a role in mediating the suppression of plasma ghrelin, since the greater glycaemic and insulinaemic responses during exposure of greater than 60 cm of the small intestine to glucose was associated with lower concentrations of plasma ghrelin when compared with glucose confined to the isolated 60 cm segment of the proximal small intestine. However, it should be noted that there was no change in plasma ghrelin when glucose was infused into the “short” segment, despite significant rises in both blood glucose and plasma insulin, suggesting that the

length of small intestinal exposure was the most important variable. This concept is further supported by the lack of relationship between the amount of glucose infused into the distal segment with the AUC for plasma ghrelin.

Antral motility was only suppressed during the "long segment infusion", whereas isolated pyloric pressure waves and pyloric tone were stimulated to a similar degree whether glucose was confined to the proximal 60 cm or whether it was allowed access to intestinal lengths greater than 60 cm. Both CCK and GLP-1, when administered exogenously, have been shown to potently suppress antral pressure waves and stimulate phasic and tonic pyloric pressures (Rayner *et al.*, 2000; Schirra *et al.*, 2000). Therefore, the additional release of GLP-1 may have enhanced the suppression of antral pressure waves during the "long segment infusion". Since isolated pyloric pressure waves were potently stimulated by the 3.5 kcal/min glucose infusion on both study days, it is likely that the response we observed was supra-maximal (i.e. approaching greater than 2-3 IPPWs per minute) (Heddle *et al.*, 1988), hence the effect of increasing the length of small intestine exposed to nutrient may have been obscured. It should, however, be noted that duodenal distension has been shown to stimulate isolated pyloric pressure waves and increase basal pyloric pressure in humans (Edelbroek *et al.*, 1994), hence our observations may be confounded by the presence of the intraintestinal balloon, a possibility we cannot discount because of the lack of a control day of saline perfusions during balloon distension. Moreover, while the suppression of antral motility and stimulation of pyloric pressures is associated with the slowing of gastric emptying (Heddle *et al.*, 1989), it is unclear whether these measures completely characterise the control of gastric emptying. Clearly,

additional investigation is required to understand how the control of gastric emptying, antropyloric motor responses and length-dependent, glucose-driven, intestinal feedback may be interrelated in humans.

In conclusion, this study has demonstrated that increasing the length of small intestine exposed to glucose stimulates the release of GLP-1 and suppresses ghrelin secretion, but has no effect on GIP and CCK. These observations have implications for the regulation of gastric emptying, postprandial glycaemia, appetite, and energy intake.

Table 8.1: Total glucose absorbed (kcal/h) from the proximal 60 cm small intestinal segment* and total amount of glucose infused distal to the occluding balloon (kcal/h)^ during infusion into either (i) a 60 cm segment of the proximal small intestine ("short segment infusion") or (ii) greater than 60 cm of the small intestine ("long segment infusion").

Subject	**"Short segment infusion"**			**"Long segment infusion"**				
	Proximal infusion			**Proximal infusion**			**Distal infusion**	
	Infused (kcal/h)	**Recovered (kcal/h)**	**Absorbed (kcal/h)***	**Infused (kcal/h)**	**Recovered (kcal/h)**	**Absorbed (kcal/h)***	**Distal infusion (kcal/h)^**	**% of short segment recovery**
1	210	36.3	173.7	210	23.2	186.8	22.7	62.5
2	210	64.8	145.2	210	21.1	188.9	56	86.4
3	210	110.2	99.8	210	69.8	140.2	60.8	55.2
4	210	72.3	137.7	210	44.2	165.8	38.1	52.7
5	210	42.3	167.7	210	3.6	206.4	3.1	7.3
6	210	48.4	161.6	210	173.1	36.9	47.2	97.5
7	210	81.9	128.1	210	27.5	182.5	24.7	30.2
8	210	74.2	135.8	210	57.3	152.7	63.8	86
Mean	210	66.3	143.7	210	52.5	157.5	39.6	59.7
SEM	0	8.5	8.5	0	18.8	18.8	7.6	10.8

* Calculated by subtracting the sum of the amount of glucose recovered in the aspirate from the proximal small intestinal segment per 10 min over the 60 min of perfusion from the total amount of glucose infused.
^ Glucose was infused at a concentration equivalent to that which was not absorbed in the proximal small intestinal segment during the "short segment infusion". The rate of infusion was determined by the amount of glucose recovered from the proximal segment during the "long segment infusion".
% of short segment recovery – the percentage of glucose that was recovered from the proximal small intestinal segment infused into the distal segment.

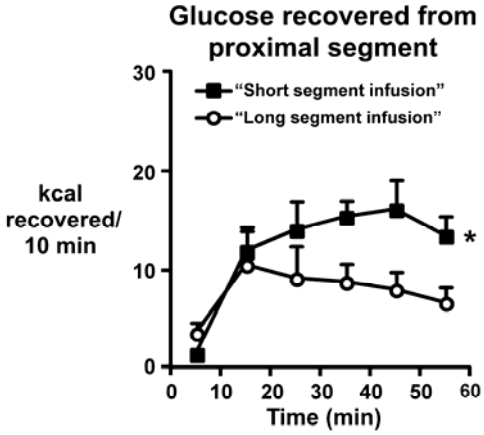

Figure 8.1: The amount of glucose (kcal) aspirated from the 60 cm segment of the proximal small intestine per 10 min during a 60 min infusion of 1M glucose to either (i) a 60 cm segment of the proximal small intestine ("short segment infusion") or (ii) greater than 60 cm of the small intestine ("long segment infusion"). Data are mean values ± SEM, n = 8. * "short segment infusion" vs. "long segment infusion", $P < 0.05$.

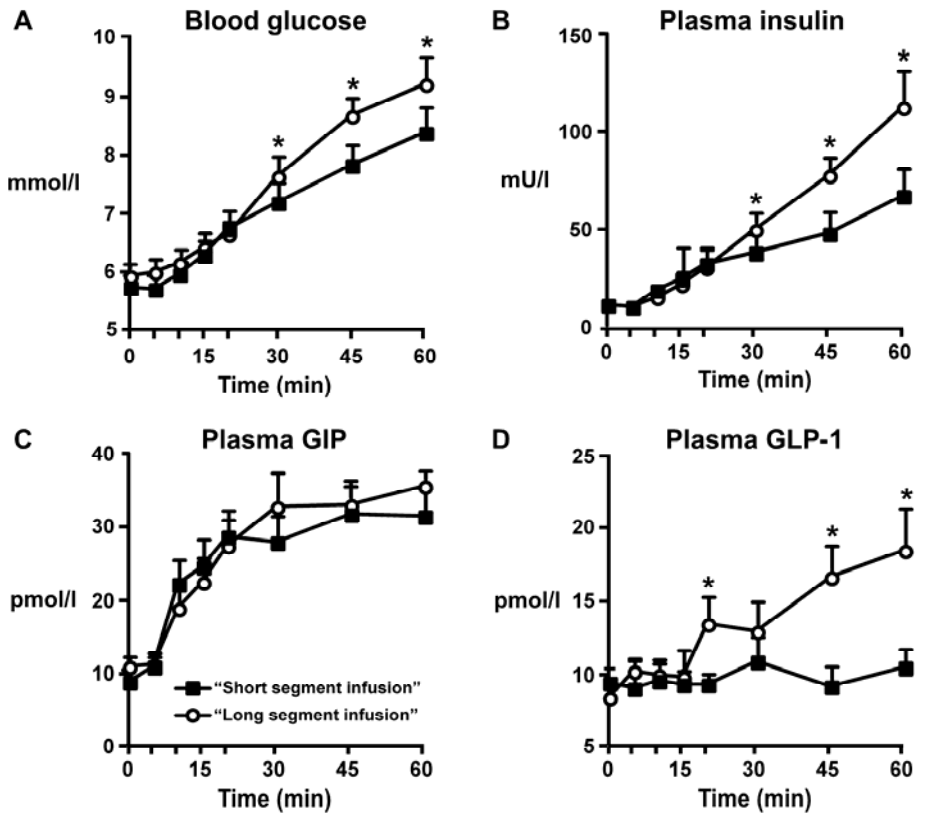

Figure 8.2: Blood glucose (A), plasma insulin (B), plasma glucose-dependent insulinotropic peptide (GIP) (C) and glucagon-like peptide-1 (GLP-1) (D) concentrations during a 60 min infusion of 1M glucose to either (i) a 60 cm segment of the proximal small intestine ("short segment infusion") or (ii) greater than 60 cm of the small intestine ("long segment infusion"). Data are mean values ± SEM, n = 8. * "short segment infusion" vs. "long segment infusion", $P < 0.05$.

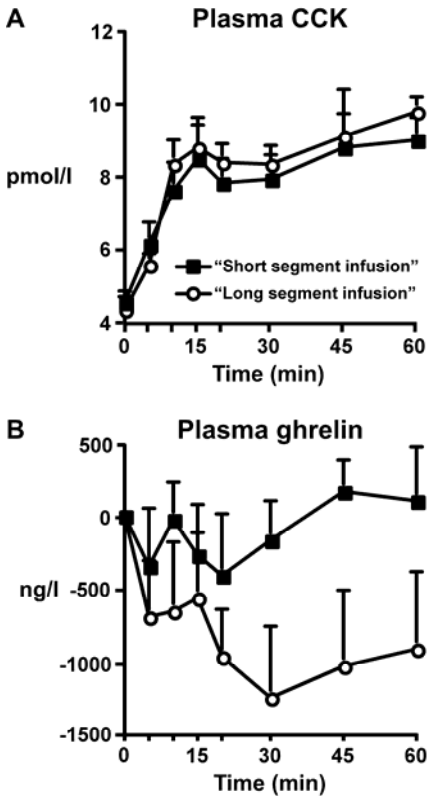

Figure 8.3: Plasma cholecystokinin (CCK) (A) and ghrelin (B) concentrations during a 60 min infusion of 1M glucose to either (i) a 60 cm segment of the proximal small intestine ("short segment infusion") or (ii) greater than 60 cm of the small intestine ("long segment infusion"). Data are mean values ± SEM, n = 8.

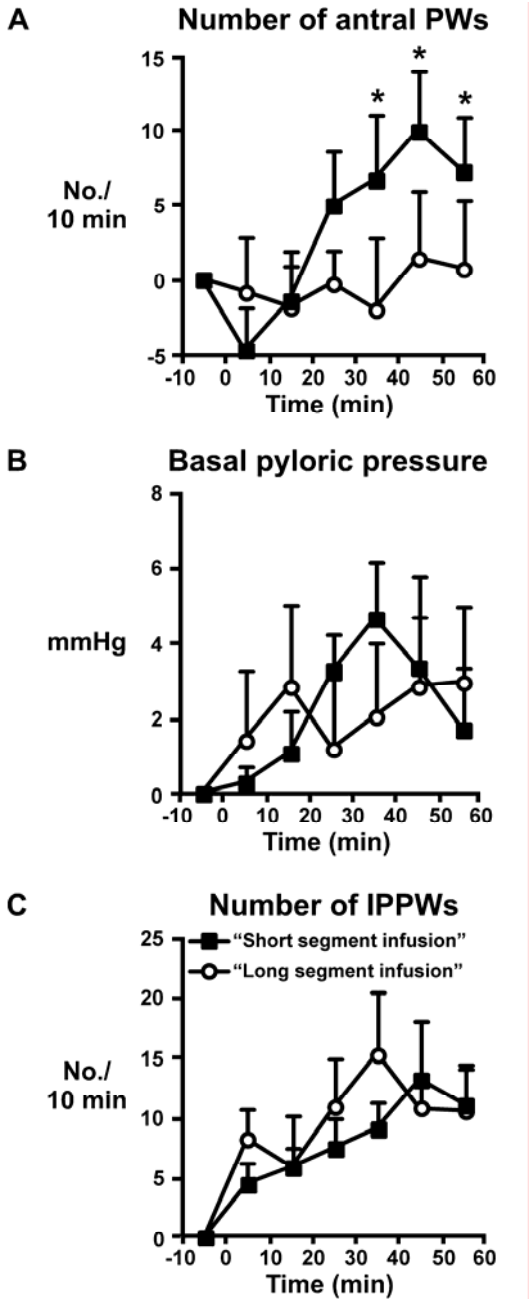

Figure 8.4: Number of antral pressure waves (A), basal pyloric pressure (B) and number of isolated pyloric pressure waves (IPPWs) (C) during a 60 min infusion of 1 M glucose into either (i) a 60 cm segment of the proximal small intestine ("short segment infusion") or (ii) greater than 60 cm of the small intestine ("long segment infusion"). Data are mean values ± SEM, n = 8. * "short segment infusion" vs. "long segment infusion", $P < 0.05$.

