Evaluation of appetite regulation in lean and obese individuals

A thesis submitted by
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<td>3-D</td>
<td>three-dimensional</td>
</tr>
<tr>
<td>AP</td>
<td>adequate-protein</td>
</tr>
<tr>
<td>APD</td>
<td>antropyloroduodenal</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CCK</td>
<td>cholecystokinin</td>
</tr>
<tr>
<td>CHO</td>
<td>carbohydrate</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<td>follicle stimulating hormone</td>
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<td>GLP-1</td>
<td>glucagon-like peptide-1</td>
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<td>IPPWs</td>
<td>isolated pyloric pressure waves</td>
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<td>LH</td>
<td>luteinizing hormone</td>
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<td>LOX</td>
<td>loxiglumide</td>
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<td>LP</td>
<td>low-protein</td>
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<td>MMC</td>
<td>migrating motor complex</td>
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<td>transmucosal potential difference</td>
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Summary

THESIS SUMMARY

The research presented within this thesis has focussed on the complex and interrelated postprandial gastrointestinal mechanisms involved in the regulation of appetite and energy intake. The three broad areas that have been investigated include: (i) the effect of gastrointestinal hormones on gastric motility, gastrointestinal hormone release/suppression, appetite and energy intake in healthy lean subjects, (ii) the effect of oral macronutrients on appetite and energy intake in both lean and obese subjects and (iii) the effects of acute energy restriction on gastrointestinal motility, gastrointestinal hormone release, appetite and energy intake in obese subjects.

Following meal ingestion, the presence of nutrients in the small intestine stimulate small intestinal receptors that trigger a number of gastrointestinal mechanisms within ~ 15 minutes; these include the modulation of gastric emptying and gastrointestinal motility and the release, or suppression, of gastrointestinal hormones i.e. cholecystokinin (CCK), peptide-YY (PYY), glucagon-like peptide-1 (GLP-1) and ghrelin. Hence, it is conceivable that interactions occur between one or more of these stimuli. The study in Chapter 5 assessed possible interactions between intravenous CCK (1.8 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min) that may modulate ghrelin and PYY release. At the doses evaluated, exogenous CCK-8 and GLP-1 had discrepant effects on the secretion of ghrelin and PYY; CCK-8
markedly suppressed ghrelin whereas GLP-1 had no effect, and the stimulation of PYY by CCK-8 was attenuated markedly by GLP-1.

Of the gastrointestinal hormones modulated following nutrient ingestion, CCK and its role in appetite regulation has been studied the most comprehensively. A recent study from our laboratory using exogenous CCK-8 suggested that the ability of CCK to suppress appetite and energy intake were mediated, at least in part, by its actions on the gastrointestinal tract. However, the plasma CCK concentrations resulting from this study were moderately supraphysiological and infusion of CCK-8 was associated with an increase, albeit modest, in nausea. The effects of increasing doses of CCK-8 on gastrointestinal motility, gut hormone release and the relationships between these effects with those on hunger and energy intake had not hitherto been assessed in humans. In Chapter 6, exogenous CCK-8 stimulated pressures in the pylorus, increased plasma PYY concentrations and suppressed desire-to-eat and energy intake in a dose-dependent manner, while all CCK-8 doses equally suppressed ghrelin. There were relationships between plasma CCK with basal pyloric pressure and isolated pyloric pressure waves, and energy intake with isolated pyloric pressure waves.

The prevalence of obesity is rapidly increasing, the cause of which is related, in part, to the readily available supply of high-fat, energy-dense foods. Recent data indicate that there are more than 250 million obese people worldwide, representing ~ 7% of the adult population. There is evidence that gastrointestinal function in obesity is modified, which may be the result of the eating habits of obese
individuals, and in turn, may also contribute to the maintenance of obesity by causing insufficient suppression of energy intake. However, much of the literature relating to gastrointestinal function in the obese is inconclusive and controversial. A better understanding of any adaptations that occur in obesity is important, particularly in regards to treatment approaches for weight loss.

Protein is considered to be the most satiating macronutrient and studies have demonstrated that consumption of dietary protein reduces appetite and ad libitum energy intake when compared with either carbohydrate or fat. One option in the dietary management of obesity has been to replace some carbohydrate in the diet with protein, which has been demonstrated to facilitate loss of fat and blunt loss of lean mass. However, there are discrepancies in the ranking of macronutrients and not all studies demonstrate that protein is more satiating than carbohydrates or fat. Furthermore, studies that have demonstrated effects of high-protein preloads on appetite and energy intake have often used preloads consisting of ~60% protein. Thus, it is plausible that the observed effects may have been due to excessive amounts of protein in the test meal; such meals would be less palatable, which may also lead to reduced energy intake. Since there may be differences in the regulation of gastrointestinal motor function, gastrointestinal hormone release, appetite and energy intake between lean and obese individuals, it is likely that ingestion of individual macronutrients may also have different effects on these parameters, which might have implications for the dietary treatment of obesity.
The study in Chapter 7 evaluated the effects of high-protein, high-fat and high-carbohydrate test meals, and increasing amounts of protein in a test meal, on appetite and energy intake in lean and obese subjects. In addition, the study compared these responses between lean and obese subjects. In lean, but not obese, subjects, hunger was less, and fullness increased, following ingestion of the HF and HP meals. In addition, energy intake was reduced in lean subjects following the HF and HP meals when compared with the HC meal, while in obese subjects, the HP and AP meals reduced energy intake when compared with the HF and HC meals, and HC meal, respectively. When these responses were compared, the percentage change in energy intake between the HF and AP test meals was significantly different between lean and obese, suggesting that obese subjects may be less sensitive to the satiating effects of fat.

The studies presented in the subsequent two chapters (Chapters 8 and 9) investigated the contribution of factors that may influence the effects of oral macronutrients on gastrointestinal function, appetite and energy intake. While young, lean males are the subject group most capable of adjusting their energy intake in response to caloric manipulation, it has been observed that significant inter-individual variation occurs within this group. Therefore, it was important to evaluate whether there was a day-to-day variability in gastrointestinal function, including gastric emptying and gastrointestinal hormone secretion, and if so, how these variations influenced temporal changes in appetite and energy intake. The study in Chapter 8 demonstrated that, in a laboratory setting, appetite perceptions and energy intake in response to a nutrient preload in healthy lean men were
Summary

highly reproducible, and that this consistency in energy intake was associated with reproducible patterns of gastric emptying and insulin and CCK secretion.

A major reason that females are used less frequently than males in research studies assessing gastrointestinal function, appetite and energy intake is the perceived confounding effect of the menstrual cycle on these parameters. There is evidence that fluctuations in hormone levels over the menstrual cycle affect energy intake, such that hunger and energy intake are less during the follicular phase and increased during the luteal phase. How this modulation of appetite and energy intake would be related to changes in gastrointestinal function, i.e. gastric emptying and gastrointestinal hormone release, remained unclear. The study described in Chapter 9 demonstrated that gastric emptying was slower, and glycaemia, plasma GLP-1 and insulin responses, hunger and energy intake were less, during the follicular when compared with the luteal phase. Moreover, energy intake and the glucose, plasma GLP-1 and insulin responses were related to gastric emptying. In addition, these parameters were reproducible when assessed twice within the follicular phase of the menstrual cycle.

There is evidence that both previous patterns of macronutrient intake and fasting affect gastrointestinal function. In the context of obesity, both are of relevance. For example, in humans after a high-fat diet for 2 weeks, gastric emptying and mouth-to-caecum transit in response to a high-fat test meal were faster. In contrast, fasting has the opposite effect and a 4-day fast slowed gastric emptying of a glucose drink in both lean and obese subjects, suggesting that a reduction in
nutrient exposure may increase the sensitivity of gastrointestinal responses to
nutrients in the obese. The study in Chapter 10 demonstrated that following a
four-day very-low calorie diet (VLCD) there was a significant increase in basal
pyloric pressure and the number and amplitude of isolated pyloric pressure waves,
and a decrease in the number of antral and duodenal pressure waves and pressure
wave sequences, during a 120 minute intraduodenal lipid infusion. In addition,
following the four-day VLCD, hunger and prospective consumption scores were
lower, and energy intake was reduced, indicating that gastrointestinal function,
appetite and energy intake in the obese can be modified over a short period of
time.

The studies reported in this thesis provide new information relating to the
regulation of appetite and energy intake by gastrointestinal motor function and
hormone release and/or suppression, in healthy lean and obese subjects. These
observations will contribute to advances in basic appetite physiology and have
clinical implications for further development of dietary interventions for
successful treatment of obesity.
STATEMENT OF ORIGINALITY

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Ixchel Brennan

June 2009
DEDICATION

This thesis is dedicated to

my mother, Kathleen Winifred Brennan

and to

my grandmother, Winifred Royal Brennan.

My first teachers.

I am indebted to your selfless, extraordinary commitment to my education. I am forever grateful to you both for giving me the courage to think for myself, for helping me learn to persevere and work hard to succeed and achieve my goals, and for instilling within me the confidence that I am capable of doing anything I put my mind to.
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powerful examples to me of many qualities you cannot be ‘taught’ – generosity, faithfulness, respect, thoughtfulness, forgiveness and patience to name a few, and for these lessons, I will be forever grateful.

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PUBLICATIONS ARISING FROM THESIS


OTHER PUBLICATIONS


CHAPTER 1

Regulation of gastrointestinal function, appetite and energy intake

1.1 INTRODUCTION

Human eating behaviour is complex and a large number of factors, including genetic, environmental and physiological, contribute to the short, and long-term, regulation of appetite and energy intake. For example, the fact that some individuals are more prone to increased perceptions of hunger (De Castro 1999), greater daily energy intake (de Castro 1993) and increased body weight (Sorensen et al. 1989) reflects, at least in part, genetic factors that influence the long-term regulation of appetite and energy intake. Environmental factors that affect acute energy intake include the palatability of food (Yeomans et al. 2001) as well as the portion size and energy density of meals (Rolls et al. 1999, Rolls et al. 2002, Kral et al. 2004). Physiological factors following meal ingestion play an important role in the acute regulation of appetite and energy intake. It is well established that the presence of nutrients in the small intestine results in a number of gastrointestinal responses, which have been proposed to mediate, at least in part, the associated reduction in hunger and increase in fullness and suppression of energy intake.
These gastrointestinal responses include the slowing of gastric emptying (Sepple and Read 1989), as a result of the modulation of antropyloroduodenal (APD) motility (Cook et al. 1997, Feinle et al. 2003), and the release, or suppression (in the case of the hormone, ghrelin), of gastrointestinal hormones (Matzinger et al. 1999, Feinle-Bisset et al. 2005, Pilichiewicz et al. 2005).

In this chapter the role of the central nervous system in the regulation of appetite and energy intake is initially summarised. The major focus of the research described in this thesis relates to the role of changes in postprandial gastrointestinal function in the regulation of appetite and energy intake. Accordingly, literature relating to the acute effects of nutrients on gastric motor function, gastrointestinal hormone release, including cholecystokinin (CCK), peptide YY (PYY), glucagon-like peptide-1 (GLP-1), ghrelin and insulin, appetite and energy intake, is revised.

1.2 ROLE OF THE CENTRAL NERVOUS SYSTEM IN THE REGULATION OF APPETITE AND ENERGY INTAKE

A complex interplay exists between the central nervous system (CNS) and the activity of numerous organs involved in energy homeostasis (Figure 1.1). Energy homeostasis consists of the interrelated processes integrated by the brain to maintain energy stores at appropriate levels for given environmental conditions. Energy homeostasis thus includes the regulation of nutrient levels in key storage organs, i.e. fat in adipose tissue and glycogen in the liver, as well as the blood, i.e.
blood glucose concentrations. To accomplish this, the brain receives continuous
information about energy stores and fluxes in critical organs, food that is being
eaten and absorbed, and basal and situational energy needs by tissues. The brain
in turn controls tissues that have important roles in energy homeostasis, such as the
liver and skeletal muscle, as well as the secretion of key metabolically active
hormones, primarily through the autonomic nervous system.

Figure 1.1: Overview of the peripheral and central sites involved in the
regulation of appetite and energy intake (Woods and D’Alessio
2008).

Following meal ingestion, distension of the stomach activates gastric
mechanoreceptors located in the wall of the proximal stomach that are responsive
to stretch, while small intestinal nutrients activate luminal mechanoreceptors and
chemoreceptors. The stimulation of these receptors activates vagal afferents,
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which signal information to the brain, specifically the hypothalamus and brainstem (Moran et al. 1997, Knauf et al. 2008). These signals interact with neurons in the nucleus of the solitary tract (NTS) where they influence satiation and meal termination (Rinaman et al. 1995, Navarro et al. 1996, Moran et al. 1997). Gastrointestinal hormones stimulate appetite centres of the hypothalamus particularly the arcuate nucleus (ARC), either directly (Cowley et al. 2003, Cummings 2006), or via vagal efferents (Blackshaw and Grundy 1990, Garlicki et al. 1990). Adiposity signals related to body fat, such as leptin (Elias et al. 1999) and insulin (Corp et al. 1986), circulate in the blood to the brain passing through the blood-brain barrier in the region of the ARC and interacting with neurons that synthesise proopiomelanocortin or neuropeptide-Y and agouti-related peptide. ARC neurons in turn, project to other hypothalamic areas, including the paraventricular nuclei (PVN) and the lateral hypothalamic area (LHA). The net output of the PVN enhances the potency of satiation signals in the hindbrain, thereby acting to inhibit energy intake (Batterham et al. 2002, Fan et al. 2004). In contrast, the net output of the LHA suppresses the activity of satiation signals, thereby increasing energy intake (Asakawa et al. 2001). Accordingly, the acute and long-term regulation of appetite and energy intake involves the complex integration of a number of signals arising from the gastrointestinal tract and adipose tissue in the CNS. Upon food ingestion, the initial signals transmitted to the CNS by nutrients originate in the gastrointestinal tract. –The effects of these signals on post-prandial gastrointestinal function represent the focus of this thesis.
1.3 ANATOMY AND FUNCTION OF THE GASTROINTESTINAL TRACT

Each region of the upper gastrointestinal tract, including the proximal and distal stomach, pylorus and small intestine, has a role that assists in the digestion and absorption of ingested nutrients.

1.3.1 Function of the stomach

The stomach is a J-shaped organ that anatomically can be divided into the fundus, corpus, antrum and pylorus (Figure 1.2). Functionally, the stomach can be divided into two regions, the proximal, and the distal, regions, and they have different functions following meal ingestion. The proximal stomach receives, and accommodates, the ingested food (Heddle et al. 1989), while the distal stomach is responsible for the mixing and grinding of solid foods into smaller particles called chyme (Holt et al. 1982).

1.3.2 Function of the pylorus

The primary function of the pylorus is to regulate the flow of gastric content from the stomach into the small intestine and this may be the most important motor mechanism involved in the regulation of gastric emptying (Anvari et al. 1995). The pylorus exhibits both tonic and phasic contractile activity, occurring over a narrow zone (~ 2 mm), either in isolation or in temporal association with antral contractions (Heddle et al. 1988, Heddle et al. 1989).
1.3.3 Function of the small intestine

The small intestine is a muscular tube, approximately 5 metres in length, which can be divided into three regions – the duodenum (~25 cm in length), the jejunum (~2 metres in length), and the ileum (~3 metres in length) (Figure 1.2). The primary site of nutrient digestion is the proximal small intestine, specifically the duodenum and jejunum. The major functions of the small intestine are to i) accommodate the delivery of chyme from the stomach, ii) mix chyme with secretions from the pancreas that aid nutrient digestion and iii) absorb digested nutrients. The process of nutrient digestion and absorption can last up to 4 – 5 hours (Borgstrom et al. 1957), hence, nutrient exposure within the small intestine continues for hours after meal ingestion.

Figure 1.2: Basic anatomy of the stomach and small intestine
1.4 ROLE OF NUTRIENTS IN THE REGULATION OF GASTROINTESTINAL MOTILITY

The motor activity of the gastrointestinal tract alternates between two distinct patterns – the interdigestive migrating motor complex (MMC) during fasting and a ‘fed motility pattern’ that is initiated following food ingestion. The following section describes the patterns of motility in specific regions of the upper gastrointestinal tract, including the stomach, the pylorus and the small intestine, during fasting, and the changes induced by a meal.

1.4.1 Fasting gastrointestinal motility

During fasting the gastrointestinal tract exhibits a distinct cyclic pattern of motility, termed the migrating motor complex (MMC) (Sarna and Otterson 1988). The MMC consists of three phases with a cycle time of approximately 120 minutes: phase I is a period of motor quiescence, which has a duration between 40 – 60 min, phase II consists of irregular phasic contractions, which has a duration of between 45 – 90 min and phase III (Sarna and Otterson 1988) (Kellow et al. 1986). Phase III, which lasts for 5 – 10 minutes, is characterised by powerful coordinated contractions, which occur at the maximal frequency of the electrical pacemaker, which in the stomach is ~ 3, and in the duodenum is ~ 12, contractions per minute. Approximately 50 % of phase III episodes commence in the stomach and the remainder occur in the small intestine ensuring that any undigested food remaining in the stomach is propelled distally (Sarna and Otterson 1988). Once ingested
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nutrients are detected, fasting motility is interrupted and altered into a ‘fed’, or postprandial, motility pattern, which aids the digestion and absorption of nutrients.

1.4.2 Postprandial gastrointestinal motility

The interaction of nutrients with chemoreceptors in the small intestine following meal ingestion is responsible for the conversion of fasting motility into postprandial motility. Characteristic postprandial motility patterns in the upper gastrointestinal tract include proximal gastric relaxation (Feinle et al. 1996), a reduction in both antral and duodenal contractions (Heddle et al. 1988) and the stimulation of tonic and phasic pyloric pressures (Kumar et al. 1987, Heddle et al. 1988, Heddle et al. 1989) (Figure 1.3). These effects, perhaps particularly the stimulation of pressure waves isolated in the pylorus (Heddle et al. 1988), underlie the slowing of gastric emptying and ensure that chyme is delivered from the stomach to the small intestine at an overall rate of approximately 2 – 3 kcal/minute (Brener et al. 1983).

1.4.2.1 Gastric emptying

Gastric emptying is regulated by coordinated activity of the proximal, distal and pyloric 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 food (Horowitz et al. 1994). Following meal ingestion, two motor responses occur in the proximal stomach. The first, termed ‘receptive relaxation’, is initiated by swallowing, lasts for ~ 20 seconds, and is associated with a decrease in intragastric pressure. This is followed by a prolonged period of relaxation,
term ‘adaptive relaxation’ which occurs so that the meal can be stored, i.e. it ‘accommodates’ the rise in intragastric volume following ingestion of a meal without a substantial increase in intragastric pressure (Azpiroz and Malagelada 1987). As food is broken down and “ground” in the distal stomach, propulsive contractions move chyme from the stomach into the small intestine. Gastric emptying is predominantly a pulsatile, rather than continuous, process and patterns of transpyloric flow reflect the integration of motor activity in the proximal stomach, antrum, pylorus and proximal small intestine (Horowitz et al. 1994). Both antegrade and retrograde flow occurs and there is considerable variation in the characteristics of individual flow pulses (Anvari et al. 1995). 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. Patterns of gastric emptying are critically dependent on the nature, i.e. liquid or solid, and the macronutrient composition, of the meal ingested (Horowitz and Dent 1991, Edelbroek et al. 1992). Gastric emptying of nutrient-containing liquids and liquefied solids approximates an overall linear pattern (Horowitz and Dent 1991). In contrast, gastric emptying of non-nutrient liquids approximates a mono-exponential pattern (Collins et al. 1991). The emptying of solids is characterised by an initial lag phase, usually 10 – 30 min before emptying commences, during which solids move from the proximal into the distal stomach and are ground into small particles followed by an emptying phase which approximates a linear pattern (Collins et al. 1991), at least until the stomach is close to empty.
That the interactions of nutrients with chemoreceptors in the small intestine is responsible for the induction of postprandial motility is attested to by the effects of infusion of nutrients directly into the small intestine which changes fasting motility into a postprandial pattern (Heddle et al. 1988) and slows gastric emptying (Heddle et al. 1989). The magnitude of small intestinal feedback is dependent on both the length and region of the small intestine exposed to nutrients (Cooke 1977, Lin et al. 1989), and is influenced by prior nutrient exposure (Horowitz et al. 1996); the latter which will be discussed in more detail in Chapter 3.

1.5 EFFECTS OF SMALL INTESTINAL NUTRIENTS ON GASTROINTESTINAL HORMONE SECRETION

Meal ingestion and the subsequent interaction of nutrients with small intestinal receptors, stimulates the secretion of a number of hormones, including cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) from the small intestine (Cook et al. 1997, Feinle et al. 2000, MacIntosh et al. 2001, Batterham et al. 2003), insulin from the pancreas (Andrews et al. 1998), and suppresses the release of ghrelin from the stomach (Cummings et al. 2001, Williams et al. 2003). The following section summarises current knowledge of the mechanisms underlying the release, or suppression, of these gastrointestinal hormones, by macronutrients.
1.5.1 **Cholecystokinin**

CCK is synthesised in the “I” cells of the duodenal and jejunal mucosa and is also present in enteric vagal afferent neurones, and in the central nervous system, i.e. in the thalamus, hypothalamus, basal ganglia and dorsal hindbrain. CCK occurs in a number of forms including CCK-5, -8, -22, -33, -39, -54 and -58 (Rehfeld 1981). CCK-8 is the most abundant form of CCK in the human brain, while in the human intestine and circulation CCK-58, CCK-33, CCK-22 and CCK-8 are all present in significant amounts (Eberlein et al. 1988, Rehfeld et al. 2001). Fat and protein are potent stimulants of CCK (Liddle et al. 1985, Lieverse et al. 1994), and recent studies have demonstrated that glucose also has an (albeit lesser) effect (Parker et al. 2005).

1.5.2 **Peptide YY**

PYY is a 36 amino acid peptide synthesised by endocrine “L” cells, predominantly located in the ileum and small intestine (Adrian et al. 1985). PYY is secreted as PYY(1-36), and rapidly degraded to PYY(3-36) by dipeptidyl peptidase (Grandt et al. 1994). The release of PYY from the gut is proportional to the caloric content of ingested nutrients (Ekblad and Sundler 2002). PYY is released in response to the presence of fat (Pappas et al. 1986, Onaga et al. 2002) and protein (Fu-Cheng et al. 1997) in the small intestine, with fatty acids being the most potent stimulant (Onaga et al. 2002), but not carbohydrate (Groger et al. 1997).
1.5.3  **Glucagon-like peptide-1**

GLP-1 is a 33-amino acid peptide product of the glucagon gene and is released from the L cells, predominantly located in the distal small intestinal mucosa. GLP-1 is rapidly biodegraded into a biologically inactive form in human serum by the enzyme dipeptidyl peptidase IV (DPP-IV) (Mentlein et al. 1993). GLP-1 is released in response to the presence of nutrients in the small intestine, predominantly by carbohydrate and fat (Näslund et al. 1998, Feinle et al. 2002, Feinle et al. 2003), but also protein (Bowen et al. 2006).

1.5.4  **Ghrelin**

Ghrelin is a 28-amino acid peptide with an n-octanoyl side-chain which is important for its actions on gastrointestinal motor function and appetite (Kojima et al. 1999). Ghrelin is secreted from the fundic region of the stomach by oxyntic cells and has been identified as the endogenous ligand for the growth-hormone secretagogue receptor (Kojima et al. 1999, Date et al. 2000). In contrast to other gastrointestinal hormones, plasma ghrelin concentrations are higher during fasting and suppressed following food ingestion (Cummings et al. 2001), supporting the concept that ghrelin has a role in meal initiation. The suppression of ghrelin occurs in response to administration of nutrients in to the stomach, duodenum or jejunum, and carbohydrate and protein appear to be more potent suppressors of ghrelin than fat (Monteleone et al. 2003, Overduin et al. 2005). Studies in both animals and humans have established that ghrelin suppression is dependent on the exposure of nutrients to the small intestine, not the stomach (Williams et al. 2003,
Overduin et al. 2005, Parker et al. 2005). Intravenous glucose, but not lipid, has been reported to suppress ghrelin secretion (Mohlig et al. 2002), suggesting that post-absorptive factors play a role in the nutrient-specific suppression of ghrelin.

### 1.5.5 Insulin

Insulin is released by β-cells in the pancreas. Its primary role relates to glucose homeostasis and it is released in response to increased plasma glucose concentrations. Insulin is transported through the blood-brain barrier (Woods et al. 2003), and gains access to neurons in the hypothalamus and elsewhere in the brain to influence energy homeostasis. The magnitude of insulin secretion in response to carbohydrate is dependent on the release of the incretin hormones, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) (Lavin et al. 1998, Pilichiewicz et al. 2007).

In summary, the release, or suppression of CCK, PYY, GLP-1, ghrelin and insulin is dependent on the interaction of nutrients with receptors in the small intestine. In addition, this nutrient-dependent modulation of gastrointestinal hormones is, in many cases, dependent on the type of macronutrient.

### 1.6 EFFECTS OF SMALL INTESTINAL NUTRIENTS ON APPETITE AND ENERGY INTAKE

Direct intestinal infusions of nutrients allow the role of specific areas of the gastrointestinal tract in the control of appetite and energy intake to be investigated.
Subjects receive no cues regarding the taste or palatability of the nutrients infused and variations in gastric emptying are not a potentially confounding factor. Studies investigating the effects of intraduodenal nutrient administration have demonstrated that the presence of nutrients in the small intestine is associated with decreased perceptions of hunger and desire-to-eat, increased fullness and reduced subsequent energy intake (Cook et al. 1997, Chapman et al. 1999, MacIntosh et al. 1999, Pilichiewicz et al. 2005, Pilichiewicz et al. 2007). For example, following intraduodenal infusion of saline or 10% Intralipid at either 0.25, 1.5 or 4 kcal/min in 16 healthy males, hunger, and subsequent energy intake, were reduced in a dose-dependent manner (Pilichiewicz et al. 2007). In contrast, intravenous administration of nutrients appear to have little, if any, effect on appetite and energy intake (Welch et al. 1985, Lavin et al. 1996). The effects of intraduodenal and intravenous carbohydrate administration (20% glucose at 4 ml/min for 90 minutes) were compared in a study using healthy male subjects. Intravenous glucose had no effect on hunger or fullness ratings or energy intake. In contrast, intraduodenal administration of glucose suppressed hunger, increased fullness and reduced energy intake at a subsequent meal, indicating that the effects of nutrients on appetite and energy intake are mediated primarily by the stimulation of small intestinal receptors.

Fat, carbohydrate and protein have all been shown to reduce subsequent energy intake in animals, and humans, when infused 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 (Burton-Freeman et
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1.7 RELATIONSHIP BETWEEN APPETITE AND ENERGY INTAKE WITH GASTROINTESTINAL MOTOR FUNCTION

As discussed under 1.6, the presence of nutrients in the small intestine decreases hunger, increases fullness and reduces subsequent energy intake (Cook et al. 1997, Chapman et al. 1999, MacIntosh et al. 2001). There is evidence that the modulation of gastric motor function by nutrients, specifically changes in gastric, pyloric and small intestinal motility, contribute to the effects on appetite and energy intake.

1.7.1 Relationship between proximal stomach function with appetite and energy intake

Studies in humans indicate that distension of the proximal stomach, reflecting the slowing of gastric emptying, contributes to the postprandial regulation of appetite.
and energy intake (Geliebter 1988, Geliebter et al. 1988, Khan et al. 1993). For example, distension of the proximal stomach with an air-filled balloon at rates between 20 – 200 ml/min was reported to be associated with increasing perceptions of fullness (Khan et al. 1993). Distending the proximal stomach with a water-filled balloon at volumes of 400, 600 and 800 ml also leads to an increase in fullness, decrease in hunger and desire to eat and a reduction in subsequent energy intake in a volume-dependent manner (Geliebter 1988), as a result of the activation of mechanoreceptors in the stomach (Feinle et al. 1996). However, there were limitations associated with both of these studies. In particular, a barostat was not used, and it is, accordingly, unclear whether the observed effects reflect changes in pressure and/or volume. In addition, the regions of the stomach distended were not clearly visualised, i.e. the balloons were not positioned precisely and it is likely that the distal stomach was also distended. While these studies suggest that gastric distension, as associated with meal ingestion, plays a role in the regulation of appetite and the suppression of energy intake, this regulation is relatively transient, because as gastric emptying progresses, the distension stimulus is reduced.

1.7.2 Relationship between distal stomach function with appetite and energy intake

Studies in humans suggest that, following meal ingestion, accommodation in the distal stomach may play a more important role than that in the proximal stomach, in the regulation of appetite and energy intake (Jones et al. 1997, Hveem et al.
2001, Sturm et al. 2004). For example, following consumption of 350 ml of a 20% glucose drink the perception of fullness was closely related to antral area, or content, in healthy subjects, such that the greater the antral area the greater the perception of fullness, while no relationship was found between fullness with either total or proximal stomach content (Jones et al. 1997, Hveem et al. 2001). Furthermore, in healthy young and older subjects, energy intake after a ‘yoghurt preload’ is inversely related to antral area, so that a larger antral area is associated with decreased energy intake (Sturm et al. 2004) (Figure 1.4). While these observations suggest strongly that, amongst the ‘intragastric’ mechanisms, the antrum plays a dominant role in the regulation of appetite and energy intake, the relationship between changes in energy intake in response to a nutrient challenge with total, proximal and distal gastric volumes has not been assessed (Chapters 8 – 9).
1.7.3 Relationship between appetite and energy intake with pyloric pressures

Studies in animals and humans suggest that stimulation of pyloric pressures contributes to the regulation of energy intake. In dogs, electrical stimulation of the pylorus, increasing both tonic and phasic pyloric pressures, is associated with a reduction in energy intake (Xu et al. 2005). Studies in humans also suggest that there is an association between the suppression of energy intake with the stimulation of pyloric pressures (Feltrin et al. 2004, Brennan et al. 2005, Pilichiewicz et al. 2007). For example, there is a significant relationship between the suppression of energy intake and the stimulation of isolated pyloric pressure waves (IPPWs) by CCK-8 (Brennan et al. 2005). This suggests that an individual, in whom there is greater stimulation of IPPWs from a given stimulus, may eat less,
potentially because small intestinal feedback is greater. However, recognition of such a relationship does not necessarily imply a causal association.

In summary, while the interaction of nutrients with chemoreceptors in the small intestine is important in the modulation of appetite and energy intake, there is evidence that the associated effects on upper gastrointestinal motility, i.e. changes in the stomach and pylorus, contribute to the effects on appetite and energy intake.

1.8 ROLE OF GASTROINTESTINAL HORMONES IN MEDIATING THE EFFECTS OF NUTRIENTS ON GASTROINTESTINAL MOTOR FUNCTION, APPETITE AND ENERGY INTAKE

There is substantial evidence that gastrointestinal hormones mediate, at least in part, the effects of nutrients on gastrointestinal motor function and energy intake (Fried et al. 1991, Abbott et al. 2005, Schirra et al. 2006). The following sections focus on the roles of CCK, PYY, GLP-1, ghrelin and insulin in the regulation of gastrointestinal motor function and energy intake. While many of the studies described have employed exogenous administration of hormones to demonstrate effects on gastrointestinal motor function and energy intake, in order to establish a physiological role, specific receptor antagonists need to be employed.
1.8.1 Cholecystokinin

It is well established that CCK modulates gastrointestinal motility and energy intake. Studies employing specific CCK₁ receptor antagonists, such as loxiglumide, have demonstrated that CCK has a physiological role in the regulation of postprandial gastrointestinal motor function and energy intake.

1.8.1.1 Effects of CCK on gastrointestinal motor function

Exogenous administration of CCK slows gastric emptying in both animals and humans (Liddle et al. 1986, McHugh and Moran 1986, Muurahainen et al. 1988). In humans, intravenous CCK-8 slows gastric emptying, associated with a decrease in antral and duodenal pressure waves and the stimulation of IPPWs and pyloric tone (Liddle et al. 1986, Rayner et al. 2000, Brennan et al. 2005). Studies in both animals and humans, using the specific CCK₁ receptor antagonist, loxiglumide, have established that endogenous CCK has an effect on gastrointestinal motor function (Fried et al. 1991, Feinle et al. 1996, Katschinski et al. 1996). For example, the inhibitory effects of fat on gastric emptying and gastroduodenal motility are attenuated by administration of loxiglumide (Feinle et al. 1996, Katschinski et al. 1996). During duodenal perfusion of a mixed liquid meal for 150 min, loxiglumide administration decreased total numbers of antral, pyloric and duodenal contractions by 44%, 74% and 41% respectively (Katschinski et al. 1996), indicating that the inhibitory effects of fat on gastric emptying and gastrointestinal motility are mediated, at least in part, by CCK.


1.8.1.2 Effects of CCK on appetite and energy intake

The role of CCK in appetite regulation has been extensively studied following the initial report by Gibbs et al that intraperitoneal administration of the biologically active, sulphated octapeptide of CCK (CCK-8) dose-dependently suppressed energy intake, and sham-feeding, in rats (Gibbs et al. 1973). In healthy young (Kissileff et al. 1981, Brennan et al. 2005), older (MacIntosh et al. 2001) and obese (Pi-Sunyer et al. 1982), individuals, intravenous administration of CCK-8 and CCK-33 increases the perception of fullness, decreases hunger and reduces subsequent energy intake. In these studies, the doses of CCK used ranged between 1 – 4 ng/kg/min resulting in supraphysiological plasma concentrations, and adverse effects, including nausea and bloating. It has been suggested that the effects of CCK to suppress appetite and energy intake is mediated, at least in part, by its actions on the gastrointestinal tract. The effects of increasing doses of CCK-8 on gastrointestinal motility and gut hormone release, and the relationships between these effects with those on hunger and energy intake, and adverse effects have, however, not hitherto been assessed in humans (Chapter 6).