Chapter 9

Effects of intravenous GLP-1 on gastric emptying and intragastric distribution in healthy subjects – relationships with postprandial glycaemic and insulinaemic responses

9.1. Abstract

The inhibitory action of glucagon-like peptide-1 (GLP-1) on gastric emptying is likely to be important in mediating its effects on glycaemia, appetite and upper gastrointestinal symptoms. The aims of this study were therefore to evaluate the effects of "low" and "high" doses of i.v. GLP-1 on gastric emptying, intragastric meal distribution, postprandial glycaemia, insulinaemia and appetite. 10 healthy males were studied on 3 separate days. Gastric emptying and intragastric meal distribution of a solid (minced beef)/liquid (glucose) meal, blood glucose, plasma insulin, glucagon and GIP, appetite perceptions and gastrointestinal symptoms were evaluated during i.v. infusion of (i) GLP-1 at 0.3 $pmol.kg^{-1}.min^{-1}$ (GLP-1 0.3), (ii) GLP-1 at 0.9 $pmol.kg^{-1}.min^{-1}$ (GLP-1 0.9) and (iii) 0.9 % saline. GLP-1 0.3 and 0.9 slowed gastric emptying of solid (intragastric retention at t = 100 min, saline: 28 ± 5 %, GLP-1 0.3: 53 ± 6 %, GLP-1 0.9: 58 ± 7 %, $P < 0.001$), and liquid (50 % emptying time (T50), saline: 28 ± 2 min, GLP-1 0.3: 42 ± 7 min, GLP-1 0.9: 50 ± 9 min, $P < 0.001$). Both doses of GLP-1 induced gastroparesis (i.e. an emptying rate for solid and/or liquid slower than a normal range) in about half the cohort. GLP-1 also increased the amount of solid and liquid in the distal stomach ($P < 0.05$). GLP-1 attenuated the rises in plasma glucose, insulin and GIP ($P < 0.05$, for all). There was an inverse relationship between blood glucose

at t = 15 min and liquid (i.e. carbohydrate) gastric emptying (T50) ($r = -0.70$, $P < 0.001$). The perception of bloating was related ($r > 0.41$, $P < 0.05$) to the retention of the meal in the distal stomach. In healthy subjects (i) the slowing of solid and liquid gastric emptying by exogenous GLP-1 is associated with increased retention in the distal stomach and, even when administered in a "low" dose can induce "gastroparesis" and (ii) the effects of GLP-1 on postprandial glycaemic and insulinaemic responses are predictable on the basis of its effect on gastric emptying, supporting the concept that gastric emptying is a major target mechanism for the clinical use of incretin mimetics.

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9.2. Introduction

It is now well recognised that exogenous administration of glucagon-like peptide-1 (GLP-1) has diverse effects on gastrointestinal function in humans (Meier *et al.*, 2003; Nauck *et al.*, 1997; Schirra and Goke, 2005; Verdich *et al.*, 2001). Intravenous GLP-1 slows gastric emptying (Delgado-Aros *et al.*, 2002; Flint *et al.*, 2001; Nauck *et al.*, 1997), and this is associated with relaxation of the proximal stomach (Delgado-Aros *et al.*, 2002; Schirra *et al.*, 2002), inhibition of antral and duodenal motility (Schirra *et al.*, 2000), and stimulation of tonic and phasic pyloric pressures (Schirra *et al.*, 2000). A recent study using the specific GLP-1 antagonist, exendin (9-39), indicates that GLP-1 is also a physiological modulator of gastric motility (Schirra *et al.*, 2006). Exogenous GLP-1 also stimulates insulin and suppresses glucagon, these effects being glucose-dependent (Kreymann *et al.*, 1987; Nauck *et al.*, 1993), and has been reported to suppress appetite and energy intake in some (Gutzwiller *et al.*, 1999; Näslund *et al.*, 1999; Verdich *et al.*, 2001), but not all (Brennan *et al.*, 2005; Long *et al.*, 1999), studies. These actions of GLP-1 have stimulated substantial interest in the potential for its use (Schirra *et al.*, 1998), and that of specific GLP-1 analogues and GLP-1 receptor agonists (incretin mimetics) (Nauck, 1998), to improve glycaemic control in type-2 diabetes (Meier *et al.*, 2003; Nauck *et al.*, 1998) and induce weight loss in obesity (Meier *et al.*, 2002; Näslund *et al.*, 1999). While there is increasing evidence that this is effective, the mechanisms by which incretin mimetics improve glycaemia and suppress energy intake remain poorly defined.

The inhibitory effect of exogenous GLP-1 on gastric emptying has been demonstrated in healthy (Delgado-Aros *et al.*, 2002; Nauck *et al.*, 1997), type-2 diabetic (Meier *et al.*, 2003) and obese (Flint *et al.*, 2001; Näslund *et al.*, 1998) subjects, and has major implications for an understanding of its potential therapeutic efficacy, as well as upper gastrointestinal adverse effects (Elbrond *et al.*, 2002; Madsbad *et al.*, 2004). It is not known whether GLP-1 has the capacity to markedly slow gastric emptying of a mixed (solid/liquid) meal when administered at "low" doses. This is of particular importance given the high prevalence of delayed gastric emptying in type-2 diabetic patients (Horowitz *et al.*, 2002). Exogenous administration of GLP-1 at 0.3 $pmol.kg^{-1}.min^{-1}$ ("low dose") has been reported to result in "physiological" concentrations, while 0.9 $pmol.kg^{-1}.min^{-1}$ ("high dose"), results in "supraphysiological" plasma concentrations (Schirra *et al.*, 2002). Infusion of GLP-1 in a dose of 1.2 $pmol.kg^{-1}.min^{-1}$ profoundly delays gastric emptying of a liquid drink in type-2 patients (Willms *et al.*, 1996). While lower doses of GLP-1 are known to slow gastric emptying in both healthy subjects (Nauck *et al.*, 1997) and type-2 diabetes (Meier *et al.*, 2003), the magnitude of this effect is uncertain, particularly as results were not compared to a control range. Scintigraphy is the "gold standard" method for evaluating gastric emptying, and allows concurrent assessment of solid and liquid emptying, as well as quantification of intragastric meal distribution (Collins *et al.*, 1983; Horowitz and Dent, 1991; Horowitz *et al.*, 2002). The majority of previous studies relating to the effects of GLP-1 on gastric emptying have, however, employed less than optimal techniques including dye dilution (Nauck *et al.*, 1997), radioisotopic breath tests (Meier *et al.*, 2003) and measurement of paracetamol absorption (Flint *et al.*, 2001;

Näslund *et al.*, 1999); gastric emptying of discrete solid and liquid meal components and the effects of GLP-1 on intragastric meal distribution have not been evaluated.

The rate of entry of carbohydrate into the small intestine is a critical determinant of postprandial glycaemia in both healthy subjects and type-2 diabetes (Horowitz *et al.*, 1993; Jones *et al.*, 1996; O'Donovan *et al.*, 2004; Rayner *et al.*, 2001). While exogenous GLP-1 stimulates fasting insulin secretion, postprandial insulin levels are reduced, rather than increased, in healthy subjects (Nauck *et al.*, 1997) and type-2 diabetes (Meier *et al.*, 2003). Hence, slowing of gastric emptying is likely to be the dominant mechanism by which exogenous GLP-1 improves postprandial glycaemia, and GLP-1 may not be a "physiological" incretin (Nauck *et al.*, 1997). This concept is supported by a recent study in which reversal of the inhibitory effect of exogenous GLP-1 on gastric emptying, by the gastrokinetic drug, erythromycin, was associated with a substantial attenuation of its glucose-lowering effect (Meier *et al.*, 2005). It is, however, not known whether there is a direct relationship between postprandial blood glucose and insulin concentrations and the magnitude of the slowing of gastric emptying induced by GLP-1.

The effects of GLP-1 on gastric emptying and intragastric meal distribution may also have implications for an understanding of its capacity to induce gastrointestinal symptoms, such as nausea and bloating (Agerso *et al.*, 2002), and suppress appetite and energy intake (Verdich *et al.*, 2001). We (Hveem *et al.*, 1996; Jones *et al.*, 1997; Sturm *et al.*, 2004), and others (Santangelo *et al.*, 1998), have reported in healthy young, and older, subjects that both the perception of

postprandial fullness (Hveem *et al.*, 1996; Jones *et al.*, 1997; Santangelo *et al.*, 1998) and energy intake (Sturm *et al.*, 2004) are more closely related to the content of the distal, than the total, stomach. There is no information about the relationship between the effects of GLP-1 on gastrointestinal symptoms and perceptions of appetite with those on gastric emptying and intragastric meal distribution.

The aims of this study were to determine the effects of "low" (0.3 $pmol.kg^{-1}.min^{-1}$) and "high" (0.9 $pmol.kg^{-1}.min^{-1}$) doses of GLP-1 on gastric emptying, intragastric distribution, glycaemia and insulinaemia, and appetite perceptions following ingestion of a radioisotopically labelled mixed solid/liquid meal. Carbohydrate was included only in the liquid component of the test meal, so that the relationship between glycaemic responses and gastric emptying could be evaluated precisely. The specific hypotheses were that in response to both "low" and "high" doses of GLP-1: (i) the magnitude of the slowing of gastric emptying would be sufficient to induce "gastroparesis" and be associated with increased meal retention in the distal stomach, (ii) the suppression of postprandial glycaemia and insulinaemia would be related to the slowing of gastric emptying of carbohydrate and (iii) postprandial appetite perceptions and gastrointestinal symptoms would be related to changes in intragastric meal distribution.

9.3. Subjects and methods

9.3.1. Subjects

10 healthy male subjects (mean age: 27 ± 2 (range: 19 - 44) years), with normal body weight for their height (body mass index: 22 ± 0.8 (range: 19 – 25) kg/m^2) were studied.

9.3.2. Study design

Each subject was studied on three occasions, separated by 3 – 10 days. The effects of 150 minute intravenous infusions of: (i) GLP-1 at 0.3 $pmol.kg^{-1}.min^{-1}$ ("GLP-1 0.3"), (ii) GLP-1 at 0.9 $pmol.kg^{-1}.min^{-1}$ ("GLP-1 0.9") (Merck Biosciences, Läufelfingen, Switzerland) and (iii) 0.9 % isotonic saline on gastric emptying, intragastric meal distribution, blood glucose, plasma insulin, glucagon and glucose-dependent insulinotropic peptide (GIP) concentrations, appetite perceptions and gastrointestinal symptoms were evaluated, in a double-blind, randomised fashion.

9.3.3. Protocol

Subjects attended the Department of Nuclear Medicine, PET and Bone Densitometry at 0845 h, after an overnight fast from 2200 h from solids and liquids. An intravenous cannula was inserted into each forearm, for the collection of blood samples and administration of the intravenous infusions, respectively. Subjects were then seated with their back upright against a gamma camera. The study protocol is summarised in **Figure 9.1**. A baseline blood sample was taken (t = -45 min) and a visual analogue scale questionnaire (VAS) assessing appetite perceptions and gastrointestinal symptoms, was completed.

After 15 min (i.e. t = -30 min), an intravenous infusion of (i) GLP-1 0.3, (ii) GLP-1 0.9 or (iii) saline was commenced and maintained for 150 min (i.e. until t = 120 min). 25 minutes later (t = -5 min), subjects completed a VAS, and then consumed a mixed solid/liquid meal over a period of 5 min. The meal consisted of a 100 g minced beef patty (270 kcal, 25 g protein, 21 g fat) labelled with 15 MBq ^{99m}Tc-sulphur colloid chicken liver, followed immediately by 150 ml of 10 % dextrose labelled with 4 MBq ^{67}Ga-EDTA (Jones *et al.*, 2002). The time of completion of the meal was defined as t = 0 min. Gastric emptying and intragastric distribution of the solid and liquid components of the meal were then measured for 2 hours (i.e. from t = 0 - 120 min). Blood samples were collected for determination of blood glucose at t = -45, -30, -15, 0, 15, 30, 45, 60, 75, 90 and 120 min. Plasma insulin, GIP and GLP-1 were measured on the blood samples obtained at t = -30, 0, 30, 60, 90 and 120 min, and plasma glucagon at t = -30, 0, 30 and 60 min; VAS were completed at 15 min intervals throughout the study.