Only a small number of studies have evaluated the role of endogenous CCK in the regulation of appetite and energy intake, using the CCK₁ receptor antagonist, loxiglumide (Lieverse et al. 1994, Matzinger et al. 1999, Matzinger et al. 2000, Beglinger et al. 2001). In healthy subjects, the suppression of energy intake and the perception of fullness, induced by intraduodenal lipid, are abolished by loxiglumide (Matzinger et al. 1999, Feinle et al. 2001). In addition, administration of loxiglumide for one hour prior to, and during, ingestion of a meal, increased
both sensations of hunger and energy intake when compared with a saline infusion (Beglinger et al. 2001), providing further evidence that CCK is an endogenous physiological satiety signal acting through CCK$_1$ receptor-mediated mechanisms. It should be recognised, however, that the acute effects of CCK$_1$ receptor antagonists on energy intake are more modest than exogenous CCK so that significantly more subjects are required to show an effect (Matzinger et al. 2000, Beglinger et al. 2001), e.g. 40 healthy subjects were included in the study that observed an increase in energy intake by $\sim$10 % following loxiglumide administration (Beglinger et al. 2001), while, exogenous administration of CCK-8 have been shown to increase fullness and suppress hunger and energy intake by $\sim$20 %, even in studies with small subject numbers ($n = 8 – 12$) (Kissileff et al. 1981, Lieverse et al. 1995, Brennan et al. 2005). These observations indicate that the physiological role of CCK, acting via CCK$_1$-receptor mechanisms, in appetite regulation requires further clarification.

### 1.8.2 Peptide YY

Exogenous administration of PYY$_{(3-36)}$ modulates gastrointestinal motor function and energy intake in both animals and humans, however, there is currently no specific receptor antagonist available to clarify the physiological role of PYY in humans.
1.8.2.1 Effects of PYY on gastrointestinal motor function

In rhesus monkeys, intramuscular infusion of PYY(3-36) dose-dependently slows gastric emptying of saline (Moran et al. 2005). Similarly, in humans, exogenous administration of PYY(3-36) at either 0.18, or 0.51, pmol/kg/min dose-dependently slowed gastric emptying and mouth-to-caecum transit (Savage et al. 1987). While the dose of 0.51 pmol/kg/min resulted in plasma PYY concentrations higher than the physiological range, plasma concentrations following the lower dose of 0.18 pmol/kg/min were comparable with those observed following a meal (Adrian et al. 1985). These observations suggest that PYY(3-36) may be involved in mediating the effects of nutrients on gastrointestinal function. Moreover, since the cells that synthesise PYY are located primarily in the distal small intestine, and the release of PYY is related to the fat-induced inhibition of distal gut motility (Pilichiewicz et al. 2007), it has been suggested that PYY acts as the primary mediator of the fat-induced “ileal brake”, i.e. the slowing of gastric emptying induced by distal small intestinal feedback (Lin et al. 1997).

1.8.2.2 Effects of PYY on appetite and energy intake

While some studies have reported that administration of exogenous PYY(3-36) decreases energy intake in lean and obese humans (Batterham et al. 2002, Batterham et al. 2003, Neary et al. 2005) observations are inconsistent. PYY was reported to have no effect on energy intake in rats (Tschop et al. 2004). Large doses of PYY(3-36), i.e. 0.4 – 0.8 pmol/kg/min, were required to suppress energy intake in humans in another study (Degen et al. 2005), which resulted in
pharmacological plasma concentrations of PYY, and induced nausea, vomiting and abdominal pain in some subjects (Degen et al. 2005). Accordingly, it remains uncertain whether the reduction in energy intake was a specific or adverse effect of PYY. A recent study in rats reported that the anorexigenic effect of PYY$_{(3-36)}$ are inhibited by a PYY receptor antagonist, BIIE0246, (Abbott et al. 2005). The lack of a specific PYY receptor antagonist suitable for use in humans currently prevents clarification of the physiological role of PYY.

1.8.3 Glucagon-like peptide-1

A number of studies have demonstrated that exogenous GLP-1 modulates gastrointestinal motor function and energy intake. However, few studies have administered the specific GLP-1 receptor antagonist, exendin(9-39), to determine its physiological role.

1.8.3.1 Effects GLP-1 on gastrointestinal motor function

Exogenous GLP-1, administered at doses between 0.3 – 1.2 pmol/kg/min, mimics the effects of nutrients on gastrointestinal motor function by slowing gastric emptying (Nauck et al. 1997, Schirra et al. 2000, Delgado-Aros et al. 2002, Little et al. 2006). This is associated with the relaxation of the proximal stomach (Schirra et al. 2000), increased meal retention in the distal stomach (Little et al. 2006), the suppression of antral and duodenal pressure waves and stimulation of tonic and phasic pyloric pressures (Schirra et al. 2000, Brennan et al. 2005). The effect of endogenous GLP-1 on gastrointestinal motor function has been evaluated
using its specific receptor antagonist, exendin(9-39). The administration of exendin(9-39) blocks the effects of GLP-1 on gastric emptying in rats (Tolessa et al. 1998), and attenuates the effects of intraduodenal glucose on the stimulation of tonic and phasic pyloric motility and the suppression of antral and duodenal pressure waves, in humans (Schirra et al. 2006), suggesting that endogenous GLP-1 plays a physiological role in mediating the effects of nutrients on gastrointestinal motility. In contrast, a recent study by Salehi et al reported that blocking GLP-1 action through exendin(9-39) administration had no effect on gastric emptying in humans, suggesting that postprandial GLP-1 may only have minimal effects on gastric emptying of oral glucose. However, in this study gastric emptying was measured using a relatively insensitive technique (D-xylose absorption), and as such, further studies are warranted.

1.8.3.2 Effects of GLP-1 on appetite and energy intake

Exogenous administration of GLP-1 increases the perceptions of fullness and decreases hunger in animals (Turton et al. 1996) and inhibits energy intake in lean (Flint et al. 1998, Neary et al. 2005), overweight (Näslund et al. 1999) and type-2 diabetic subjects (Gutzwiller et al. 1999). However, observations relating to the effects of GLP-1 on appetite and energy intake are inconsistent (Long et al. 1999, Brennan et al. 2005) and, accordingly, the anorexigenic effect of exogenous GLP-1, even in pharmacological doses, is uncertain (Verdich et al. 2001). In rats, exendin(9-39) markedly increased food intake (Turton et al. 1996) suggesting that, at least in animals, GLP-1 plays a physiological role in the regulation of appetite
and energy intake. However, since no studies to date in humans have evaluated the effect of exendin(9-39) on energy intake, the role of endogenous GLP-1 in the regulation of appetite and energy intake in humans remains unclear.

1.8.4 Ghrelin

Exogenous administration of ghrelin affects gastrointestinal function and energy intake, and in contrast to the other gastrointestinal hormones, ghrelin may have a role in the regulation of fasting gastrointestinal motor function and meal initiation. Since currently, there is no specific receptor antagonist available for use in humans, the physiological role of ghrelin remains uncertain.

1.8.4.1 Effects of ghrelin on gastrointestinal motor function

In both animals and humans, exogenous administration of ghrelin induces phase III activity in the stomach (Tack et al. 2006), increases proximal gastric tone, accelerates gastric emptying, and dose-dependently stimulates gastric motility (Masuda et al. 2000, Levin et al. 2006, Tack et al. 2006). However, as previously discussed, ghrelin release is suppressed following meal ingestion (Monteleone et al. 2003, Greenman et al. 2004), hence, the relevance of the effect of ghrelin on gastric emptying is unclear. Tranzyme Pharma (TZP-101) is a small molecular agonist with potent binding affinity and full agonist activity at the common ghrelin and human recombinant growth hormone secretagogue (GRLN-R) receptor. In rats, TZP-101, administered either intracerebroventricularly or intravenously, accelerated the rate of gastric emptying of a liquid meal and stimulated
spontaneous food intake in a concentration-dependent manner, consistent with the classification of ghrelin as a brain-gut peptide. However, until the effects of a ghrelin receptor antagonist have been evaluated in humans, the role of endogenous ghrelin in the regulation of gastrointestinal motility remains uncertain.

1.8.4.2 **Effects of ghrelin on appetite and energy intake**

Ghrelin is the most potent known circulating orexigen. In humans, exogenous administration of ghrelin at 5.0 pmol/kg/min for 270 minutes decreased fullness and increased hunger (Wren et al. 2001). Moreover, energy intake at a subsequent meal was increased by ~ 28 % during ghrelin infusion when compared with saline (Wren et al. 2001), though the plasma ghrelin concentrations achieved were substantially higher than normal circulating levels. In rats, chronic intraperitoneal injection of 10 nmol of ghrelin for seven days increased cumulative energy intake which was associated with a marked increase in body weight gain (~ 2.2 g/day) (Wren et al. 2001). Since no studies have used a specific ghrelin receptor antagonist to investigate the role of endogenous ghrelin on energy intake, the physiological role of ghrelin in feeding behaviour remains uncertain.

1.8.5 **Insulin**

The role of insulin in the modulation of gastrointestinal motor function and energy intake is not well understood since, to date, studies in humans have not clearly discriminated the effects of insulin from those of hypoglycaemia.
1.8.5.1 Effects of insulin on gastrointestinal motility

In dogs, intravenous infusion of insulin has been reported to stimulate gastric motility (Nelsen et al. 1966), possibly due to vagal stimulation induced by hypoglycaemia (Walker et al. 1974). Fraser et al., investigated the effect of hypoglycaemia on gastrointestinal motility, induced by intravenous infusion of insulin, in healthy humans and reported that there was no significant difference in the number of antral, pyloric or duodenal pressure waves after the insulin injection (hypoglycaemia) when compared with 45 min after the saline injection (euglycaemia) (Fraser et al. 1991). Hyperinsulinemia suppresses antral PWs (Gielkens et al. 1997) and inhibits fed jejunal motility, therefore delaying small intestinal transit time (Kong et al. 1998). Furthermore, hyperinsulinemia under euglycaemic conditions slows solid and liquid gastric emptying in healthy humans (Eliasson et al. 1995, Kong et al. 1998), but has no effect in type-1 or type-2 diabetes patients (Kong et al. 1999).

1.8.5.2 Effects of insulin on appetite and energy intake

The role of insulin in the regulation of appetite is unclear and controversial. In animals, in the absence of hypoglycaemia, elevated levels of circulating insulin appear to decrease food intake (Nicolaidis and Rowland 1976). In addition, when insulin is administered chronically to baboons both energy intake and body weight are decreased (Woods et al. 1979). In humans, infusion of insulin at either 0.8 or 1.6, mU/kg/min for 150 minutes did not alter appetite or energy intake (Chapman et al. 1998). However the two doses of insulin used in this study produced
relatively low ‘physiological’ concentrations. Hence, an influence on energy intake may have possibly been observed with the administration of more insulin to produce higher, but still ‘physiological’, concentrations. However, in a study by Woo et al, lean humans were intravenously administered either 0.03 mU/kg/min of insulin and 0.25 g/kg/min of glucose or an insulin bolus of 12 mU/kg/min followed by 0.03 mU/kg/min of insulin and 0.125 g/kg/min of glucose during a meal (Woo et al. 1984). While the changes induced by the insulin-glucose infusions mimicked normal insulin and glucose levels observed before satiation is complete, they had no effect on either meal size or duration suggesting postprandial elevation of insulin is unlikely to signal satiety in humans (Woo et al. 1984). In contrast, endogenous increases in insulin concentrations following a meal have been reported to be inversely related to energy intake at a subsequent meal in lean, but not in obese, human subjects (Speechly and Buffenstein 2000, Verdich et al. 2001), suggesting that insulin concentrations may have a role in the acute regulation of energy intake.

In summary, exogenous administration of a number of gastrointestinal hormones, including CCK, PYY and GLP-1, mimic the effect of small intestinal nutrients on gastrointestinal motor function and energy intake. In contrast, exogenous ghrelin affects fasting motility and may play a role in meal initiation. While a physiological role in the regulation of gastrointestinal motor function and energy intake has been clearly established for CCK, further studies utilising specific receptor antagonists are required to clarify the physiological roles of PYY, GLP-1, ghrelin and insulin in the regulation of gastrointestinal function and energy intake.
1.9 INTERACTIONS BETWEEN GASTROINTESTINAL STIMULI WITH APPETITE AND ENERGY INTAKE

As discussed previously, meal ingestion triggers a number of gastrointestinal mechanisms, including gastric distension, stimulation of small intestinal receptors and the release, or suppression, of gastrointestinal hormones, within ~ 15 min of meal ingestion. Hence, it is conceivable that interactions between these stimuli occur, potentially enhancing their individual effects on appetite and energy intake further. The following section reviews the outcome of studies that have investigated these potential interactions, including those between small intestinal nutrients with gastric distension and CCK, and interactions between gastrointestinal hormones.

1.9.1 Interactions between gastric distension with small intestinal nutrients

While gastric distension, achieved by inflating a balloon in the stomach, causes a sensation of epigastric pressure, when combined with a concurrent lipid infusion, at a rate similar to normal gastric emptying (~ 2 kcal/min), the outcome is a more ‘meal-like’ fullness (Feinle et al. 1997). In addition, the combination of intraduodenal nutrients (1 ml/min over 90 minutes) and gastric distension (induced by consumption of a nutrient drink) suppresses energy intake more than gastric distension alone both in animals and humans (Pappas et al. 1989, Castiglione et al. 1998). These studies indicate that the combination of gastric distension and small
intestinal nutrient stimulation has a greater effect to modify appetite sensations and suppress energy intake, than the individual stimuli alone.

1.9.2 Interactions between gastric distension and CCK

The effect of the combination of gastric distension and intravenous CCK has also been investigated (Muurahainen et al. 1994, Kissileff et al. 2003). For example, CCK (225 ng/ml) or saline were intravenously infused for 5 min before and 5 min during a pasta meal, served 20 min after consumption of either a 100 or 500 g soup preload (Muurahainen et al. 1994). Energy intake was significantly lower when CCK was administered, following the larger preload, when compared with the other treatments (Muurahainen et al. 1994). Furthermore, concurrent gastric distension (300 ml distilled water) and intravenous CCK-8 (112 ng/ml for 23 min) reduced energy intake by 200 g, which was greater than the sum of the effects of either gastric distension (3 g), or CCK-8 (96 g), alone (Kissileff et al. 2003), indicating that gastric distension and intravenous CCK interact synergistically to suppress energy intake. However, although gastric distension appears to be a likely stimulus for the interactive effect with CCK-8, other factors such as the activation of nutrient-sensitive sites cannot be eliminated.

1.9.3 Interactions between small intestinal nutrients and CCK

In healthy males, the effects of a nutrient ‘preload’, consisting of a 400 ml banana shake, and intravenous CCK-8 (0.7 μg infused over 10 minutes) on sensations of appetite and energy intake have been investigated (Gutzwiller et al. 2000). The
combination of the preload and CCK-8 resulted in a greater decrease in hunger and increase in fullness, and a subsequent 510 kJ decrease in energy intake (total energy intake: 7002 kJ), than when the preload was administered alone (total energy intake: 7512 kJ). This study indicates there is an interaction between intravenous CCK and small intestinal nutrients, which enhances the effects of either to modulate appetite sensations and suppress energy intake.

1.9.4 Interactions between gastrointestinal hormones

While the majority of previous studies evaluating the effects of gut hormones have focused on the effects of gastrointestinal hormones when administered in isolation, it is now increasingly evident that there are interactions between gastrointestinal hormones, perhaps particularly those released from different regions of the small intestine. Investigation of these interactions provides insights into the potential mechanisms through which gut hormones are able to modulate gastric motor function, appetite and energy intake. For example, administration of intravenous PYY has been demonstrated to suppress plasma ghrelin concentrations in both lean and obese subjects (Batterham et al. 2003), and in-vitro studies in rodents suggest that GLP-1 may also have this effect (Lippl et al. 2004). In dogs, the stimulation of PYY by the presence of fat in the proximal small intestinal is mediated, at least in part, by CCK (Lin et al. 2000). Conversely, there is evidence that GLP-1 may inhibit the release of PYY (Naslund et al. 1999). Furthermore, when an intraperitoneal injection of CCK-8 was administered simultaneously with
ghrelin, the orexigenic effect of ghrelin is attenuated, suggesting that CCK may modify the action of ghrelin (Kobelt et al. 2005).

1.9.4.1 Interaction between CCK and GLP-1

A study in humans has suggested that the reduction of energy intake by intraduodenal lauric acid, a fatty acid with 12 carbon atoms, but not decanoic acid, a fatty acid with 10 carbon atoms, may reflect an interaction between CCK and GLP-1 (Feltrin et al. 2004). Intraduodenal administration of lauric acid markedly increased plasma CCK and GLP-1, while, decanoic acid only slightly increased plasma concentrations of CCK, and had no effect on GLP-1. This is consistent with the concept that the combined actions of CCK and GLP-1 (possibly with other gut peptides) are, in part, responsible for the more potent suppressive effects of lauric acid, compared with decanoic acid, on energy intake. Clarification as to whether there is an interaction between CCK-8 and GLP-1 is relevant for an understanding of the mechanisms regulating energy intake and gastrointestinal motility and, potentially, the pathogenesis of obesity.

Two studies have investigated the effects of combined intravenous infusions of CCK and GLP-1 on appetite and energy intake (Gutzwiller et al. 2004, Brennan et al. 2005). Gutzwiller and colleagues reported that the combined infusions of CCK-33 (0.2 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min) had a synergistic effect to decrease hunger (Gutzwiller et al. 2004). In contrast, the combination of CCK-33 and GLP-1 did not decrease energy intake to a greater extent that either CCK-
33 or GLP-1 alone, so that there was no synergistic, or additive, effect of the combination of CCK-33 and GLP-1 on energy intake (Gutzwiller et al. 2004). The author then reported the effects of CCK-8 (1.8 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min), administered alone and in combination, on antropyloroduodenal motility, appetite and energy intake (Brennan et al. 2005). Infusion of CCK-8 decreased perceptions of appetite, energy intake, the number of antral and duodenal pressure waves, and the number of pressure waves sequences, and increased both the number and amplitude of isolated pyloric pressure waves. In contrast, infusion of GLP-1 did not suppress appetite or energy intake or stimulate pyloric pressures, but decreased antral and duodenal PWs to an extent comparable to CCK-8. Although the combination of CCK-8 and GLP-1 decreased the number of duodenal PWs more than CCK-8 or GLP-1 alone, it did not have any additive effects on appetite, energy intake or other motility parameters. However, the doses of CCK-8 and GLP-1 used in this study may have exerted near maximal effects on APD motility and if this were the case, the magnitude of the individual effects of CCK-8 and GLP-1 given alone may have been too great to allow a demonstration of a synergistic interaction when the two peptides were administered concurrently. To date, there is no information as to possible interactions between CCK and GLP-1 that may modulate ghrelin and PYY release (Chapter 5).

Observations of possible interactions between gastrointestinal hormones may provide insights into the potential mechanisms through which they modulate gastric motor function and energy intake (Chapters 5 and 6). Furthermore,
establishing how relationships between gut hormones may vary between lean and obese is potentially important for an improved understanding of any adaptations in gastrointestinal function, and the modulation of appetite and energy intake, that may exist the obese.

1.10 CONCLUSIONS

This chapter has reviewed the role of post-prandial gastrointestinal function, specifically the modulation of gastric emptying, antropyloroduodenal motility, gut hormone release and/or suppression, and their interactions, in the acute regulation of appetite and energy intake. Studies described in subsequent chapters of this thesis address the following aims:

(i) to assess the effects of exogenous CCK-8 and GLP-1, given alone and in combination, on ghrelin and PYY release (Chapter 5) and

(ii) to evaluate the effects of increasing doses of exogenous CCK-8 on antropyloroduodenal motility, gastrointestinal hormone release, appetite and energy intake (Chapter 6).
CHAPTER 2

The effects of dietary excess, or restriction, on gastrointestinal function, appetite and energy intake

2.1 INTRODUCTION

As discussed in Chapter 1, signals arising from the gastrointestinal tract play a fundamental role in the regulation of appetite and energy intake. There is evidence that these signals may be modified by dietary excess, or restriction. Hence, the development of obesity may, at least in part, reflect decreased sensitivity to the gastrointestinal effects of nutrients to favour an increase in hunger and energy intake. Currently, the most common non-pharmacological method of weight loss in obesity is dietary restriction, which is usually not sustained in the long-term. This has been attributed to a lack of compliance, however, it may also potentially reflect adaptive changes in gastrointestinal function. In this chapter current knowledge relating to the effects of dietary excess and restriction on gastrointestinal function, including gastrointestinal motor function and hormone secretion, appetite and energy intake, is reviewed. Because the effects of dietary excess and restriction have implications for weight loss, hence, obesity and adaptations in gastrointestinal function in the obese are also discussed.
2.2 ROLE OF HIGH DIETARY FAT INTAKE IN THE DEVELOPMENT OF EXPERIMENTALLY INDUCED HYPERPHAGIA

Although the causes of obesity are heterogeneous, it is widely accepted that one of the environmental factors contributing to the current obesity epidemic is the increased availability and over-consumption of high-fat, energy-dense foods, with a concomitant decrease in physical activity (Catford and Caterson 2003). Animal studies have 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). In humans, epidemiological studies have demonstrated a direct relationship between the incidence of overweight and obesity and dietary fat consumption (Rolls 1995, Golay and Bobbioni 1997). For example, in those countries in which the incidence of obesity is increasing rapidly, ~ 45 % of the daily energy intake is provided by fat (Golay and Bobbioni 1997). In addition, there is evidence that obese individuals have an increased preference for the consumption of fatty foods (Mela and Sacchetti 1991) and that the proportion of dietary fat is higher in obese, than in lean, individuals (Miller et al. 1990). The consumption of a high-fat diet has also consistently been shown to promote an increase in energy intake (Lissner et al. 1987, Tremblay et al. 1989) and body weight.
2.3 EFFECTS OF EXPERIMENTALLY-INDUCED DIETARY EXCESS ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE

The precise mechanisms by which dietary excess promotes weight gain remain unclear, and these may potentially relate to an increase in either the overall energy content of a diet, and/or the macronutrient composition of a diet, i.e. high-fat diets. For example, consumption of a high-fat diet results in significant metabolic changes (Fleming 2002, Miller et al. 2009), and has the capacity to modify the effects of nutrients on gastric emptying, gastrointestinal transit, antropyloroduodenal motility and the secretion, and action, of gastrointestinal hormones. These observations may be of fundamental relevance to the pathogenesis of obesity. The following sections discuss the effects of experimental dietary excess on gastric motor function, gastrointestinal hormones and appetite and energy intake.

2.3.1 Effects of experimentally-induced dietary excess on gastrointestinal motor function

Studies in animals indicate that the gastrointestinal motor response to fat is attenuated after acute energy excess, i.e. a 2 – 4 week diet containing between 40 – 60 % of energy from fat. For example, in rats, infusion of palm oil into the ileum at 0.3 ml/hour for 3 hours per day on 3 days per week for 4 weeks was associated with a reduction in the lipid induced slowing of the stomach-to-caecum transit of a meal (lactose bean meal) (Brown et al. 1994). This acceleration of stomach-to-
caesum transit was still evident 4 weeks after cessation of the palm oil infusions, indicating that since changes in gastrointestinal function persists beyond the period of dietary manipulation, these adaptations may, in fact, occur more rapidly than their reversal. In healthy humans, consumption of a high-fat diet (19.26 MJ/day) for a period of 14 days has been reported to result in marked acceleration of gastric emptying and mouth-to-caecum transit of a high-fat test meal (1.4 MJ), when compared with a low-fat diet (9 MJ/day) (Cunningham et al. 1991) (Figure 2.1).

Figure 2.1: Gastric emptying (A) and mouth to caecum transit (B) of a high-fat test meal (1.4 MJ) following consumption of a low-fat (9 MJ/day) or high-fat (19.2 MJ/day) diet for 14 days in healthy subjects (n=12). * vs low-fat, P < 0.05. Adapted from (Cunningham et al. 1991).

In another study, consumption of a high-fat diet (55 % energy from fat/day) for 14 days led to the acceleration of gastric emptying of a high-fat (55% energy from fat), but not high-carbohydrate (62 % energy from carbohydrate), test meal, indicating that the changes in gastric emptying following a high-fat diet may be
Effects of dietary intake on gut function and appetite

2.3.2 Effects of experimentally-induced dietary excess on the sensitivity to, and the release of, gastrointestinal hormones

There is evidence that experimentally induced energy excess has the capacity to modify both the sensitivity to, and the release, of gastrointestinal hormones. For example, when rats were exposed to a high-fat (30 % energy from fat), high-protein (71 % energy from protein), or low-fat (64 % energy from carbohydrate), diet for 14 days the inhibitory effects of two doses of CCK-8 (0.125 and 0.250 μg/kg) on energy intake were shown to be less following the high-fat and high-protein diets, when compared with the low-fat diet (Covasa et al. 2001), suggesting that sustained elevation of CCK by dietary-induced elevation of plasma...
CCK contributes to the reduced sensitivity to exogenous CCK. No studies have, however, determined whether exposure to a high-fat diet modifies the actions of endogenous or exogenous GLP-1, PYY or ghrelin on gastrointestinal function and/or energy intake.

The release of CCK, PYY, GLP-1 and ghrelin has been reported to be modulated after a high-fat diet. In mice, that had become obese in response to a 16 week high-fat diet (60% energy from fat), plasma concentrations of PYY were lower in both the fasting and postprandial states, when compared with mice maintained on a low-fat diet (2.6% energy from fat) (le Roux et al. 2006). In addition, rats who gained weight when fed a hypercaloric, high-fat diet (70% energy from fat) for 14 weeks, fasting plasma ghrelin concentrations decreased by ~30% when compared with rats fed a control diet (Beck et al. 2002). The levels of ghrelin were, therefore, related to the fat content of the diet and the low ghrelin concentrations observed in rats ingesting a high-fat diet may serve to limit the energy intake provided by this energy-rich diet.

In humans, relatively few studies have investigated the responses of gastrointestinal hormones to diets high in a particular nutrient. Twelve male subjects participated in a study to investigate the effects of overfeeding a high-fat diet (58% energy from fat) for 14 days on plasma CCK concentrations (French et al. 1995). Elevated postprandial CCK concentrations in response to a standard breakfast were reported following the high-fat diet, when compared with the pre-diet condition, suggesting that CCK receptors may be ‘desensitized’ by the
exposure to large amounts of fat. However, Boyd et al., found no change in the CCK response to a duodenal lipid infusion (6.28 kJ/min) following a high-fat diet (Boyd et al. 2003). This apparent discrepancy may be attributed to differences in methodology, since the study by French et al., (French et al. 1995), reported that differences in plasma CCK may be accounted for by changes in the rate of gastric emptying, i.e. an increased rate of nutrient delivery to the small intestine would be anticipated to increase CCK release. Given this, in the study by Boyd et al, which eliminated the influence of gastric emptying by directly infusing nutrients into the small intestine, the plasma CCK response to the duodenal lipid infusion did not differ in response to the high-fat diet. This suggests that a high-fat diet does not result in changes in CCK release when gastric emptying is taken into account.

The secretion of GLP-1, in response to a duodenal lipid infusion, also does not appear to be influenced by a high-fat diet, when compared with a low-fat diet (Boyd et al. 2003). In humans, exposure to a high-fat diet has been reported to decrease plasma ghrelin concentrations, although observations are inconsistent (Robertson et al. 2004, Paul et al. 2005). For example, in subjects instructed to ingest a high-fat dietary supplement (125 ml cream and 50 g roasted peanuts; 88 g fat/day) each day for 3-weeks there was an increase in postprandial ghrelin suppression following oral fat (125 ml cream; 60 g fat). In contrast, following ingestion of predominantly high-carbohydrate (30% energy from carbohydrate), high-protein (30% energy from protein) or high-fat (42% energy from fat), drinks for 16-weeks preprandial ghrelin concentrations were not affected by macronutrient intake (Paul et al. 2005). In summary, observations derived from
animal and human studies reviewed in this section demonstrate that changes in the nutrient-induced modulation of gastrointestinal hormones occur following experimental dietary manipulation, which are likely to have important implications for the regulation of gastrointestinal function, appetite and energy intake.

### 2.3.3 Effect of experimentally-induced dietary excess on appetite and energy intake

There is some evidence that periods of dietary excess are capable of modifying appetite and energy intake. For example, in healthy males who had gained ~ 2 kg of weight, appetite perceptions were modified after exposure to a high-fat diet (58 % energy from fat) for 14 days with hunger increasing and fullness decreasing (French et al. 1995). Subjects also reported an increase in average daily energy intake (0.66 MJ/day), as measured by food diaries, during the 14-day period (French et al. 1995). In healthy females, manipulation of dietary fat content for 14 days resulted in an increase in total daily energy intake during consumption of a high-fat diet (45 – 50 % energy from fat), by 15 % when compared with the consumption of a medium-fat diet (30 – 35 % energy from fat) and by 23 % when compared with a low-fat diet (15 – 20 % energy from fat), which was associated with weight gain (~ 0.32 kg on the high-fat diet) (Lissner et al. 1987). Similarly, in healthy males, acute energy intake markedly increased following a 2-day diet with increased fat content (an increase from 30 % to 45 % energy from fat) (Tremblay et al. 1989).
In summary, the studies discussed provide a persuasive rationale for the hypothesis that experimental dietary excess modulates changes in gastrointestinal function that promote increased energy intake and predispose to weight gain, which make these factors are of interest and relevance in the development of obesity.

2.4 EFFECTS OF EXPERIMENTAL DIETARY RESTRICTION ON GASTROINTESTINAL FUNCTION AND APPETITE

As previously discussed, experimentally-induced dietary excess, i.e. high-fat diets, is associated with the modulation of gastrointestinal function, increased energy intake and subsequent weight gain. Based on these findings, there has been a growing interest in understanding how weight loss can be achieved through dietary restriction. Current literature relating to these effects can be divided into two categories; studies which have investigated the effects of either short-, or longer-, term dietary restriction. The following sections discuss what is known about the differential effects of these periods of dietary restriction on gastric motor function, gastrointestinal hormones and appetite.

2.4.1 Effects of short-term experimental dietary restriction

Very few studies have assessed the effects of acute dietary restriction on feedback signals from the gut. One study in both lean and obese subjects assessed gastric emptying before and after a 4-day fast (Corvilain et al. 1995). Gastric emptying of a 75 g glucose load (320 ml) was slower following the 4-day fast in both lean and obese subjects, when compared with a 12-hour fast (Corvilain et al. 1995),
suggesting that a dramatic reduction in nutrient exposure may increase the 
sensitivity of the gastrointestinal tract to the actions of nutrients. However, 
subjects in this study were fasted and, observed effects may, accordingly, not 
necessarily mimic what is observed while nutrients are still being consumed.

NOTE:  
This figure is included on page 46 of the print copy of 
the thesis held in the University of Adelaide Library.

**Figure 2.2:** Gastric emptying of a 75 g glucose load (320 ml) before and 
after a 4-day fast in lean (n=12) and obese (n=11) subjects. * vs 
overnight fast, $P < 0.05$. Adapted from (Corvilain et al. 1995).

In the only study to investigate this premise, Doucet *et al* demonstrated, in 15 
healthy males, that both fasting and postprandial total serum ghrelin did not appear 
to be influenced by a 4-day period of energy restriction (-800 kcal/day of an 
individuals daily energy requirements) (Doucet *et al.* 2004). While the effects of 
fasting on gastric emptying have been assessed, the effects of acute energy 
restriction on antropyloroduodenal motility, gastrointestinal hormone release, e.g. 
CCK, and energy intake remains unknown (Chapter 10). If short-term energy 
restriction has the capacity to increase the sensitivity of gastrointestinal responses
to nutrients, for example the slowing of gastric emptying, it may also have the potential to modify other gastrointestinal functions. More specifically, changes in gastrointestinal motility and gastrointestinal hormone secretion that would normally favour an increase in appetite and energy intake in the obese could potentially be modified by short-term energy restriction.

2.4.2 Effects of long-term experimental dietary restriction

The most common non-pharmacological method of weight loss in obesity is long-term dietary restriction, i.e. fat-restricted diets (Foster et al. 2003, Claessens et al. 2009), and high-protein low-carbohydrate diets (Noakes et al. 2005, Clifton et al. 2008). However, there is some evidence that ‘positive’ changes in response to acute dietary restriction, i.e. the slowing of gastric emptying following a 4-day fast suggesting an increased sensitivity of gastrointestinal responses to nutrients, may not be sustained in the longer-term. In a study by Lieverse et al, six obese subjects consumed a very-low-calorie diet (240 kcal/day), which induced 23 kg weight loss, and a 75 % reduction in hunger scores over a 10-week period (Lieverse et al. 1993). However, post prandial plasma CCK concentrations were not affected by the 10-weeks of dieting (Lieverse et al. 1993), suggesting that any changes in the sensitivity of gastrointestinal responses to nutrients induced during a period of longer-term dietary restriction do not effect CCK release. There is evidence, however, that plasma GLP-1 is modulated following weight reduction through dietary restriction (Verdich et al. 2001, Adam et al. 2005). For example, following a 6-week period of dietary restriction (608 kcal/day) associated with ~ 6.1 kg
weight loss, both fasting and postprandial GLP-1 concentrations were less, when compared with before the period of dietary restriction (Adam et al. 2005). While there is currently no literature relating to the effects of weight loss through dietary restriction on plasma PYY, a study conducted with 73 obese children reported that PYY concentrations were increased in patients with more effective weight loss following a 1-year out-patient weight reduction programme (Roth et al. 2005). Circulating concentrations of ghrelin over a 24-hour period markedly increased after a 3-month period of diet-induced weight loss – a finding which suggests that ghrelin may play a role in the adaptive responses to dietary restriction that affects the amount of weight an individual can lose while dieting (Cummings et al. 2002). Furthermore, while not ‘experimentally’ induced, ~ 50 % of patients with anorexia nervosa, a condition associated with dramatic dietary restriction, have delayed gastric emptying which is reversed by reintroduction of oral nutrition and before significant weight gain (Rigaud et al. 1988). In addition, in critically ill patients chronic nutrient deprivation is associated with delayed gastric emptying and increased plasma CCK and PYY concentrations (Nguyen et al. 2007).