9.3.4. Gastric emptying

Radioisotopic data were acquired in 1 min frames for the first hour and in 3 min frames for the remaining 60 min. Data were corrected for subject movement, radionuclide decay and gamma-ray attenuation, and analysed as described previously (Collins *et al.*, 1983). Regions-of-interest were drawn for the total stomach and proximal and distal gastric regions, with the proximal region corresponding to the fundus and proximal corpus, and the distal region corresponding to the distal corpus and antrum. Gastric emptying curves, expressed as percent retention over time, were derived for total, proximal and

distal stomach regions (Collins *et al.*, 1983). For both the solid and the liquid meal components, the amount remaining in the total, proximal and distal stomach between t = 0 – 120 min was derived at 15-minute intervals. The lag phase for solid and liquid meal components was determined as the time period between meal completion and the appearance of radioactivity in the proximal small intestine (Collins *et al.*, 1983). The amount of solid remaining in the stomach at t = 100 min and the time for 50 % of the liquid to empty (T50) from the stomach was calculated (Collins *et al.*, 1983). Gastric emptying was classified as delayed when the solid % retention at t = 100 min was > 61 % and/or the liquid T50 was > 31 min, based on an established normal range (Jones *et al.*, 2002).

9.3.5. Data and statistical analysis

The amount of the solid and liquid components of the meal remaining in the total, proximal and distal stomach, absolute blood glucose, plasma insulin, glucagon, GIP and GLP-1 concentrations and VAS data were analysed by repeated measures analysis of variance (ANOVA) with time and treatment as factors. For plasma insulin, one subject was excluded from analysis due to high basal values. Areas under the curve (AUC) were calculated (using the trapezoidal rule) for the magnitude of the rise in blood glucose and plasma insulin between t = 0 - 60 min. The T50 of liquid and the % retention of solid at t = 100 min were analysed by one-way ANOVA. Post-hoc paired comparisons, corrected for multiple comparisons by Bonferroni's correction, were performed if ANOVAs revealed significant effects. Correlations, corrected for repeated measures, were determined between (i) the solid % retention at t = 100 min and the liquid T50, (ii) the change in blood glucose at t = 15 min (i.e. glucose at t = 15 min – glucose

at t = 0 min), the area under the curve for the change in blood glucose and the area under the curve for the change in plasma insulin from t = 0 – 60 min with the liquid T50, and (iii) appetite perceptions and gastrointestinal symptoms with the amount of solid (ml) and liquid (ml) in the total, proximal and distal stomach for pooled data (overall group), and for each treatment. Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

9.4. Results

The study protocol was tolerated well by all subjects. No adverse effects were reported during infusion of GLP-1 or saline.

9.4.1. Gastric emptying

9.4.1.1. Solid

Total stomach: There was no difference in the lag phase between the three treatments. The % retention at t = 100 min was greater during infusion of both GLP-1 0.3 and 0.9 when compared with saline ($P < 0.001$, saline: 29 ± 5 %, GLP-1 0.3: 53 ± 6 %, GLP-1 0.9: 58 ± 7 %), with no difference between the two doses of GLP-1. During infusion of saline, gastric emptying was within the normal range in all subjects; gastric emptying was delayed, as assessed by the % retention at t = 100 min, with GLP-1 0.3 in 5 subjects, and with GLP-1 0.9 in 4 subjects. There was a treatment * time interaction for the amount of solid remaining in the total stomach ($P < 0.001$) (**Figure 9.2A**). The intragastric retention of solid was greater during infusion of GLP-1 0.3 and 0.9 when compared with saline ($P < 0.01$) between t = 45 – 120 min, with no difference between the two doses of GLP-1. At t = 120 min intragastric retention was slightly, but significantly, greater for GLP-1 0.9 than GLP-1 0.3 (30.1 ± 5.4 % vs. 22.1 ± 5.6 %, $P < 0.05$).

Proximal stomach: There was no difference in the amount of the solid meal remaining in the proximal stomach during treatment with GLP-1 0.3 or 0.9 compared with control (**Figure 9.2B**).

Distal stomach: There was a treatment * time interaction for the amount of solid in the distal stomach ($P < 0.001$) (**Figure 9.2C**). More of the solid meal was retained in the distal stomach during treatment with GLP-1 0.3 between t = 60 - 120 min, and with GLP-1 0.9 between t = 75 – 120 min ($P < 0.001$, for both) when compared with saline, with no difference between the two doses of GLP-1.

9.4.1.2. Liquid

Total stomach: There was no difference in the lag phase between the three treatments. The T50 was longer following infusion of both GLP-1 0.3 and 0.9 when compared with saline ($P < 0.01$, saline: 28 ± 2 min, GLP-1 0.3: 42 ± 7 min, GLP-1 0.9: 50 ± 9 min), with no difference between the two doses of GLP-1. During infusion of saline gastric emptying was normal in all subjects; gastric emptying was delayed, as assessed by the T50, with GLP-1 0.3 in 6 subjects, and with GLP-1 0.9 in 7 subjects. There was a treatment * time interaction for the amount of liquid remaining in the total stomach ($P < 0.001$) (**Figure 9.2D**). The intragastric retention of liquid was greater during infusion of GLP-1 0.3 and 0.9 between t = 15 - 120 min when compared with saline ($P < 0.05$), with the effect of GLP-1 0.9 being greater than that of GLP-1 0.3 between t = 30 – 120 min ($P < 0.01$). At t = 120 min, intragastric retention was greater for GLP-1 0.9 than for GLP-1 0.3 (15.4 ± 4.4 % vs. 7.0 ± 3.0 %, $p < 0.05$).

Proximal stomach: There was a treatment * time interaction for the amount of liquid in the proximal stomach ($P < 0.001$) (**Figure 9.2E**). GLP-1 0.3 increased the amount of liquid remaining in the proximal stomach between t = 15 – 75 min when compared with saline ($P < 0.05$). GLP-1 0.9 increased the amount of liquid

present in the proximal stomach between t = 15 – 120 when compared with saline (P < 0.001), with the effect of GLP-1 0.9 being greater than that of GLP-1 0.3 between t = 15 – 60 min (P < 0.05).

Distal stomach: There was an effect of treatment on the amount of liquid in the distal stomach (P<0.05) (**Figure 9.2F**). Both GLP-1 0.3 and GLP-1 0.9 increased the amount of liquid present in the distal stomach when compared with saline, with no difference between the two doses of GLP-1.

9.4.1.3. Relationship between solid and liquid gastric emptying

There was a positive relationship between solid and liquid GE (i.e. solid % retention at t = 100 min vs. liquid T50 (min)), such that the slower the rate of solid GE, the slower the rate of liquid GE in the overall group (r = 0.66, P < 0.001) and for GLP-1 0.3 (r = 0.81, P < 0.01), but not for GLP-1 0.9 or saline.

9.4.2. Blood glucose and plasma hormone responses

9.4.2.1. Blood glucose

There was no difference in baseline blood glucose concentrations between the three study days. There was a treatment * time interaction for blood glucose concentrations (P = 0.001) (**Figure 9.3A**). GLP-1 0.3 (P < 0.01) and 0.9 (P < 0.05) slightly decreased fasting blood glucose concentrations at t = -15 min (i.e. prior to ingestion of the meal) when compared with saline (saline: 5.5 ± 0.2 mmol/l, GLP-1 0.3: 5.1 ± 0.2 mmol/l, GLP-1 0.9: 5.0 ± 0.1 mmol/l). Postprandially, while glucose concentrations increased with all treatments (P < 0.05), GLP-1 0.3 attenuated the postprandial rise in blood glucose between t = 15

– 45, and at t = 105, min, and GLP-1 0.9 from t = 15 - 30 min, when compared with saline ($P < 0.05$ for all), with the effect of GLP-1 0.9 being greater than that of GLP-1 0.3 at t = 30 min ($P < 0.01$). There was an effect of treatment on the AUC for the change in blood glucose between t = 0 – 60 min ($P < 0.05$), so that the AUC was less during infusion of GLP-1 0.3 and 0.9, when compared with saline ($P < 0.05$), with no difference between the two doses of GLP-1. There was also an effect of treatment on peak blood glucose concentrations ($P < 0.01$), with the peak being greater for saline (7.9 ± 0.2 mmol/l) than for GLP-1 0.3 (6.7 ± 0.3 mmol/l) and GLP-1 0.9 (6.3 ± 0.2 mmol/l).

9.4.2.2. Plasma insulin

There was a treatment * time interaction for plasma insulin ($P < 0.05$) (**Figure 9.3B**). Plasma insulin concentrations did not differ between the study days at baseline, or during the 25-minute infusion of GLP-1 0.3 and 0.9 prior to ingestion of the test meal. Postprandial plasma insulin concentrations were lower during infusion of GLP-1 0.3 and 0.9 at t = 30 min ($P < 0.05$), with the effect of GLP-1 0.9 being greater than that of GLP-1 0.3 ($P < 0.05$). There was an effect of treatment on the AUC of the change in plasma insulin between t = 0 – 60 min ($P < 0.05$), so that the AUC was less during infusion of GLP-1 0.9 compared with GLP-1 0.3 and saline ($P < 0.01$).

9.4.2.3. Glucagon

There was no effect of treatment, or time, on fasting (saline: 82.5 ± 4.4 pg/ml, GLP-1 0.3: 75.7 ± 6.9 pg/ml, GLP-1 0.9: 84.5 ± 6.3 pg/ml), or mean postprandial

(saline: 80.4 ± 7.0 pg/ml, GLP-1 0.3: 83.9 ± 6.1 pg/ml, GLP-1 0.9: 91.2 ± 7.8 pg/ml), plasma glucagon concentrations (data not shown).

9.4.2.4. GIP

There was no effect of treatment on fasting GIP concentrations. While GIP increased after the meal with all treatments ($P < 0.001$), the rise at t = 60 min was less for GLP-1 0.3 ($P < 0.01$), and tended to be less for GLP-1 0.9 ($P = 0.09$) when compared with saline (**Figure 9.3C**).

9.4.2.5. GLP-1

In response to intravenous infusion of GLP-1, plasma concentrations increased in the first 30 min, to reach steady-state levels thereafter; the effect of 0.9 was greater than that of 0.3 ($P < 0.001$) (**Figure 9.4**). Peak plasma GLP-1 concentrations during infusion of GLP-1 0.3 and 0.9 were 25.3 ± 5.3 pmol/l and 43.8 ± 8.4 pmol/l, respectively. There was no relationship between gastric emptying of solid or liquid with the AUC of plasma GLP-1 from t = 0 – 120 min (data not shown).

9.4.3. Relationship between gastric emptying, blood glucose and plasma insulin

There was an inverse relationship between the magnitude of the postprandial rise in blood glucose and gastric emptying (T50) of liquid (i.e. the carbohydrate component of the meal). The increase in blood glucose between t = 0 - 15 min was related to the T50 during saline ($r = -0.73$, $P < 0.01$), GLP-1 0.3 ($r = - 0.63$, $P < 0.05$), and GLP-1 0.9 ($r = -0.69$, $P < 0.05$) infusions, as well as overall ($r = -0.70$, $P < 0.001$), i.e. the increase in blood glucose was greater when gastric

emptying of liquid was relatively more rapid (**Figure 9.5A**). The AUC for the change in blood glucose from t = 0 - 60 min was also related to the liquid T50 (r = -0.46, $P < 0.01$) in the overall group.

There were significant relationships between the magnitude of the postprandial rise in plasma insulin with both gastric emptying of liquid (T50) and blood glucose. The AUC for the change in plasma insulin from t = 0 – 60 min was inversely related to the T50 (r = -0.42, $P < 0.05$), i.e. plasma insulin was greater when GE was relatively more rapid (**Figure 9.5B**), and directly related to the AUC for the change in blood glucose from t = 0 - 60 min during GLP-1 0.3 (r = 0.70, $P < 0.05$), as well as overall (r = 0.50, $P < 0.01$), but not during GLP-1 0.9 or saline, i.e., the rise in plasma insulin was greater when blood glucose was greater (**Figure 9.5C**).

9.4.4. Appetite perceptions and gastrointestinal symptoms

There was no effect of treatment on ratings of appetite, i.e. hunger and fullness, or gastrointestinal symptoms, i.e. nausea and bloating (**Figure 9.6**). There was an effect of time on hunger and fullness ($P < 0.05$). Hunger increased from baseline prior to meal ingestion ($P < 0.05$); following the meal hunger ratings decreased below baseline ($P < 0.05$) and then increased progressively to levels greater than those at baseline. Fullness did not change prior to meal ingestion; following the meal fullness increased ($P < 0.05$), and then progressively decreased over time, but did not return to baseline. There was no effect of meal ingestion on nausea or bloating.

9.4.5. Relationship between appetite perceptions and gastrointestinal symptoms with intragastric meal distribution

There was no relationship between hunger, fullness or nausea with the amount of solid or liquid remaining in the total, proximal or distal stomach. There was no relationship between bloating and the amount of solid or liquid in the proximal stomach. At t = 105 min there was a positive relationship between the score for bloating and the amount of solid and liquid remaining in the total ($r = 0.46$, $P < 0.01$ and $r = 0.40$, $P < 0.05$, respectively), and distal, stomach ($r = 0.41$, $P < 0.05$ and $r = 0.43$, $P < 0.05$, respectively).

9.5. Discussion

Our study provides a number of novel insights into the effects of exogenous GLP-1 on gastric emptying which have relevance to an understanding of its glycaemic, and potentially, appetite-suppressant, properties. In particular: (i) the magnitude of the slowing of solid and liquid gastric emptying by GLP-1 was sufficient to induce "gastroparesis" in a substantial proportion of subjects, even when administered in a "low" dose, and was associated with increased meal retention in the distal stomach and (ii) the suppressive effect of GLP-1 on postprandial glycaemic and insulinaemic responses could be predicted by the rate of gastric emptying of carbohydrate. It is implicit that our observations also have implications for the use of incretin mimetics, such as liraglutide and exenatide (Meier *et al.*, 2005; Nauck, 1998).

The magnitude of the observed slowing of gastric emptying by exogenous GLP-1 was substantial – gastroparesis was induced in more than 50 % of the subjects during infusion of low dose GLP-1. The prevalence of abnormally delayed gastric emptying in patients with longstanding type-2 diabetes is 30–50 % (Horowitz *et al.*, 2002), and further slowing of gastric emptying in such patients has the potential to induce, or exacerbate, gastrointestinal symptoms and affect oral drug absorption (Horowitz *et al.*, 2002). Accordingly, the acute and chronic effects of GLP-1 on gastric emptying in type-2 diabetic patients, with and without gastroparesis, warrant investigation. The inhibitory effect of GLP-1 on gastric emptying may be mediated by vagal mechanisms (Delgado-Aros *et al.*, 2002; Imeryuz *et al.*, 1997; Schirra *et al.*, 1998), and it is possible that the effect

of GLP-1 to slow gastric emptying will be attenuated in diabetic patients with autonomic neuropathy (Delgado-Aros *et al.*, 2002).

The relative importance of the mechanisms by which exogenous GLP-1 reduces postprandial glycaemia remains controversial, and there is ongoing debate as to whether GLP-1 has a physiological role as an incretin hormone (Meier *et al.*, 2005; Nauck, 1999; Nauck *et al.*, 1997; Schirra and Goke, 2005). The reduction in fasting blood glucose concentrations by GLP-1 is well documented (Kreymann *et al.*, 1987; Nauck *et al.*, 1997), as is the attenuation of the postprandial rise (Meier *et al.*, 2005; Nauck *et al.*, 1997). This improvement in postprandial glycaemia occurred despite diminished insulin secretion, as has been noted previously (Nauck, 1998; Nauck *et al.*, 1997). Recent observations strongly suggest that the inhibitory effect of GLP-1 on gastric emptying represents the dominant mechanism mediating the improvement in postprandial glycaemia (Meier *et al.*, 2005; Nauck *et al.*, 1997) Our novel observation that there is a direct relationship between postprandial glycaemic and insulinaemic responses with the slowing of gastric emptying of carbohydrate by GLP-1 adds to this. We also observed a decrease in the postprandial GIP response during infusion of GLP-1, as has been reported previously (Meier *et al.*, 2005) and this probably reflects the slowing of gastric emptying, resulting in reduced glucose-mediated GIP secretion from the K cells in the proximal small intestine. This reduction in GIP may potentially contribute to the observed reduction in postprandial insulinaemia. In studies using the specific GLP-1 receptor antagonist, exendin (9-39), to examine the role of endogenous GLP-1 in postprandial glycaemia, in healthy humans (Edwards *et al.*, 1999; Schirra and Goke, 2005), gastric emptying

was not quantified. Edwards *et al.* (1999) reported that exendin (9-39) impaired the glycaemic response to an oral glucose load, which was associated with an increase in plasma insulin, which they postulated reflected a more rapid rate of gastric emptying. More recently, Schirra *et al*. (2006) reported that the plasma insulin response to intraduodenal glucose was attenuated by exendin (9-39), but as the effects of gastric emptying were bypassed it is likely that the contribution of GLP-1 to insulin secretion was overestimated (Schirra *et al.*, 2006).

Exogenous GLP-1 has been reported to suppress appetite and energy intake in a number of studies (Flint *et al.*, 1998; Flint *et al.*, 2001; Näslund *et al.*, 1999) and "intragastric" mechanisms are known to play an important role in the regulation of appetite and energy intake in humans (Geliebter, 1988; Sturm *et al.*, 2004). While proximal stomach distension increases the perception of fullness (Feinle *et al.*, 1997), there is increasing evidence that antral distension plays a more important role (Hveem *et al.*, 1996; Jones *et al.*, 1997). For example, in healthy young, and older, subjects energy intake after a 400 ml "preload" was shown to be inversely related to antral area (Sturm *et al.*, 2004). The current observation that the perception of bloating was related to the amount of the meal retained in the distal, but not the proximal, stomach is, accordingly, of interest. It should, however, be recognised that our study design was less than optimal to evaluate the effects of GLP-1 on appetite perceptions and gastrointestinal symptoms and this was a secondary aim. Limitations include: (i) the relatively small number of subjects (n = 10) compared with previous studies in which an inhibitory effect of GLP-1 on appetite and energy intake was observed (Verdich *et al.*, 2001), (ii) the test meal was of small volume, given that it was designed to quantify gastric

emptying in patients with and without gastroparesis (Jones *et al.*, 2002), and postprandial changes in hunger and fullness were, accordingly, modest and (iii) no gastrointestinal adverse effects were reported. Further studies are indicated to evaluate the relationships between appetite and gastrointestinal symptoms with gastric emptying and intragastric meal distribution in response to GLP-1.

In conclusion, we have demonstrated that the slowing of gastric emptying of a mixed solid/liquid meal by GLP-1 is a major determinant of its effects on postprandial glycaemia and insulinaemia in healthy subjects, and that the magnitude of the slowing of gastric emptying, even by a low dose of GLP-1, is substantial and associated with a relative increase in the content of the distal stomach.

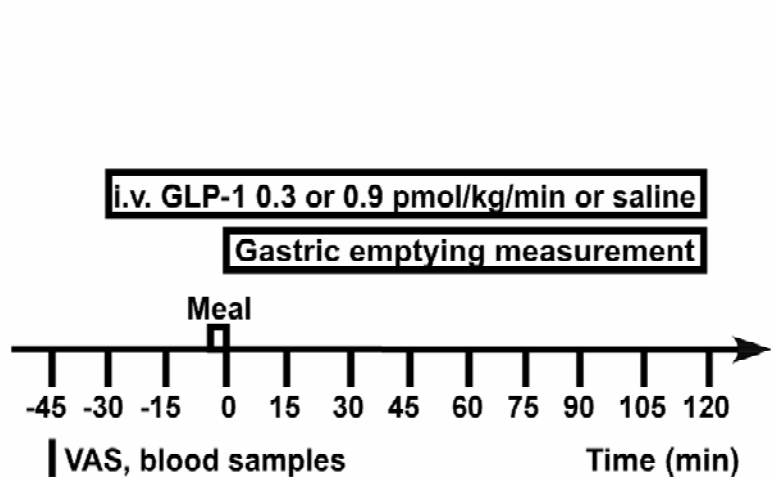


Figure 9.1: Outline of the study protocol. At t = -30 min an i.v. infusion of GLP-1 at 0.3 or 0.9 pmol.kg^{-1}.min^{-1}, or 0.9 % saline was commenced and maintained for 150 min. At t = -5 min, subjects consumed a radioisotopically labelled mixed solid/liquid meal (100 g minced beef patty, 150 ml 10 % dextrose). Gastric emptying and intragastric meal distribution were measured between t = 0 - 120 min. Blood samples for analysis of blood glucose, plasma insulin, glucagon, GIP and GLP-1 were collected and visual analogue scale (VAS) questionnaires for the evaluation of appetite and gastrointestinal symptoms administered at each of the time points indicated.

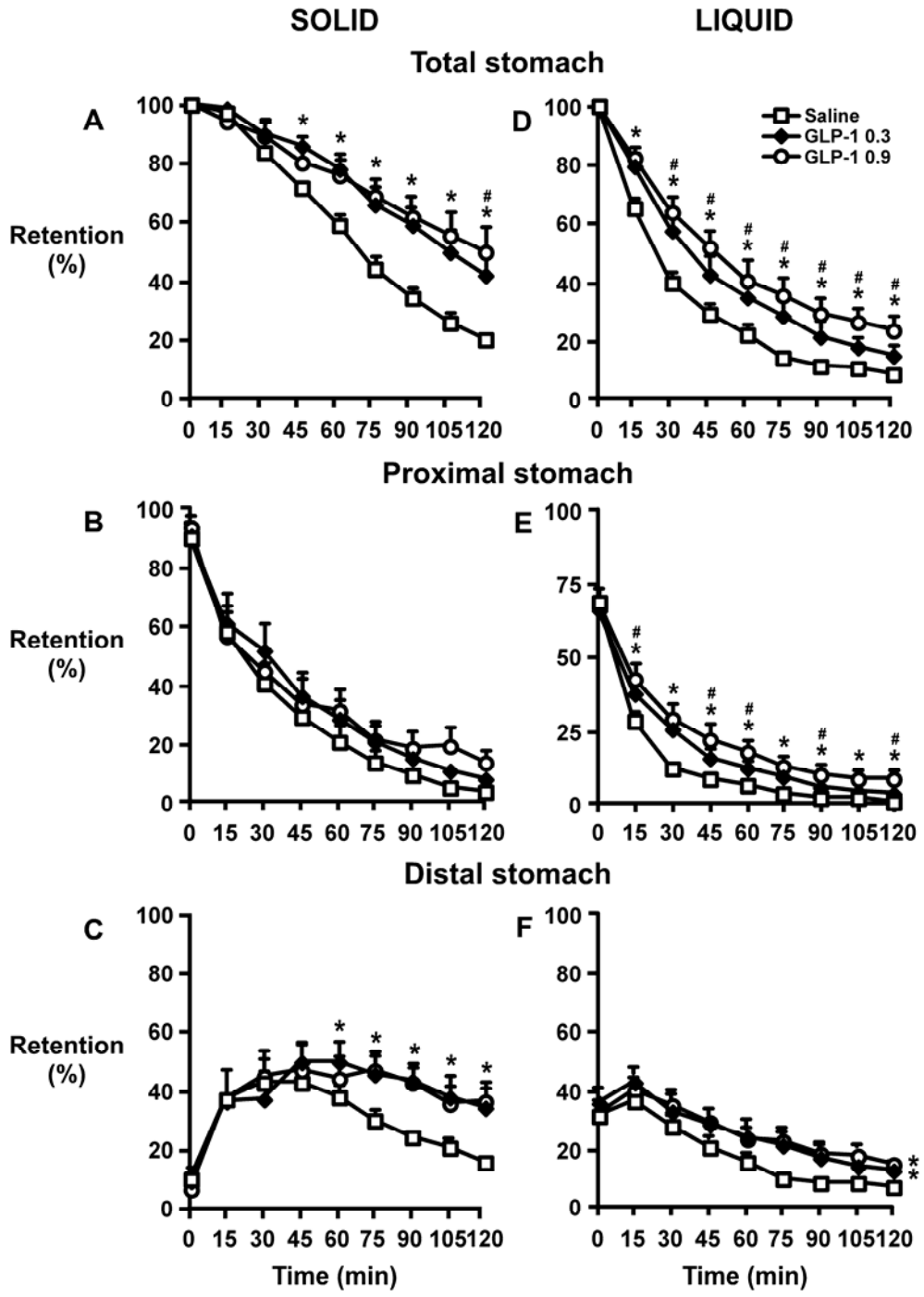


Figure 9.2: Gastric emptying curves for the solid and liquid components of the meal for the total, proximal and distal stomach during i.v. infusion of GLP-1, at 0.3 and 0.9 pmol.kg^{-1}.min^{-1}, or 0.9 % saline. Data are means ± SEM, n = 10. * GLP-1 0.3 and 0.9 vs. saline: $P < 0.05$, # GLP-1 0.9 vs. GLP-1 0.3: $P < 0.05$.