In summary, the studies discussed above highlight the importance of acute nutrient exposure on gastrointestinal function by demonstrating changes can occur over a short period of time, i.e. following acute energy restriction there is evidence of increased sensitivity in the gastrointestinal responses to nutrients. However, the short-, and longer-, term changes, and the profile of changes, that might occur during periods of energy restriction, have not been characterised systematically. Any potential modulation in gastrointestinal function, including gastric emptying,
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antropyloroduodenal motility and gastrointestinal hormone secretion, during a period of dietary restriction, and the relationship with subsequent changes in appetite and energy intake, may have major implications for the obese, particularly in regards to treatment approaches for weight loss.

2.5 PATHOPHYSIOLOGY OF OBESITY

Obesity can be considered to be the result of energy intake that exceeds energy expenditure, i.e. dietary excess. The most common method used for the assessment of overweight and obesity is body mass index (BMI). BMI is calculated by dividing an individual’s weight (kg) by the square of their height (m). Individuals with a BMI less than 19 kg/m² are considered underweight, 19 – 25 kg/m² healthy weight, 25 – 30 kg/m² overweight and individuals with a BMI over 30 kg/m² are considered clinically obese (National Task Force on the Prevention and Treatment of Obesity, 2000). The use of BMI has limitations; BMI is a measurement of body size, not body fat, as it does not distinguish between a person’s body fat or lean/muscle mass directly, nor does it provide information relating to the distribution of fat throughout the body.

Numerous pharmacological treatments for obesity have been, and are currently, available, however, most are associated with limited efficacy and often adverse effects. In addition, the available therapies have largely ignored the role of the gastrointestinal tract in the regulation of appetite, as well as the important
relationship between gastrointestinal function and energy intake, and how these mechanisms may be compromised in obesity.

2.5.1 Significance of obesity

Recent data indicate that there are more than 250 million obese people worldwide, representing ~ 7% of the adult population (Bray 2003). Between 1976 – 1980 and 1988 – 1994, the prevalence of overweight increased from 46% to 54% in the United States of America (Flegal 1996), and during the same period, the prevalence of obesity increased from 15% to 23% (Flegal 1996). In Australia, 19% of men and 22% of women are obese (Cameron et al. 2003). Linear extrapolation of the historical rate of the increase in obesity indicates that there may be as many as 7.2 million obese Australians, that is 28.9% of the population, by 2025 (Access Economics for Diabetes Australia, 2006). The health, economic and psychosocial consequences of obesity are substantial and associated with considerable, although poorly defined, costs to the health care system, particularly in Western countries. In Australia alone, the total financial cost of obesity in 2005 was estimated at $3.77 billion (Access Economics for Diabetes Australia, 2006). Obesity is most commonly characterized by either slow progression throughout adult life or by periods of weight stability or short-term weight loss followed by relapse and affects virtually all age and socioeconomic groups, both in developed and developing countries. Obesity is associated with common causes of morbidity and mortality, such as type 2 diabetes mellitus, coronary heart disease, hypertension and dyslipidemia (Must et al. 1999). In addition, obesity is
associated with a greater prevalence of gallbladder disease, liver disease, gout, musculoskeletal diseases, reproductive abnormalities, sleep apnoea and asthma (Must et al. 1999, Guh et al. 2009). There is also evidence that obesity may be of pathophysiological significance in cancer, particularly hormone-dependent cancers such as prostate, breast and uterine, as well as colorectal and kidney cancers (Guh et al. 2009, Pan and DesMeules 2009). A greater understanding of the mechanisms that contribute to the pathophysiology of obesity is required, and may result in the identification of targets for the treatment of obesity.

2.5.2 Gastrointestinal function in obesity

As previously discussed, the gastrointestinal tract plays a pivotal role in the regulation of appetite and energy intake in healthy individuals and, therefore, it is important to characterise disturbances in gastrointestinal function in the obese, which may be a contributing factor in the development, and maintenance of, obesity, however, currently these studies are both limited and controversial.

2.5.2.1 Modulation of gastrointestinal motor function in obesity

Much of the available literature relating to gastrointestinal motor function in obesity is inconclusive. For example, studies that have assessed gastric emptying in obese individuals have reported that gastric emptying is faster (Tosetti et al. 1996), similar (French et al. 1993) or slower (Maddox et al. 1989), in comparison with lean subjects. These inconsistent results may be attributable to, at least in part, inadequate subject matching or standardisation of test meals (Verdich et al.
2000), or to other factors known to influence gastric emptying, i.e. changes in weight or the previous diet of an individual (Cunningham et al. 1991, Cunningham et al. 1991). Currently, only one study has evaluated whether changes in interdigestive motility occur in the obese when compared with lean subjects. This study demonstrated disturbed motility during periods of fasting, including diminished Phase I, increased Phase II, and a more distal and less frequent occurrence of Phase III activity in obese, when compared with lean subjects, however, no differences in antral and duodenal fasting motility were reported (Pieramico et al. 1992). The significance of these changes, and studies evaluating potential disturbances in postprandial gastrointestinal motility, are yet to be determined.

2.5.2.2 Modulation of gastrointestinal hormones in obesity

There is evidence that the secretion of gastrointestinal hormones, including CCK, PYY, GLP-1 and ghrelin, may be altered in obese individuals. For example, increased fasting, and postprandial, CCK concentrations have been found in obese compared with lean individuals (French et al. 1993, Baranowska et al. 2000, Zwirska-Korczala et al. 2007). Since postprandial CCK functions as a ‘meal-terminating’ satiety signal, an increase in the obese state may reflect changes in sensitivity to CCK and be responsible, at least in part, for disturbed satiety. Lower levels of GLP-1 have been reported in obese, when compared with lean, individuals (Ranganath et al. 1996, Verdich et al. 2001), and more specifically, there was a reduced GLP-1 response to oral fat, but not carbohydrate (Ranganath
et al. 1996), while no difference have been observed following intraduodenal fat or glucose administration (Feinle et al. 2002). Since the secretion of GLP-1 release is dependent on the interaction of the small intestine with nutrients, it is possible that the reported differences in GLP-1 release between lean and obese subjects reflect changes in gastric emptying, rather than impaired stimulation of GLP-1. It has been reported that fasting plasma PYY concentrations are reduced in obese subjects (Batterham et al. 2003, le Roux et al. 2006) (Figure 2.3).

Figure 2.3: Plasma PYY concentrations in lean (n=12) and obese (n=12) subjects during and after infusion of saline. * vs lean, P < 0.001. Adapted from (Batterham et al. 2003).

Furthermore, obese individuals may have a diminished rise in PYY concentrations, in comparison with lean subjects, who had a stepwise increase in PYY concentrations following meals of increasing energy content (le Roux et al. 2006), suggesting that obese individuals may have reduced sensitivity to the presence of
nutrients, when compared with lean individuals. Fasting ghrelin concentrations have been reported to be less in the obese (Cummings et al. 2001, Tschop et al. 2001). In addition, following ingestion of a meal, plasma ghrelin concentrations are not further suppressed when compared with preprandial concentrations, suggesting obese individuals may not reach a fasted state (Cummings et al. 2002, English et al. 2002). Given the role ghrelin has in appetite stimulation, the fact that concentrations of ghrelin are not further suppressed in obese individuals may account for decreased feelings of satiety, suggesting a possible role for ghrelin in the maintenance of obesity.

In summary, as discussed previously, changes in gastrointestinal hormone concentrations, i.e. CCK, PYY, GLP-1 and ghrelin, occur concurrently with modifications in appetite and subsequent energy intake in lean subjects. Therefore, any disturbances in the secretion of, or sensitivity to, these hormones following meal ingestion are likely to contribute to an attenuated suppression of energy intake in obesity, i.e. nutrient-sensing mechanisms are compromised in the obese, implying that obese subjects may increase their food intake to gain the same satisfaction from their dietary intake as lean subjects.

2.6 CONCLUSIONS

This chapter has discussed the effects of experimentally induced dietary excess, and dietary restriction, on gastrointestinal function, appetite and energy intake. Investigation of these factors will allow further characterisation of changes in the
sensitivity of the gastrointestinal tract to the actions of nutrients that may occur during periods of energy excess or energy restriction, which are particularly relevant for obese subjects considering weight loss regimes. This chapter then discussed gastrointestinal function in obesity. The study described in a subsequent chapter of this thesis addresses the following aim:

(i) to assess the effects of a 4-day very-low calorie diet, i.e. acute energy restriction, on antropyloroduodenal motility, gastrointestinal hormone release, appetite and energy intake responses to an intraduodenal lipid infusion in obese subjects (Chapter 10).
CHAPTER 3

The effects of oral macronutrient ingestion on gastrointestinal function, appetite and energy intake

3.1 INTRODUCTION

As discussed previously, meal ingestion triggers a number of gastrointestinal responses that interact to induce satiety. Satiety can be defined as the absence of hunger and the sensation that prevents an individual from commencing a meal (Blundell and Halford 1994, Rolls and Hammer 1995) and is dependent on a number of factors, particularly the type of food ingested, i.e. the specific macronutrient composition of the meal (Rolls and Hammer 1995, Poppitt et al. 1998). This chapter initially summarises what is known about the comparative effects of fat, protein and carbohydrate on gastric motor function, gastrointestinal hormones, appetite and energy intake in lean and obese subjects. This chapter then focuses on two factors that may influence the effects of oral macronutrients on gastrointestinal function, appetite and energy intake; specifically, the day-to-day variability of gastrointestinal function, appetite and energy intake in both males and females, and the effect of the menstrual cycle on these parameters in females.
3.2 COMPARATIVE EFFECTS OF FAT, CARBOHYDRATE AND PROTEIN ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE IN LEAN SUBJECTS

It is well established that changes in gastrointestinal function following the interaction of nutrients with the small intestine play an important role in the regulation of appetite and energy intake particularly by both fat and carbohydrate. In contrast, only limited information is available about the effects of protein on gastrointestinal function. The following sections review current knowledge of the role of macronutrients in the modulation of gastric motor function, gastrointestinal hormones, appetite and energy intake in lean subjects.

3.2.1 Effect of macronutrients on gastric emptying

There are few studies in which the effects of all macronutrients on gastric motor function have been systematically compared (Cecil et al. 1998, Goetze et al. 2007). These studies differ fundamentally since one study only evaluated the effects of carbohydrate and fat (Cecil et al. 1998), whereas the other evaluated all three macronutrients simultaneously (Goetze et al. 2007). Cecil et al. reported the effect of ingestion of a high-fat or high-carbohydrate soup (400 kcal in 425 mL) on gastric emptying in nine healthy men (Cecil et al. 1998); gastric emptying profiles of the two soups differed significantly, with the high-fat soup emptying more slowly from the stomach than the high-carbohydrate soup. Within the same study, there was no difference in gastric emptying when the same soups were infused into the stomach (Cecil et al. 1998), bypassing orosensory stimulation, suggesting that
subtle differences in sensory stimulation prior to ingestion of either high-fat or high-carbohydrate ‘preloads’ may modulate gastric emptying. Using magnetic resonance imaging, Goetze et al reported that initial increases in stomach and meal volumes, and thus in stomach relaxation, were more pronounced following ingestion of 500 ml of glucose (400 kcal) compared with equivolumetric and isocaloric fat and protein emulsions (Goetze et al. 2007). These changes were associated with slower ‘early phase’, i.e. $t = 0 – 45$ min, gastric emptying of glucose, whereas gastric emptying was faster for fat when compared with protein or glucose during the ‘late phase’, i.e. $t = 45 – 90$ min (Goetze et al. 2007).

However, in this study there was a major difference in the osmolality of the glucose preload (1110 mosm/l) compared with both fat and protein (308 mosm/l), therefore, whether gastric emptying was modulated specifically by the macronutrient content of the preloads, or by the higher osmolality of the glucose preload, remains unclear.

### 3.2.2 Effect of macronutrients on gastrointestinal hormones

Meal ingestion stimulates the secretion of a number of hormones, including CCK, PYY and GLP-1 from the small intestine, and suppresses the release of ghrelin from the stomach. However, the magnitude of the release and/or suppression of these hormones is modulated, in part, by specific macronutrients. Lipid and protein are stronger stimuli for CCK release than carbohydrates (Hopman et al. 1985, Liddle et al. 1985, Blom et al. 2006). For example, to determine the relative contribution of protein, fat, carbohydrates and amino acids to CCK secretion,
plasma levels of CCK were measured after ingestion of 100 g of either casein, corn oil, glucose or mixed amino acid (Liddle et al. 1985). While each food component stimulated CCK, fat, protein and amino acids were the most potent stimuli, when compared with glucose, which resulted in a small, and transient, elevation in plasma CCK (Liddle et al. 1985). Dietary fat, carbohydrate and protein all stimulate PYY release, but to different degrees and with different time-courses (Adrian et al. 1985). Adrian et al. demonstrated that fat elicited the largest increase in postprandial PYY concentrations, protein a more moderate increase, whereas a glucose solution caused only a transient and minor release (Adrian et al. 1985). Higher PYY concentrations following ingestion of lipid, compared with carbohydrate, have also been reported by others (MacIntosh et al. 1999, Essah et al. 2007). That carbohydrates are strong stimuli for GLP-1 release is consistent with the role of GLP-1 as an incretin hormone (Herrmann et al. 1995, Brubaker and Anini 2003). GLP-1 concentrations also increase after protein and fat (Herrmann et al. 1995, Blom et al. 2006, Lejeune et al. 2006) (Elliott et al. 1993), although the stimulation is delayed when compared with carbohydrates. In contrast to CCK and PYY, carbohydrates may be the most effective in suppressing ghrelin (Shiiya et al. 2002, Monteleone et al. 2003, Blom et al. 2006, Tannous dit El Khoury et al. 2006). Following oral ingestion of a high-fat meal, ghrelin concentrations have been reported to decrease (Monteleone et al. 2003, Greenman et al. 2004), or increase (Erdmann et al. 2004). The reported effects of protein on ghrelin are also conflicting, with some studies demonstrating no change (Greenman et al. 2004), or increases (Erdmann et al. 2003), in ghrelin concentrations following ingestion of a high-protein meal. Furthermore, a high-
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protein breakfast, or liquid protein preloads caused a more prolonged suppression of ghrelin when compared with carbohydrate (Al Awar et al. 2005, Blom et al. 2006, Bowen et al. 2006). The data summarised above establish that macronutrients have a marked influence on gastrointestinal hormone release, or suppression; while CCK and PYY are potently modulated by enteral fat and protein, GLP-1 and ghrelin are modulated more strongly by carbohydrate.

3.2.3 Effect of macronutrients on appetite and energy intake

There is evidence that the satiating capacity of protein, carbohydrate and fat differ. Laboratory studies in rats, utilising nutrient infusions into the stomach, suggest that kJ for kJ the satiating hierarchy is protein > carbohydrate > fat (Geliebter 1979, Walls and Koopmans 1992). In humans, a number of studies, using preloads rich in fat, carbohydrate or protein, have compared the effects of these macronutrients on appetite and energy intake (Johnson and Vickers 1993, Porrini et al. 1995, Latner and Schwartz 1999, Batterham et al. 2006, Bellissimo et al. 2008). For example, following ingestion of isocaloric liquid ‘preloads’ (450 kcal) that were either high-protein (72 % of energy from protein) or high-carbohydrate (99 % energy from carbohydrate), there was a 24 % (296 kcal) reduction in energy intake at a subsequent meal following the high-protein, when compared with the high-carbohydrate, ‘preload’ (Latner and Schwartz 1999). The small number of studies that have compared all three macronutrients simultaneously have reported that consumption of dietary protein reduces appetite and ad libitum energy intake more when compared with either carbohydrate or fat (Rolls et al. 1988, Poppitt et
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al. 1998). For example, in a group of twelve lean women, subjects were less hungry, and had a lower energy intake, following consumption of a high-protein preload compared with a high-carbohydrate, -fat or alcohol, preload (Poppitt et al. 1998) (Figure 3.1).

Figure 3.1: Hunger (A) and energy intake (B) following consumption of a high-protein, high-carbohydrate, high-fat or alcohol preload in lean subjects (n=12). * vs high-carbohydrate, high-fat and alcohol preloads, P < 0.05. Adapted from (Poppitt et al. 1998).

However, there are discrepancies in this ranking of macronutrients and not all studies have reported that protein is more satiating than carbohydrates or fat (de-Graaf et al. 1992, Vozzo et al. 2003, Blom et al. 2006, Bowen et al. 2006). In another study, 16 healthy, lean males consumed iso-energetic yoghurt ‘preloads’ that were either high in fat, carbohydrate or protein, or were given no ‘preload’ (Vozzo et al. 2003). The ‘preloads’, controlled for palatability, volume ingested
and energy density, delayed the first spontaneous request for food by the same length of time ~ 1.6 h, and exerted comparable suppressive effects on hunger and subsequent energy intake. These data suggest that the acute satiating efficiency of foods may also result from their sensory properties, i.e. variations in palatability, rather than macronutrient composition per se.

In summary, the effects of fat, carbohydrate and protein on gastrointestinal function, i.e. gastrointestinal hormone release, and the relationship between these effects and changes in appetite and subsequent energy intake have both been poorly characterised. This will represent the focus of the study presented in Chapter 9. Further studies investigating the role of macronutrients in the regulation of eating behaviour provide a better understanding of the regulation of appetite in healthy lean individuals.

### 3.3 COMPARATIVE EFFECTS OF FAT, CARBOHYDRATE AND PROTEIN ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE IN OBESE SUBJECTS

In Chapter 2, differences in gastrointestinal motor function, gastrointestinal hormone release, appetite and energy intake between lean and obese individuals were discussed. It is likely that differences may be dependent on the type of macronutrients ingested and this has implications for satiety and, potentially, the development of improved dietary treatments for obesity. The following sections review literature relating to the role of macronutrients in the modulation of gastric
motor function, gastrointestinal hormones, appetite and energy intake in obese subjects.

3.3.1 Effect of macronutrients on gastric motor function

There is little information about the effects of different macronutrients on gastric motor function in the obese so that, to date, only one study has hitherto assessed the effect of the three macronutrients on gastric emptying concurrently (Segura Molina et al. 2006). In this study, subjects ingested an isocaloric breakfast (507 kcal) that was either high in fat (50 % energy from fat), protein (30 % energy from protein) or carbohydrate (60 % energy from carbohydrate). Gastric emptying was shown to be modified depending on the macronutrient composition, i.e. gastric emptying was slower following the high-protein, when compared with the high-fat and high-carbohydrate, meals (Segura Molina et al. 2006). This is in contrast to the effects of macronutrients observed in lean, where fat slowed gastric emptying to a greater extent than either protein or carbohydrate (Cecil et al. 1998, Goetze et al. 2007), suggesting that there are differences in nutrient-related feedback by specific macronutrients which influence gastric emptying between lean and obese.

3.3.2 Effect of macronutrients on gastrointestinal hormones

A number of studies have investigated the effects of macronutrients on gastrointestinal hormone release, or suppression, in the obese, however, definitive conclusions are limited. Similarly to lean subjects, CCK concentrations remain elevated for longer after liquid whey, casein, soy and gluten ingestion, when
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compared to either glucose or lactose (all preloads ~ 1.1 MJ, 450 ml) (Bowen et al. 2006, Bowen et al. 2006) (Figure 3.2). However, the increase in CCK concentrations following carbohydrates appear to be shorter lived when compared with lean subjects, and returns close to baseline within 1 hour (Bowen et al. 2006).

NOTE:
This figure is included on page 64 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.2: Plasma CCK concentrations following ingestion of protein, lactose and glucose preloads (1MJ) in obese subjects (n=19). * vs lactose and glucose, P < 0.01. Adapted from (Bowen et al. 2006).

More recently, Batterham et al. reported that a high-protein meal (65 % energy from protein) induced the greatest release of PYY, when compared with a high-fat (65 % energy from fat) and high-carbohydrate (65 % energy from carbohydrate) meal (Batterham et al. 2006). This differs from lean subjects, in whom fat elicits the largest increase in PYY concentrations (Onaga et al. 2002), suggesting that in obese subjects, PYY release in response to high-fat meals may be attenuated. In obese subjects who consumed a glucose drink (300 kcal), after an initial decline,
ghrelin concentrations started to return towards baseline earlier when compared with lean subjects (Greenman et al. 2004). Ghrelin levels were lower in obese subjects following a lipid meal (400 kcal), while a protein load (240 kcal) did not affect ghrelin concentrations (Greenman et al. 2004). In contrast, a subsequent study reported that liquid ‘preloads’ of whey, soy and gluten (~ 50 % energy from protein) prolong the postprandial suppression of ghrelin (~ 180 min), when compared with glucose (~ 120 min) (Bowen et al. 2006), and these responses were not affected by the type of protein consumed. Since gastrointestinal hormones modulate, at least in part, the regulation of appetite and energy intake, a greater understanding of what chemical components of nutrients stimulate specific receptors in the gastrointestinal tract to release hormones and neurotransmitters that affect receptors in the brain and periphery and subsequently reduce or enhance food intake, is required.

3.3.3 Effect of macronutrients on appetite and energy intake

Obese individuals may differ from lean individuals in their ability to adjust appetite and subsequent energy intake in response to different macronutrients (Rolls et al. 1994, Rolls and Hammer 1995, Bellissimo et al. 2008). For example in one study (Rolls et al. 1994), lean and obese subjects consumed yoghurts which varied in fat (65 % of energy as fat) and carbohydrate (81 % of energy as carbohydrate) composition, 30 min before they were offered lunch. Lean subjects responded similarly to the variations in fat or carbohydrate, whereas in the obese, the high-fat yoghurt suppressed energy intake at lunch less than the high-
carbohydrate yogurts. These observations suggest that obese individuals may be relatively insensitive to the satiety effect of fat (Rolls et al. 1994). In another study, energy intake after a lunch meal was higher following a whey-protein ‘preload’ (837 kcal, 56 g whey protein isolate) compared with a glucose ‘preload’ (837 kcal, 55 g glucose) (Bellissimo et al. 2008). In contrast, appetite scores, i.e. hunger, fullness and desire-to-eat, were not affected by the type of ‘preload’ (Bellissimo et al. 2008), suggesting that obese subjects may be less responsive to changes in appetite perceptions compared with lean subjects. The fact that the whey-protein preload did not reduce energy intake when compared with glucose could reflect differences in the release of, or sensitivity to, gastrointestinal hormones in the obese.

As discussed above, it is apparent that while there is some evidence that macronutrients elicit different effects on the gastrointestinal tract, particularly the modulation of gastrointestinal hormones, the role of macronutrients in the regulation of appetite and subsequent energy intake has been poorly characterised in the obese. Relationships between gastrointestinal function with appetite and energy intake, in response to the ingestion of a high-protein and/or high-fat meals, comparable to those typically consumed in Western society, need to be determined in order to improve dietary strategies for the management of obesity (Chapter 7).
3.4 FACTORS THAT MAY INFLUENCE THE EFFECTS OF ORAL MACRONUTRIENTS ON GASTROINTESTINAL FUNCTION, APPETITE AND ENERGY INTAKE

3.4.1 Day-to-day variation in appetite and energy intake

As previously discussed in Chapter 1, gastric emptying, as well as intragastric meal distribution, and gastrointestinal hormones, are important physiological mechanisms involved regulation of appetite and energy intake. However, it is possible that temporal variations in these factors may confound the interpretations of research studies that investigate these parameters. In this chapter, current knowledge of the inter-individual variations in appetite and energy intake, in healthy lean males, and the underlying factors are discussed, as well as the implications of these factors for studies in which energy intake is a primary outcome.

3.4.1.1 Inter-individual variation in appetite and energy

The diverse responses to a treatment, observed between individuals, is referred to as ‘inter-individual variation’. Previous studies have suggested that healthy, lean, young males have a greater capacity to adjust their energy intake in response to dietary manipulation when compared with elderly men, healthy females, or obese individuals (Rolls et al. 1994, Shide et al. 1995, MacIntosh et al. 2001). For example, normal-weight, unrestrained males were able to accurately adjust their energy intake to compensate for the ingestion of yoghurt preloads that varied in their fat and carbohydrate content, i.e. they decreased the amount of calories eaten
at a subsequent meal within 98 – 103 % of baseline, when compared with normal-weight unrestrained females and both normal-weight, and obese, restrained males and females (Rolls et al. 1994). Despite evidence that males may most accurately make compensations in energy consumption following preload ingestion, in studies performed by our group, appetite perceptions and energy intake responses have been shown to vary considerably between individuals. For example, in a study investigating the effects of intraduodenal saline (control) and lipid infusion on energy intake, large inter-individual variation was observed with regards to the ability of subjects to make compensatory changes in energy intake in response to either treatment (Figure 3.3) (Feinle-Bisset and Horowitz 2006). In addition, the magnitude of the reduction in energy intake following lipid administration (2.8 kcal/min for 120 min), varied markedly between subjects. For example, while some subjects showed a large reduction in response to lipid, in others the reduction was minimal, and in a few of these there was an increase in energy intake.

Energy intake in a laboratory setting is commonly assessed using a standardised buffet-style meal, containing a range of food items, varying in macronutrient composition, and provided in excess of what the subject would be expected to consume (Arvaniti et al. 2000, MacIntosh et al. 2001, Feltrin et al. 2004). It has been suggested that the presentation of a meal in excess could potentially result in spontaneous over-consumption (Kral et al. 2004, Norton et al. 2006), thereby confounding the results of studies attempting to demonstrate subtle changes in energy intake in response to a treatment. To our knowledge, only one study has addressed this issue (Arvaniti et al. 2000). This study evaluated, on two separate
Intraduodenal saline
Intraduodenal lipid

Figure 3.3: Energy intake at a cold, buffet-style meal in healthy males (n = 13) consumed immediately after a 120 min duodenal infusion of either saline (control) or lipid (rate: 2.8 kcal/min). Duodenal lipid significantly reduced energy intake when compared with saline (*P < 0.05). The bars on the right are means ± SD for the respective study condition. Adapted from (MacIntosh et al. 2001).

occasions, energy intake and macronutrient composition of food consumed from a standardised, buffet-style meal, and reported that energy intake did not vary between the two days (although this does not exclude the possibility that subjects did not over-consume on both days) (Arvaniti et al. 2000).

In summary, while young males may be the subject group with the greatest capacity to adjust their energy intake in response to dietary manipulation, significant inter-individual variation in energy intake is evident in this group. In regards to optimising study outcomes, it is important to determine whether there are temporal changes in appetite and energy intake and, if so, how these variations
are related to gastrointestinal function, including gastric emptying and gastrointestinal hormone secretion in healthy young males, and females (Chapters 8 and 9).

### 3.4.2 Effects of the menstrual cycle on gastrointestinal function, appetite and energy intake

Studies in both animals and humans suggest that fluctuations in hormone levels during the menstrual cycle modulate gastric emptying and energy intake. Consequently, a major reason why females may be used less frequently, or not at all, in research studies assessing gastrointestinal function, appetite and energy intake is the perceived confounding factor of the menstrual cycle on these parameters. The following discussion reviews the changes in hormones that occur throughout the menstrual cycle and the effect of the menstrual cycle on gastric emptying, gastrointestinal hormones, glycaemia, appetite and energy intake.

#### 3.4.2.1 Hormonal changes during the menstrual cycle

The standardised model of the menstrual cycle is comprised of three phases: (i) the follicular phase: days 1 – 12 (with menstruation days 1 – 7), (ii) periovulation: days 13 – 15, and (iii) the luteal phase: days 16 – 28. The menstrual cycle is regulated primarily by the complex interaction between four hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), produced by the pituitary gland and the female sex hormones, oestrogen and progesterone, produced by the
ovaries. Changes in these hormone levels can be used as markers to characterise each of the four phases (Figure 3.4).

![Figure 3.4: Schematic diagram of changes that occur across the menstrual cycle, specifically hormone concentrations and ovarian and endometrial histology (durations and values may differ between different females or different cycles) (Source: http://commons.wikimedia.org/wiki/File:MenstrualCycle.png).](image)

During menses follicle stimulating hormone (FSH) is relatively elevated (days 1 – 5). FSH rises again at the end of the follicular phase, during which time LH peaks (days 12 – 14). Oestrogen concentrations increase during the end of the follicular phase and peak at the onset of the LH surge (days 5 – 12). Thereafter, oestrogen concentrations decline and rise again, though not to the same extent, during the
luteal phase (days 16 – 25). Progesterone concentrations remain low throughout the follicular phase and rise after ovulation for the next 6 – 10 days (days 16 – 28). Over and above this general pattern of cyclical hormone changes, all four of these hormones show pulsatile rhythms throughout the day (Ferin et al. 1984). Furthermore, the range of plasma concentrations of the two ovarian hormones, estrogen and progesterone, vary considerably as a function of age, with the greatest variability in young women under 25 years old and in those over 40 years old (Chiazze et al. 1968, Schweiger et al. 1987). Ovarian hormone concentrations are also influenced by diet, e.g. vegetarian compared with omnivorous diet (Pirke et al. 1986), energy intake (Pirke et al. 1985), and physical exercise (Shangold 1982). In one study, eighteen healthy lean women who had regular menstrual cycles consumed either a vegetarian, or non-vegetarian, diet for six weeks. Throughout the vegetarian diet, average LH, estradiol and progesterone values were significantly decreased during the luteal phase. In contrast, in women consuming the non-vegetarian diet there was no change in hormone concentrations during any phase of the menstrual cycle, indicating that dietary composition has the capacity to influence the menstrual cycle over a short period of time.

### 3.4.2.2 Effect of the menstrual cycle on gastric motor function

Previous studies that have assessed the effect of the menstrual cycle on gastric emptying have yielded conflicting information (Horowitz et al. 1985, Gill et al. 1987, Degen and Phillips 1996). For example, some studies found no difference in the emptying of a mixed solid-liquid meal between the follicular and luteal phase
Effects of oral macronutrients of gut function and appetite

Chapter 3

(Horowitz et al. 1985), whereas in other studies, gastric emptying of a solid meal was slower during the luteal phase and correlated with plasma levels of progesterone (Wald et al. 1981, Gill et al. 1987). Studies assessing the effect of the menstrual cycle on antropyloroduodenal motility are warranted since this has not previously been evaluated.

3.4.2.3 Effects of the menstrual cycle on gastrointestinal hormones and glycaemia

Few studies have directly investigated the effect of the menstrual cycle on gastrointestinal hormone secretion. Dafopoulos et al., studied eight healthy women in whom daily blood samples were collected over one menstrual cycle. Plasma ghrelin concentrations did not change (Dafopoulos et al. 2008), suggesting that the magnitude of physiologic changes of the sex steroids during the menstrual cycle has no major effect on ghrelin secretion. Studies in rats indicate that oestrogen may increase the sensitivity to the inhibitory effect of CCK on energy intake (Geary 2001), however, no studies have evaluated this in humans or indeed, whether the effects of the menstrual cycle on energy intake are associated with changes in the release of CCK. Studies investigating the effect of the menstrual cycle on glucose tolerance are also inconsistent (Jarrett and Graver 1968, Fioretti et al. 1975, Bonora et al. 1987). For example, some studies using an oral glucose tolerance test have demonstrated better glucose tolerance during the follicular phase (Jarrett and Graver 1968, Fioretti et al. 1975), whereas others have shown no effect (Bonora et al. 1987). The inconsistencies in the results from these studies
may potentially reflect differences in the days of menstrual cycle on which subjects were studied. Gastric emptying was not assessed in any of these studies.

-Since the rate of gastric emptying is a major determinant of postprandial blood glucose homeostasis any changes in gastric emptying during the menstrual cycle may have substantial implications for the diagnosis of diabetes using oral glucose tolerance testing in pre-menopausal women.

3.4.2.4 Effects of the menstrual cycle on appetite and energy intake

There is evidence from studies in both animals (Blaustein and Wade 1976, Kemnitz et al. 1984), and humans (Dalvit-McPhillips 1983, Lissner et al. 1988, Johnson et al. 1994, Barr et al. 1995, Li et al. 1999) that energy intake is reduced immediately prior to ovulation, when oestrogen is at its peak, and increased during the luteal phase, when progesterone is elevated, suggesting, that fluctuations in hormone levels over the menstrual cycle affect energy intake. In a study of 26 normal weight women who recorded their food consumption, exercise and eating patterns in a diet diary over one menstrual cycle, energy intake was increased by 686 ± 101 kJ/day during the luteal, when compared with the follicular, phase (Johnson et al. 1994). In a similar study, females recorded their food intake in a three-day diet diary during both the mid-follicular and mid-luteal phases and mean daily energy intake was higher during the luteal (6978 ± 1847 kJ), than in the follicular (6095 ± 1174 kJ), phase (Li et al. 1999). A review of all the literature relating to the effect of the menstrual cycle on energy intake concluded that the consistent trend in changes in energy intake across the menstrual cycle suggests
that the observed pattern reflects true biological events (Buffenstein et al. 1995), given that energy intake in most women varies with cyclical fluctuations in hormones and increases by ~ 10% during the luteal phase.