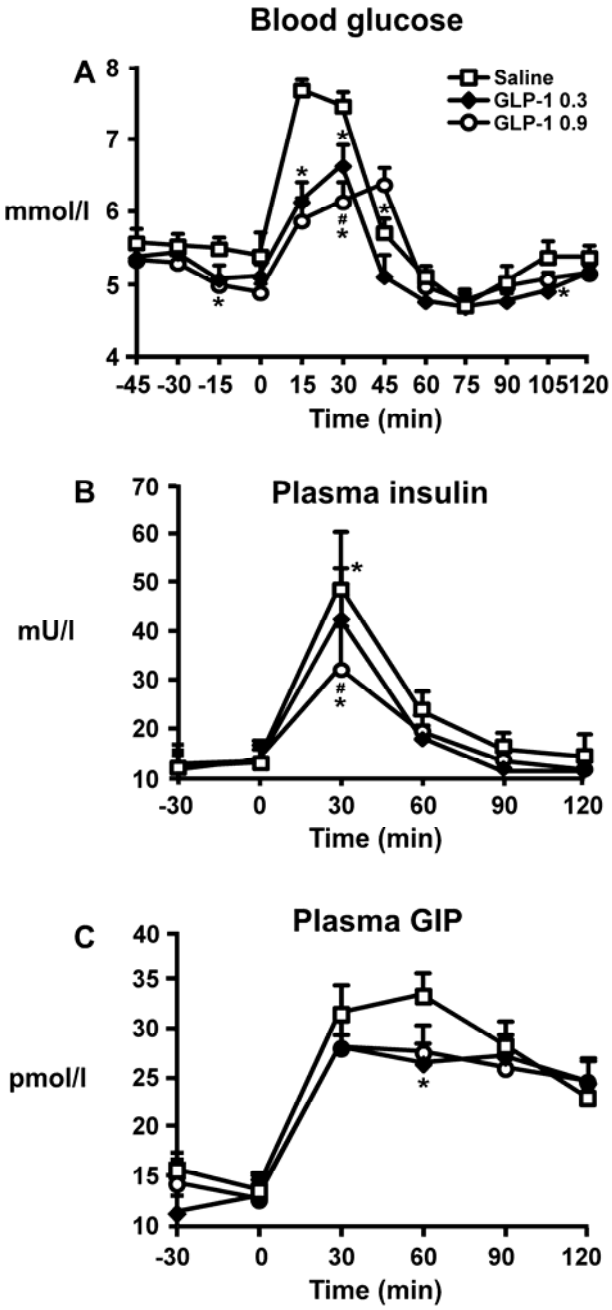


Figure 9.3: (A) Blood glucose, (B) plasma insulin and (C) plasma glucose-dependent insulinotropic-peptide (GIP) concentrations during i.v. infusion of GLP-1, at 0.3 and 0.9 pmol.kg^{-1}.min^{-1}, or 0.9 % saline. Data are means ± SEM, n = 10. * GLP-1 0.3 and 0.9 vs. saline: P<0.05, # GLP-1 0.9 vs. GLP-1 0.3: P<0.05.

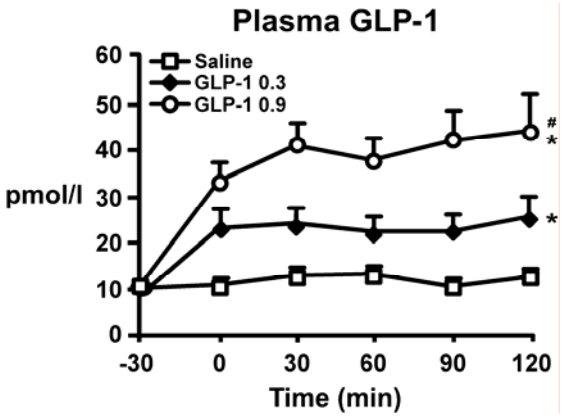


Figure 9.4: Plasma glucagon-like peptide-1 (GLP-1) concentrations during i.v. infusion of GLP-1, at 0.3 and 0.9 pmol.kg^{-1}.min^{-1}, or 0.9 % saline. Data are means ± SEM, n = 10. * GLP-1 0.3 and 0.9 vs. saline: $P < 0.05$, # GLP-1 0.9 vs. GLP-1 0.3: $P < 0.05$.

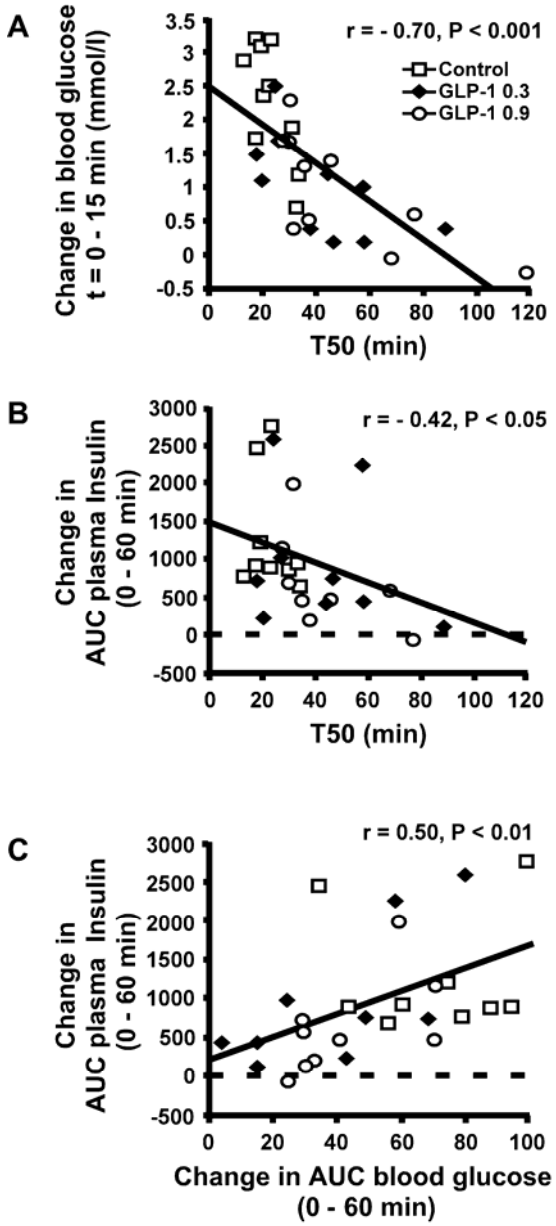


Figure 9.5: Relationships between (A) the increase from baseline (t = 0 min) in blood glucose at t = 15 min and gastric emptying (T50), (B) incremental plasma insulin (AUC of the change in plasma insulin from t = 0 - 60 min) and gastric emptying (T50) and (C) incremental plasma insulin (AUC of the change in plasma insulin from t = 0 – 60 min) and incremental blood glucose (AUC of the change in blood glucose from t = 0 – 60 min).

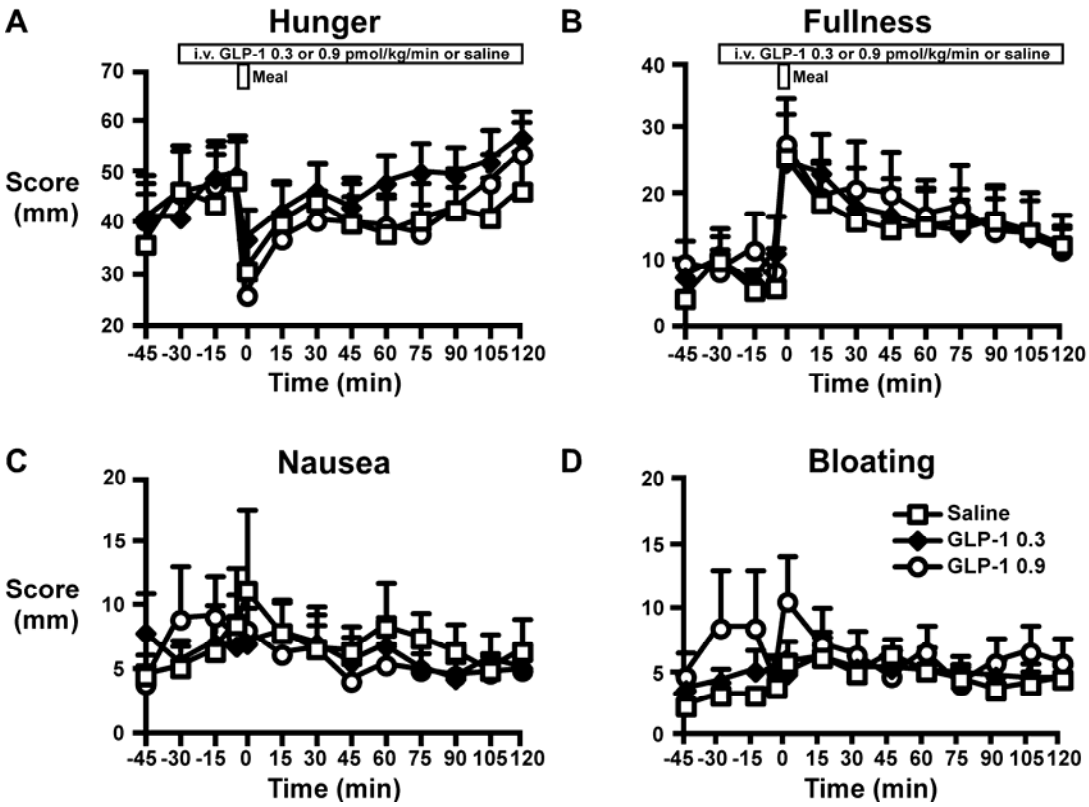


Figure 9.6: Perceptions of (A) hunger, (B) fullness, (C) nausea and (D) bloating during infusion of GLP-1, at 0.3 and 0.9 pmol.kg^{-1}.min^{-1}, or 0.9 % saline. Data are means ± SEM, n = 10.

Chapter 10

Consumption of a high-fat diet for 3 weeks increases fasting plasma cholecystokinin concentrations but does not diminish the effects of exogenous CCK-8 on APD motility, appetite and energy intake in healthy lean men

10.1. Abstract

There is evidence in animals that the effects of both fat and cholecystokinin (CCK) on gastrointestinal function and energy intake are attenuated by a high-fat diet. We hypothesised that in humans, the effects of exogenous CCK-8 on antropyloroduodenal motility, appetite and energy intake are attenuated by a high-fat diet. 10 healthy lean males consumed isocaloric diets (~ 15,400 kJ per day), comprising either 44 % ("high-fat") or 9 % ("low-fat") fat, each for 21 days in single-blind, randomised, cross-over, fashion. Immediately following each diet (i.e. on day 22), the effects of a 45-min intravenous infusion of CCK-8 (2 ng/kg/min) on antropyloroduodenal motility, appetite perceptions and plasma CCK concentrations were assessed. At t = 30 min during the infusion, subjects were offered a buffet-style meal, from which energy intake (kJ) was quantified. Body weight was unaffected by the diets. Baseline CCK concentrations were greater following the high-fat diet, when compared with the low-fat diet (high-fat: 4.3 ± 0.4 pmol/l, low-fat: 3.1 ± 0.3 pmol/l, $P < 0.05$). Infusion of CCK-8 stimulated phasic and tonic pyloric pressures (time effect: $P < 0.01$) and suppressed antral and duodenal pressures (time effect: $P < 0.05$), with no

difference between the two diets. Energy intake in response to CCK-8 did not differ between the two diets. Consumption of a high-fat diet increases fasting plasma CCK concentrations but the effects of exogenous CCK-8 on antropyloroduodenal motility and energy intake do not appear to be affected by exposure to a high-fat for three weeks. This latter observation suggests that, in healthy lean men, sensitivity to exogenous CCK-8 is unchanged following a short period on a high-fat diet.

10.2. Introduction

There is evidence from rodent studies, and, to a limited degree, in humans, that the gastrointestinal mechanisms involved in the suppression of appetite and energy intake are attenuated following exposure to a high-fat diet (Boyd *et al.*, 2003; Covasa and Ritter, 2000, 1999; Cunningham *et al.*, 1991). For example, in humans, the effects of fat on gastric emptying (Cunningham *et al.*, 1991), tonic and phasic pyloric pressures (Boyd *et al.*, 2003) and cholecystokinin (CCK) release (French *et al.*, 1995) have been reported to be attenuated following exposure to a high-fat diet. Understanding the mechanisms by which dietary fat impairs the regulation of energy intake is important to our understanding of the pathophysiology of obesity.

The gastrointestinal hormone, CCK, has a number of physiological effects, including the slowing of gastric emptying (Valenzuela and Defilippi, 1981) and regulation of gastrointestinal motility (Brennan *et al.*, 2005). As CCK plays an important role in the acute regulation of meal size and energy intake (Beglinger *et al.*, 2001; Lieverse *et al.*, 1995), it is conceivable that alterations in the secretion and/or action of CCK may predispose to hyperphagia and obesity. In rats, following exposure to a high-fat diet the CCK response to an intraduodenal triglyceride infusion was approximately 1.7-fold greater, than in rats on a low-fat diet (Spannagel *et al.*, 1996). Furthermore, the inhibitory effects of intraperitoneal administration of CCK on gastric emptying (Covasa and Ritter, 2000) and food intake (Covasa *et al.*, 2001; Covasa and Ritter, 1998) have been reported to be attenuated following exposure to high-fat diets (containing 34 or 54 % energy as fat), when compared with a low-fat diet (5 % energy as fat),

suggesting that a high-fat diet alters the sensitivity of animals to the effects of CCK.

Only two studies have hitherto investigated the effects of a high-fat diet on plasma CCK concentrations in humans (Boyd *et al.*, 2003; French *et al.*, 1995). While fasting concentrations did not differ, the CCK response to a standardised breakfast was greater following exposure to a high-fat diet, when compared with a pre-diet, condition (French *et al.*, 1995). In contrast, the CCK response to intraduodenal infusion of lipid did not differ following exposure to a high-fat (44 % energy as fat), when compared with a low-fat (10 % energy as fat), diet for two weeks (Boyd *et al.*, 2003), suggesting that the increased postprandial plasma CCK concentrations following consumption of a high-fat diet (French *et al.*, 1995) reflect more rapid gastric emptying (Cunningham *et al.*, 1991), and hence a greater amount of nutrients present within the proximal small intestine to stimulate the secretion of CCK. Interestingly, Boyd *et al.* (2003) reported that consumption of a high-fat diet attenuated the stimulatory effects of intraduodenal lipid on tonic and phasic pyloric pressures, despite a similar increase in plasma CCK concentrations. Since pyloric motility is mediated, at least in part, by the activation of CCK_1 receptors located on the pylorus (Yamagishi and Debas, 1978), it is possible that, despite unchanged CCK secretion, the sensitivity of the antropyloroduodenal region to CCK is reduced following exposure to a high-fat diet. This hypothesis has, hitherto, not been investigated in humans. Furthermore, all previous studies conducted in humans have compared the effects of a high-energy, high-fat diet with either a pre-diet condition (i.e. the subjects habitual diet) or a low-energy, low-fat diet. Thus, whether the observed changes

in gastrointestinal function are due to an increase in the fat content of the diet *per se*, or due to the increased caloric intake is unclear.

The aims of this study were, therefore, to evaluate in healthy lean male subjects the hypothesis that exposure to a high-fat diet for a period of three weeks would increase fasting plasma CCK concentrations and attenuate the effects of an intravenous infusion of CCK-8 on antropyloroduodenal motility, appetite perceptions and energy intake.

10.3. Subjects and methods

10.3.1. Subjects

Ten healthy male subjects (age: 22.4 ± 1.0 (range 18 – 28) years) with normal body weight for their height (body mass index: 22.1 ± 0.8 kg/m^2) were studied. Prior to inclusion in the study, subjects completed a 5-day diet diary (3 weekdays and a weekend) to ascertain that they habitually consumed between 25 – 35 % energy from fat as recommended in the NHMRC Dietary guidelines for adult Australians (**Table 10.1**).

10.3.2. Study design

Subjects completed two 21-day diet periods during which they consumed, in single-blind randomised, cross-over fashion, isocaloric diets (~ 15,400 kJ per day) that were either high in fat ("high-fat"), where fat contributed ~ 44 % of the total daily energy, or low in fat ("low-fat"), where fat contributed ~ 9 % of the total daily energy intake. The two diet periods were separated by a 14-day 'washout' period during which subjects consumed their habitual diet. Immediately after each dietary period (i.e. day 22), the effects of a 45-minute intravenous infusion of cholecystokinin-8 (CCK-8 sulphated, Clinalfa, Merck Biosciences AG, Läufelfingen, Switzerland) at 2ng/kg/min on antropyloroduodenal (APD) pressures, appetite perceptions and energy intake were evaluated. The dose of CCK was selected on the basis of previous studies, which indicated that it would have sub-maximal effects on energy intake (MacIntosh *et al.*, 2001) and APD pressures (Rayner *et al.*, 2000), while resulting in plasma concentrations comparable to those observed postprandially. Blood samples were collected before, during and after the infusion at t = -10, 0, 10, 20,

30, 60 and 90 minutes for subsequent determination of plasma concentrations of CCK.

10.3.3. Diets

The difference in fat intake between the two diets was approximately 150 g/day, the protein content of the diets was matched; however, carbohydrate content differed in order to make the diets isocaloric, i.e. the carbohydrate content of the "low-fat" diet was greater than that of the "high-fat" diet. The planned total daily energy and macronutrient intakes are summarised in **Table 10.2**. Six daily meal plans were designed for each diet to increase variety and, thus, optimise the potential for compliance with the prescribed diets; an example of a daily menu for each diet is shown in **Table 10.3**. These menus were rotated over the 21-day periods. The macronutrient distribution of the food provided was achieved by supplying subjects with similar high-fat or low-fat varieties of foods (cheeses, yoghurts, biscuits, butter, margarine, etc.), respectively (Boyd *et al.*, 2003). Subjects were also provided with (pre-packaged) high-fat or low-fat snacks, such as potato crisps or biscuits, to be eaten between main meals (Boyd *et al.*, 2003). Commercially-available foods were repackaged to remove nutritional information so that the subjects were not formally aware of the macronutrient composition of the foods consumed during the respective diets, and were supplied to subjects at 3 – 4 day intervals. A detailed plan outlining what, and when, to eat was provided with the food, and subjects were required to document, in a diary, that they had consumed all of the food provided. These diaries were reviewed regularly during each diet period to determine the compliance of the subjects to the prescribed diet.

10.3.4. Protocol

On the day immediately following each of the diet periods (i.e. day 22), subjects attended the Department of Medicine at 0830 h following an overnight fast from both solids and liquids from 2200 h. Subjects were intubated with a 16-channel manometric catheter (Dentsleeve, Adelaide, Australia) via an anaesthetised nostril. The catheter was allowed to pass through the stomach and into the small intestine by peristalsis. Two intravenous cannulae were inserted, one into each forearm, for the intravenous infusion, and blood sampling, respectively. A baseline blood sample was taken immediately, and the subject rated perceptions of appetite on a visual analogue scale questionnaire (VAS) (t = -10 min). At t = 0 min, an intravenous infusion of CCK-8 at 2 ng/kg/min was commenced and continued for 45 min. APD pressures were recorded continuously from t = 0 - 30 min. Blood samples were taken and VAS completed throughout the infusion at 10 min intervals. At t = 30 min, the subject was extubated and immediately offered a cold, buffet-style (Feltrin *et al.*, 2004). Subjects were allowed 30 min to eat (t = 30 – 60 min), and were instructed to eat until comfortably full. The CCK-8 infusion was terminated 15 minutes after the commencement of the meal (i.e. at t = 45 min). At t = 60 min and t = 90 min, further blood samples were taken and VAS completed. The intravenous cannulae were then removed and the subject was then free to leave the laboratory.

10.3.5. Data and statistical analysis

Baseline values were calculated as the means of values obtained between t = -10 and 0 min for VAS scores, number of antral and duodenal PWs, number of IPPWs, basal pyloric pressure and plasma CCK concentrations. The total

number and amplitude of antral and duodenal pressure waves were expressed as means over the 30 minute recording period. The number and amplitude of IPPWs, and basal pyloric pressures were expressed as means over 10 min periods during the 30 min recording period. All data were expressed as changes from baseline.

The number and amplitude of IPPWs, basal pyloric pressure, VAS scores and plasma CCK concentrations were analysed using repeated measures analysis of variance (ANOVA) with treatment, and time, as factors. Antral and duodenal PWs and energy intake were analysed using one-way ANOVA. Post-hoc paired comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed if ANOVAs revealed significant effects. Data are presented as means ± SEM. Statistical significance was accepted at $P < 0.05$.

10.4. Results

The study protocol was tolerated well by all subjects. There was no change in body weight on either the high-fat, or the low-fat, diets (baseline: high-fat: 69.5 ± 3.0 kg, low-fat: 69.9 ± 3.0 kg; day 22 of diet: high-fat: 69.5 ± 3.5 kg, low-fat: 69.3 ± 3.2 kg).

10.4.1. Antropyloroduodenal pressures

There was no effect of treatment on the number, or amplitude, of antral, pyloric or duodenal pressure waves, or basal pyloric pressure (**Figure 10.1**). Following both the high-fat and low-fat diets, administration of CCK-8 was associated with an increase in the number and amplitude of antral and duodenal PWs, and stimulation of basal pyloric pressure and isolated pyloric pressure waves (time effect: $P < 0.05$, for all), when compared with baseline.

10.4.2. Plasma CCK concentrations

Baseline concentrations of CCK were greater following the high-fat, when compared with the low-fat diet (high-fat: 4.3 ± 0.4 pmol/l, low-fat: 3.1 ± 0.3 pmol/l, $P < 0.05$). Plasma concentrations of CCK increased during infusion of CCK-8 to reach a peak at t = 10 min, with peak levels being maintained between t = 10 – 30 min (time effect: $P < 0.001$), with no difference in the magnitude of the response between the two diets (**Figure 10.2**). The peak plasma concentrations of CCK may be considered to be moderately supraphysiological. Following ingestion of the meal, plasma CCK concentrations fell and were lower at t = 60 min, when compared with t = 30 min ($P < 0.001$), with a further fall at t = 90 min.

10.4.3. VAS scores and energy intake

There was no effect of treatment on ratings of hunger or fullness. Hunger remained at baseline throughout the infusion. During infusion of CCK-8 fullness increased from baseline between t = 10 – 30 min (time effect: $P < 0.05$). CCK-8 did not induce bloating or nausea (data not shown).

There was no difference in energy intake (kJ), amount (g) or the macronutrient distribution of food consumed at the buffet meal following the high-fat, when compared with the low-fat, diet (**Table 10.4**).

10.5. Discussion

This study has evaluated in healthy lean men the effects of increasing the fat content of the diet on the antropyloroduodenal motility and energy intake responses to intravenous CCK-8. The novel observation is that consumption of a high-fat (44 % energy as fat) diet was associated with increased fasting concentrations of CCK, suggesting that exposure to a high-fat diet modulates the secretion of CCK. However, contrary to our hypothesis, and previous data from studies in rodents, the effects of exogenous CCK-8 on antropyloroduodenal motility and energy intake were maintained following exposure to a high-fat diet, when compared with an isocaloric low-fat diet (9 % energy as fat), for a period of three weeks, in the absence of a change in body weight, suggesting that, in lean men, the sensitivity to exogenous CCK-8 is not changed following a short period on a high-fat diet.

Our observation of increased fasting concentrations of CCK following exposure to the high-fat diet indicates that the response of CCK to fat is modulated, suggesting that changes in the mechanisms that mediate CCK secretion, metabolism and sensitivity may occur. In agreement with this, a previous study has reported increased postprandial CCK concentrations following exposure to a high-fat diet, when compared with a pre-diet condition (integrated plasma CCK: high-fat: 1285 ± 153 pM/min, pre-diet: 897 ± 78 pM/min; $P < 0.01$) (French *et al.*, 1995). However, in response to direct intraduodenal infusion of lipid (2.8 kcal/min), which bypasses the influence of gastric emptying, CCK levels did not differ following exposure to a high-fat, when compared with a low-fat, diet (Boyd *et al.*, 2003). It is, therefore, likely that the increased postprandial plasma

CCK concentrations observed following consumption of a high-fat diet (French *et al.*, 1995) are reflective of more rapid gastric emptying (Cunningham *et al.*, 1991). The mechanisms by which fasting concentrations of CCK are increased are unclear. Similar observations have previously been reported in patients with anorexia (Baranowska *et al.*, 2000; Phillipp *et al.*, 1991), and in healthy elderly subjects (MacIntosh *et al.*, 2001), and have been suggested to relate to impaired clearance of CCK from the circulation. Increased fasting concentrations of CCK have also been suggested to attenuate the effects of CCK on satiety. For example, MacIntosh *et al*. reported an inverse relationship between plasma CCK concentrations and hunger ratings in young, but not older, men during intraduodenal lipid infusion, suggesting reduced sensitivity to the satiating effects of CCK (MacIntosh *et al.*, 1999).