In summary, given the inconsistencies in current literature relating to the effects of the menstrual cycle on gastric emptying and gastrointestinal hormones, studies directly assessing these parameters, and the relationships between them with appetite and energy intake, are likely to yield novel insights into appetite regulation in women (Chapter 9). More information relating to the effects of the menstrual cycle may also benefit the design of future studies so that any variations in gastrointestinal factors across the menstrual cycle can be controlled for, particularly in protocols where energy intake is a primary outcome.

3.5 CONCLUSIONS

This chapter has reviewed the effects of orally ingested macronutrients on gastrointestinal function, appetite and energy intake in both lean and obese individuals, with a particular focus on two factors, i.e. day-to-day variation and the menstrual cycle, that may influence the effect of oral macronutrients on gastrointestinal function, appetite and energy intake. Studies described in subsequent chapters of this thesis address the following aims:

(i) to compare the effects of high-fat, high-carbohydrate and high-protein meals, and the effects of increasing amounts of protein in a
(ii) to assess day-to-day variations in gastric emptying, gastrointestinal hormone release, appetite and energy intake, in response to an oral ‘preload’ in male (Chapter 8) and female subjects (Chapter 9) and,

(iii) to assess the effect of the menstrual cycle on gastric emptying, gastrointestinal hormone release, appetite and energy intake, in response to an oral ‘preload’ (Chapter 9).
CHAPTER 4

Common methodologies

4.1 INTRODUCTION

This chapter describes the methods and techniques that were used in the studies presented within this thesis in Chapters 5 – 10. All of these techniques have been validated and are well established for the assessment of gastrointestinal luminal pressures, gastric emptying, gastrointestinal hormone concentrations, appetite and energy intake.

4.2 SUBJECTS

Subjects were recruited from an existing pool of volunteers, or through the combined use of flyers placed around the Royal Adelaide Hospital, University of Adelaide and University of South Australia and advertisements placed in ‘The Sunday Mail’ or ‘The Messenger’.
4.2.1 **Healthy subjects**

Young male (Chapters 5 – 8) and female (Chapter 9) subjects, aged 18 – 55 years old, who were of normal body weight for their height (body mass index (BMI) 19 – 25 kg/m²) were studied.

4.2.2 **Obese subjects**

Young males, aged 18 – 55 years old, who were obese (BMI 30 – 35) (Chapters 7 and 10) were studied. The BMI range for obese subjects was chosen to ensure inclusion of a relatively ‘homogenous’ group (i.e. excluding the morbidly obese). All subjects were required to be weight-stable (i.e. < 5 % fluctuation in their body weight) at study entry, as determined by their weight in the preceding 12 weeks, and were asked to maintain their normal physical activity over the course of the study.

4.2.3 **Inclusion/Exclusion criteria**

Each subject was questioned prior to their inclusion into a study to exclude:

(i) significant gastrointestinal symptoms, disease or surgery
(ii) diabetes mellitus
(iii) gallbladder or pancreatic disease
(iv) cardiovascular or respiratory disease, or other significant illness
(v) epilepsy
(vi) current use of medications known to have the potential to affect gastrointestinal motor function, body weight or appetite (e.g. metoclopramide, erythromycin, hyoscine, orlistat)

(vii) consumption of > 20 g of alcohol per day

(viii) smoking > 10 cigarettes per day

(ix) high performance athletes

(x) lactose intolerance

(xi) in female subjects, pregnancy or lactation. A pregnancy test was performed in all female subjects, using a urine sample, prior to commencement of a study (Chapter 9)

The degree of eating restraint in an individual, i.e. the tendency of some persons to restrict their food intake in order to control their body weight, was measured in all subjects, using the Three Factor Eating questionnaire (Stunkard and Messick 1985) (Appendix I), but only used as an exclusion criterion (score > 12) in lean subjects (Chapters 5 – 9), since, in our experience, obese subjects were likely to have some degree of eating restraint.

Subjects in the studies described in Chapters 5 and 6 were also excluded if their haemoglobin concentrations were outside of the normal range: 135 – 175 g/l or plasma biochemical measurements of liver/renal function were outside the following ranges:

- alkaline aminotransferase: 0 – 55 U/l
- alkaline phosphate: 30 – 110 U/l
- aspartate transaminase: 0 – 45 U/l
- bilirubin: 6 – 24 μmol/l
- creatinine: 0.05 – 0.12 mmol/l

All subjects were required to provide written, informed consent prior to their inclusion into a study and were informed of their right to withdraw at any time. All subjects were offered an honorarium for their participation ($15 – $18 per hour)

### 4.3 ETHICS COMMITTEE APPROVAL

All study protocols were approved by the Royal Adelaide Hospital Ethics Committee, and all experiments were carried out in accordance with the Declaration of Helsinki. Approvals from the Royal Adelaide Hospital Investigational Drug Sub-Committee and Therapeutic Goods Administrations were obtained for the intravenous administrations of the hormones CCK and GLP-1 (Chapters 5 and 6).

### 4.4 STUDY ENVIRONMENT

Studies were conducted within the clinical rooms of the University of Adelaide Discipline of Medicine, Royal Adelaide Hospital (Chapters 5 – 10). Every attempt was made to exclude environmental factors, specifically social interaction or influence by any other individual. In studies that assessed APD motility, subjects were positioned supine on a hospital bed (Chapters 6 and 10). In studies that
assessed gastric emptying, subjects were seated comfortably in an upright position (Chapters 8 and 9). In studies that assessed the effect of meal ingestion on appetite and energy intake, subjects were seated at a table (Chapter 7). Subjects were allowed to read (subject material unrelated to food) or listen to music, except when blood samples were taken and/or questionnaires being completed. During the consumption of the buffet meal to assess energy intake, subjects were seated at a table and were not permitted to read, listen to music or converse.

4.5 MEASUREMENT OF ANTROPYLORODUODENAL PRESSURES

High-resolution perfusion manometry is a technique used to measure pressures in the gastrointestinal tract, including the antrum, pylorus and duodenum (Dent 1976, Heddle et al. 1989). Pressures in the APD region were measured using a 3.5 mm (outer diameter), 17-channel silicone manometric catheter (originally manufactured by Dentsleeve Pty Ltd, Adelaide, Australia (Chapter 6), and subsequently by Dentsleeve International Ltd, Ontario, Canada (Chapter 10)).

4.5.1 Catheter design

The manometric catheter utilised in Chapters 6 and 10 consisted of sixteen side-holes (0.1 mm diameter) separated by 1.5 cm intervals, for the recording of luminal pressures. Six side-holes (channels 1 – 6) were positioned in the antrum, a 4.5 cm sleeve sensor (channel 7), with two side-holes 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 11.75 cm distal to the pylorus, for the administration of intraduodenal infusions (Chapter 10).

**Figure 4.1:** Schematic drawing of the multi-lumen catheter used for the measurement of antropyloroduodenal pressures (Chapters 6 and 10) and the intraduodenal nutrient infusion (Chapter 10).

### 4.5.2 Nasoduodenal intubation and manometry

Subjects were intubated with the manometric catheter via an anaesthetised nostril into the stomach, which was allowed to pass into the duodenum via peristalsis. The correct positioning of the catheter, so that the sleeve sensor straddled the pylorus, was maintained by continuous measurement of the transmucosal potential difference (TMPD) between the most distal antral (channel 6) (~ 40 mV), and the most proximal duodenal (channel 10) (~ 0 mV), channel (Figure 4.1) (Heddle et
al. 1989). For this purpose, an intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode (Heddle et al. 1989). All manometric channels were perfused with degassed, distilled water, except for two TMPD channels, which were perfused with degassed 0.9% saline (Heddle et al. 1989). Once the catheter was in the correct position, fasting motility was monitored until the occurrence of a phase III of the interdigestive MMC. All study interventions began ~15 min after the phase III activity had passed, and during a period of motor quiescence (phase I of the MMC).

### 4.5.3 Data acquisition and analyses

Manometric pressures were digitised and recorded on a computer-based system: Dimension 2004; Dell Computer Corporations, Round Rock, Texas, USA, running commercially available software (Oakdale, Flexisoft, Associate Professor G Hebbard, Melbourne, Australia), written in Labview 3.1.1 (National Instruments). Data obtained were stored for subsequent analysis. APD pressures were analyzed for the: (i) the number and amplitude of PWs in the antrum and duodenum, (ii) basal pyloric pressure (pyloric ‘tone’), (iii) the number and amplitude of IPPWs and (iv) pressure wave sequences (PWSs). PWs in the antrum, pylorus and duodenum were defined by an amplitude $\geq 10$ mmHg, with a minimum interval of 15 s between peaks for antral and pyloric waves, and 3 s for duodenal waves, and analyzed using custom-written software (by Professor A Smout, Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The
Netherlands) (Samsom et al. 1998). Basal pyloric pressures were calculated by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side hole from the mean basal pressure recorded at the sleeve (Hedde et al. 1988), using custom-written software (by Professor A Smout). PWs in the antrum, pylorus and duodenum were considered related and defined as PWSs, if their rate of travel between side-holes was between 9 - 160 mm/s. PWSs were characterized according to the distance travelled, i.e. over at least two (1.5 < 3 cm), three (3 < 4.5 cm), four (4.5 < 6 cm), five (6 < 7.5 cm), six (7.5 < 9 cm), seven (9 < 10.5 cm) eight (10.5 < 12 cm), nine (12 < 13.5 cm), ten (13.5 < 15 cm), eleven (15 < 16.5 cm), twelve (16.5 < 18 cm), thirteen (18 < 19.5 cm), fourteen (19.5 < 21 cm) and fifteen (21 < 22.5 cm) channels, and expressed as total number of waves using custom-written software (by Professor A Smout).

4.6 MEASUREMENT OF GASTRIC EMPTYING

Several techniques can be used for the measurement of gastric emptying. In Chapters 8 and 9, 3-D ultrasonography was used, as described below.

4.6.1 Three dimensional (3-D) ultrasonography

3-D ultrasonography measurements were acquired in Chapters 8 and 9 using a Logiq (Logiq™9 ultrasound system; GE Healthcare Technologies, Sydney, New South Wales, Australia), a technique that has been validated against the ‘gold standard’ scintigraphy, as an accurate measure of the gastric emptying of liquids in healthy subjects. This technique allows evaluation of total, proximal and distal
gastric volumes (i.e. gastric emptying and intragastric meal distribution). While scintigraphy may be regarded as the ‘gold standard’ for the measurement of gastric emptying in clinical and research studies (Collins et al. 1983), the associated radiation burden limits its use and the use of 3-D ultrasonography has now been validated against scintigraphy, as an accurate measure of gastric emptying of liquids in healthy subjects (Gentilcore et al. 2006). For 3-D positioning and orientation measurement, a transmitter was placed next to the subject, and a 3-D sensor was attached to a 3.5C broad spectrum 2.5 - 4 MHz convex transducer. All metal objects were removed from both the subject and surrounding area to avoid the possibility of interference during acquisition. 3-D sweeps of the total stomach were taken to evaluate total gastric volume. Subjects were instructed to hold their breath at the end of an inspiration, not move, and the stomach was scanned by a continuous translational movement along its long axis, starting proximally at the left subcostal margin, and moving distally to the gastroduodenal junction, to produce transverse sections of the entire stomach. The total scanning time was ~10 seconds.

4.6.1.1 Data acquisition and analyses

The raw data (original scan planes) were used for 3-D reconstructions of the stomach using EchoPAC-3D software® (GE Vingmed Sound, Horten, Norway). The proximal and distal gastric segments were separated by vertically slicing the 3-D stomach reconstruction from the incisura angularis at the lesser gastric curvature sagittally towards the greater curvature. Total, proximal and distal
gastric volumes at each time point were derived and expressed as percentages of the volumes at $t = 0$ min (volume immediately following preload ingestion), with total gastric volume at $t = 0$ min defined as 100%. Gastric emptying profiles were then constructed, and the time at which 50% of the meal had emptied from the stomach (50% gastric emptying time ($T_{50}$)) was derived (Collins et al. 1983).

4.7 MEASUREMENT OF HORMONES

4.7.1 Measurement of gastrointestinal hormones

A cannula was placed into an antecubital vein to obtain blood samples for subsequent assessment of gastrointestinal hormones. Blood samples (10 ml) were collected into ice-chilled EDTA-treated tubes containing 400 kIU aproptinin per ml blood (Trayslol; Bayer Australia Ltd, Pymble, Australia). 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.

4.7.1.1 Plasma cholecystokinin

Plasma CCK concentrations (Chapters 6, 8 and 9) were determined following ethanol extraction, using an established radioimmunoassay. A commercially available antibody raised in rabbits against synthetic sulfated CCK-8 was used (C258, Lot 105H4852; Sigma-Aldrich, St Louis, Missouri, USA). This antibody binds to all CCK peptides containing the sulfated tyrosine residue in position 7, has a 26% cross-reactivity with unsulfated CCK-8, less than 2% cross-reactivity with
human gastrin, and does not bind to structurally unrelated peptides. Intra- and inter-assay coefficients of variations (CVs) were 6.2 and 14.8 %, respectively, with a detection limit of 2.5 pmol/L

### 4.7.1.2 Plasma ghrelin

In Chapter 5, total plasma ghrelin was measured by a commercially available radioimmunoassay (Phoenix Pharmaceuticals, Mountain View, California, USA) that uses $^{125}$I-labeled bioactive ghrelin as a tracer and a polyclonal antibody raised in rabbits against the C-terminal end of human ghrelin. No cross-reactivities with any relevant molecules (i.e. secretin, vasoactive intestinal peptide, prolactin-releasing-peptide-31, galanin, growth hormone releasing factor, neuropeptide Y (NPY), orexin A, orexin B) have been found. All samples from individual subjects were measured in the same run. Intra- and inter-assay CVs were 5.3 % and 13.6 %, respectively, with a detection limit of 64 pg/ml.

### 4.7.1.3 Plasma peptide YY

Two different radioimmunoassays were utilised for the determination of plasma PYY concentrations. In Chapter 5, total plasma PYY was measured by a commercially available radioimmunoassay (Linco Research, Missouri, USA) using $^{125}$I-labeled bioactive PYY as tracer and a PYY antiserum to determine the level of active PYY by the double antibody/PEG technique. The PYY antibody was raised in guinea pigs and recognizes both the PYY (1-36) and PYY (3-36) forms of human PYY. No cross-reactivities with any relevant molecules (i.e.
NPY, human leptin, glucagon, ghrelin, insulin, GLP-1) have been found. All samples from individual subjects were measured in the same run. Intra- and inter-assay CVs were 5.3 % and 7.0 %, respectively, with a detection limit of 10 pg/ml.

In Chapter 6, plasma PYY concentrations were determined 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); i.e., the assay does not distinguish between PYY-1–36 and PYY-3–36. This antiserum showed <0.001% cross-reactivity with human pancreatic polypeptide or sulfated CCK-8 and 0.0025% cross-reactivity with human neuropeptide Y. Tracer (NEX3410) was purchased from Perkin-Elmer (Boston, MA, USA). Intra- and inter-assay CVs were 12.3 and 16.6 %, respectively, with a detection limit of 1.5 pmol/L.

4.7.1.4 Plasma insulin

In Chapters 8 and 9, plasma insulin concentrations were measured by ELISA (Diagnostics Systems Laboratories Inc. Webster, Texas, USA). The intra-assay and inter-assay CVs were 2.6 and 6.2 %, respectively, with a detection limit of 0.26 mU/L.

4.7.2 Serum oestradiol and progesterone

In Chapter 9 blood samples (8 ml) were collected into serum clot activator tubes. Oestradiol and progesterone concentrations were both determined by the Institute
of Medical and Veterinary Science (Royal Adelaide Hospital, Adelaide, South Australia, Australia) using chemiluminescent microparticle immunoassay (ARCHITECT System, Abbott Laboratories, Abbott Park, Illinois, USA).

For oestradiol, the intra-assay and inter-assay CVs were 1.8 and 2.3 %, respectively, with a detection limit of 37 pmol/L. For progesterone, the intra-assay and inter-assay CVs were 1.5 and 2.1 %, respectively, with a detection limit of 1 nmol/L.

4.8 MEASUREMENT OF APPETITE PERCEPTIONS AND ENERGY INTAKE

4.8.1 Visual analogue scale questionnaires

Perceptions of appetite, including hunger, fullness, desire-to-eat and prospective consumption were measured using validated visual analogue scale questionnaires (VAS) (Parker et al. 2004) (Appendix II) (Chapters 6 – 10). The gastrointestinal symptoms, nausea and bloating, were also assessed. Other perceptions, including anxiety, happiness and drowsiness, were assessed to distract the subjects from the main purpose of the questionnaire, but were not evaluated formally. Each VAS consisted of 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 place a vertical mark on the 100 mm line to indicate how they felt at a particular point in time.
4.8.2 Energy intake

Energy intake was assessed by quantifying the amount consumed by a subject at an *ad libitum* cold, buffet-style meal (Chapters 6 – 10). The meal comprised 4 slices (125 g) wholemeal bread, 4 slices (125 g) white bread, 100 g sliced ham, 100 g sliced chicken, 85 g sliced cheddar cheese, 100 g lettuce, 100 g sliced tomato, 100 g sliced cucumber, 20 g mayonnaise, 20 g margarine, 170 g apple, 190 g banana, 200 g strawberry yogurt, 150 g chocolate custard, 140 g fruit salad, 375 ml iced coffee, 300 ml orange juice and 600 ml water. The energy (kJ), amount (g) and macronutrient distribution (i.e. the percentage of energy from fat, carbohydrates and protein), of each item in the buffet meal are detailed in Appendix III. The amount of food offered was in excess of what the subject was expected to eat and each subject was allowed up to 30 min to consume from the buffet meal freely until comfortably full.

The amount (g) of food consumed from the buffet meal was determined by weighing the meal immediately before and after consumption. Energy intake (kJ) and macronutrient composition were analysed using commercially available software (Foodworks 3.01, Xyris Software, Highgate Hill, QLD, Australia).
4.9 PREPARATION AND ADMINISTRATION OF STUDY INTERVENTIONS

4.9.1 Hormones for intravenous infusion

4.9.1.1 Cholecystokinin

CCK-8 was purchased from Clinalfa®, Merck Biosciences, Laeufelfingen, Switzerland (Chapter 5). In Chapter 5 the CCK-8 was dissolved in 0.9 % sterile saline and infused at a rate of 2 ng/kg/min for 150 minutes. In Chapter 6 the CCK-8 was dissolved in 0.9 % sterile saline and infused at a rate of (i) 0.33 ng/kg/min, (ii) 0.66 ng/kg/min, (iii) 1.33 ng/kg/min and (iv) 2 ng/kg/min for 120 minutes.

4.9.1.2 Glucagon-like peptide-1

GLP-1 was purchased from Clinalfa®, Merck Biosciences, Laeufelfingen, Switzerland (Chapter 5). The GLP-1 was in 0.9 % sterile saline and infused at a rate of 0.9 pmol/kg/min for 150 minutes.

The doses of CCK-8 and GLP-1 were selected on the basis of previous studies, which indicated that they had submaximal effects on gastric emptying, energy intake and APD pressures, while resulting in apparently physiological plasma concentrations (Schirra et al. 2000, MacIntosh et al. 2001).
4.9.2 Test meal and preload preparation

Orally administered treatments, called ‘test meals’ or ‘preloads’, allow the effects of nutrients on gastrointestinal function, appetite and energy intake to be assessed (Chapters 7 – 9).

4.9.2.1 Test meals

In Chapter 7, subjects ingested four test meals, which were administered in a randomised fashion. The four meals were isocaloric and matched for volume, palatability, smell, texture and appearance. Each meal comprised varying quantities of conventional pasta (San Remo Macaroni Pty Ltd, Windsor Gardens, South Australia, Australia), with a tomato-based sauce (Leggo’s pasta sauce, Simplot Australia Pty Ltd, Mentone, Victoria, Australia) consisting of olive oil, lean beef mince, onion and mixed dry herbs, and a vanilla yoghurt dessert (Yoplait, National Foods Ltd, Melbourne, Victoria, Australia). To achieve the described macronutrient compositions, whey protein isolate (Pure Nutrition, Nexus Pty Ltd, Ermington, New South Wales, Australia) was used to increase the protein content, pure cream (Bulla, Regal Cream Products Pty Ltd, Colac, Victoria, Australia) to increase the fat content, and sucrose (Bundaberg Sugar, Enoggera, Queensland, Australia) and corn flour (White Wings Food, Macquarie Park, New South Wales, Australia) to increase the carbohydrate content, of the meals. All test meals were prepared by the same investigator on the morning of each study day. The macronutrient composition of each test meal is detailed in Table 7.1.
4.9.2.2 **Glucose preload**

In Chapters 8 and 9, subjects consumed a preload consisting of 50 g of glucose dissolved in 300 ml of water (200 kcal, 926 mosmol/l). Each subject received the preload at the same time on each of the three study days, and the preload was consumed at a constant rate, using a straw, within ~ 2 minutes.

4.9.3 **Intraduodenal nutrient infusion**

Treatments given directly into the small intestine via an intraduodenal catheter allow the effects of nutrients on gastrointestinal function, appetite and energy intake to be assessed without the influence of gastric distension and gastric emptying.

A commercially available lipid emulsion, Intralipid (10 %, 300 mOsmol/kg, 1.1 kcal/ml, Baxter Healthcare Pty Ltd, Old Toongabbie, New South Wales, Australia), which consists predominantly of long-chain tryglycerides extracted from soy bean oil (50 g/500 ml), egg phospholipids (1.2 g/500 ml) and glycerol anhydrous (2.25 g/500 ml), was used as the nutrient infusion in Chapter 10. The lipid infusion was administered at a rate of 2.86 kcal/min for 120 minutes.

4.9.4 **Very-low calorie diet**

In Chapter 10, to achieve a period of acute energy restriction, subjects were placed on a four-day very-low calorie diet (VLCD). The VLCD was 30 % of an
individual’s daily energy requirements calculated using the Harris Benedict equation (with a physical activity factor between 1.4 – 1.5 based on an individual’s daily activity) (Harris and Benedict 1918). The VLCD consisted of both liquid meal replacements (KicStart, Pharmacy Health Solutions Pty Ltd, Frenchs Forest, New South Wales, Australia) and standard food items, i.e. sliced ham, wholemeal bread, salad items and pre-packaged frozen meals (Lean Cuisine beef lasagne/chicken stirfry, Nestle Inc, Sydney, New South Wales, Australia). Each subject was provided with a meal plan diary which detailed the food items and amount (g) of these items to be consumed at each meal. An example of the VLCD meal plan diary is provided in Appendix IV (based on an individual with 4000 kJ/day energy requirements). Throughout the VLCD, each subject was permitted to consume an unlimited quantity of non-caloric beverages; subjects were required to document the name and quantity of any beverages consumed in their meal plan diary each day.

4.10 DATA AND STATISTICAL ANALYSES

Data analyses performed in each study are described in detail in the individual chapters. Data were analysed using commercially available statistical software (i) SuperANOVA Version 1.1 (Abacus Concepts Inc., Berkeley, California, USA), (ii) Statview Version 5 (SAS Institute Inc., North Carolina, USA) or (iii) SPSS Version 16.0 (SPSS Inc, Chicago, Illinois, USA). In accordance with appropriate statistical practise, significant effects are reported as time-by-treatment interactions, treatment and/or time effects, in this hierarchy, i.e. by definition,
when a treatment effect is reported, no time-by-treatment interaction is evident. Statistical significance was accepted at $P < 0.05$ and data are presented as means ± SEM.
CHAPTER 5

Intravenous CCK-8, but not GLP-1, suppresses ghrelin and stimulates PYY release in healthy men

5.1 SUMMARY

We have investigated the effects of exogenous CCK-8 and GLP-1, alone and in combination, on ghrelin and PYY secretion. Nine healthy males were studied on 4 occasions. Plasma ghrelin and PYY concentrations were measured during 150 min intravenous infusions of: (i) isotonic saline, (ii) CCK-8 at 1.8 pmol/kg/min, (iii) GLP-1 at 0.9 pmol/kg/min, or (iv) CCK-8 and GLP-1 combined. CCK-8 markedly suppressed ghrelin and stimulated PYY when compared with control between $t = 0 – 120$ min (P < 0.001 for both). GLP-1 had no effect on ghrelin, but decreased PYY slightly at 120 min (P < 0.05). During infusion of CCK-8+GLP-1, there was comparable suppression of ghrelin (P < 0.001), but the stimulation of PYY was less (P < 0.001), than that induced by CCK-8, between $t = 20 – 120$ min. In conclusion, in healthy subjects, in the doses evaluated, exogenous CCK-8 suppresses ghrelin and stimulates PYY, and exogenous GLP-1 has no effect on ghrelin and attenuates the effect of CCK-8 on PYY.
5.2 INTRODUCTION

Following ingestion of a meal, a number of hormones, including CCK (Lilja et al. 1984), are released from the proximal small intestine, and other hormones, including GLP-1 (Herrmann et al. 1995) and PYY (Adrian et al. 1985), are released from the distal small intestine. In contrast, meal ingestion suppresses the release of ghrelin (Cummings et al. 2001), which is synthesized predominantly in the stomach (Kojima et al. 1999). Studies in which these hormones have been administered exogenously (Flint et al. 1998, MacIntosh et al. 2001, Batterham et al. 2002), or in which specific antagonists have been employed (Fried et al. 1991, Schirra et al. 1998, Beglinger et al. 2001), have established their capacity to modulate a number of postprandial gastrointestinal functions, including gastric emptying (Konturek et al. 1990, Anvari et al. 1998, Inui et al. 2004) and energy intake (MacIntosh et al. 2001, Wren et al. 2001, Batterham et al. 2002).

The majority of previous studies have focused on the effects of gastrointestinal hormones when administered in isolation. However, it is increasingly evident that there are interactions between gastrointestinal hormones, perhaps particularly those released from different regions of the small intestine (Lin et al. 2000, Lin and Chey 2003, Lippl et al. 2004). Intravenous (iv) PYY suppresses ghrelin in humans (Batterham et al. 2003), and in-vitro studies in rodents suggest that GLP-1 may also have this effect (Lippl et al. 2004). Somewhat surprisingly, the effect of CCK on ghrelin secretion has, to our knowledge, not been evaluated. In dogs, the stimulation of PYY by the presence of fat in the proximal small intestine is mediated, at least in part, by CCK (Lin et al. 2000). Conversely, there is evidence
in humans that GLP-1 may inhibit the release of PYY (Naslund et al. 1999). There is no information as to possible interactions between CCK and GLP-1 that may modulate ghrelin and PYY release.

We recently reported the effects of iv administration of CCK and GLP-1, alone and in combination, on appetite, antropyloroduodenal motility and energy intake in healthy male subjects (Brennan et al. 2005). Infusion of CCK-8 decreased perceptions of appetite, energy intake, the number of antral and duodenal PWs, and the number of PWSs, and increased both the number and amplitude of IPPWPs. In contrast, infusion of GLP-1 did not suppress appetite or energy intake or stimulate pyloric pressures, but decreased antral and duodenal PWs to an extent comparable with CCK-8. Although the combination of CCK-8 and GLP-1 decreased the number of duodenal PWs more than CCK-8 or GLP-1 alone, it did not have any additional effects on appetite, energy intake or other motility parameters. We have now assayed the remaining plasma samples to evaluate the hypotheses that (i) iv CCK decreases plasma ghrelin and increases plasma PYY, and (ii) iv GLP-1 attenuates the effect of CCK-8 on ghrelin and PYY release.

5.3 SUBJECTS AND METHODS

5.3.1 Subjects

9 healthy males, aged 22 ± 1 (range 18 – 37) years, and of normal body weight for their height (body mass index 23 ± 0.5 (range 20 – 25.2) kg/m², body weight 75 ±
0.2 (range 63.5 – 81.5) kg) were recruited according to guidelines described in Chapter 4.2.

5.3.2 Study design

Each subject attended the laboratory on four occasions, each separated by 3 – 10 days, in which they received, in randomised, double-blind fashion, 150 min iv infusions of: (i) isotonic saline (“control”), (ii) CCK-8 at 1.8 pmol/kg/min (2 ng/kg/min) (“CCK-8”), (iii) GLP-1 at 0.9 pmol/kg/min (“GLP-1”) (both from Merck Biosciences, Läufelfingen, Switzerland) or (iv) both (ii) and (iii) (“CCK-8+GLP-1”). The doses of CCK-8 and GLP-1 used were selected on the basis of their submaximal effects on gastric emptying, appetite and antropyloroduodenal motility in other studies (Nauck et al. 1997, Rayner et al. 2000, Schirra et al. 2000, MacIntosh et al. 2001).

5.3.3 Protocol

Subjects attended the laboratory at 0830 h after fasting from 2200 h the previous night. Intravenous cannulae were placed in each arm for infusion and blood sampling, respectively. A baseline blood sample (t = 0 min) was taken, and the infusion of either control, CCK-8, GLP-1 or CCK-8+GLP-1 commenced. Plasma concentrations of CCK and GLP-1 achieved during the infusions, as previously reported (Brennan et al. 2005), were as follows: plasma CCK; control: baseline: 3.8 ± 0.3 pg/ml, 120 min: 4.6 ± 0.5 pg/ml, CCK-8: baseline: 7.1 ± 2.8 pg/ml, 120
min: 26.8 ± 3.0 pg/ml, GLP-1: baseline: 4.2 ± 0.4 pg/ml, 120 min: 5.1 ± 0.7 pg/ml
and CCK-8+GLP-1: baseline: 4.3 ± 0.3 pg/ml, 120 min: 25.8 ± 2.5 pg/ml, and
plasma GLP-1; control: baseline: 9.4 ± 1.2 pg/ml, 120 min: 10.2 ± 1.7 pg/ml,
CCK-8: baseline: 9.4 ± 2.1 pg/ml, 120 min: 20.5 ± 1.8 pg/ml, GLP-1: baseline: 8.9
± 1.5 pg/ml, 120 min: 34.1 ± 7.7 pg/ml and CCK-8+GLP-1: baseline: 10.3 ± 1.5
pg/ml, 120 min: 49.9 ± 8.1 pg/ml. At \( t = 120 \) min (with the iv infusions
continuing), subjects were offered a standardised, cold, buffet-style meal. During
the iv infusions blood samples were obtained at \( t = 0, 10, 20, 30, 45, 60, 90 \) and
120 min and after the meal, at \( t = 150 \) and \( t = 180 \) min.

5.3.4 Measurements

5.3.4.1 Plasma hormone concentrations

Blood sample collection and analysis of plasma ghrelin and PYY were performed
as described in Chapters 4.7.1.2 and 4.7.1.3.

5.3.5 Statistical analysis

Data were analyzed by repeated measures analysis of variance (ANOVA) with
time (i.e. \( t = 0, 10, 20, ..., 120 \) min) and treatment as factors. Post-hoc paired
comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were
performed when ANOVAs revealed significant effects. Plasma ghrelin and PYY
concentrations immediately following the buffet meal (i.e. \( t = 150 \) min) and 30
min later (\( t = 180 \) min) were compared with pre-meal (\( t = 120 \) min) concentrations
using Student’s paired t-test. Statistical significance was accepted at P < 0.05, and data are presented as means ± SEM.

5.4. RESULTS

5.4.1 Plasma ghrelin concentrations (Figure 5.1A)

5.4.1.1 Effect of intravenous infusion

There was no difference in baseline ghrelin concentrations between study days (Table 5.1). There was a treatment by time interaction (P < 0.001) for plasma ghrelin concentrations. Infusion of CCK-8 reduced plasma ghrelin over the entire 120 min infusion period when compared with control (P < 0.001), between t = 30 – 120 min when compared with GLP-1 (P < 0.001), and between t = 60 – 120 min when compared with CCK-8+GLP-1 (P < 0.05). GLP-1 did not affect plasma ghrelin. CCK-8+GLP-1 reduced plasma ghrelin between t = 20 – 120 min when compared with control (P < 0.001), and between t = 30 – 120 min when compared with GLP-1 (P < 0.01). The suppression of ghrelin during CCK-8 and CCK-8+GLP-1 was progressive and evident within 10 and 20 min, respectively, upon commencement of the infusion (P < 0.001 for both). Plasma ghrelin concentrations at baseline and t = 120 min are presented in Table 5.1.

5.4.1.2 Effect of meal

Immediately after the buffet meal (i.e. t = 150 min), plasma ghrelin was less when compared with pre-meal concentrations (i.e. t = 120 min), following control (P <
0.01), but not CCK-8, GLP-1 or CCK-8+GLP-1. At $t = 180$ min, plasma ghrelin was less following control ($P < 0.001$), GLP-1 ($P < 0.001$) and CCK+GLP-1 ($P < 0.05$), but not CCK, when compared with concentrations at $t = 120$ min.

### 5.4.2 Plasma PYY concentrations (Figure 5.1B)

#### 5.4.2.1 Effect of intravenous infusion

There was no difference in baseline PYY concentrations between study days (Table 5.1). There was a treatment by time interaction ($P < 0.001$) for plasma PYY concentrations. Infusion of CCK-8 increased plasma PYY over the entire infusion period when compared with both control and GLP-1 ($P < 0.001$) and between $t = 20 – 120$ min when compared with CCK-8+GLP-1 ($P < 0.001$). There was no difference in plasma PYY concentrations between GLP-1 and control, except at $t = 120$ min, when PYY was less during infusion of GLP-1 than control ($P < 0.05$). Plasma PYY was greater during CCK-8+GLP-1 between $t = 60 – 120$ min when compared with control and between $t = 45 – 120$ min when compared with GLP-1 ($P < 0.01$ for both). The increase of PYY during CCK-8 was progressive and evident within 10 min of commencement of the infusion ($P < 0.001$). Plasma PYY concentrations at baseline and $t = 120$ min are presented in Table 5.1.
5.4.2.2 Effect of meal

Immediately after the buffet meal (i.e. $t = 150$ min), plasma PYY was higher following control ($P = 0.001$), CCK-8 ($P = 0.01$) and GLP-1 ($P = 0.01$) when compared with pre-meal concentrations ($t = 120$ min). At $t = 180$ min, plasma PYY was higher following all treatments ($P < 0.01$) when compared with concentrations at $t = 120$ min.
Table 5.1: Plasma ghrelin and PYY concentrations at $t = 0$ (baseline) and $t = 120$ min during intravenous infusion of saline (control), CCK-8, GLP-1, or CCK-8+GLP-1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$t = 0$ min</th>
<th>$t = 120$ min</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(t = 120 min vs t = 0 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma ghrelin concentrations (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>231.7 ± 10.0</td>
<td>219.6 ± 12.0</td>
<td>ns</td>
</tr>
<tr>
<td>CCK-8</td>
<td>227.0 ± 13.8</td>
<td>168.7 ± 13.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GLP-1</td>
<td>232.9 ± 21.2</td>
<td>221.8 ± 11.2</td>
<td>0.05</td>
</tr>
<tr>
<td>CCK-8+GLP-1</td>
<td>231.3 ± 7.3</td>
<td>183.1 ± 11.1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

| **Plasma PYY concentrations (pg/ml)** | | | |
| Control            | 204.3 ± 16.9 | 174.5 ± 7.8  | < 0.001   |
| CCK-8              | 198.8 ± 12.1 | 364.4 ± 37.3 | < 0.001   |
| GLP-1              | 201.9 ± 13.1 | 149.4 ± 7.4  | < 0.001   |
| CCK-8+GLP-1        | 201.9 ± 15.7 | 218.6 ± 15.7 | ns        |

Data are means ± SEM, n = 9. ns, not significant
Figure 5.1: Plasma concentrations of (A) ghrelin and (B) PYY during intravenous infusion of saline (control), CCK-8, GLP-1, or CCK-8+GLP-1. A: φ CCK-8 vs control, P < 0.001; * CCK-8 vs GLP-1, P < 0.001; Δ CCK vs CCK-8+GLP-1, P < 0.05; # CCK-8+GLP-1 vs control, P < 0.001; α CCK-8+GLP-1 vs GLP-1, P < 0.01; β vs t = 120 min, P < 0.05. B: φ CCK-8 vs control and GLP-1, P < 0.001; Δ CCK vs CCK-8+GLP-1, P < 0.001; * GLP-1 vs control: P < 0.03, # CCK-8+GLP-1 vs control, P < 0.01; α CCK-8+GLP-1 vs GLP-1, P < 0.01; β vs t = 120 min, P < 0.05. Data are means ± SEM, n = 9.
5.5 DISCUSSION

This study indicates that exogenous CCK-8 and GLP-1 have discrepant effects on the secretion of ghrelin and PYY. In particular (i) CCK-8 markedly suppressed ghrelin, whereas GLP-1 had no effect, and (ii) the stimulation of PYY by CCK-8 was attenuated markedly by GLP-1.

This study has demonstrated for the first time in humans that intravenous administration of CCK-8 has the capacity to suppress plasma ghrelin concentrations and, in contrast to what has been suggested in animal studies (Lippl et al. 2004), GLP-1 has no effect. Hence, the suppression of ghrelin observed during the concurrent infusion of CCK-8 and GLP-1 is likely to be attributable to the effect of CCK-8 alone. It is well established that CCK-8 and ghrelin exert opposite effects on appetite and energy intake (MacIntosh et al. 2001, Wren et al. 2001), accordingly, the observed effects of CCK-8 are perhaps not surprising. In rats, peripheral administration of CCK-8 abolishes ghrelin-induced food intake, possibly by modulating vagal afferent function (Kobelt et al. 2005). There are a number of possible mechanisms which may account for the postprandial suppression of ghrelin, including hyperinsulinemia (Flanagan et al. 2003), elevation of blood glucose (Little et al. 2006), and the presence of nutrients in the stomach and small intestine (Feinle-Bisset et al. 2005, Parker et al. 2005). Clearly, only the latter is relevant to the observed effects of CCK-8.
The observed stimulation of PYY by CCK-8 is consistent with previous observations (Lin et al. 2000). CCK-8, at the dose used, stimulated PYY more than what has been observed after a large meal (Degen et al. 2005). Nevertheless, the levels were comparable with the postprandial levels seen in our study after the other infusions. The rise in plasma PYY occurs within 15 min of a meal (Adrian et al. 1985), well before there can be direct contact of nutrients with the distal gut. Observations in dogs have confirmed that the release of PYY is not dependent on direct contact of fat with the distal gut (Lin et al. 2000, Lin and Chey 2003). A probable explanation for this stimulation of PYY is that CCK, also released within 15 min of nutrient ingestion (Lilja et al. 1984), serves as a foregut signal, linking the presence of nutrients in the proximal, with the subsequent release of PYY from the distal, small intestine. During infusion of GLP-1, PYY levels were less at only one time point ($t = 120$ min) when compared with control. While this finding does not allow us to conclude that GLP-1 suppresses PYY secretion – elevated PYY concentrations, for example induced by nutrients would probably be required in the first place – the stimulation of PYY by CCK-8 was abolished by concurrent administration of GLP-1. The mechanism(s) by which GLP-1 attenuates the effects of CCK-8 on PYY release are currently unclear, but studies utilizing specific receptor antagonists may help to elucidate this. Nevertheless, our observations are consistent with those of others (Naslund et al. 1999, Gutzwiller et al. 2004), suggesting that GLP-1 inhibits PYY release in humans. The reasons for the slight, but statistically significant, reduction of PYY during the control infusion is unclear, but is possibly attributable to fasting for a prolonged period.
Our observations provide insights into the potential mechanisms through which gut hormones are able to modulate energy intake as well as gastrointestinal function, including gastrointestinal motor activity. The capacity for CCK-8 to suppress ghrelin, and stimulate PYY, release is likely to represent a mechanism through which CCK plays an important role in the short-term regulation of feeding (Kissileff et al. 1981, MacIntosh et al. 2001). Furthermore, the changes in ghrelin and PYY may also have contributed to the effects of CCK-8 on the stimulation of tonic and phasic pyloric pressures, described in the original manuscript (Brennan et al. 2005), an effect not seen during infusion of GLP-1. Both the suppression of ghrelin and stimulation of PYY were progressive throughout the infusion, without evidence of a plateau. Hence, the immediate effect of CCK-8 to inhibit ghrelin secretion, evident within 10 minutes of commencing the infusion, may also be related to the effect of CCK-8 on PYY, also evident within 10 minutes. In a recent study from our group (Little et al. 2006), ghrelin was suppressed following a small intestinal glucose infusion when there was access to the entire small intestine, but not when glucose was infused into an isolated 60 cm-segment of the proximal small intestine, suggesting that exposure of the more distal part of the small intestine to nutrients is important for the suppression of ghrelin. In contrast, the stimulation of CCK by glucose was not length-dependent, supporting the concept that PYY plays a role in the post-prandial suppression of ghrelin.

It is important to recognize that we only evaluated the effects of one dose of CCK-8 (1.8 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min); these doses were based on previous studies suggesting that ‘physiological’ plasma concentrations would be
achieved (Schirra et al. 2000, MacIntosh et al. 2001), but, in fact, the plasma concentrations proved to be supraphysiological, albeit only moderately (Brennan et al. 2005). Hence, it is possible that the observed effects might only be evident when CCK-8 and/or GLP-1 are administered at supraphysiological doses. Future studies, potentially using specific CCK and GLP-1 antagonists, are required to clarify the physiological relevance of our observations.

5.6 CONCLUSIONS

In conclusion, this study has demonstrated that in healthy humans, at the doses evaluated, exogenous CCK-8 suppresses ghrelin and stimulates PYY, and exogenous GLP-1 has no effect on ghrelin and attenuates the effect of CCK-8 on PYY. These observations provide insights into the potential mechanisms through which gastrointestinal hormones interact to modulate gut function, appetite and energy intake.
CHAPTER 6

Dose-dependent effects of cholecystokinin-8 on antropyloroduodenal motility, gastrointestinal hormones, appetite and energy intake in healthy men

6.1 SUMMARY

CCK mediates the effects of nutrients on gastrointestinal motility and appetite. Intravenously administered CCK stimulates pyloric pressures, increases plasma PYY and suppresses ghrelin, all of which may be important in the regulation of appetite and energy intake. The dose-related effects of exogenous CCK on gastrointestinal motility and gut hormone release, and the relationships between these effects and those on energy intake, are uncertain. We hypothesised that: (i) intravenous CCK-8 would have dose-dependent effects on APD pressures, plasma PYY and ghrelin concentrations, appetite and energy intake; and (ii) the suppression of energy intake by CCK-8 would be related to the stimulation of pyloric motility. Ten healthy men (age 26 ± 2 yrs) were studied on four separate occasions in double-blind, randomised fashion. APD pressures, plasma PYY, and plasma ghrelin and appetite were measured during 120-min intravenous infusions of (i) saline (‘control’), or CCK-8 at (ii) 0.33 (‘CCK0.33’), (iii) 0.66 (‘CCK0.66’).
or (iv) 2.0 (‘CCK2.0’) ng/kg/min. After 90 min, energy intake at a buffet meal was quantified. CCK-8 dose-dependently stimulated phasic and tonic pyloric pressures, and plasma PYY concentrations ($r > 0.70$, $P < 0.05$), and reduced desire-to-eat and energy intake ($r > -0.60$, $P < 0.05$), without inducing nausea. There were relationships between basal pyloric pressure and IPPWs with plasma CCK ($r > 0.50$, $P < 0.01$), and between energy intake with IPPWs ($r= -0.70$, $P < 0.05$). Therefore, our study demonstrates that exogenous CCK-8 has dose-related effects on APD motility, plasma PYY, desire-to-eat and energy intake and suggests that the suppression of energy intake is related to the stimulation of IPPWs.

### 6.2 INTRODUCTION

The presence of nutrients in the small intestine is associated with the release of a number of gastrointestinal hormones, including CCK and PYY (Matzinger et al. 1999, Feinle et al. 2000, Feinle et al. 2003), and the suppression of ghrelin (Williams et al. 2003, Parker et al. 2005), all of which modulate appetite and energy intake (MacIntosh et al. 2001, Wren et al. 2001, Batterham et al. 2002) and gastrointestinal motility (Feinle et al. 1996, Schirra et al. 1998, MacIntosh et al. 2001). Of these hormones, CCK and its role in appetite regulation have been studied the most comprehensively.

CCK is released from endocrine I cells of the duodenal and jejunal mucosa, in the presence of fat, protein and carbohydrate (Liddle et al. 1985, Lieverse et al. 1994, Parker et al. 2005). CCK has a number of physiological functions, as established
by studies using specific CCK receptor antagonists (Fried et al. 1991, Matzinger et al. 1999), including stimulation of gallbladder contraction and pancreatic secretion, slowing of gastric emptying and suppression of food intake. For example, intravenous administration of the CCK1 receptor antagonist, loxiglumide, attenuates the increase in fullness, reduction in hunger and subsequent energy intake following intraduodenal lipid infusion (Feinle et al. 1996, Matzinger et al. 1999). CCK-58, CCK-33/-39 and CCK-8 are the main biologically active forms of CCK found in the human brain, intestine and circulation. While CCK-58 and CCK-33/-39 are the most abundant forms in humans (Eysselein et al. 1990), CCK-8 has frequently been used in research studies, and its intravenous administration mimics the effects of intraduodenal lipid on gastrointestinal motility, including suppression of antral and duodenal, and stimulation of tonic and phasic pyloric, pressures (Fraser et al. 1993, Rayner et al. 2000, Brennan et al. 2005), the slowing of gastric emptying (Fried et al. 1991), increase in plasma PYY (Lin et al. 2000), suppression of plasma ghrelin (Brennan et al. 2007), and the reduction of hunger and subsequent energy intake (Kissileff et al. 1981, McIntosh et al. 2001, Brennan et al. 2005).

There is some evidence that the modulation of gastrointestinal motor function may contribute to the short-term regulation of energy intake. For example, a recent study from our laboratory, in healthy lean males, demonstrated that the suppression of energy intake by a single dose of CCK-8 (2 ng/kg/min) was inversely related to the stimulation of IPPWs (Brennan et al. 2005). In contrast, intravenous infusion of GLP-1 at 0.9 pmol/kg/min did not stimulate IPPWs or
reduce energy intake (Brennan et al. 2005). While these observations support evidence in dogs that pyloric electrical stimulation reduces energy intake (Xu et al. 2005), the plasma CCK concentrations resulting from the infusion were moderately supraphysiological, and infusion of CCK-8 was associated with an increase, albeit modest, in nausea (Brennan et al. 2005). We have, accordingly, now evaluated the effects of increasing doses of CCK-8 on APD motility and gut hormone release, and the relationships between these effects with those on hunger and energy intake. The hypotheses were that (i) intravenous CCK-8 would have dose-dependent effects on these parameters and (ii) the suppressive effects of CCK-8 on energy intake would be related to the stimulation of pyloric motility, but independent of nausea.

6.3 SUBJECTS AND METHODS

6.3.1 Subjects

10 healthy males, aged 26 ± 2 years (range 21 – 36 years) and of normal body weight for their height (BMI 23 ± 0.5 kg/m², range 20 – 25 kg/m²) were recruited according to guidelines described in Chapter 4.2.

6.3.2 Study design

Each subject attended the laboratory on four occasions, each separated by 4 – 10 days, where they received, in randomised, double-blind fashion, intravenous infusions of CCK-8 (Merck Biosciences, Läufelfingen, Switzerland) at either (i) 0.33 ("CCK0.33"), (ii) 0.66 ("CCK0.66") or (iii) 2.0 ("CCK2.0") ng/kg/min, or
(iv) isotonic saline (“control”). CCK-8 was dissolved in 0.9 % sterile saline. In all studies APD motility, plasma CCK, PYY and ghrelin concentrations, appetite and energy intake were evaluated.

6.3.3 Protocol

Subjects attended the laboratory at 0830 h after fasting from solid and liquid food from 2200 h the previous night. Subjects were intubated, via an anaesthetized nostril, with a 17-channel manometric catheter, as described in Chapter 4.5. Intravenous cannulae were placed in each arm for the intravenous infusion and blood sampling, respectively.

Once the catheter was positioned correctly, a baseline (t = -15 min) blood sample was taken and a VAS, assessing perceptions of appetite (Parker et al. 2004) (Appendix II), administered. At t = 0 min, infusion of either (i) control, (ii) CCK0.33, (iii) CCK0.66 or (iv) CCK2.0 was commenced and continued for 120 min. During the infusion, blood samples were obtained and VAS completed at regular intervals, i.e. every 10 min between t = 0 – 30 min, every 15 min until t = 60 min, and every 30 min until t = 150 min. At t = 90 min, subjects were extubated and immediately offered a standardised, cold, buffet-style meal. Detailed information on the types of food, as well as the macronutrient composition and energy content, of the meal is provided in Appendix III. At t = 120 min the infusion was ceased. Subjects were then monitored for a further 30
min and, after removal of the intravenous cannulae, allowed to leave the laboratory.

6.3.4 Measurements

6.3.4.1 Antropyloroduodenal pressures
APD pressures were analyzed for (i) the number and amplitude of PWs in the antrum and duodenum, (ii) basal pyloric pressure (pyloric ‘tone’) and (iii) the number and amplitude of IPPWs, as described in Chapter 4.5.

6.3.4.2 Plasma hormone concentrations
Blood sample collection and analysis of plasma CCK, PYY and ghrelin were performed as described in Chapters 4.7.1.1, 4.7.1.3 and 4.7.1.2.

6.3.4.3 Appetite
Appetite ratings (desire-to-eat, fullness) were assessed using validated VAS (Parker et al. 2004) as described in Chapter 4.8.1. Nausea and bloating were also assessed.

6.3.4.4 Energy intake
Assessment of energy intake is described in Chapter 4.8.2.
6.3.5 Statistical analysis

Baseline values (‘0’) were calculated as the mean of values obtained at \( t = -15 \) and 0 min for VAS and plasma hormone concentrations, and between \( t = -15 \) to 0 min for the total number and mean amplitude of antral and duodenal PWs, IPPWs and basal pyloric pressures. The number and amplitude of antral and duodenal PWs were expressed as total and mean values, respectively, during the first 90 min of the infusion period. IPPWs and basal pyloric pressure were expressed as mean values of 15 min intervals between 0 - 90 min (i.e. 0 - 15, 15 - 30, … , 75 - 90 min). All data, with the exception of plasma hormone concentrations, were expressed as changes from baseline. VAS, plasma hormone concentrations, IPPWs and basal pyloric pressures were analyzed by repeated-measures ANOVA, with time and treatment as factors. Areas under the curves (AUCs) for basal pyloric pressure, number and amplitude of IPPWs and plasma hormone concentrations were determined using the trapezoidal rule. The number and amplitude of antral and duodenal PWs, and energy intake were analyzed by one-way ANOVA. Post-hoc paired comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed when ANOVAs revealed significant effects. Plasma hormone concentrations at \( t = 90, 120 \) and 150 min were compared using Student’s \( t \)-test. Correlations, corrected for repeated-measures, were determined for 1) the total number and mean amplitude of antral and duodenal PWs, AUCs (between \( t = 0 - 90 \) min) for basal pyloric pressures, number and amplitude of IPPWs and plasma hormone concentrations, and energy intake with the natural logarithm (ln)-transformed CCK-8 doses; and 2) energy intake with AUCs for APD pressures and plasma hormone concentrations using
the method described by Bland and Altman (Bland and Altman 1995). Only $r$ values $> 0.5$ were considered physiologically relevant. Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

6.4 RESULTS

All subjects completed the four randomised study days and tolerated the experimental conditions well.

6.4.1 Antypyloroduodenal pressures

6.4.1.1 Antral pressures

There was a significant effect of treatment on both the number and amplitude of antral PWs ($P < 0.01$ for both) (Table 6.1). The number of antral PWs was lower during both CCK0.66 and CCK2.0 when compared with control ($P < 0.01$ for both), with no difference between CCK0.33 and control, and lower during CCK2.0 when compared with CCK0.33 ($P < 0.05$), with no difference between CCK2.0 and CCK0.66. The amplitude of antral PWs was lower during all three treatments when compared with control ($P < 0.001$ for all), with no differences between treatments (Table 6.1).
6.4.1.2 Pyloric pressures

**Basal pressure (‘tone’)***

There was a treatment by time interaction for basal pyloric pressure (P < 0.01) (Figure 6.1A). Basal pyloric pressure rose within 15 min of the start of each of the CCK infusions (P < 0.05) and was higher during CCK0.33 between \( t = 15 \) – 90 min (P < 0.05), during CCK0.66 between \( t = 30 \) – 90 min (P < 0.05), and during CCK2.0 between \( t = 0 \) – 90 min (P < 0.01), when compared with control. Basal pyloric pressure was also higher during CCK2.0 between \( t = 60 \) – 90 min when compared with CCK0.33 (P < 0.05), and between \( t = 45 \) – 90 min when compared with CCK0.66 (P < 0.05), with no difference between CCK0.66 and CCK0.33.

**Phasic pressures***

There was a treatment by time interaction for the number of IPPWs (P < 0.001) (Figure 6.1B). The number of IPPWs reached a peak at 15 – 30 min during all CCK infusions, after which time the response declined (P < 0.01). The number of IPPWs was higher during CCK0.66 and CCK2.0 between \( t = 0 \) – 90 min (P < 0.01 for both), and during CCK0.33 between \( t = 0 \) – 45 min and \( t = 60 \) – 75 min (P < 0.05), when compared with control. The number of IPPWs was higher during CCK0.66 between \( t = 0 \) – 30 min and \( t = 60 \) – 75 min (P < 0.05), when compared with CCK0.33, and during CCK2.0 between \( t = 0 \) – 30 min and \( t = 45 \) – 90 min (P < 0.05), when compared with CCK0.33, and between \( t = 30 \) – 45 min (P < 0.001), when compared with CCK0.66.
There was an effect of treatment on the amplitude of IPPWs (P < 0.001), which was higher during all three treatments when compared with control (P < 0.001 for all), with no differences between treatments (mean values between $t = 0 – 90$ min: Control: $11 \pm 3$ mmHg, CCK0.33: $31 \pm 6$ mmHg, CCK0.66: $36 \pm 5$ mmHg, CCK2.0: $38 \pm 7$ mmHg).

### 6.4.1.3 Duodenal pressures

There was a significant effect of treatment on both the number and amplitude of duodenal PWs (P < 0.05 for both) (Table 6.1). The number of duodenal PWs was lower during both CCK0.66 and CCK2.0 when compared with control (P < 0.01 for both), with no difference between CCK0.33 and control, or between CCK0.33, CCK0.66 and CCK2. The amplitude of duodenal PWs was lower during CCK2.0 when compared with control (P < 0.01), with no difference between CCK0.33 or CCK0.66 and control, or between CCK0.33, CCK0.66 and CCK2 (Table 6.1).

### 6.4.2 Gastrointestinal hormone concentrations

#### 6.4.2.1 Plasma CCK

There was no difference in baseline CCK-8 concentrations between study days. *Effect of intravenous infusion:* There was a treatment by time interaction for plasma CCK concentrations (P < 0.001) (Figure 6.2A). Plasma CCK remained at baseline concentrations during the control infusion and was higher during all three treatments when compared with baseline (P < 0.001). Plasma CCK concentrations were higher during all three treatments between $t = 10 – 90$ min when compared
with control (P < 0.001), during CCK0.66 at $t = 90$ min when compared with CCK0.33 (P < 0.05), and during CCK2.0 between $t = 10 - 90$ min when compared with both CCK0.33 and CCK0.66 (P < 0.001 for both).

*Effect of meal:* Immediately after the buffet meal (i.e. $t = 120$ min), plasma CCK concentrations were higher following control (P < 0.001), and lower following CCK0.66 and CCK2.0 (P < 0.05 for both), when compared with pre-meal concentrations (i.e. $t = 90$ min). At $t = 150$ min, plasma CCK concentrations were higher following control and lower following CCK0.66 and CCK2.0 (P < 0.05 for all), when compared with concentrations at $t = 90$ min, while there was no difference between treatments.

### 6.4.2.2 Plasma PYY

There was no difference in baseline PYY concentrations between study days.

*Effect of intravenous infusion:* There was a treatment by time interaction for plasma PYY concentrations (P < 0.01) (Figure 6.2B). Plasma PYY remained at baseline concentrations during control and CCK0.33 and was higher during CCK0.66 and CCK2.0 when compared with baseline values (P < 0.01). Plasma PYY concentrations were higher during both CCK0.66 and CCK2.0, reaching significance during CCK0.66 at $t = 90$ min when compared with control (P < 0.001), and during CCK2.0 between $t = 30 - 90$ min when compared with control and CCK0.33 (P < 0.01 for both) and between $t = 10 - 30$ min when compared with CCK0.66 (P < 0.05).

*Effect of meal:* Immediately after the buffet meal (i.e. $t = 120$ min), and at $t = 150$ min, plasma PYY concentrations were higher following all infusions (P < 0.01),
when compared with pre-meal concentrations (i.e. \( t = 90 \) min), while there was no difference between treatments.

6.4.2.3 Plasma ghrelin

Plasma ghrelin was analysed in only six subjects. There was no difference in baseline ghrelin concentrations between study days.

*Effect of intravenous infusion:* There was a treatment by time interaction for plasma ghrelin concentrations (P < 0.01) (Figure 6.2C). Plasma ghrelin concentrations increased slightly during the control infusion, and were lower during all three treatments, when compared with baseline values (P < 0.01). Plasma ghrelin concentrations were lower during CCK0.33 between \( t = 10 – 90 \) min (P < 0.05), and during CCK0.66 and CCK2.0 between \( t = 30 – 90 \) min (P < 0.01), when compared with control, and during CCK2.0 between \( t = 30 – 90 \) min (P < 0.05) when compared with CCK0.66.

*Effect of meal:* Immediately after the buffet meal (i.e. \( t = 120 \) min), there was no difference between treatments in plasma ghrelin concentrations when compared with pre-meal concentrations (i.e. \( t = 90 \) min). At \( t = 150 \) min, plasma ghrelin was lower following control, CCK0.66 and CCK2.0 (P < 0.05 for all), but not CCK0.33, when compared with concentrations at \( t = 90 \) min, while there was no difference between treatments.
6.4.3 Appetite

There was a treatment by time interaction for desire-to-eat \( (P < 0.001) \) (Figure 6.3). Desire-to-eat continued to increase during control, and decreased during CCK2.0, over the entire infusion period when compared with baseline values \( (P < 0.01 \) for both), with no changes occurring during CCK0.33 and CCK0.66. Desire-to-eat was less during CCK0.33 between \( t = 20 – 30 \) min and \( t = 60 – 90 \) min \( (P < 0.01) \), during CCK0.66 between \( t = 20 – 90 \) min \( (P < 0.05) \), and during CCK2.0 between \( t = 10 – 90 \) min \( (P < 0.05) \), when compared with control. Desire-to-eat was less during CCK2.0 at \( t = 30 \) min and between \( t = 60 – 90 \) min when compared with both CCK0.33 and CCK0.66 \( (P < 0.05 \) for both).

There was a significant effect of treatment on fullness \( (P < 0.05) \) (data not shown). Fullness was higher during CCK0.33, CCK0.66 and CCK2.0 when compared with control \( (P < 0.05 \) for all), with no difference between treatments.

There was no effect of treatment on nausea, which increased less than 8 % from baseline. There was a significant effect of time, but not treatment, on bloating (time effect: \( P < 0.05 \) ) (data not shown). Bloating was \( \sim 10 \) % higher than baseline scores during CCK2.0 between \( t = 0 – 20 \) min and \( t = 45 – 90 \) min when compared with baseline \( (P < 0.05) \).

6.4.4 Energy intake

There was a significant effect of treatment on both the amount eaten (g) \( (P < 0.05) \) and energy intake (kJ) \( (P < 0.01) \) at the buffet meal (Table 6.2). The amount eaten
was less during CCK0.66 and CCK2.0 when compared with control and CCK0.33 (P < 0.05 for both), with no differences between CCK0.33 and control, or between CCK0.66 and CCK2.0. Energy intake was less during CCK2.0 when compared with control (P < 0.01), CCK0.33 (P < 0.01) and CCK0.66 (P < 0.05), with no significant differences between CCK0.33, CCK0.66 and control or between CCK0.33 and CCK0.66.

6.4.5 Relations between antropyloroduodenal motility, plasma hormones, appetite and energy intake

6.4.5.1 Relationships between antropyloroduodenal motility, plasma hormones, appetite and energy intake with the dose of CCK-8 administered

There were direct relationships between basal pyloric pressure (r = 0.80, P < 0.01), the number of IPPWs (r = 0.70, P < 0.01), plasma CCK (r = 0.80, P < 0.01) and plasma PYY (r = 0.70, P < 0.05), and inverse relationships between desire-to-eat (r = -0.60, P < 0.05) and the amount of food (r = -0.70, P < 0.05) and energy (r = -0.70, P < 0.01) consumed at the buffet meal, with the dose of CCK-8 administered.

6.4.5.2 Relationships between antropyloroduodenal motility with plasma hormones

There were direct relationships between both basal pyloric pressure (r = 0.50, P < 0.01) and the number of IPPWs (r = 0.60, P < 0.01) with plasma CCK, and between the number of IPPWs with plasma PYY (r = 0.60, P < 0.05).
6.4.5.3 Relationships between energy intake with antropyloroduodenal motility and plasma hormones

There was an inverse relationship between energy intake with the number of IPPWs \( r = -0.70, P < 0.05 \), but no other motility parameter, or gastrointestinal hormones.
**Table 6.1:** Number and amplitude of antral, pyloric and duodenal pressure waves during 90 min intravenous infusion of saline (control) or CCK-8 at 0.33, 0.66 or 2.0 ng/kg/min.

<table>
<thead>
<tr>
<th></th>
<th>Saline (control)</th>
<th>CCK-8 (ng/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>Antral pressure waves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>92 ± 18</td>
<td>75 ± 14</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>75 ± 13</td>
<td>28 ± 3*</td>
</tr>
<tr>
<td>Duodenal pressure waves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>589 ± 83</td>
<td>490 ± 76</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>29 ± 2</td>
<td>25 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 10). * vs control, P < 0.05, # vs CCK0.33, P < 0.05.
Table 6.2: Energy intake at the buffet meal following 90 min intravenous infusion of saline (control) or CCK-8 at 0.33, 0.66 or 2.0 ng/kg/min.

<table>
<thead>
<tr>
<th></th>
<th>Saline (control)</th>
<th>CCK-8 (ng/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.33</td>
<td>0.66</td>
</tr>
<tr>
<td>Amount eaten (g)</td>
<td>1347 ± 77</td>
<td>1217 ± 85</td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>5184 ± 533</td>
<td>5388 ± 490</td>
</tr>
<tr>
<td>Energy (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>31 ± 2</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>47 ± 4</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>Protein</td>
<td>22 ± 2</td>
<td>22 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 10). * vs control, P < 0.05.
Figure 6.1: Mean basal pyloric pressure (A) and total number (B) of isolated pyloric pressure waves (IPPWs), occurring during 15 min intervals, during intravenous infusion of (i) saline (control) or CCK-8 at (ii) 0.33 (‘CCK0.33’), (iii) 0.66 (‘CCK0.66’), or (iv) 2.0 (‘CCK2.0’) ng/kg/min. * CCK0.33, CCK0.66 or CCK2.0 vs control: P < 0.01; φ CCK0.66 vs CCK0.33 P < 0.05; # CCK2.0 vs CCK0.33: P < 0.05; § CCK2.0 vs CCK0.66: P < 0.001. Data are means ± SEM (n = 10).
Figure 6.2: Plasma CCK (n = 10) (A), PYY (n = 10) (B) and ghrelin (n = 6) (C) during intravenous infusion of saline (control) or CCK-8 at (ii) 0.33 (‘CCK0.33’), (iii) 0.66 (‘CCK0.66’) or (iv) 2.0 (‘CCK2.0’) ng/kg/min. * CCK0.33, CCK0.66 or CCK2.0 vs control: P < 0.001; \( \phi \) CCK0.66 vs CCK0.33 P < 0.05; # CCK2.0 vs CCK0.33: P < 0.001; \( \$ \) CCK2.0 vs CCK0.66: P < 0.001; \( \Delta \) vs t = 90 min: P < 0.05. Data are means ± SEM.
Figure 6.3: Scores for desire-to-eat during intravenous infusion of saline (control) or CCK-8 at (ii) 0.33 (‘CCK0.33’), (iii) 0.66 (‘CCK0.66’) or (iv) 2 (‘CCK2.0’) ng/kg/min. * CCK0.33, CCK0.66 or CCK2.0 vs control: P < 0.05; # CCK2.0 vs CCK0.33 and CCK0.66: P < 0.05. Data are means ± SEM (n = 10).
Our study is the first to comprehensively evaluate the dose-related effects of CCK-8 on APD motility, gastrointestinal hormone release, appetite and energy intake. The observations establish that exogenous administration of CCK-8, at the doses evaluated, has discrepant effects on APD motility, PYY and ghrelin, appetite and energy intake in healthy, male subjects, and that energy intake is inversely related to the stimulation of IPPWs.

Clarification of the dose-related effects of CCK-8 is relevant for a greater understanding of the mechanisms relating to the regulation of APD motility, PYY and ghrelin and appetite and energy intake. Healthy, young males were studied since they have been reported to have a greater capacity to adjust energy intake in response to dietary manipulation, when compared with elderly men, healthy females or obese individuals (Rolls et al. 1994, Shide et al. 1995), and the number of subjects included was based on power calculations derived from our previous studies (MacIntosh et al. 2001, Feinle et al. 2003, Brennan et al. 2005). The doses of CCK-8 were selected on the basis of previous studies, in which CCK-8 at a dose of 2 ng/kg/min resulted in moderately supraphysiological plasma concentrations and was shown to have submaximal effects on APD motility and energy intake (MacIntosh et al. 2001, Brennan et al. 2005). That the doses of CCK-8 used in this study were physiological is also supported by the postprandial concentrations observed.
Intravenous CCK-8 modulates gastroduodenal motility and slows gastric emptying (Muurahainen et al. 1988, Fraser et al. 1993). This study has demonstrated for the first time that CCK-8 at a dose as low as 0.33 ng/kg/min stimulates pyloric pressure waves, while higher doses of CCK-8, i.e. 0.66 and 2.0 ng/kg/min, were required for suppression of antral and duodenal PWs. These observations indicate that there are differences in the sensitivity of the APD region to CCK. Some studies conducted in animals support these findings (Isenberg and Csendes 1972, Grider and Makhlouf 1987). For example, regional heterogeneity of CCK receptors on smooth muscle and neurons in the gut has been observed in the guinea pig (Grider and Makhlouf 1987). In humans, CCK released from the small intestine acts directly at CCK1 receptors, which are expressed by vagal afferent neurons of the stomach and small intestine. Studies employing the specific CCK1 receptor antagonist, loxiglumide, have established that endogenous CCK has a physiological role in the regulation of gastrointestinal motility (Fried et al. 1991, Fried et al. 1991).