Our hypothesis, that the sensitivity to CCK may be reduced by a high-fat diet in humans, was based primarily on the outcome of a previous study in healthy lean men demonstrating that exposure to a hyper-caloric high-fat diet (40 % energy from fat, 20,123 kJ per day) for a period of 14 days attenuated the stimulatory effects of an intraduodenal lipid infusion on tonic and phasic pyloric pressures, when compared with a low-fat diet (11 % energy from fat, 11,191 kJ per day), despite similar elevations in plasma CCK (Boyd *et al.*, 2003). Contrary to this hypothesis, there was no difference in the effects of an intravenous infusion of CCK-8 on antropyloroduodenal motility or energy intake. This is in contrast to data from rodent studies reporting that the inhibitory effects of an i.p. injection of CCK on both gastric emptying (Covasa and Ritter, 2000) and food intake (Covasa *et al.*, 2001; Covasa and Ritter, 1998) are attenuated following exposure

to a high-fat diet (containing 34 or 54 % energy as fat), when compared with exposure to an isocaloric low-fat diet (containing 5 % energy as fat), for 2 weeks, in the absence of any change in body weight or adiposity. There is, however, some inconsistency in the available information. For example, the inhibitory effect of i.p. CCK-8 on energy intake has been reported to be maintained following consumption of a 34 % fat diet for a period of two weeks in rats (Torregrossa and Smith, 2003), and in rats consuming a 60 % fat diet for two weeks sensitivity to the satiating effects of CCK has been reported to be increased, rather than decreased, when compared with rats maintained on a low-fat diet (Torregrossa and Smith, 2003). The reason(s) for the discrepant observations are unclear, however, it should be noted that the rats consuming the 60 % fat diet gained more weight and adipose mass when compared to rats on the 5 or 34 % diets, and that the effects of CCK on energy intake may, accordingly, be dependent on body weight. Furthermore, the observations from studies in animals, particularly rodents, may not be applicable to humans. It is important to note that in animal studies the magnitude of the fat supplementation is far greater than in human studies as the diet can be enriched to almost 100 % with a particular macronutrient, whereas in human studies the degree of fat supplementation is limited by the need to provide normal foods.

Previous studies have indicated that exposure to a high-fat diet modulates gastrointestinal function and energy intake in humans (Boyd *et al.*, 2003; Castiglione *et al.*, 2002; Cunningham *et al.*, 1991; French *et al.*, 1995). For example, in healthy lean male subjects consumption of a high-energy, high-fat diet (2340 kJ of fat, 19.3 MJ energy daily) for 14 days resulted in acceleration of

the gastric emptying and mouth-to-caecum transit of a high-fat test meal, when compared with a low-fat diet (105 kJ of fat, 9.1 MJ energy daily) (Cunningham *et al.*, 1991). Exposure to a high-fat diet has also been reported to result in an increase in energy intake, as measured from food diaries (French *et al.*, 1995). Given the important role of CCK in the regulation of gastric emptying (Fried *et al.*, 1991) and energy intake (Beglinger *et al.*, 2001) it would seem that exposure to a high-fat diet may modulate the actions of CCK, however, currently available data from human studies are unable to directly relate the changes in gastrointestinal function and energy intake to a change in CCK sensitivity.

The methodological approach taken in the current study warrants some discussion. In contrast to previous studies in humans, which have evaluated the effects of high-fat diets that were also high in energy, and compared them to low-energy, low-fat diets (Boyd *et al.*, 2003; Cunningham *et al.*, 1991; French *et al.*, 1995), we employed isocaloric high-fat and low-fat diets. It is, therefore, possible that the increased energy content of the high-fat diet, rather than the fat content *per se*, mediated the changes in gastric emptying (Cunningham *et al.*, 1991), antropyloroduodenal motility (Boyd *et al.*, 2003), cholecystokinin secretion (French *et al.*, 1995) and appetite (Boyd *et al.*, 2003; French *et al.*, 1995) observed in previous studies. Furthermore, while we matched the protein content of the diets, in attempting to increase the fat content of the diet, the carbohydrate content decreases, and vice-versa, thus it is difficult to discriminate between an effect of fat, as opposed to carbohydrate. The interpretation of our current observations is limited by the administration of only one dose of CCK, which was necessary in order to study the effects of CCK on the day immediately

following the diets. Nevertheless, this dose has previously been demonstrated to modulate antropyloroduodenal motility (Rayner *et al.*, 2000) and suppress energy intake (MacIntosh *et al.*, 2001), and our observations of the effect on gastrointestinal motility were consistent with previous reports. Furthermore, due to the lack of a control (i.e. intravenous infusion of saline following each diet) we cannot determine if the magnitude of the response to CCK was attenuated, when compared with baseline, following both diets, as they were both hypercaloric in comparison to the subject's habitual diet. It is unclear why plasma CCK concentrations did not rise following ingestion of the buffet-meal, however, it has previously been reported that exogenous administration of CCK-8 suppresses endogenous CCK secretion (MacIntosh *et al.*, 2001).

In conclusion, our study suggests that while exposure to a high-fat diet results in increased fasting plasma CCK concentrations, gastrointestinal function and energy intake in response to exogenous CCK-8, at the dose administered, is not changed, when compared with an isocaloric low-fat diet, for a period of three weeks in healthy lean male subjects.

Table 10.1: Energy intake and dietary macronutrient composition over the 5-day baseline period, as obtained from diet diaries.

% Fat	***% CHO***	***% Protein***	***Energy intake (kJ)***	***Amount (g)***
32 ± 4	50 ± 4	18 ± 1	9720 ± 313	1983 ± 193

Data are mean ± SEM (n = 10). CHO = carbohydrate.

Table 10.2: Prescribed daily energy and macronutrient intakes during the 21-day dietary periods and dietary compliance (%).

Diet	***Energy (kJ)***	***Fat***	***CHO***	***Protein***
High-fat	15,546 ± 432	185 ± 7 g	371 ± 30 g	115 ± 7 g
% energy		44	38	12
Compliance (%)	96	95	97	96
Low-fat	15,335 ± 530	36 ± 4 g	674 ± 40 g	121 ± 3 g
% energy		9	70	14
Compliance (%)	95	98	98	94

Data are mean ± SEM of the energy and macronutrient composition of the 6 daily meal plans. The dietary compliance (%) was determined from food diaries completed during each 21-day diet period. CHO = carbohydrate.

Table 10.3: Example of a menu for the high-fat, and low-fat, diets, respectively on one day.

High-fat diet		**Low-fat diet**	
Breakfast	***Amount***	***Breakfast***	***Amount***
Toasted muesli	80 g	Low-fat muesli	120 g
Full-cream milk	200 ml	Skim milk	300 ml
		Tinned fruit	220 g
		Orange fruit juice	250 ml
Lunch		***Lunch***	
Quiche Lorraine	2 (~240 g)	Baked beans	220 g
Chocolate milk (full-cream)	600 ml	Bread roll	60g
		Chocolate milk (low-fat)	525 ml
Snacks		***Snacks***	
Plain potato crisps	40 g	Oven baked fruit bars	90 g
Popcorn	30 g	Popcorn	40 g
Salted peanuts	45 g	Apple	~ 210 g
Crunchy muesli bar	32 g	Jelly snakes	100 g
Dinner		***Dinner***	
Beef chilli with beans	425 g	Lean beef chilli	425 g
White rice, boiled	2 cups (cooked)	White rice, boiled	2 cups (cooked)
Diet lemonade	600 ml	Lemonade	600 ml
Bread roll, white	~30 g		
Butter	10 g		

Table 10.4: Energy intake, amount and macronutrient distribution of food consumed at a buffet-style meal immediately after an intravenous infusion of CCK-8 at 2ng/kg/min following either a high-fat (44 % energy as fat) or a low-fat (9 % energy as fat) diet for a period of 21 days.

Diet	***Energy intake (kJ)***	***Amount (g)***	***% Fat***	***% CHO***	***% Protein***
High-fat	4545 ± 620	1133 ± 135	30 ± 2	48 ± 3	21 ± 1
Low-fat	4246 ± 648	1013 ± 191	32 ± 2	47 ± 3	21 ± 2

Data are mean ± SEM (n = 10). CHO, carbohydrate.

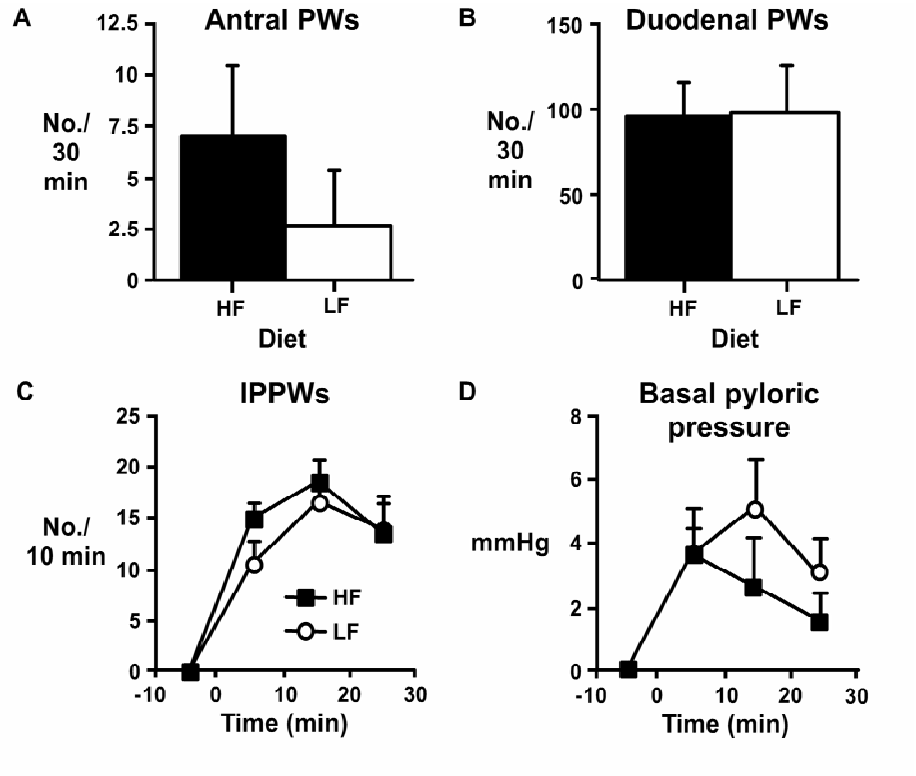


Figure 10.1: Number of (A) antral and (B) duodenal pressure waves (PWs), (C) isolated pyloric pressure waves (IPPWs) and (D) basal pyloric pressure during intravenous infusion of CCK-8 at 2 ng/kg/min following ingestion of high-fat (44 % energy as fat, HF) and low-fat (9 % energy as fat, LF) diets each for a period of 21 days. Data are mean ± SEM (n = 10).

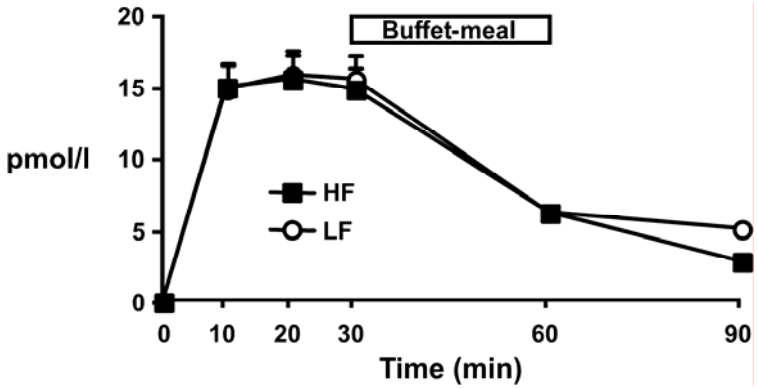


Figure 10.2: Plasma CCK concentrations during intravenous infusion of CCK-8 at 2 ng/kg/min following ingestion of high-fat (44 % energy as fat, HF) and low-fat (9 % energy as fat, LF) diets each for a period of 21 days. Data are expressed as change from baseline and presented as mean ± SEM (n = 10).