Following the ingestion of a meal, a number of peptides other than CCK are released, including PYY (Adrian et al. 1985) and GLP-1 (Herrmann et al. 1995), and ghrelin is suppressed (Williams et al. 2003). There is evidence of interactions between these hormones that may enhance their effects on gastrointestinal motor function and energy intake. For example, intravenous PYY suppresses ghrelin in humans (Batterham et al. 2003), and in-vitro studies in rodents suggest that GLP-1 also has this effect (Lippl et al. 2004). In dogs, the stimulation of PYY by the presence of fat in the proximal small intestine is mediated, at least in part, by CCK.
CCK-8 effects on gut motility, hormones and appetite Chapter 6

(Lin et al. 2000). Conversely, there is evidence in humans that GLP-1 may inhibit the release of PYY (Naslund et al. 1999). Our recent study, in healthy men, demonstrated that exogenous CCK-8, but not GLP-1, markedly stimulated the release of PYY and suppression of ghrelin (Chapter 5) (Brennan et al. 2007), supporting work of others (Lin et al. 2000, Degen et al. 2007). The current study confirms and extends these findings by demonstrating that the effect of exogenous CCK-8 on plasma PYY concentrations is dose-dependent. A previous study demonstrated that the fat-induced suppression of ghrelin release was reversed, and the fat-induced stimulation of PYY abolished, by the CCK1 receptor antagonist, deloxiglumide, suggesting that both effects are mediated via the CCK1 receptor (Degen et al. 2007). As expected, the buffet meal stimulated an increase in plasma PYY concentrations, and while mean plasma ghrelin concentrations were less following the meal, this reduction was not statistically significant, which may well reflect the smaller sample size (i.e. n=6). The observation that CCK affects other gastrointestinal hormones provides additional insight into potential mechanisms through which CCK modulates gut motility, appetite and energy intake.

It appears that the acute effects of endogenous CCK on energy intake are more modest and require significantly more subjects, in comparison with exogenous CCK, which more frequently result in ‘supraphysiological’ plasma concentrations. For example, 40 healthy subjects were included in a study that intravenously infused loxiglumide for 1 hr prior to, and during, ingestion of a mixed nutrient meal and demonstrated an ~10 % increase in energy intake when compared with the control infusion (Beglinger et al. 2001). In contrast, exogenous administration
of CCK-8 potently increases fullness and suppresses hunger and energy intake, even in studies with small subject numbers \((n = 8 – 12)\) (Kissileff et al. 1981, MacIntosh et al. 2001, Brennan et al. 2005). However, only few studies have demonstrated that CCK-8, when infused intravenously to produce ‘physiological’ plasma concentrations, i.e. concentrations comparable with those observed after a mixed-nutrient meal in humans, suppresses energy intake (Ballinger et al. 1995, Lieverse et al. 1995). In the current study, involving 10 healthy subjects, CCK2.0 markedly reduced energy intake by 20 % when compared with control, comparable in magnitude with that reported by Ballinger et al (Ballinger et al. 1995). Since exogenous administration of CCK can induce nausea, possibly as a result of elevated plasma CCK concentrations, the potent suppression of appetite and energy intake has been attributed to nausea. In our study intravenous CCK reduced appetite and energy intake in the absence of nausea. It should be recognised that the absence of a significant effect of the lower doses of CCK-8 on energy intake may reflect the timing of the meal. Since CCK is known to be released during meal ingestion, and changes in basal pyloric pressure, IPPWs and plasma hormones concentrations were all observed to peak within ~ 30 minutes after commencement of the CCK-8 infusions, individuals may have consumed less energy if the buffet meal had been consumed earlier e.g. at \(t = 30\) min rather that \(t = 90\) min.

In dogs, electrical stimulation of the pylorus, increasing both tonic and phasic pressures, has been reported to be associated with suppression of energy intake (Xu et al. 2005). Given the association between energy intake and pyloric motility
demonstrated in our previous work (Brennan et al. 2005), in the current study we anticipated that changes in energy intake would be related to a greater modulation of antropyloroduodenal motility, particularly pyloric pressures. That there was an inverse relationship between energy intake and the number of isolated pyloric pressure waves, i.e. the reduction in energy intake was associated with the stimulation of pyloric motility, is consistent with this concept. This suggests that an individual, in whom there is greater stimulation of IPPWs from a given stimulus, should eat less, potentially because small intestinal feedback is greater. Certainly, any effects of CCK on gastrointestinal motility that are relevant to its appetite-suppressant properties warrant further investigation.

6.6 CONCLUSIONS

In summary, this study has demonstrated that in healthy males, CCK-8 stimulates pressures in the pylorus, increases plasma PYY concentrations and suppresses desire-to-eat and energy intake in a dose-dependent manner, while all CCK-8 doses equally suppressed ghrelin. There were relationships between plasma CCK with basal pyloric pressure and IPPWs, and energy intake with IPPWs. Hence, these data provide evidence that enhanced stimulation of IPPWs is associated with suppression of energy intake. In view of this, the relationship between energy intake and pyloric motility, as well as the combination of CCK with other peptides to suppress appetite and potentially induce weight loss, warrants further investigation.
CHAPTER 7

Comparative effects of fat, protein and carbohydrate, and different protein loads, on appetite and subsequent energy intake in lean and obese subjects

7.1 SUMMARY

The relative effects of fat, protein and carbohydrate on appetite and energy intake, and ‘appetite-related’ hormones, remain controversial. While protein is often considered to be the most satiating macronutrient, many studies have employed test meals (or preloads) that contain very high and unsustainable amounts of protein, i.e. ~ 1.85 – 2.3 g/kg. The aims of this study were to evaluate the acute effects of (i) high-fat, high-protein and high-carbohydrate test meals, and (ii) increasing amounts of protein, on gastrointestinal hormones, appetite and subsequent energy intake in lean and obese subjects, and to (iii) compare these responses between lean and obese. Sixteen healthy (aged 29 ± 2 yr; BMI 24 ± 0.4 kg/m²), and fourteen obese (aged 38 ± 4 yr; BMI 33 ± 0.5 kg/m²), males were studied on four separate occasions where, following a standardised breakfast, they received for lunch, in randomised order, a i) high-fat (‘HF’), ii) high-protein (‘HP’), iii) high-carbohydrate (low-protein (‘LP’)) (‘HC’/‘LP’) or iv) adequate-
Effects of macronutrients on appetite and energy intake

Chapter 7

7.1 PROTEIN (‘AP’), TEST MEAL. Hunger and fullness were then measured for 180 min (following test meal ingestion) and energy intake at a buffet meal consumed between $t = 180 – 210$ min, was quantified. In lean subjects, hunger was less, and fullness greater, following ingestion of the HF and HP meals ($P < 0.05$), whereas there was no difference between the macronutrients in the obese. In lean subjects, energy intake was reduced following the HF and HP meals when compared with the HC meal ($P < 0.01$ for both), with no difference between the HF and HP meals. In obese subjects, the HP and AP meals reduced energy intake when compared with the HF and HC meals ($P < 0.05$ for both), and HC meal ($P < 0.01$), respectively. The percentage change in energy intake between the HF and AP test meals was significantly different between lean and obese (lean: $-12 \pm 6$; obese $4 \pm 4$ %; $P < 0.05$). In the lean, but not obese, there were relationships between energy intake with hunger ($r = 0.70$, $P < 0.001$) and fullness ($r = -0.50$, $P < 0.001$) at $t = 180$ min. In conclusion, our study demonstrates that HP test meals suppress energy intake in both lean and obese subjects, but obese appear less sensitive to the satiating effects of fat.

7.2 INTRODUCTION

Currently, more than 250 million people worldwide, representing ~ 7 % of the adult population, are obese (Bray 2003), a condition which results from excess energy intake relative to energy expenditure over a period of time (Catford and Caterson 2003). Diet is an important factor in both the management and prevention of obesity, however, what constitutes the optimal diet composition for
sustained weight loss remains unclear. In particular, the relative effects of fat, protein and carbohydrate, and of protein load, on appetite and energy intake remain controversial. The current average Western diet derives approximately 50 % of energy from carbohydrate, 35 % from fat, and 15 % from protein (Murtaugh et al. 2007) which differs considerably from the diet of our ancestors who derived 22 – 40 % of energy from carbohydrate, 28 – 47 % from fat and 19 – 35 % from protein. This suggests that a shift towards an increased carbohydrate, and reduced protein, diet, may contribute responsible, at least in part, to high levels of obesity in Western populations. Therefore, one dietary strategy for the management of obesity has been to replace some carbohydrate in the diet with fat or protein (Baba et al. 1999), however, the benefit of this approach remains uncertain.

Laboratory studies, utilizing pure macronutrient infusions in rats, have demonstrated that kJ for kJ the satiating hierarchy is protein > carbohydrate > fat (Geliebter 1979, Walls and Koopmans 1992). In humans, however, only a small number of studies have evaluated the effects of all three macronutrients (Rolls et al. 1988, Poppitt et al. 1998). Studies that have reported that high protein meals (or preloads) can suppress appetite and energy intake have frequently enriched them to contain ~ 60 % of energy as protein (~ 185 g/meal) (Rolls et al. 1988, Poppitt et al. 1998, Batterham et al. 2006). The use of such excessive amounts of protein makes it difficult, if not impossible, to covertly manipulate the test meals without introducing differences in the texture, taste, appearance and smell. For example, while in a group of twelve lean women, subjects were less hungry and had a lower energy intake following consumption of a high-protein, compared
with high-carbohydrate, -fat or alcohol, test meals (Poppitt et al. 1998), the high-protein test meal was rated as the least pleasant to consume when compared with the other meals. In addition, there are discrepancies in the ranking of macronutrients and not all studies that have compared the satiating efficiency of the three macronutrients have demonstrated that protein is more satiating than fat or carbohydrates (de-Graaf et al. 1992, Vozzo et al. 2003, Blom et al. 2006). For example, isocaloric yoghurt preloads (~ 3 MJ) enriched with either fat (40 % energy as fat), carbohydrate (60 % energy as carbohydrate) or protein (30 % energy as protein), and controlled for palatability, volume and energy density, exerted similar suppressive effects on hunger and subsequent energy intake (Vozzo et al. 2003).

The aims of this study were to evaluate concurrently the acute effects of (i) high-fat, high-protein and high-carbohydrate test meals, and (ii) increasing amounts of protein, on gastrointestinal hormone release, appetite and subsequent energy intake in lean and obese subjects, and to (iii) compare these responses in lean and obese.

### 7.3 SUBJECTS AND METHODS

#### 7.3.1 Subjects

16 healthy males, aged 29 ± 2 (range 20 – 47) years, and of normal body weight for their height (BMI 24 ± 0.4 (range 20 – 25) kg/m²), and 14 obese males, aged 38 ± 4 (range 18 – 55) years, (BMI 33 ± 0.6 (range 30 – 35) kg/m²) were recruited
according to guidelines described in Chapter 4.2. We calculated that assuming a within-subject standard deviation of 1700 kJ, and an average correlation between treatments of 0.9, an effect size in energy intake between treatments of 0.9 would be detectable with a sample size of at least 15 subjects at 80% power. Allowing for post-hoc pair-wise comparisons provides power of 68% for a difference of 786 kJ between any two specific treatments. To distract from the primary aims of the study, each subject was informed that the study was designed to evaluate the effects of the test meals on gastrointestinal hormone secretion.

### 7.3.2 Study design

Subjects attended the laboratory on four occasions, each separated by 3 – 7 days. At the same time (1150 h) on each of the study days four test meals were administered and were ingested within 20 minutes, in a single-blind, randomised fashion. The test meals were designed to compare the effects of high amounts of (i) fat (‘HF’)(% energy prescribed as fat/protein/carbohydrate 60/15/25), (ii) protein (‘HP’)(30/50/20) or (iii) carbohydrate (‘HC’)(also low-protein ‘LP’)(30/7.5/62.5) on gastrointestinal hormone release, appetite and energy intake. The fourth test meal, i.e. (iv) ‘adequate’ protein (‘AP’)(30/30/40), was given to evaluate the effect of increasing the protein load of the test meals.

### 7.3.2.1 Meal preload preparation

Test meals were prepared as described in Chapter 4.9.2.1. The total energy content of each preload represented ~ 30% of each subject’s estimated daily
energy requirement (using the Harris Benedict equation and a physical activity factor between 1.4 – 1.6 based on each subject’s daily activity) (Harris and Benedict 1918). Subjects were asked to maintain their normal physical activity over the course of the study. The actual macronutrient composition of each preload is detailed in Table 7.1.

7.3.3 Protocol

To standardise study conditions, subjects were provided with a ‘ready-to-eat’ dinner (Beef Lasagne, 2472 kJ, McCain Foods Pty Ltd, Victoria, Australia) to be consumed at 1900 h on the evening prior to each study day, after which time they were required to fast. Subjects were also provided with standardised breakfast on the morning of each study, consisting of a cup of white coffee or tea with a maximum of 1 teaspoon of sugar, a glass of orange juice and 2 slices of wholemeal toast with butter and jam, which they had 15 min to consume. The energy content of the breakfast comprised of ~ 10 % of each subject’s daily energy requirements (lean: 1205 ± 19; obese 1423 ± 26 kJ).

Each subject attended the laboratory in the Discipline of Medicine, Royal Adelaide Hospital, at 0830 h. Subjects were comfortably seated, and an intravenous cannula was placed in a forearm vein for blood sampling. At 0845 h subjects were presented with the standard breakfast.
At 1130 h ($t = -30$ min), a blood sample was taken and VAS, to assess appetite-related perceptions, nausea and bloating, administered. After a 10 min baseline period, i.e. at $t = -20$ min, subjects ingested, in randomised order either the i) high-fat (HF), ii) high-protein (HP), iii) high-carbohydrate (HC) (low-protein (LP)) or (iv) adequate-protein (AP), test meals. Immediately following ingestion of the test meal ($t = 0$ min), a 10 ml blood sample was taken and VAS scores were obtained. Subjects were also asked to rate the ‘pleasantness’ of each test meal. Subsequently, blood samples were taken, and VAS scores obtained, at 15 minute intervals until $t = 90$ min, and at 30 minute intervals until $t = 180$ min. At $t = 180$ min, subjects were presented with a cold, buffet-style meal. The types of food, as well as the macronutrient composition and energy content, of the meal are described in detail in Appendix III. At $t = 210$ min, a final blood sample was taken and VAS administered. The intravenous cannula was then removed, and the subject was allowed to leave the laboratory.

7.3.4 Measurements

7.3.4.1 Plasma hormone concentrations

Blood samples were collected for the measurement of plasma CCK, PYY and ghrelin, as described in Chapters 4.7.1.1, 4.7.1.3 and 4.7.1.2. The results of these analyses are not yet available but will available in the published manuscript.
7.3.4.2 Appetite

Appetite ratings (hunger, fullness) were assessed using validated VAS (Parker et al. 2004) as described in Chapter 4.8.1. Nausea and bloating were also assessed.

7.3.4.3 Energy intake

Assessment of energy intake is described in Chapter 4.8.2.

7.3.5 Statistical analysis

The statistical analyses were completed in two parts, according to the aims of the study. Hence, the effects of the three different macronutrients, i.e. HF, HP and HC meals, and the effects of varying the protein content, i.e. HP, AP and LP meals, were evaluated separately. All VAS data were expressed as changes from baseline. Repeated-measures analysis of variance (ANOVA) was used to evaluate VAS scores, with time and treatment as factors. One-way ANOVA was used to analyse energy intake (kJ), amount eaten (g), and macronutrient distribution (%). Post hoc-paired comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed when ANOVAs revealed significant effects. Relationships between energy intake with VAS scores at $t = 180$ min were calculated using the method described by Bland and Altman (Bland and Altman 1995). Only $r$ values $> 0.5$ were considered physiologically relevant. AUCs for VAS scores were calculated using the trapezoidal rule. Differences in the % change in AUC of VAS scores, and in energy intake, between lean and obese
subjects, were compared using independent sample unpaired t-tests. Statistical significance was accepted at P < 0.05. Data are presented as mean values ± standard error of the mean (SEM).

7.4 RESULTS

All subjects completed the four randomised study days and tolerated the experimental conditions well. Following consumption of the test meals, ‘pleasantness’ scores did not differ between test meals or between lean and obese subjects (Lean: HF: 76 ± 5, HP: 75 ± 4, HC: 80 ± 4, AP: 76 ± 6 mm; Obese: HF: 80 ± 3, HP: 74 ± 5, HC: 76 ± 6, AP: 77 ± 6 mm).

7.4.1 Appetite

7.4.1.1 Lean subjects

There was no difference in baseline hunger, fullness, bloating or nausea scores between study days. Hunger increased, and fullness decreased, progressively between t = 0 – 180 min on all study days (time effect: P < 0.01 for both).

Effect of meals high in fat, protein and carbohydrate

There was a significant treatment by time interaction for hunger (P < 0.05) (Figure 7.1A). Hunger was less following the HF meal between t = 150 – 180 min (P < 0.05), and following the HP meal between t = 0 – 30 min and t = 120 – 180 min (P < 0.05), when compared with the HC meal, with no difference between
the HF, and HP, meals. There was a significant effect of treatment on fullness (P < 0.01) (Figure 7.1B). Fullness was greater following the HF, and HP, meals when compared with the HC meal (P < 0.05 for both), with no difference between the HF, and HP, meals.

Effect of meals containing different protein loads

There was a significant treatment by time interaction for hunger (P < 0.01) (Figure 7.1A). Hunger was less following the HP meal between t = 0 – 30 min and t = 90 – 180 min (P < 0.05), and following the AP meal between t = 150 – 180 min (P < 0.05), when compared with the LP meal, with no difference between the HP, and AP, meals. There was a significant treatment by time interaction for fullness (P < 0.01) (Figure 7.1B). Fullness was greater following the HP meal between t = 120 – 180 min when compared with the AP, and LP, meals (P < 0.05 for both), with no difference between the AP, and LP, meals.

On all study days, hunger decreased, and fullness rose, in response to the buffet meal (time effect: P < 0.01 for both), with no difference in the magnitude of change between the macronutrients.

7.4.1.2 Obese subjects

There was no difference in baseline hunger, fullness, bloating or nausea scores between study days. Hunger increased (Figure 7.2A), and fullness decreased
(Figure 7.2B), progressively between $t = 0 – 180$ min on all study days (time effect: $P < 0.05$ for both).

**Effect of meals high in fat, protein and carbohydrate**

There was no effect of treatment on *hunger* or *fullness* scores.

**Effect of meals containing different protein loads**

There was no effect of treatment on *hunger* or *fullness* scores.

On all study days, *hunger* decreased, and *fullness* rose, in response to the buffet meal (time effect: $P < 0.01$ for both), with no difference in the magnitude of change between the macronutrients.

### 7.4.1.3 Comparison between lean and obese subjects

There was no difference in baseline *hunger* or *fullness* scores between lean and obese subjects. While there was a trend for the percentage change in AUC of *hunger* between the HP and HC/LP test meals to be different between $t = 0 – 180$ min ($P = 0.10$), there were no significant differences in *hunger* or *fullness* scores, between lean and obese subjects. The percentage change in AUC of *fullness* between the HF and HC/LP ($P < 0.01$), and HP and HC/LP ($P < 0.05$), test meals at $t = 180$ min, i.e. immediately prior to the buffet meal, was different between lean and obese subjects. There was no difference at $t = 210$ min, i.e. following the buffet meal, in *hunger* or *fullness* scores between lean and obese subjects.
7.4.2 Energy intake

7.4.2.1 Lean subjects

Effect of meals high in fat, protein and carbohydrate

There was a significant effect of treatment on energy intake (kJ) at the buffet meal (P < 0.01) (Table 7.2). Energy intake was less following both the HF, and HP, meals when compared with the HC meal (P < 0.01 for both), with no significant difference between the HF, and HP, meals. The amount (g) eaten at the buffet meal was not significantly different between any of the treatments. The percentage of energy consumed from fat was significantly greater following the HF meal when compared with the HP, and HC, meals (P < 0.05 for both); there was no difference in the percentage of energy from carbohydrate and protein consumed at the buffet meal, between treatments.

Effect of meals containing different protein loads

There was a significant effect on energy intake (kJ) at the buffet meal (P < 0.01) (Table 7.2). Energy intake was less following the HP meal when compared with the AP, and LP, meals (P < 0.01 for both), with no significant difference between the AP, and LP, meals. Neither the amount (g) eaten at the buffet meal, nor the macronutrient distribution, were significantly different between any of the treatments.
7.4.2.2 **Obese subjects**

*Effect of meals high in fat, protein and carbohydrate*

There was a significant effect of treatment on energy intake (kJ) at the buffet meal (P < 0.05) ([Table 7.2](#)). Energy intake was less following the HP meal when compared with the HF, and HC, meals (P < 0.05), with no difference between the HF, and HC, meals. There was a trend for the amount (g) eaten at the buffet meal to be different (P < 0.07), but no difference in the macronutrient distribution.

*Effect of meals containing different protein loads*

There was a significant effect of treatment on energy intake (kJ) at the buffet meal (P < 0.05) ([Table 7.2](#)). Energy intake was less following the HP, and AP, meals when compared with the LP meal (P < 0.01 for both) with no significant difference between the HP, and AP, meals. There was a trend for the amount (g) eaten at the buffet meal to be different (P < 0.07). The percentage of energy consumed from carbohydrates following the HP meal was significantly less when compared with the AP meal (P < 0.05); there was no difference in the percentage of energy from fat and protein consumed at the buffet meal, between treatments.

7.4.2.3 **Comparison of lean and obese subjects**

When the energy intake responses in lean and obese subjects were compared, the percentage change in energy intake between the HF and AP test meals was significantly different (lean: -12 ± 5; obese 4 ± 4 %; P < 0.05). While there was a trend for the percentage change in energy intake between the HC and AP test
meals to be different (P = 0.10), there were no other significant differences between lean and obese subjects.

### 7.4.3 Relationships between energy intake with appetite in lean and obese subjects

In lean subjects, there were direct relationships between energy intake with hunger at \( t = 180 \) min, i.e. immediately prior to the buffet meal, \( (r = 0.70, P < 0.001) \) (Figure 7.3A), and an inverse relationship between energy intake with fullness at \( t = 180 \) min \( (r = -0.50, P < 0.001) \) (Figure 7.3B). In contrast, there were no relationships between energy intake with either hunger (Figure 7.3C), or fullness (Figure 7.3D), in the obese.
Table 7.1: Macronutrient composition of the isocaloric high-fat (HF), high-protein (HP), high-carbohydrate/low-protein (HC/LP) and adequate-protein (AP) test meals consumed by lean and obese subjects.

<table>
<thead>
<tr>
<th></th>
<th>HF Lean</th>
<th>HF Obese</th>
<th>HP Lean</th>
<th>HP Obese</th>
<th>HC/LP Lean</th>
<th>HC/LP Obese</th>
<th>AP Lean</th>
<th>AP Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% E</td>
<td>16 ± 0</td>
<td>17 ± 0</td>
<td>54 ± 1</td>
<td>56 ± 1</td>
<td>8 ± 0</td>
<td>8 ± 0</td>
<td>33 ± 1</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>total g</td>
<td>36 ± 1</td>
<td>37 ± 1</td>
<td>119 ± 2</td>
<td>123 ± 2</td>
<td>18 ± 0</td>
<td>18 ± 0</td>
<td>71 ± 1</td>
<td>74 ± 1</td>
</tr>
<tr>
<td>g/kg</td>
<td>0.5 ± 0*#Δ 0.4 ± 0*#Δ</td>
<td>1.5 ± 0*#Δ</td>
<td>1.2 ± 0*#Δ</td>
<td>0.2 ± 0*Δ</td>
<td>0.2 ± 0*Δ</td>
<td>0.9 ± 0</td>
<td>0.7 ± 0</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% E</td>
<td>65 ± 1</td>
<td>67 ± 1</td>
<td>33 ± 1</td>
<td>34 ± 1</td>
<td>33 ± 1</td>
<td>34 ± 1</td>
<td>33 ± 1</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>total g</td>
<td>62 ± 1</td>
<td>64 ± 1</td>
<td>31 ± 1</td>
<td>32 ± 1</td>
<td>31 ± 1</td>
<td>32 ± 1</td>
<td>31 ± 1</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>Carb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% E</td>
<td>27 ± 0</td>
<td>28 ± 0</td>
<td>22 ± 0</td>
<td>22 ± 0</td>
<td>69 ± 1</td>
<td>71 ± 1</td>
<td>44 ± 1</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>total g</td>
<td>56 ± 1</td>
<td>58 ± 1</td>
<td>45 ± 1</td>
<td>46 ± 1</td>
<td>141 ± 2</td>
<td>146 ± 3</td>
<td>90 ± 2</td>
<td>92 ± 2</td>
</tr>
</tbody>
</table>

% E, % of energy; g/kg, g/kg of body weight. Data are means ± SEM (n = 16 lean and 14 obese). * vs HP, † vs LP, Δ vs AP, P < 0.001.
Table 7.2: Food intake at the buffet meal following ingestion of high-fat (HF), high-protein (HP), high-carbohydrate/low-protein (HC/LP) or adequate-protein (AP) test meals in lean and obese subjects.

<table>
<thead>
<tr>
<th></th>
<th>HF</th>
<th>HP</th>
<th>HC/LP</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lean subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>4021 ± 353*</td>
<td>3724 ± 394*#</td>
<td>4372 ± 333</td>
<td>4430 ± 396</td>
</tr>
<tr>
<td>Amount eaten (g)</td>
<td>943 ± 76</td>
<td>957 ± 87</td>
<td>1019 ± 74</td>
<td>1059 ± 65</td>
</tr>
<tr>
<td>Energy (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>34 ± 1*Δ</td>
<td>31 ± 1</td>
<td>31 ± 1</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>43 ± 2</td>
<td>48 ± 3</td>
<td>45 ± 2</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>Protein</td>
<td>23 ± 1</td>
<td>21 ± 1</td>
<td>24 ± 1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td><strong>Obese subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>5107 ± 372</td>
<td>4429 ± 389*#</td>
<td>5444 ± 369</td>
<td>4871 ± 369*</td>
</tr>
<tr>
<td>Amount eaten (g)</td>
<td>1183 ± 79</td>
<td>1053 ± 75</td>
<td>1250 ± 88</td>
<td>1207 ± 85</td>
</tr>
<tr>
<td>Energy (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>34 ± 1</td>
<td>35 ± 2</td>
<td>34 ± 1</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>42 ± 1</td>
<td>40 ± 2#</td>
<td>41 ± 2</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Protein</td>
<td>24 ± 1</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
<td>23 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 16 lean and 14 obese). * vs HC/LP, # vs AP, Δ vs HP and HC/LP † vs HF, P < 0.05.
**Figure 7.1:** Scores for *hunger* (A) and *fullness* (B) following ingestion of high-fat (HF), high-protein (HP), high-carbohydrate/low-protein (HC/LP) or adequate-protein (AP) test meals in lean subjects. Data are means ± SEM (n = 16). * vs HC/LP, † vs AP, P < 0.05.
Figure 7.2: Scores for hunger (A) and fullness (B) following ingestion of high-fat (HF), high-protein (HP), high-carbohydrate/low-protein (HC/LP) or adequate-protein (AP) test meals in obese subjects. Data are means ± SEM (n = 14).
Figure 7.3: Relationships between energy intake with hunger (A) and fullness (B) at $t = 180$ min following ingestion of high-fat (HF), high-protein (HP), high-carbohydrate/low-protein (HC/LP) or adequate-protein (AP) test meals in lean subjects ($n = 16$). There was no relationship between energy intake with hunger (C) or fullness (D) in obese subjects ($n = 14$).


7.5 DISCUSSION

This study has evaluated the effects of i) high-protein, high-fat and high-carbohydrate test meals, and ii) increasing amounts of protein in a test meal, on appetite and energy intake in lean and obese subjects, and (iii) compared these effects in lean and obese subjects. In both lean and obese subjects, hunger increased, and fullness decreased, progressively on all study days. In lean, but not obese, subjects, hunger was less, and fullness greater, following ingestion of the HF and HP meals, whereas there was no differences between macronutrients in the obese. In addition, energy intake was reduced in lean subjects following the HF and HP meals when compared with the HC meal, while in obese subjects, the HP and AP meals reduced energy intake when compared with the HF and HC meals, and HC meal, respectively. In both groups, energy intake was less following ingestion of the greatest protein load. The percentage change in energy intake between the HF and AP test meals was significantly different between lean and obese, so that obese subjects appeared to be less sensitive to the satiating effects of fat. The magnitude of the observed differences was substantial and these observations have implications for the dietary management of obesity.

Previous work in healthy lean subjects, including that by the author, has demonstrated an association between energy intake with changes in appetite scores (Chapters 6, 9 and 10) (Feltrin et al. 2004, Pilichiewicz et al. 2007). Consistent with this concept, in the current study there was a relationship between energy intake with hunger and fullness at \( t = 180 \) min, i.e. immediately prior to the buffet
meal. In contrast, despite reductions in energy intake, there were no reported differences between macronutrients in appetite scores in obese subjects. This observation supports previous findings that, compared with lean individuals, obese are more reactive to external cues, i.e. time, the presence and quality of food and situational events, and less sensitive to internal hunger and satiety signals (Blundell and Gillett 2001, Mela 2001), and this is likely to reflect one way in which the regulation of food intake is adjusted in weight-gaining, or obese, individuals.

To the author’s knowledge, this is the first study to assess the effect of macronutrients on appetite and energy intake responses, using isocaloric test meals designed to account for the individual daily energy requirements of the lean and obese subjects. Rather than potentially over-, or under-, feeding subjects, this approach ensures they are fed at a level appropriate for their metabolic requirements. In lean, but not obese, subjects on the HF meal reduced energy intake (by ~ 8 %) when compared with the HC meal, as previously reported (Batterham et al. 2006). Furthermore, the percentage change in energy intake between the HF and AP test meals was significantly different between lean and obese subjects, suggesting that obese subjects may be less sensitive to the satiating effects of fat. Epidemiologic studies have reported a direct relationship between the incidence of obesity and dietary fat consumption (Golay and Bobbioni 1997), and there is evidence that a high-fat diet modulates gastrointestinal function (Cunningham et al. 1991, Boyd et al. 2003), and increases appetite and energy intake (Lissner et al. 1987, French et al. 1995). Hence, reduced sensitivity to the
satiating effects of fat in the obese may reflect their habitual high-dietary fat intake.

That protein content of a meal is likely to be a key factor for satiety and appetite regulation (Schoeller and Buchholz 2005, Weigle et al. 2005). The current recommended dietary advice for adequate protein intake is 0.8 g/kg/day and this is defined as ‘the minimum daily needs for protein to maintain short-term nitrogen balance (i.e. intake = excretion) in healthy people with moderate physical activity’ (Layman 2009). In this study, the HP test meal resulted in a reduction in energy intake by ~17 % when compared with the HC/LP test meal, in both lean and obese subjects, and this is in line with previous studies (Rolls et al. 1988, Poppitt et al. 1998, Batterham et al. 2006). Furthermore, in obese subjects, the AP meal also markedly reduced energy intake, indicating that even a moderate load of protein, i.e. ~0.8 g/kg, was sufficient to suppress energy intake. Some studies that have evaluated the satiating potential of protein can be criticised for employing test meals (or preloads) that contain much higher quantities of protein than recommended dietary advice, i.e. ~1.85 – 2.3 g/kg from protein (Rolls et al. 1988, Poppitt et al. 1998, Batterham et al. 2006). In the context of the dietary management of obesity, there is no evidence that such levels of protein are safe for chronic consumption. In contrast, there is evidence for a lack of detrimental effects of protein intake up to 1.4 g/kg on renal or cardiovascular function, as well as bone metabolism, when consumed for up to 15 months (Brinkworth et al. 2004, Keogh et al. 2007). Studies to investigate the chronic effects of protein intake in the range of 0.8 – 1.6 g/kg on appetite and energy intake, as well as the
physiological functions that regulate appetite and energy intake, including gastrointestinal function, are now indicated.

The mechanisms underlying the observed effects of macronutrients on appetite and energy intake remain unclear. Differences between lean and obese individuals in the release and/or suppression of gastrointestinal hormones, including CCK, PYY and ghrelin, by macronutrients may be important, and the results from these analyses are awaited. Moreover, there is evidence of differences between lean and obese in nutrient-related feedback by specific macronutrients that influence gastric emptying (Segura Molina et al. 2006). These differences may relate to the observations of this study, but remain poorly characterised. It will be important to characterise the relationship between specific macronutrients and gastrointestinal hormone release with appetite and energy intake and also to evaluate potential differences between lean and obese individuals; the latter may have major implications for dietary strategies for obesity.

Some limitations of the study should be recognised. Firstly, data relating to the habitual dietary intake of subjects (using food frequency questionnaires) have not yet been analysed, and these will be an important consideration in the interpretation of the results. Given that the timing of subsequent food intake following previous nutrient ingestion may be an important determinant of the relative satiating effects of different macronutrients (Rolls et al. 1991, Blundell et al. 1993); further studies investigating this issue are now warranted. In addition, the buffet-style meal used is suitable for the evaluation of total energy intake but,
possibly, less effective in evaluating macronutrient distribution, or food choice (Chapter 8). Only male volunteers were studied since they have been reported to have a greater capacity to adjust their energy intake in response to dietary manipulation when compared with elderly men, healthy females and obese individuals (Rolls et al. 1994, Shide et al. 1995). Hence, the observations may not be applicable to females. Moreover, since all subjects were either lean or obese, it remains unknown if the observations apply to individuals who are overweight or morbidly obese. Finally, only the acute effects of macronutrients were evaluated. Whether the observed effects on appetite and energy intake are sustained following chronic dietary manipulation is unknown.