Chapter 11

Conclusions

Obesity and its co-morbidities, including type 2 diabetes and coronary heart disease, represent a major health, social and economic burden in Australia and other Western countries. While the causes of obesity are poorly defined, it is clear that a number of factors, including genetic, environmental, physiological and psychological, contribute. In order to understand how disordered gastrointestinal function may contribute to the pathophysiology of obesity, and to design effective treatments for obesity, it is important to appreciate the fundamental role of signals arising from the gastrointestinal tract in the regulation of appetite and energy intake. The suppression of appetite and energy intake is mediated, at least in part, by a number of gastrointestinal factors, including gastric distension, the modulation of gastric emptying, gastrointestinal motility and gastrointestinal peptides, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY) and ghrelin.

The research presented within this thesis has focused on the complex and interrelated gastrointestinal mechanisms involved in the regulation of appetite and energy intake. The main aims of the studies were to assess the mechanisms by which nutrients modulate the function of the gastrointestinal tract, and consequently influence appetite and energy intake. In particular (i) the comparative effects of free fatty acids and triglycerides (Chapter 5), (ii) the effects of the dose, energy load and concentration of the free fatty acid, lauric

acid (C12) (Chapters 6 and 7) (iii) the effect of increasing the length of small intestine exposed to nutrient (Chapter 8), (iv) the relative importance of intragastric meal distribution to the regulation of appetite and energy intake (Chapter 9) and (v) the effects of exposure to a high-fat diet (Chapter 10), were evaluated.

It is now well established that the effects of fat on gastrointestinal function, appetite and energy intake are mediated by the presence of free fatty acids in the small intestinal lumen following fat digestion (Feinle *et al.*, 2003; Feinle *et al.*, 2001; Feinle-Bisset *et al.*, 2005; Pilichiewicz *et al.*, 2003; Schwizer *et al.*, 1997). However, relatively little data regarding the direct comparative effects of free fatty acids and triglycerides on gastrointestinal function and energy intake are available. Animal studies suggest that the effects of free fatty acids on energy intake are more potent than those of triglycerides (Cox *et al.*, 2004; Woltman *et al.*, 1995). The study described in Chapter 5 demonstrated, in healthy lean male subjects, that the free fatty acids empty more slowly from the stomach, stimulate the release of CCK and suppress appetite and energy intake much more potently than triglycerides. These data suggest that the more potent effects of free fatty acids on energy intake are related to their actions on gastrointestinal function.

We had previously demonstrated that intraduodenal infusion of lauric acid (C12) (at 0.375 kcal/min, 106 mM; total energy delivered: 141 kJ) stimulates isolated pyloric pressure waves (IPPWs), inhibits antral and duodenal pressure waves (PWs), stimulates the release of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), and suppresses energy intake, and that these effects are much

greater than those seen in response to isocaloric decanoic acid (C10) infusion. However, C12 was associated with nausea, confounding interpretation of these results. In order to determine whether the effects we had observed were physiological, or related to nausea, we assessed the effects of a range of doses of C12 on the above parameters. We demonstrated that intraduodenal infusion of very small amounts of C12 potently modulates gastrointestinal motility, gut hormone secretion and suppresses energy intake at a subsequent meal in a dose-dependent fashion (Chapter 6). However, as both the load and the concentration of the infusions varied in parallel, it was unclear whether these effects were load-, or concentration-, dependent. We, therefore, examined the independent effects of load and concentration of C12 on these variables, and demonstrated that the effects of C12 on gastrointestinal motility, gut hormone release and energy intake are dependent upon the load, but not the concentration of C12 administered to the small intestine (Chapter 7).

Animal studies have indicated that the effects of nutrients on gastrointestinal function and energy intake are dependent upon the length of small intestine exposed to nutrient (Lin *et al.*, 1989; Lin *et al.*, 1990; Meyer *et al.*, 1998). In the study described in Chapter 8, we were able to demonstrate that in humans, the modulation of gastrointestinal motility and gut hormone secretion is critically dependent upon the length of small intestine exposed to nutrient; specifically, the suppression of antral motility, the release of GLP-1 and the suppression of plasma ghrelin concentrations is dependent upon greater than 60 cm of the small intestine being exposed to glucose. These observations have implications for the regulation of gastric emptying, postprandial glycaemia, appetite, and energy

intake. However, a confounding factor in the study design was that on both study days, glucose was infused into the proximal isolated 60 cm segment of the small intestine. Therefore, whether the effects were dependent upon the recruitment of receptors along a certain length of small intestine, or were dependent upon exposure of a specific region (i.e. the distal small intestine), is unclear. The effects of small intestinal infusions of nutrients into different regions of the small intestine, i.e. proximal versus distal, should be determined in future studies to evaluate this possibility. Furthermore, the effects of exposing different lengths, and regions of the small intestine to nutrients on appetite and energy intake warrant investigation.

The inhibitory action of glucagon-like peptide-1 (GLP-1) on gastric emptying (GE) is likely to be important in mediating its effects on glycaemia, appetite and upper gastrointestinal symptoms. In the study described in Chapter 9, we demonstrated that in healthy subjects (i) the slowing of solid and liquid gastric emptying by exogenous GLP-1 is associated with increased retention of both solid and liquid in the distal stomach and, even when administered in a "low" dose can induce "gastroparesis" and (ii) the effects of GLP-1 on postprandial glycaemic and insulinaemic responses are predictable on the basis of its effect on gastric emptying, supporting the concept that gastric emptying is a major target mechanism for the clinical use of incretin mimetics. The inhibitory effects of exogenous GLP-1 on energy intake are likely to relate to the increased retention of the meal in the antrum, as a close relationship has been demonstrated between the antral area and the perception of fullness and subsequent energy intake.

Further studies are indicated to evaluate the relationship between the slowing of gastric emptying by GLP-1 with energy intake.

An understanding of the physiological adaptations that occur in obesity is essential to the development of successful therapies for this condition. There is increasing evidence that consumption of a high-fat diet is associated with the development of obesity. The precise mechanisms by which this occurs are unclear, however, studies in animals suggest that adaptations in the gastrointestinal mechanisms involved in the regulation of appetite and energy intake occur (Covasa and Ritter, 2000, 1998; Spannagel *et al.*, 1996), and may, therefore, predispose to obesity. In particular, studies have demonstrated that the acute effects of exogenous CCK, a hormone that potently suppresses energy intake, are attenuated following exposure to a high-fat diet in rats (Covasa and Ritter, 2000, 1998). In our study (Chapter 10), healthy lean male volunteers were exposed to a high-fat diet, and an isocaloric low-fat diet, for a period of 3 weeks, following which the effects of an intravenous infusion of CCK on gastrointestinal motility and energy intake were evaluated. Fasting plasma concentrations of CCK were increased following the high-fat diet. In contrast, we did not observe any differences in the response to CCK following ingestion of either diet, suggesting, that at least in the short-term, in healthy lean male subjects consumption of a high-fat diet does not alter the sensitivity to the effects of CCK on antropyloroduodenal motility and energy intake. The observations of this study are limited by the administration of only one dose of CCK; future studies evaluating the effects of low doses of CCK on gastrointestinal function and energy intake following consumption of a high-fat diet are indicated.

The observations of the studies presented in this thesis provide an insight into the gastrointestinal mechanisms by which nutrients modulate appetite and energy intake, and provide potential targets for the development of treatments for weight loss in obesity. There is, however, some evidence that gastrointestinal motor function, gut hormone release and energy intake may be disturbed in obesity (Delgado-Aros *et al.*, 2004; Park and Camilleri, 2005; Speechly and Buffenstein, 2000; Tschop *et al.*, 2001; Verdich *et al.*, 2001). The observations presented in this thesis therefore need to be extended in obese individuals.

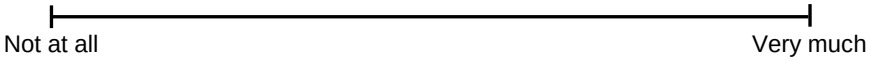
Appendix 1

Visual Analogue Scale questionnaire

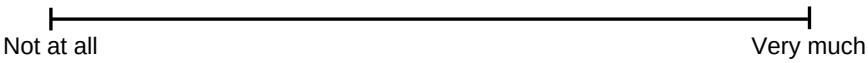
Name (Initials): **Visit:** **Time:**

Please indicate how you are feeling at this moment by placing a vertical mark at the appropriate point on each scale below. Furthest LEFT means you do not feel the sensation in question, furthest RIGHT means you feel it very much. Please, mark all scales.

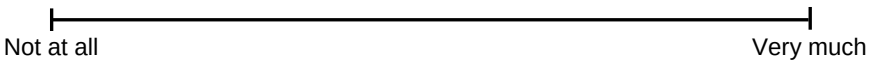
I feel nauseated



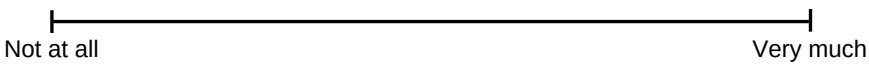
I feel drowsy



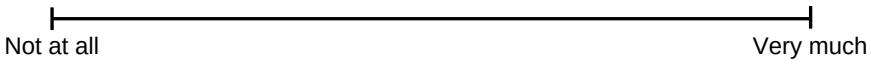
I feel bloated



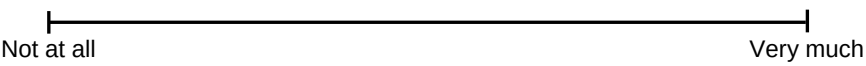
I feel anxious



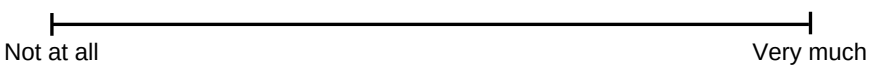
I feel hungry



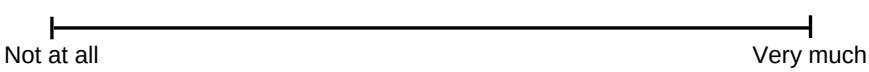
I feel full



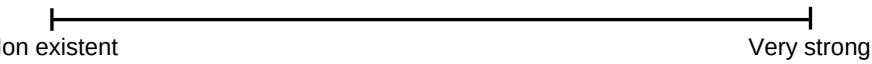
I feel happy



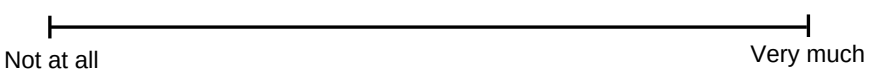
I feel energetic



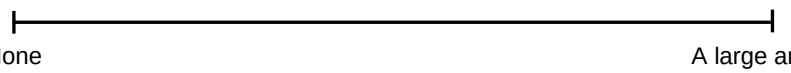
How strong is your desire to eat?



I feel comfortable



How much food do you think you could eat?



Appendix 2

Composition of the buffet-style meal used to quantify energy intake

Food items	Amount (g)	Energy (kJ)	Fat (g)	Carbohydrate (g)	Protein (g)
Wholemeal bread, 4 slices[a]	125	1,304	3.6	50.0	12.6
White bread, 4 slices[a]	125	1,295	2.9	56.4	11.8
Ham, sliced[b]	100	453	3.6	0	18.8
Chicken sliced[c]	100	677	7.0	0	24.6
Cheese, sliced[d]	85	1,436	28.3	0.9	21.9
Tomato, sliced	100	56	0.1	1.9	1.0
Lettuce	100	27	0	0.4	0.9
Cucumber, sliced	100	44	0.1	1.9	0.5
Strawberry yoghurt[e]	200	966	6.2	33.8	9.4
Fruit salad[f]	140	343	0.1	19.3	0.6
Chocolate custard[g]	150	662	5.3	22.7	4.8
Apple	170	359	0.2	21.3	0.5
Banana	190	680	0.2	37.8	3.2
Orange juice [h]	500	800	5.0	42.5	5.0
Iced coffee[i]	600	1,788	10.2	61.8	21.0
Water	600	0	0	0	0
Margarine[j]	20	609	16.4	0.1	0.1
Mayonnaise[k]	20	310	6.5	4.0	0.2
Total	***3,425***	***11,808***	***95.7***	***354.6***	***136.9***

[a]Sunblest, Tiptop, Australia; [b]Deli leg ham, Woolworths, Australia; [c]Virginian chicken, Woolworths, Australia; [d]Coon Tasty Cheese slices, Australian Cooperative Foods Ltd., Australia; [e]Yoplait, National Foods Ltd., Australia; [f]Goulburn Valley, SPC, Ardmona Operations Ltd., Australia; [g]Yogo, National Foods Ltd., Australia; [h]Daily Juice Company, Australia; [i]Farmers Union, Balemar Pty. Ltd., Australia; [j]Flora, Unilever Australasia, Australia; [k]Kraft, Kraft Foods Ltd., Australia.

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