7.6 CONCLUSIONS

In summary, this study has demonstrated that HP test meals suppress energy intake in both lean and obese subjects, but obese subjects appear less sensitive to the satiating effects of fat. A successful weight loss diet should result in a sustained suppression of appetite and food intake. In the context of obesity, chronic studies are now required to investigate if the acute effects of a moderate protein intake, i.e. ~0.8 – 1.6 g/kg, are maintained over prolonged periods of time.
CHAPTER 8

Reproducibility of energy intake, gastric emptying, blood glucose, plasma insulin and cholecystokinin responses in healthy young males

8.1 SUMMARY

Gastric emptying, as well as intragastric meal distribution, and gastrointestinal hormones, including CCK, play an important role in appetite regulation. The evaluation of gastrointestinal factors regulating food intake is commonly performed in healthy, lean, young male participants. It has, however, been suggested that there is marked inter-individual variability in the effects of nutrient ‘preloads’ on energy intake in this group. Whether there is significant intra-individual variation in acute energy intake after a nutrient preload, and, if so, how this relates to day-to-day variations in gastric emptying and gastrointestinal hormone release, is unclear. We hypothesised that energy intake after a nutrient preload would be reproducible and associated with reproducible patterns of gastric emptying, intragastric distribution and gastrointestinal hormone release. Fifteen healthy men (age 25 ± 5 yr) consumed a glucose preload (50 g glucose in 300 ml
solution; 0.17 g/ml) on three occasions. Gastric emptying and intragastric meal distribution (using 3-D ultrasound), blood glucose, plasma insulin and CCK concentrations and appetite perceptions, were evaluated over 90 minutes, and energy intake from a cold, buffet-style, meal was then quantified. Energy intake was highly reproducible within individuals between visits (intra-class correlation coefficient, \( r_i = 0.9 \)). Gastric emptying, intragastric meal distribution, blood glucose, plasma insulin and CCK concentrations and appetite perceptions did not differ between visits (\( r_i > 0.7 \) for all). Therefore, our study demonstrated that in healthy males, energy intake is highly reproducible, at least in the short-term, and associated with reproducible patterns of gastric emptying, glycaemia, insulinaemia and CCK release.

8.2 INTRODUCTION

Studies evaluating appetite and energy intake in a laboratory setting have frequently utilised ‘preload’ paradigms (Cook et al. 1997, Cecil et al. 1998, Cecil et al. 1999, Feinle et al. 2003, O'Donovan et al. 2003, Sturm et al. 2003, Norton et al. 2006), in which subjects receive a standardised amount of a test meal that can vary in macronutrient composition, physical state, volume and energy density, either orally, or infused directly into the stomach or small intestine, and subsequent energy intake is assessed. Healthy, lean, young, male subjects are frequently used in such studies, as they have been reported to have a greater capacity to adjust energy intake in response to a caloric preload (i.e. by decreasing the amount of calories eaten at a subsequent meal), when compared with elderly
men, healthy females, or obese individuals (Rolls et al. 1994, Shide et al. 1995). Nevertheless, within this group, there appears to be substantial inter-individual variability in both the total amount of food consumed and the magnitude of the reduction in their energy intake in response to caloric manipulation (Feinle-Bisset and Horowitz 2006). For example, while a significant overall (mean) reduction in energy intake was evident in response to an intraduodenal lipid infusion, when compared with a control infusion of saline, the magnitude of the decrease in energy intake in individual subjects was highly variable, and some subjects failed to compensate for the energy infused (Feinle-Bisset and Horowitz 2006).

Energy intake in a laboratory setting is commonly assessed using a standardised buffet-style meal, containing a range of food items, varying in macronutrient composition, and provided in excess of what subjects would be expected to consume (Cook et al. 1997, Arvaniti et al. 2000, MacIntosh et al. 2001, Feltrin et al. 2004). The presentation of a meal in excess could potentially result in spontaneous over-consumption (Kral et al. 2004, Norton et al. 2006), thereby confounding the results of studies designed to detect subtle differences in energy intake in response to a treatment. To our knowledge, only two studies have hitherto addressed this issue (Arvaniti et al. 2000, Gregersen et al. 2008). Arvaniti et al. evaluated energy intake and macronutrient composition of food consumed from a standardised, buffet-style meal, on two separate occasions, and reported that energy intake did not vary between the two days (Arvaniti et al. 2000). Since such studies frequently consist of more than two study conditions (Castiglione et al. 1998, French et al. 2000, MacIntosh et al. 2001, Feltrin et al. 2004), we
considered it important to evaluate intra-individual reproducibility of energy intake on three, rather than two, occasions.

Gastrointestinal factors, including gastric distension (occurring as a result of slowing of gastric emptying) (Sepple and Read 1989), intragastric meal distribution (Jones et al. 1997) and the release of gastrointestinal hormones, including CCK (Kissileff et al. 1981), play an important role in the regulation of energy intake. It is currently unclear whether the consistency in energy intake that has been described previously (Arvaniti et al. 2000) is associated with reproducible patterns of gastric emptying and gastrointestinal hormone release. A second aim of our study was, therefore, to evaluate whether gastrointestinal changes in response to an orally ingested glucose ‘preload’ would also be reproducible when assessed repeatedly within an individual. We employed a glucose drink as the preload, as we have recently shown that its gastric emptying can be accurately assessed with a novel three-dimensional (3-D) ultrasound technique (Gentilcore et al. 2006). Finally, we reasoned that the determination of intra-individual variations in response to the same treatment would also allow us to calculate the minimum changes in energy intake and gastrointestinal function that would be required to detect statistically significant treatment effects, should a treatment be given, for which there is relatively little information; this formed the third aim of our study.
8.3 SUBJECTS AND METHODS

8.3.1 Subjects

15 healthy males, with a mean age of 25 ± 5 (range 18 - 30) years, and of normal body weight for their height (BMI 22.5 ± 3.0 (range 19 - 25) kg/m²), were recruited according to guidelines described in Chapter 4.2. The number of subjects included was based on power calculations derived from previous work (MacIntosh et al. 1999, Feinle et al. 2003, Gentilcore et al. 2006). To distract from the primary aim of the study, the subjects were informed that the study was designed to evaluate the effects of a glucose drink on gastric emptying and gut hormone secretion.

8.3.2 Study design

Each subject participated on three occasions, separated by 7 – 10 days. To further standardise study conditions, subjects were provided with a ‘ready-to-eat’ dinner (Beef Lasagne, 2472 kJ, McCain Foods Pty Ltd, Victoria, Australia) to be consumed at 2000h on the evening prior to each study day, after which time they were required to fast. On each of the three occasions, subjects consumed a ‘preload’ consisting of 50 g glucose dissolved in 300 ml solution (200 kcal, 0.17 g/ml). On all study days gastric emptying and intragastric meal distribution, blood glucose and plasma CCK and insulin concentrations, appetite and energy intake were evaluated.
8.3.3 Protocol

On the study days, subjects attended the laboratory in the Discipline of Medicine at either 0800h or 1100h, i.e. two subjects could potentially be studied on one day; each subject attended at the same time of day on each visit. Upon arrival, an intravenous cannula was placed into a forearm vein for blood sampling, and the subject was seated comfortably in an upright position for the duration of the study. At $t = -15$ min, an image of the fasted stomach was acquired using 3-D ultrasound, a baseline blood sample collected and a VAS, assessing perceptions of appetite, completed. At $t = -2$ min, subjects were instructed to consume the glucose preload within 2 minutes. At $t = 0$ min, immediately following ingestion of the preload, another 3-D image of the stomach was acquired, a blood sample collected and a VAS completed. Subsequently, 3-D ultrasound scans, blood samples and VAS scores were obtained at 15 minute intervals until $t = 90$ min. At $t = 90$ min, subjects were immediately offered a standardised, cold, buffet-style meal. The types of food, as well as the macronutrient composition and energy content, of the meal are described in detail in Appendix III. A final blood sample was collected and VAS completed following the meal ($t = 120$ min), after which the intravenous cannula was removed, and the subject was free to leave the laboratory.

8.3.4 Measurements

8.3.4.1 Gastric emptying and intragastric meal distribution

Gastric emptying and intragastric meal distribution was assessed using 3-D ultrasonography as described in Chapter 4.6.
8.3.4.2 Blood glucose, plasma insulin and plasma CCK concentrations

Blood sample collection and analysis of plasma insulin and CCK were performed as described in Chapters 4.7.1.4 and 4.7.1.1.

8.3.4.3 Appetite perceptions and energy intake

Appetite perceptions (hunger, desire-to-eat) were rated on VAS, as described in Chapter 4.8.1 (Parker et al. 2004). Nausea and bloating were also assessed.

8.3.4.4 Energy intake

Assessment of energy intake is described in Chapter 4.8.2.

8.3.5 Statistical analysis

AUCs for gastric emptying, blood glucose, plasma insulin and CCK concentrations were calculated using the trapezoidal rule. Peak concentrations for blood glucose, plasma insulin and CCK were defined as the greatest mean change from baseline in each subject for each visit. Intra-subject reproducibility (i.e. the agreement within each individual’s data) between the 3 visits for $T_{50}$, AUCs, peak concentrations and times to peak concentrations for blood glucose, plasma insulin and CCK, energy intake (kJ), weight of food consumed (g), macronutrient distribution and weight of individual food items consumed (g) from the buffet meal, was evaluated by determining intra-class correlation coefficients, $r_i$ (Shrout and Fleiss 1979). An $r_i \geq 0.8$ was considered to indicate excellent agreement, 0.8
> r_i ≥ 0.7 as indicating good agreement, and 0.7 > r_i ≥ 0.6 as indicating moderate agreement (Choi et al. 2000). For variables measured over time (% retention for total gastric volume, intragastric meal distribution, plasma hormone concentrations and VAS scores), repeated-measures analysis of variance (ANOVA) was used to evaluate any differences between visits, and over time. Relationships between gastric emptying, T_{50}, peak concentrations, and concentrations at t = 90 min of blood glucose, plasma insulin and CCK with energy intake, and between gastric emptying and T_{50} with blood glucose and plasma insulin concentrations, were calculated using partial correlations (Bland and Altman 1995). Statistical significance was accepted at P < 0.05. Data are presented as raw values and expressed as means ± SEM. Based on the day-to-day variations observed in our sample, we calculated the minimum effect sizes required to detect a hypothetical treatment effect (with 80 % power) for our measured parameters (i.e. gastric emptying, blood glucose, plasma insulin and CCK concentrations and mean energy intake). Since this was a repeated-measures study design, correlation between these measures can be assumed, and for each of our measured parameters, the intraclass correlation coefficient was estimated. This value was then used in the SAS macro fpower (SAS 9.1, Cary, NC, USA) to calculate the minimum difference between the data points of interest that would be detected by a sample of n = 15.
8.4 RESULTS

The study protocol was well tolerated, and all subjects completed all visits. Mean data for baseline values and in response to treatment are summarised in Table 8.1 and Table 8.2, respectively. There were no differences in baseline values for any of the parameters, including desire-to-eat, between the 0800h or 1100h visits, nor did the timing of the commencement of the studies (i.e. 0800h or 1100h) affect any of the outcome measures (data not shown).

8.4.1 Gastric emptying

8.4.1.1 Total gastric emptying

Gastric emptying occurred in an overall linear pattern (Figure 8.1A), resulting in an average emptying rate of 2.1 ± 0.4 kcal/min for visit 1, 2.8 ± 0.1 kcal/min for visit 2 and 1.7 ± 0.1 kcal/min for visit 3 (\(r_i = 0.90\)). There was no difference in gastric emptying profiles between visits. Less than 30 % (~ 15 g glucose) of the drink remained in the stomach at the end of the study on all visits. There was significant intra-individual variation between visits in the T50 (\(r_i = 0.12\)) and the AUC of gastric emptying (\(r_i = 0.23\)).

8.4.1.2 Intragastric meal distribution

There was no difference in the amount of glucose retained in the proximal or distal stomach between visits (Figure 8.1B). The amount of glucose in the proximal stomach decreased progressively over time (time effect: P < 0.001), reflecting total
gastric emptying, while the amount in the distal stomach remained relatively constant.

8.4.2 Blood glucose and plasma hormone concentrations

8.4.2.1 Blood glucose

There was no difference in overall blood glucose concentrations between visits (Figure 8.2A). Blood glucose rose within 15 minutes of glucose ingestion (time effect $P < 0.01$) and, from $t = 60$ min, decreased. There was good intra-individual agreement for AUCs under the blood glucose profiles ($r_i = 0.72$) and for peak blood glucose concentrations ($r_i = 0.72$). In contrast, the time taken for blood glucose concentrations to peak varied between visits ($r_i = 0.33$). Blood glucose concentrations returned to baseline values following the buffet meal, with no differences in values between visits.

8.4.2.2 Plasma insulin

There was no difference in overall plasma insulin concentrations between visits (Figure 8.2B). Plasma insulin rose within 15 min of glucose ingestion (time effect $P < 0.01$) and decreased from $t = 60$ min. There was excellent intra-individual agreement for AUCs under plasma insulin profiles ($r_i = 0.88$) and for peak insulin concentrations ($r_i = 0.81$). In contrast, the time taken for plasma insulin concentrations to peak varied between visits ($r_i = 0.41$). Plasma insulin rose again
in response to the buffet meal (time effect $P < 0.01$), with no difference in the magnitude of the increase between visits.

### 8.4.2.3 Plasma CCK

There was no difference in overall plasma CCK concentrations between visits (Figure 8.2C). Plasma CCK rose within 15 min of glucose ingestion (time effect: $P < 0.01$) and subsequently reached a plateau. There was excellent intra-individual agreement for AUCs under plasma CCK profiles ($r_i = 0.84$) and for the time-to-peak of plasma CCK concentrations ($r_i = 0.89$), and good agreement for peak plasma CCK concentrations ($r_i = 0.70$) between visits. Plasma CCK rose again in response to the meal (time effect: $P < 0.01$), with no difference in the magnitude of the increase between visits.

### 8.4.3 Appetite and energy intake

#### 8.4.3.1 Appetite

There was no effect of treatment on scores for fullness or desire-to-eat, between visits. Fullness increased (time effect: $P < 0.05$) in response to glucose ingestion until $t = 15$ min, after which time scores gradually returned to baseline. Desire-to-eat remained unchanged from baseline (Figure 8.3). Fullness rose, and desire-to-eat decreased, in response to the buffet meal (time effect: $P < 0.001$), with no differences between visits. No subject experienced nausea or bloating (data not shown).
8.4.3.2 Energy intake

Both energy intake ($r_1 = 0.89$) and the amount of food consumed ($r_1 = 0.80$) showed excellent agreement between visits. Agreement between visits for macronutrient composition was good for protein ($r_1 = 0.77$), moderate for fat ($r_1 = 0.68$) and low for carbohydrate ($r_1 = 0.54$).

For ~50% of the foods contained in the buffet meal there was either an excellent (for margarine, mayonnaise, custard, chicken and banana) or good (for tomato, ham, cucumber and iced coffee) agreement, and for approximately 16% of the foods (cheese, fruit salad and fruit yoghurt) there was a moderate agreement, between visits. Agreement between visits was low for five buffet meal items (wholemeal and white breads, orange juice, lettuce and water) (Table 8.3).

8.4.4 Relations between gastric emptying, blood glucose, hormones and energy intake

8.4.4.1 Relationships between gastric emptying, blood glucose, and plasma insulin and CCK with energy intake

There were no significant relationships between energy intake with the amount of glucose remaining in the stomach at $t = 90$ min, i.e. immediately prior to the buffet meal, $T_{50}$, peak concentrations of blood glucose, plasma insulin and CCK blood glucose, plasma insulin or CCK concentrations at $t = 90$ min.
8.4.4.2 Relationships between blood glucose and plasma insulin concentrations with gastric emptying

There were inverse relationships between blood glucose concentrations at $t = 30$ min ($r = -0.33$, $P < 0.05$) and $t = 45$ min ($r = -0.29$, $P = 0.05$) with the amount of glucose remaining in the stomach at these times, and direct relationships between blood glucose concentrations between $t = 15 – 90$ min ($r > 0.38$, $P < 0.01$) with $T_{50}$, and the change in blood glucose from baseline at $t = 30$ min ($r = 0.32$, $P < 0.05$) and at $t = 45$ min ($r = 0.32$, $P < 0.05$) with the rate of gastric emptying (kcal/min).

There were inverse relationships between plasma insulin concentrations at $t = 30$ min ($r = -0.35$, $P < 0.05$) and $t = 45$ min ($r = -0.38$, $P < 0.01$) with the amount of glucose drink retained in the stomach at these times, and direct relationships between the change in plasma insulin from baseline at $t = 30$ min ($r = 0.31$, $P < 0.05$), $t = 45$ min ($r = 0.30$, $P < 0.05$) and $t = 90$ min ($r = 0.38$, $P = 0.01$) with the rate of gastric emptying (kcal/min).

There was a direct relationship between blood glucose and plasma insulin concentrations at $t = 15$ min ($r = 0.53$, $P = 0.001$).
8.4.5 Calculation of minimum effect sizes for the parameters assessed, based on observed intra-individual variations

Based on the day-to-day variations observed in our sample, in order to detect a treatment effect, we calculated that minimum mean effect sizes for gastric emptying AUC would have to be $\geq 866 \text{ min.}\%$, for gastric emptying $T_{50} \geq 13.2$ min, for blood glucose AUC $\geq 130 \text{ min.mmol/L}$, time to peak blood glucose $\geq 6.8$ min and peak blood glucose concentration $\geq 1.97 \text{ mmol/L}$, plasma insulin AUC $\geq 2230 \text{ min.mU/L}$, time to peak plasma insulin $\geq 20.3$ min and peak plasma insulin concentration $\geq 33.5 \text{ mU/L}$, plasma CCK AUC $\geq 162 \text{ min.pmol/L}$, time to peak plasma CCK $\geq 11.9$ min and peak plasma CCK concentration $\geq 2.20 \text{ pmol/L}$, and energy intake $\geq 916 \text{ kJ}$. 
Table 8.1: Baseline values for blood glucose and plasma insulin and CCK concentrations and appetite and symptom ratings.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit 1</th>
<th></th>
<th>Visit 2</th>
<th></th>
<th>Visit 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>5.4</td>
<td>0.2</td>
<td>5.0</td>
<td>0.2</td>
<td>5.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Plasma insulin (mU/L)</td>
<td>10.6</td>
<td>0.1</td>
<td>11.1</td>
<td>2.3</td>
<td>8.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Plasma CCK (pmol/L)</td>
<td>4.0</td>
<td>0.3</td>
<td>4.0</td>
<td>0.3</td>
<td>4.0</td>
<td>0.4</td>
</tr>
<tr>
<td>VAS ratings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desire-to-eat (mm)</td>
<td>54</td>
<td>8</td>
<td>62</td>
<td>8</td>
<td>63</td>
<td>8</td>
</tr>
<tr>
<td>Fullness (mm)</td>
<td>10</td>
<td>4</td>
<td>9</td>
<td>3</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Nausea (mm)</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Bloating (mm)</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

CCK, cholecystokinin; VAS, visual analogue scale.
Table 8.2: Mean values for gastric half-emptying time, blood glucose, plasma insulin and CCK concentrations and food intake.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit 1</th>
<th></th>
<th>Visit 2</th>
<th></th>
<th>Visit 3</th>
<th></th>
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<td>SEM</td>
<td>Mean</td>
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</tr>
<tr>
<td>Gastric emptying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half emptying time (T&lt;sub&gt;50&lt;/sub&gt;) (min)</td>
<td>58.6</td>
<td>4.7</td>
<td>61.0</td>
<td>3.0</td>
<td>64.5</td>
<td>3.9</td>
</tr>
<tr>
<td>AUC (min.% retention)</td>
<td>5228</td>
<td>271</td>
<td>5508</td>
<td>242</td>
<td>5574</td>
<td>263</td>
</tr>
<tr>
<td>Blood glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak concentration (mmol/L)</td>
<td>9.8</td>
<td>0.7</td>
<td>9.7</td>
<td>0.6</td>
<td>9.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Time to peak concentration (min)</td>
<td>35</td>
<td>3</td>
<td>36</td>
<td>2</td>
<td>37</td>
<td>2</td>
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<tr>
<td>AUC (min.mmol/L)</td>
<td>697</td>
<td>49</td>
<td>698</td>
<td>40</td>
<td>689</td>
<td>34</td>
</tr>
<tr>
<td>Plasma insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak concentration (mU/L)</td>
<td>69.0</td>
<td>10.0</td>
<td>77.0</td>
<td>13.0</td>
<td>63.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Time to peak concentration (min)</td>
<td>47</td>
<td>4</td>
<td>49</td>
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<td>6</td>
</tr>
<tr>
<td>AUC (min.mU/L)</td>
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<td>580</td>
<td>4896</td>
<td>915</td>
<td>3874</td>
<td>563</td>
</tr>
<tr>
<td>Plasma CCK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak concentration (pmol/L)</td>
<td>6.8</td>
<td>0.4</td>
<td>7.6</td>
<td>0.5</td>
<td>7.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Time to peak concentration (min)</td>
<td>23</td>
<td>4</td>
<td>22</td>
<td>4</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>AUC (min.pmol/L)</td>
<td>563</td>
<td>36</td>
<td>599</td>
<td>38</td>
<td>572</td>
<td>41</td>
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<tr>
<td>Food intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>3811</td>
<td>289</td>
<td>3632</td>
<td>338</td>
<td>3919</td>
<td>296</td>
</tr>
<tr>
<td>Weight of food (g)</td>
<td>915</td>
<td>70</td>
<td>896</td>
<td>92</td>
<td>937</td>
<td>97</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>21.0</td>
<td>1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>29</td>
<td>2</td>
<td>30</td>
<td>2</td>
<td>30.0</td>
<td>1</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>51</td>
<td>2</td>
<td>50</td>
<td>3</td>
<td>49.0</td>
<td>1</td>
</tr>
</tbody>
</table>

CCK, cholecystokinin; AUC, area under the curve.
### Table 8.3: Day-to-day reproducibility of food items consumed at the buffet meal.

<table>
<thead>
<tr>
<th>Food item</th>
<th>( r_i )</th>
<th>Food item</th>
<th>( r_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Margarine</td>
<td>0.93</td>
<td>10 Cheese</td>
<td>0.66</td>
</tr>
<tr>
<td>2 Mayonnaise</td>
<td>0.88</td>
<td>11 Fruit salad</td>
<td>0.66</td>
</tr>
<tr>
<td>3 Custard</td>
<td>0.86</td>
<td>12 Fruit yoghurt</td>
<td>0.66</td>
</tr>
<tr>
<td>4 Chicken</td>
<td>0.84</td>
<td>13 Wholemeal bread</td>
<td>0.56</td>
</tr>
<tr>
<td>5 Banana</td>
<td>0.82</td>
<td>14 Orange juice</td>
<td>0.50</td>
</tr>
<tr>
<td>6 Tomato</td>
<td>0.77</td>
<td>15 White bread</td>
<td>0.41</td>
</tr>
<tr>
<td>7 Ham</td>
<td>0.74</td>
<td>16 Lettuce</td>
<td>0.36</td>
</tr>
<tr>
<td>8 Cucumber</td>
<td>0.72</td>
<td>17 Water</td>
<td>0.30</td>
</tr>
<tr>
<td>9 Iced coffee</td>
<td>0.71</td>
<td>18 Apple</td>
<td>not consumed</td>
</tr>
</tbody>
</table>

\( r_i \), intraclass correlation coefficient, defined as follows: \( r_i \geq 0.8 \) excellent agreement, \( 0.8 > r_i \geq 0.7 \) good agreement, and \( 0.7 > r_i \geq 0.6 \) moderate agreement between visits.
Figure 8.1: Total (A) and proximal and distal (B) gastric emptying (% retention) of a ‘preload’ containing 50 g glucose in a 300 ml solution on 3 different days. Data are mean values ± SEM (n = 15).
Figure 8.2: Blood glucose (A), plasma insulin (B) and plasma CCK (C), concentrations following ingestion of a ‘preload’ containing 50 g glucose in 300 ml solution on 3 different days. Data are mean values ± SEM (n = 15).
Figure 8.3: Scores for fullness (A) and desire-to-eat (B) following ingestion of a ‘preload’ containing 50 g glucose in 300 ml solution on 3 different days. Data are mean values ± SEM (n = 15).
8.5 DISCUSSION

Our observations indicate that, in a laboratory setting; (i) appetite perceptions and energy intake in response to a nutrient preload in healthy lean men are highly reproducible, and (ii) this consistency in energy intake is associated with reproducible patterns of gastric emptying and insulin and CCK secretion. In addition, to our knowledge, our study is the first to provide information about minimum effect sizes for our measured parameters required to detect a hypothetical treatment effect based on our data on intra-individual variations in response to the same treatment.

In the present study, both energy intake (kJ) and the amount of food consumed (g) from a test meal in response to a glucose preload showed very good reproducibility between the 3 visits, supporting the hypothesis that, at least in a laboratory setting, acute energy intake does not markedly change on a day-to-day basis in healthy, lean men. This observation is in agreement with previous studies that demonstrated good reproducibility of energy intake using a standardised buffet meal (Arvaniti et al. 2000, Gregersen et al. 2008). It had been suggested that subjects tend to over-consume energy when allowed *ad libitum* access to a variety of sandwiches (Norton et al. 2006), and that this could potentially relate to the sense of novelty that is associated with a selection of appetising foods, as in the case of a buffet-style meal. Conversely, subjects may experience a sense of boredom when presented with the same meal over a number of occasions, resulting in a reduction in energy intake at later visits. Hence, a limitation of the
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study by Arvaniti et al., (Arvaniti et al. 2000) is that energy intake was only assessed on two occasions. In the current study we were able to demonstrate that energy intake is reproducible when assessed on three occasions, thus, neither the variety of food presented with the buffet meal, nor providing the subjects with the same foods on multiple occasions appeared to influence energy intake. The excellent agreement in energy intakes between days in both the current and previous (Arvaniti et al. 2000, Gregersen et al. 2008) studies, therefore, indicates that the use of a buffet-style meal produces a reliable measure of energy intake. Taken together, the data suggest that the magnitude of the effects on energy intake observed previously in response to either an oral nutrient preload or duodenal nutrient infusion (Feinle et al. 2003, Sturm et al. 2004) are significant, and that the number of subjects required to observe these effects is sufficient.

While energy intake and the amount of food consumed were both highly reproducible between study days, the macronutrient composition of the food consumed, although still showing moderate to good agreement, varied more between the three visits. The greater variation in the macronutrient composition, despite very little variation in energy intakes between visits, may reflect the selection of foods available, i.e. individuals tended to select different items with varying macronutrient contents from the meal on each occasion to achieve their overall energy intake. Hence, our data suggest that buffet-style meals, as used in our study, are highly suitable for the evaluation of total energy intake, but less effective in evaluating macronutrient distribution, or food choice. How isocaloric,
buffet style meals varying in their food composition may affect energy intake and macronutrient composition has not been evaluated, but warrants investigation.

The consistency in energy intake was associated with reproducible patterns of gastric emptying, intragastric meal distribution, glycaemia, insulinaemia and CCK secretion, suggesting that these factors may have contributed to reproducible energy intakes in response to the nutrient preload. In contrast, the statistical analysis indicated a lack of agreement between visits for the time taken for 50% of the meal to empty from the stomach. Gastric half-emptying time ($T_{50}$) has been used widely as a measure of gastric emptying, particularly in scintigraphic studies (Sidery et al. 1994, Hveem et al. 1996, Jones et al. 1998, Naslund et al. 2000), however, it is not as well established for 3-D ultrasound studies. For example, while agreement has been demonstrated between scintigraphy and 3-D ultrasound, the limits of agreement for the $T_{50}$ of 300 ml 25% dextrose solution, as measured by 3-D ultrasound for gastric emptying profiles, were -35.3 min to +47.6 min, which, while statistically not different from the data obtained scintigraphically, were highly variable (Gentilcore et al. 2006). It is important to recognise that 3-D ultrasonography is associated with some limitations. The presence of intragastric air, particularly in the fundus (Gilja et al. 1997), has the potential to compromise visualisation of the gastric outline, and this may have contributed to the lack of agreement in $T_{50}$ between visits.

In this study, there were direct relationships between the blood glucose and plasma insulin responses with the rate of gastric emptying, consistent with previous
observations (Horowitz et al. 1993, Rayner et al. 2001). It is well established that CCK mediates, at least in part, the effects of nutrients, particularly fat, on gastrointestinal motility and energy intake (Katschinski et al. 1996, Matzinger et al. 1999). Thus, while our finding that both gastric emptying and energy intake in response to the glucose preload were unrelated to plasma CCK concentrations may appear surprising, it is likely to reflect relatively modest stimulation of CCK by glucose. Dietary lipid and protein are known to be much more potent stimuli for CCK release than glucose (Liddle et al. 1985).

Our study has calculated effect sizes based on intra-individual variations in response to a standardised treatment on repeated occasions. Our data, from a relatively small sample of n = 15 subjects, suggest that quite large effect sizes in response to a treatment are required to detect a significant treatment effect, indicating that, while there was very good statistical agreement for most parameters between study days, variability is still substantial. Thus, in circumstances where small differences between treatments need to be detected, the sample size may need to be quite large, particularly in studies evaluating energy intake.

Some limitations of the present study warrant discussion. Firstly, we only evaluated healthy, lean males, hence, our observations may not be applicable to females and other subject groups, i.e. under- or over-weight and obese. While the number of subjects included was based on power calculations derived from our previous studies, it is nonetheless possible that small differences in some of the
outcome measures may not have been detected. Finally, potential changes in individual food preferences were not evaluated over the three visits, although it is clear that, in general, subjects selected the majority of food items included in the buffet meal.

8.6 CONCLUSIONS

In conclusion, we have demonstrated that, when measured repeatedly in a laboratory setting, energy intake in response to a nutrient preload is reproducible in healthy lean males, and this consistency is associated with reproducible patterns of gastric emptying and gastrointestinal hormone secretion.

Acknowledgement: IM Brennan and NS Nair conducted the study described in this chapter with equal contribution, hence, it was also submitted as part of an Honours degree by NS Nair, University of Adelaide, 2006.
CHAPTER 9

Effects of the phases of the menstrual cycle on gastric emptying, glycaemia, plasma GLP-1 and insulin, and energy intake in healthy lean women

9.1 SUMMARY

There is evidence that the menstrual cycle affects appetite, such that energy intake is lower during the follicular, compared with the luteal, phase. Gastric emptying influences energy intake, glycaemia and plasma GLP-1, insulin and CCK release. We hypothesised that (i) gastric emptying of a glucose drink is slower, and glycaemia, plasma hormones, hunger and energy intake are less, during the follicular, compared with the luteal, phase, (ii) the reduction in the latter parameters during the follicular phase are related to slower gastric emptying, and (iii) these parameters are reproducible when assessed twice within a particular phase of the menstrual cycle. Nine healthy, lean females were studied on 3 separate occasions; twice during the follicular (days 6 - 12), and once during the luteal (days 18 - 24), phase. Following consumption of a 300 ml glucose drink (0.17 g/ml), gastric emptying, blood glucose, plasma hormone concentrations and
hunger were measured for 90 min, after which energy intake at a buffet meal was quantified. During the follicular phase, gastric emptying was slower ($P < 0.05$), and blood glucose ($P < 0.01$), plasma GLP-1 and insulin ($P < 0.05$), hunger ($P < 0.01$) and energy intake ($P < 0.05$) were lower compared with the luteal phase, with no differences for CCK or between the two follicular phase visits. There were inverse relationships between energy intake, blood glucose and plasma GLP-1 and insulin concentrations with the amount of glucose drink remaining in the stomach at $t = 90$ min ($r < -0.6$, $P < 0.05$). In conclusion, in healthy women (i) gastric emptying of glucose is slower, and glycaemia, plasma GLP-1 and insulin, hunger and energy intake are less, during the follicular, when compared with the luteal, phase, (ii) energy intake, glycaemia and plasma GLP-1 and insulin are related to gastric emptying, and (iii) these parameters are reproducible when assessed twice during the follicular phase.

9.2 INTRODUCTION

Following meal ingestion, the presence of nutrients in the small intestine results in slowing of gastric emptying (Sepple and Read 1989), which increases gastric distension, and modulates the release of a number of gastrointestinal hormones, including CCK and GLP-1 (Feinle et al. 2003). These effects are important in the acute regulation of energy intake (Jones et al. 1997, MacIntosh et al. 2001). Studies evaluating appetite and energy intake, accordingly, frequently utilize a ‘preload’ paradigm (Cecil et al. 1999, Feinle et al. 2003, Sturm et al. 2004), whereby subjects are presented with a standardised test meal of variable
macronutrient composition, volume and/or energy density, and subsequent energy intake is assessed. Healthy, lean, young, male subjects are traditionally used in such studies, as they have been reported to have a greater capacity to adjust energy intake in response to a nutrient preload when compared with healthy females, elderly males and obese individuals (Rolls et al. 1994, Shide et al. 1995), although even within this group there is substantial inter-individual variability (Feinle-Bisset and Horowitz 2006). However, our recent study in which healthy males consumed a glucose preload on three separate days, acute energy intake was shown to be highly reproducible and associated with reproducible patterns of gastric emptying (Nair et al. 2008). It is unknown whether this applies to females.

One of the major reasons that females are used less frequently in research studies assessing gastrointestinal function, appetite and energy intake is the perceived confounding effect of the menstrual cycle on these parameters. There is evidence from studies in both animals (Czaja and Goy 1975, Kemnitz et al. 1984) and humans (Lissner et al. 1988, Johnson et al. 1994, Martini et al. 1994) that fluctuations in hormone levels over the menstrual cycle affect energy intake, such that energy intake is less during the follicular phase, when oestrogen is relatively high, and increased during the luteal phase, when progesterone is at its peak. There is also evidence from studies in rats that oestrogen increases the sensitivity to the inhibitory effect of CCK on energy intake (Geary 2001), consistent with the concept that energy intake is reduced when plasma estrogen is higher. It is not known whether the effects of the menstrual cycle on energy intake in humans are related to changes in the release of CCK.
There is persuasive evidence that both the rate of gastric emptying (Sepple and Read 1989) and intragastric meal distribution (Jones et al. 1997) affect energy intake. Accordingly, differences in energy intake across the menstrual cycle may potentially be related to changes in gastric emptying. However, previous studies that have assessed the effect of the menstrual cycle on gastric emptying have yielded conflicting information (Horowitz et al. 1985, Gill et al. 1987, Mones et al. 1993, Degen and Phillips 1996, Caballero-Plasencia et al. 1999). The rate of gastric emptying is recognised as a major determinant of postprandial blood glucose homeostasis, so that when gastric emptying is relatively slower, the initial (Jones et al. 1996), and potentially overall (Russo et al. 2003), glycaemic response to a carbohydrate-containing meal is reduced. In addition, enteral (as opposed to intravenous) glucose stimulates the release of the incretin hormone, GLP-1, the release of which is dependent on the rate of gastric emptying into the small intestine (O'Donovan et al. 2004, Pilichiewicz et al. 2007), and the so-called ‘incretin effect’ accounts for approximately 50 % of the rise in plasma insulin following oral glucose. Hence, if there are changes in gastric emptying during the menstrual cycle, these have the potential to affect glycaemia, with implications for the diagnosis of diabetes using oral glucose tolerance testing (Jarrett and Graver 1968, Fioretti et al. 1975).

Our study was designed to evaluate the hypotheses that in healthy women (i) gastric emptying of a glucose ‘preload’ would be slower, and blood glucose, plasma GLP-1, insulin and CCK responses, hunger and energy intake less, during the follicular, when compared with the luteal, phase, (ii) the reduction in hunger
and energy intake during the follicular phase would be related to slower gastric emptying and (iii) gastric emptying, blood glucose and plasma hormone concentrations, hunger and energy intake would be reproducible when assessed twice within a particular phase of the menstrual cycle i.e. during the follicular phase.

9.3 SUBJECTS AND METHODS

9.3.1 Subjects

9 healthy females, aged 31 ± 1 (range 26 – 38) years, of normal body weight for their height (BMI 21 ± 0.5 (range 19 – 24) kg/m²) were recruited according to guidelines described in Chapter 4.2. The number of subjects included was based on power calculations derived from previous work addressing within-subject variability of energy intake (Nair et al. 2008). Assuming a within-subject standard deviation of 553 kJ, a mean difference in energy intake between phases of 700 kJ is detectable with a sample size of 9 subjects at 80 % power and a Bonferroni adjusted significance level of 5 %, consistent with the reported differences in energy intake between the follicular and luteal phases (Martini et al. 1994, Li et al. 1999). To distract from the primary aim of assessing energy intake, subjects were informed that the study would evaluate the effects of a glucose drink on gastric emptying and blood glucose concentrations. The investigator who performed the studies and analysed the data (Brennan) was blinded to the phase of the menstrual cycle on each study day; this information was obtained, and study visits were
coordinated, by investigators (Feltrin, Little), who were not involved in primary data analysis.

9.3.2 Study design

Each subject participated on 3 occasions, twice during the follicular (between days 6 – 12; “FOL-P1” and “FOL-P2”), and once during the luteal (between days 18 – 24; “LUT-P”), phase of the menstrual cycle (where day 1 is the first day of menstrual bleeding). To standardize study conditions, subjects were provided with a ‘ready-to-eat’ dinner (Beef Lasagne (2472 kJ), McCain Foods Pty Ltd, Wendouree, Victoria, Australia) to be consumed at 1900 h on the evening prior to each study day, after which time they were required to fast. On each occasion, subjects consumed a ‘preload’ consisting of a 300 ml glucose solution containing 50 g glucose (200 kcal, 0.17 g/ml). To exclude potential order effects, the study was conducted in randomised fashion, with visits scheduled across consecutive menstrual cycles. On all study days gastric emptying and intragastric meal distribution, blood glucose, serum estradiol (E2) and progesterone (P4), and plasma CCK and insulin concentrations, appetite and energy intake were evaluated.

9.3.3 Protocol

On each study day, subjects attended the laboratory in the Discipline of Medicine, Royal Adelaide Hospital at either ~ 0800 or 1100 h i.e. two subjects could potentially be studied on one day; each subject attended at the same time of day on
each visit. Upon arrival an intravenous cannula was inserted into an antecubital vein in one arm for blood sampling and kept patent using 0.9 % saline. Subjects were seated comfortably in an upright position for the duration of the study. At $t = -15$ min, an image of the fasted stomach was acquired using 3-D ultrasound, a baseline blood sample collected and a VAS, assessing perceptions of appetite (hunger and fullness) and gastrointestinal symptoms (nausea and bloating), completed. At $t = -2$ min, subjects consumed the glucose preload within 2 minutes. The glucose preload employed in this study was chosen as we have demonstrated that gastric emptying of this drink can be assessed accurately using 3-D ultrasonography (Gentilcore et al. 2006). At $t = 0$ min, immediately following ingestion of the preload, another 3-D image of the stomach was acquired, a blood sample collected and a VAS completed. Subsequently, 3-D ultrasound scans, blood samples and VAS were obtained at 15 minute intervals until $t = 90$ min. At $t = 90$ min, subjects were immediately offered a standardised, cold, buffet-style meal. The types of food, as well as the macronutrient composition and energy content, of the meal are described in detail in Appendix III. A final blood sample was collected and VAS completed after the meal ($t = 120$ min), after which the intravenous cannula was removed, and the subject was allowed to leave the laboratory.
9.3.4 Measurements

9.3.4.1 Gastric emptying and intragastric meal distribution
Gastric emptying and intragastric meal distribution was assessed 3-D ultrasonography as described in Chapter 4.6.

9.3.4.2 Blood glucose, plasma GLP-1, insulin and CCK, and serum estradiol and progesterone concentrations
Blood sample collection and analysis of plasma GLP-1, insulin and CCK and serum estradiol and progesterone were performed as described in Chapters 4.7.1 and 4.7.2.

9.3.4.3 Appetite
Appetite perceptions (hunger, fullness) were rated on VAS, as described in Chapter 4.8.1 (Parker et al. 2004). Nausea and bloating were also assessed.

9.3.4.4 Energy intake
Assessment of energy intake is described in Chapter 4.8.2.

9.3.5 Statistical analysis
AUCs for gastric emptying, proximal and distal gastric volumes, blood glucose and plasma hormone concentrations were calculated using the trapezoidal rule.
Repeated-measures analysis of variance (ANOVA) was used to evaluate variables measured over time (% retention for total, proximal and distal gastric volumes, blood glucose and plasma hormones and VAS scores), with time and visit as factors. One-way ANOVA was used to analyse energy intake. Post-hoc paired comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed when ANOVAs revealed significant effects. Blood glucose and plasma hormone concentrations at $t = 90$ and 120 min were compared using Student’s paired $t$-test. Relationships between gastric emptying with blood glucose and plasma hormone concentrations, and between energy intake with gastric emptying, $T_{50}$ and scores for hunger, were calculated using the method described by Bland and Altman (Bland and Altman 1995). Only $r$ values $> 0.5$ were considered physiologically relevant. Intra-subject reproducibility (i.e. the agreement within each individual’s data) between FOL-P1 and FOL-P2 for $T_{50}$, energy intake, and AUCs for gastric emptying, proximal and distal gastric volumes, blood glucose and plasma hormone concentrations, were evaluated by determining intra-class correlation coefficients, $r_i$. An $r_i \geq 0.8$ was considered to indicate ‘excellent’ agreement, $0.8 > r_i \geq 0.7$ to indicate ‘good’ agreement, and $0.7 > r_i \geq 0.5$ to indicate ‘moderate’ agreement (Nair et al. 2008). Intra-subject variability in energy intake within, and between, phases was assessed by calculating the coefficients of variation ($CV = \text{standard deviation} / \text{mean} \times 100\%$). Statistical significance was accepted at $P < 0.05$. Data are presented as mean values $\pm$ standard error of the mean (SEM).
9.4 RESULTS

The study protocol was well tolerated, and all subjects completed all visits. Subjects reported regular menstrual cycles ranging 25 – 30 days in length. While there were no differences in serum estradiol concentrations (mean concentrations (pmol/L); FOL-P1: 499 ± 85, FOL-P2: 466 ± 72, LUT-P: 544 ± 73; mean differences (pmol/L); FOL-P1 vs LUT-P: 45 ± 133; FOL-P2 vs LUT-P: 78 ± 124; FOL-P1 vs FOL-P2: 33 ± 59), there were significant differences in progesterone concentrations between study days (mean concentrations (nmol/L); FOL-P1: 2 ± 0, FOL-P2: 2 ± 1, LUT-P: 48 ± 4; P < 0.05; mean differences (nmol/L); FOL-P1 vs LUT-P: 46 ± 4; FOL-P2 vs LUT-P: 46 ± 4; FOL-P1 vs FOL-P2: -1 ± 1). There were no differences in baseline values between the 0800 h and 1100 h visits, nor did the timing of the commencement of the studies affect any of the outcome measures (data not shown). No subject reported pre-menstrual gastrointestinal symptoms.

9.4.1 Gastric emptying

9.4.1.1 Total gastric emptying

The profile of gastric emptying approximated an overall linear pattern on all study days (Figure 9.1A), so that the volume of glucose retained in the stomach decreased progressively over time (time effect: $F_{(6, 48)} = 136.10$, $P < 0.001$). There was a significant effect of the phase of the menstrual cycle on gastric emptying ($F_{(2, 16)} = 3.89$, $P < 0.05$) (Table 9.1). Gastric emptying was slower during FOL-
P1 and FOL-P2 when compared with LUT-P (P < 0.05), with no difference between FOL-P1 and FOL-P2 (mean AUC differences (%·min); FOL-P1 vs LUT-P: 762 ± 313; FOL-P2 vs LUT-P: 718 ± 250; FOL-P1 vs FOL-P2: 45 ± 261).

There was also a significant effect of the phase of the menstrual cycle on the 50% gastric emptying time, T50 (F(2, 16) = 5.20, P < 0.05), so that the T50 was greater during FOL-P1 (76 ± 4 min) and FOL-P2 (75 ± 5 min) when compared with LUT-P (61 ± 6 min) (P < 0.05), with no difference between FOL-P1 and FOL-P2 (mean differences (min); FOL-P1 vs LUT-P: 17 ± 5; FOL-P2 vs LUT-P: 18 ± 4; FOL-P1 vs FOL-P2: -1 ± -2).

### 9.4.1.2 Intragastric meal distribution

There was no significant effect of the phase of the menstrual cycle on the volume of glucose retained in the proximal or distal stomach (Figure 9.1B), although the mean values for volumes in the proximal stomach were greater during FOL-P1 and FOL-P2 when compared with LUT-P. The volume of glucose retained in the proximal (time effect: F(6, 46) = 82.52, P < 0.05) and distal stomach (time effect: F(6, 46) = 5.60, P < 0.05) decreased progressively over time, reflecting total gastric emptying.

### 9.4.2 Blood glucose and plasma hormone concentrations

Examples of differences in the magnitude of responses in blood glucose and plasma GLP-1 and insulin concentrations from 3 subjects, i.e. individuals with
smaller, moderate or larger responses, following consumption of the glucose solution, are provided in Figure 9.3. There were uniformly no differences in plasma CCK concentrations in response to the glucose solution (data not shown).

9.4.2.1 Blood glucose

There was no difference in baseline blood glucose concentrations between visits. There was a treatment by time interaction ($F_{(12, 96)} = 1.95, P < 0.05$) (Figure 9.2A) so that after the glucose load, blood glucose concentrations were less during FOL-P1 and FOL-P2 when compared with LUT-P between $t = 30 – 90$ min ($P < 0.01$), with no difference between FOL-P1 and FOL-P2. There was a substantial difference in the maximum peak blood glucose concentrations during FOL-P1 and FOL-P2 (~7.6 mmol/L for both) when compared with LUT-P (~9.4 mmol/L). The AUC between $t = 0 – 90$ min (Table 9.1) was less during FOL-P1 and FOL-P2 when compared with LUT-P ($P < 0.05$), with no difference between FOL-P1 and FOL-P2 (mean AUC differences (mmol/L.min); FOL-P1 vs LUT-P: 92 ± 50; FOL-P2 vs LUT-P: 109 ± 56; FOL-P1 vs FOL-P2: 17 ± 38). Following the buffet meal ($t = 120$ min), blood glucose concentrations returned to baseline values during FOL-P1 and FOL-P2, but not LUT-P.

9.4.2.2 Plasma GLP-1

There was no difference in baseline plasma GLP-1 concentrations between visits. There was a treatment by time interaction ($F_{(12, 96)} = 1.95, P < 0.05$) (Figure 9.2B)
so that after the glucose load plasma GLP-1 concentrations were less during FOL-P1 and FOL-P2 when compared with LUT-P between $t = 15 – 75$ min ($P < 0.01$), with no difference between FOL-P1 and FOL-P2. The AUC between $t = 0 – 90$ min (Table 9.1) was less during FOL-P1 and FOL-P2 when compared with LUT-P ($P < 0.01$), with no difference between FOL-P1 and FOL-P2 (mean AUC differences (pmol/L.min); FOL-P1 vs LUT-P: $340 \pm 140$; FOL-P2 vs LUT-P: $297 \pm 109$; FOL-P1 vs FOL-P2: $-43 \pm 84$). Plasma GLP-1 concentrations increased following the buffet meal (time effect: $P < 0.01$), with no difference between visits.

### 9.4.2.3 Plasma insulin

There was no difference in baseline plasma insulin concentrations between visits. There was a treatment by time interaction ($F_{(12, 96)} = 1.83$, $P < 0.05$) (Figure 9.2C) so that after the glucose load plasma insulin concentrations were less during FOL-P1 and FOL-P2 when compared with LUT-P between $t = 15 – 90$ min ($P < 0.05$), with no difference between FOL-P1 and FOL-P2. The AUC between $t = 0 – 90$ min (Table 9.1) was less during FOL-P1 and FOL-P2 when compared with LUT-P ($P < 0.05$), with no difference between FOL-P1 and FOL-P2 (mean AUC differences (mU/L.min); FOL-P1 vs LUT-P: $1073 \pm 608$; FOL-P2 vs LUT-P: $1184 \pm 456$; FOL-P1 vs FOL-P2: $111 \pm 334$). Plasma insulin concentrations increased following the buffet meal (time effect: $P < 0.01$), with no difference between visits.
9.4.2.4 Plasma CCK

There was no difference in baseline CCK concentrations between visits, and no effect of the phase of the menstrual cycle on overall plasma CCK concentrations, or the AUC between $t = 0 - 90$ min (Table 9.1) (mean AUC differences (pmol/L.min); FOL-P1 vs LUT-P: 11 ± 11; FOL-P2 vs LUT-P: 7 ± 13; FOL-P1 vs FOL-P2: 5 ± 11) (Figure 9.2D). Plasma CCK rose slightly within 15 minutes of glucose ingestion (time effect: $P < 0.01$) and subsequently reached a plateau. Plasma CCK increased further following the buffet meal (time effect: $P < 0.01$), with no difference between visits.

9.4.3 Appetite and energy intake

9.4.3.1 Appetite

There was a trend for baseline hunger scores to be less during FOL-P1 and FOL-P2 when compared with LUT-P ($P = 0.07$). There was a treatment by time interaction ($F_{(12, 96)} = 3.10, P < 0.01$) (Figure 9.4). Hunger was less during FOL-P1 and FOL-P2 over the entire study period when compared with LUT-P ($P < 0.01$), with no difference between FOL-P1 and FOL-P2 (Table 9.1) (mean AUC differences (mm.min); FOL-P1 vs LUT-P: 1133 ± 420; FOL-P2 vs LUT-P: 981 ± 360; FOL-P1 vs FOL-P2: 485 ± 358).
There was no difference in baseline scores, and no effect of the menstrual cycle, on scores for fullness, nausea or bloating, which all increased less than 10% from baseline (data not shown).

### 9.4.3.2 Energy intake

There was a significant effect of the menstrual cycle on both the amount eaten (g) ($F(2, 16) = 5.35, P < 0.05$), and energy consumed (kJ) ($F(2, 16) = 8.17, P < 0.05$), at the buffet meal ([Table 9.2](#)). Both were less during FOL-P1 and FOL-P2 when compared with LUT-P ($P < 0.05$ for both), with no difference between FOL-P1 and FOL-P2 P2 (mean differences in amount eaten (g); FOL-P1 vs LUT-P: 70 ± 63; FOL-P2 vs LUT-P: 72 ± 89; FOL-P1 vs FOL-P2: 2 ± 84; mean differences in energy intake (kJ); FOL-P1 vs LUT-P: 588 ± 245; FOL-P2 vs LUT-P: 766 ± 230; FOL-P1 vs FOL-P2: 178 ± 130). Differences ranged from -174 – 854 kJ for FOL-P1 – FOL-P2, from 674 – 1966 kJ for LUT – FOL-P1, and from 18 – 1937 kJ for LUT-P – FOL-P2 ([Figure 9.5](#)). There was no difference between visits in the percentage of macronutrients consumed ([Table 9.2](#)).
9.4.4  Relations between gastric emptying, hormones, blood glucose, appetite and energy intake

9.4.4.1  Relationships between hormones and blood glucose with gastric emptying

There were inverse relationships between plasma GLP-1 at $t = 45, 60, 75$ and $90$ min ($r > -0.60, P < 0.05$ for all), and blood glucose and plasma insulin at $t = 60, 75$ and $90$ min ($r > -0.60, P < 0.05$ for all), but not plasma CCK concentrations, with the volume of glucose remaining in the stomach at these times. There was an inverse relationship between $T_{50}$ with serum progesterone, but not estradiol ($r > -0.60, P < 0.05$).

9.4.4.2  Relationships between energy intake with gastric emptying and hunger

There were inverse relationships between energy intake with the amount of glucose remaining in the stomach at $t = 90$ min (i.e. immediately prior to the buffet meal), $T_{50}$ and scores for hunger at $t = 90$ min ($r > -0.60, P < 0.05$ for all).

9.4.4.3  Relationships between hunger and energy intake with hormones and blood glucose

There were significant relationships between energy intake with blood glucose concentrations at $t = 45, 60, 75$ and $90$ min ($r > 0.50, P < 0.05$ for all) (Figure 9.6A), and plasma GLP-1 concentrations at $t = 15, 30, 45,$ and $75$ min ($r > 0.50, P < 0.05$ for all) (Figure 9.6B), but not with plasma insulin and CCK concentrations.
There was no relationship between blood glucose, plasma GLP-1, insulin or CCK concentrations with scores for hunger at any time point. There was a relationship between scores for hunger at $t = 90$ min and energy intake with serum progesterone ($r > 0.60$, $P < 0.05$ for both), but not serum estradiol, concentrations.

### 9.4.5 Intra-subject reproducibility between FOL-P1 and FOL-P2

There was excellent agreement between FOL-P1 and FOL-P2 for $T_{50}$ ($r_i = 0.81$), AUCs of plasma CCK profiles ($r_i = 0.91$) and energy intake ($r_i = 0.94$), good agreement for AUCs of hunger ($r_i = 0.71$) and plasma GLP-1 ($r_i = 0.70$) profiles and moderate agreement for AUCs of plasma insulin ($r_i = 0.65$) and blood glucose ($r_i = 0.50$) profiles. In contrast, there was no agreement for AUCs of proximal ($r_i = 0.11$) and distal ($r_i = 0.12$) gastric volume profiles. Intra-subject variability of energy intake was less during FOL-P1 and FOL-P2 (CV = 6.6 %) compared with FOL-P1 vs LUT-P (CV = 15.0 %) and FOL-P2 vs LUT-P (CV = 16.0 %).
Table 9.1: Mean AUC values for gastric emptying, blood glucose, plasma GLP-1, plasma insulin and plasma CCK concentrations and hunger during the follicular ("FOL-P1" and "FOL-P2"), and luteal ("LUT-P"), phases of the menstrual cycle.

<table>
<thead>
<tr>
<th></th>
<th>FOL-P1</th>
<th>FOL-P2</th>
<th>LUT-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean AUC values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric emptying (%.min)</td>
<td>6705 ± 318*</td>
<td>6659 ± 216*</td>
<td>5711 ± 188</td>
</tr>
<tr>
<td>Blood glucose (mmol/L.min)</td>
<td>651 ± 30*</td>
<td>634 ± 42*</td>
<td>743 ± 49</td>
</tr>
<tr>
<td>Plasma GLP-1 (pmol/L.min)</td>
<td>1081 ± 99#</td>
<td>1124 ± 116#</td>
<td>1421 ± 100</td>
</tr>
<tr>
<td>Plasma insulin (mU/L.min))</td>
<td>4162 ± 403*</td>
<td>4052 ± 394*</td>
<td>5235 ± 519</td>
</tr>
<tr>
<td>Plasma CCK (pmol/L.min)</td>
<td>455 ± 37</td>
<td>449 ± 37</td>
<td>444 ± 32</td>
</tr>
<tr>
<td>Hunger (mm.min)</td>
<td>3513 ± 332#</td>
<td>3831 ± 593#</td>
<td>4812 ± 431</td>
</tr>
</tbody>
</table>

Data are mean values ± SE, n = 9; * vs LUT-P: P < 0.05, # vs LUT-P: P < 0.01.
Table 9.2: Mean values for energy intake (kJ), amount eaten (g) and macronutrient distribution (%) after ingestion of a buffet-style lunch during the follicular (“FOL-P1” and “FOL-P2”), and luteal (“LUT-P”), phases of the menstrual cycle.

<table>
<thead>
<tr>
<th></th>
<th>FOL-P1</th>
<th>FOL-P2</th>
<th>LUT-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kJ)</td>
<td>3181 ± 348*</td>
<td>3003 ± 339*</td>
<td>3769 ± 468</td>
</tr>
<tr>
<td>Weight of food (g)</td>
<td>827 ± 77*</td>
<td>825 ± 128*</td>
<td>877 ± 99</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20 ± 1</td>
<td>19 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>33 ± 1</td>
<td>34 ± 1</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>47 ± 1</td>
<td>47 ± 2</td>
<td>48 ± 1</td>
</tr>
</tbody>
</table>

Data are mean values ± SE, n = 9; * vs LUT-P: P < 0.05.
Figure 9.1: Total (A) and proximal and distal (B) gastric emptying (% retention) of a preload containing 50 g glucose in 300 ml water during the follicular phase visit 1 (“FOL-P1”) and visit 2 (“FOL-P2”) and the luteal phase (“LUT-P”). Data are means ± SEM (n = 9). * vs LUT-P, P < 0.05.
Figure 9.2: Blood glucose (A), plasma GLP-1 (B), plasma insulin (C) and plasma CCK (D), concentrations following ingestion of a preload containing 50 g glucose in 300 ml water and after ingestion of a buffet-style lunch during the follicular phase visit 1 (“FOL-P1”) and visit 2 (“FOL-P2”) and the luteal phase (“LUT-P”). Data are means ± SEM (n = 9). * vs LUT-P, P < 0.05.
Figure 9.3: Examples of (A) small, (B) moderate and (C) large differences in effects on blood glucose, plasma GLP-1 and plasma insulin concentrations following ingestion of a preload containing 50 g glucose in 300 ml water and after ingestion of a buffet-style lunch during the follicular phase visit 1 (“FOL-P1”) and visit 2 (“FOL-P2”) and the luteal phase (“LUT-P”).
Figure 9.4: Scores for hunger following ingestion of a preload containing 50 g glucose in 300 ml water and after ingestion of a buffet-style lunch during the follicular phase visit 1 ("FOL-P1") and visit 2 ("FOL-P2") and the luteal phase ("LUT-P"). Data are means ± SEM (n = 9). * vs LUT-P, P < 0.05.
Figure 9.5: Cumulative frequency plot of the differences in energy intake (kJ) after ingestion of a buffet-style lunch between follicular phase visit 1 and visit 2 “FOL-P1 – FOL-P2”, the luteal phase visit and follicular phase visit 1 “LUT-P – FOL-P1”, and the luteal phase visit and follicular phase visit 2 “LUT-P – FOL-P2” ($n = 9$).
Figure 9.6: Relationships between energy intake (MJ) with (A) blood glucose and (B) plasma GLP-1 at $t = 45$ min after ingestion of a preload containing 50 g glucose in 300 ml water ($n = 9$).
9.5 DISCUSSION

Our observations establish that in healthy women after ingestion of a 50 g glucose ‘preload’, (i) gastric emptying is slower, and glycemic, plasma GLP-1 and insulin responses, hunger and energy intake are less, during the follicular, when compared with the luteal, phase, (ii) energy intake and the glucose, plasma GLP-1 and insulin responses are related to gastric emptying, and (iii) these parameters are reproducible when assessed twice within one phase of the menstrual cycle i.e. the follicular phase.

To our knowledge, this is the first study that quantified the effects of the menstrual cycle on acute energy intake prospectively within the laboratory setting. Our observation that energy intake varies across the menstrual cycle is consistent with existing literature which includes studies in both animals (Czaja and Goy 1975, Kemnitz et al. 1984) and humans (Lissner et al. 1988, Johnson et al. 1994, Martini et al. 1994). Specifically, in our study, both the amount eaten (g) and energy consumed (kJ) at the buffet meal, were less during the follicular, when compared with the luteal, phase, with a substantial mean difference in energy intake of 700 kJ. Previous studies have observed a difference of between 660 – 1000 kJ in daily (24-h) energy intake, using dietary recall methods, between the follicular and luteal phases (Martini et al. 1994, Li et al. 1999), while our study quantified acute energy intake following a nutrient ‘preload’. There is evidence that daily energy expenditure fluctuates across the menstrual cycle, i.e. energy expenditure is lower in the latter half of the follicular phase and higher during the luteal phase.
Effect of the menstrual cycle on gastric emptying and appetite

Chapter 9

(Solomon et al. 1982, Webb 1986). Therefore, an adjustment of appetite and energy intake ensures that energy balance is maintained. In contrast to others (Martini et al. 1994, Li et al. 1999), we did not observe any effect of the menstrual cycle on the amount of energy derived from individual macronutrients. It should, however, be recognised that this may reflect the composition of our buffet-style meal, i.e. a meal which contains a selection of foods that vary substantially in their macronutrient composition is likely to be more suitable to evaluate effects on macronutrient distribution or food choice.

We have demonstrated that gastric emptying of glucose is slower during the follicular ($T_{50} \sim 75$ min), when compared with the luteal ($T_{50} \sim 60$ min), phase. This observation contrasts with previous studies that have reported either no difference in the rate of emptying of the solid and liquid phases of a mixed meal between the follicular and luteal phases (Horowitz et al. 1985, Degen and Phillips 1996), or slower gastric emptying of a solid meal during the luteal phase (Gill et al. 1987). In some studies gastric emptying was evaluated using low-nutrient meals, e.g. beef soup and mashed potato (~ 99 kcal) (Gill et al. 1987), which are known to empty rapidly from the stomach, since they do not stimulate mechanisms that retard gastric emptying effectively. Of particular interest is that our study demonstrates that the changes in appetite and energy intake observed across the menstrual cycle are related to varying patterns of gastric emptying, although this is not surprising given that intragastric factors are important in the regulation of energy intake (Feinle et al. 1997, Jones et al. 1997, Sturm et al. 2004). While mechanisms underlying the observed effects of the menstrual cycle on gastric
emptying and appetite remain to be defined, the observed relationship between the rate of gastric emptying with the serum progesterone concentration suggests that changes in sex steroids, particularly progesterone, are likely to be important.

It is well established that there is a close relationship between the initial rise in blood glucose and gastric emptying after an oral glucose load and carbohydrate-containing meals (Horowitz et al. 1993, Jones et al. 1996). In some (Gonlachanvit et al. 2003, Russo et al. 2003), but not all (Hidalgo et al. 2002), studies the total glycemic response to a meal was also diminished when gastric emptying was slowed. In this current study, the observed direct relationship between the initial and overall glycemic response and the rate of gastric emptying was, accordingly, predictable, given that even modest changes in gastric emptying of glucose are associated with substantial changes in the glycemic response (Horowitz et al. 1993, Rayner et al. 2001). Our study has demonstrated for the first time that oral glucose-induced GLP-1 release is greater during the luteal, compared with the follicular, phase of the menstrual cycle and is related to more rapid gastric emptying. Given the observed changes in gastric emptying, this is not surprising as previous studies have shown that the stimulation of GLP-1 (and the other incretin hormone, GIP) are dependent on the rate of entry of glucose into the small intestine (O'Donovan et al. 2004, Chaikomin et al. 2005, Pilichiewicz et al. 2007). Hence, the observed reduction in insulinemia during the follicular phase is likely to reflect the decreases in both glycaemia and GLP-1 secretion. CCK has an established role in appetite regulation (Feinle et al. 1996, Matzinger et al. 1999) and mediates, at least in part, the effects of nutrients on gastrointestinal motility.
and energy intake (Katschinski et al. 1996, Matzinger et al. 1999). Glucose, in comparison with both fat or protein (Liddle et al. 1985), stimulates plasma CCK only modestly, and it is, therefore, not surprising that the differences in gastric emptying, hunger and energy intake, observed between the follicular and luteal phases, were not associated with changes in plasma CCK concentrations. It would, therefore, be of interest to evaluate potential variations in CCK release across the menstrual cycle using test meals known to stimulate CCK potently. Alternatively, given the evidence, albeit from animal studies, that the sensitivity to CCK is increased when estrogen is high (Geary 2001), it is possible that while concentrations of CCK did not change during the follicular phase, subjects may be more sensitive to the effects of CCK. While we did not measure plasma ghrelin in this study, it has been reported previously that plasma ghrelin concentrations remain unchanged over the menstrual cycle (Dafopoulos et al. 2008).

Buffet-style meals that contain a range of food items, varying in macronutrient composition, and provided in excess of what subjects would be expected to consume, are used frequently to assess energy intake in the laboratory setting (Arvaniti et al. 2000, Brennan et al. 2005). It has been suggested that the availability of a meal in excess could result in spontaneous overeating (Kral et al. 2004), thereby confounding the capacity to detect small changes in energy intake in response to a treatment. On the other hand, individuals may experience a sense of boredom when presented with the same meal on multiple occasions, resulting in a reduction in energy intake at later visits because of disinterest. Few studies have assessed the potential variability that may occur when energy intake is assessed
repeatedly using a buffet-style meal (Arvaniti et al. 2000, Nair et al. 2008), and, to our knowledge, no study has assessed this in females. We have now demonstrated that energy intake is highly reproducible when young adult females are assessed twice during the follicular phase of the menstrual cycle. This observation supports the use of a buffet-style meal as a reliable measure of energy intake, since providing subjects with the same foods repeatedly did not appear to influence energy intake.

Some limitations of our study warrant discussion. Firstly, as we have only evaluated healthy, lean women, our observations may not be applicable to other female subject groups i.e. under- or over-weight and obese. While the number of subjects included was based on power calculations derived from our previous work (Nair et al. 2008), and the observed differences in primary endpoints appear clear-cut, it is possible that potential differences in intragastric meal distribution remained undetected.

9.6 CONCLUSIONS
In summary, we have demonstrated that in healthy females the reduction in energy intake during the follicular phase is related to slower gastric emptying and reduced hunger. Furthermore, during the follicular phase the glycaemic and plasma GLP-1 and insulin responses to an oral glucose load are attenuated and related to the slower rate of gastric emptying. Hence, our observations strongly suggest that the menstrual cycle should be controlled for in research studies investigating
gastrointestinal function, appetite and energy intake, glycaemia and gastrointestinal hormone concentrations, i.e. female subjects included in multiple visit research studies should ideally be assessed during the same phase of the menstrual cycle. The substantial effects of the menstrual cycle on glycaemia secondary to gastric emptying may have important implications for oral glucose tolerance testing for the diagnosis of diabetes mellitus in pre-menopausal women – our data suggest that consideration should be given to the timing of oral glucose tolerance tests, since it is likely that more subjects will be diagnosed with impaired glucose tolerance and diabetes during the luteal phase. Further studies are required to assess the time course of changes in variables across the menstrual cycle and longer-term studies are desirable to better characterize the significance of our findings.
CHAPTER 10

Effects of acute energy restriction on antropyloroduodenal motor, appetite and energy intake responses to small intestinal fat in the obese

10.1 SUMMARY

The presence of nutrients in the small intestine modulates gastrointestinal function and suppresses appetite and energy intake. There is evidence that previous patterns of energy intake, including excess and restriction, may affect these parameters. It was hypothesised that acute energy restriction would ‘sensitise’ small intestinal nutrient feedback so that in response to small intestinal fat infusion the suppression of antral and duodenal PWs would be less, the stimulation of basal pyloric pressure and IPPWs would be greater, and the suppression of appetite and energy intake would be increased, in obese individuals. Eight obese males (aged 50 ± 1 yr; BMI 34 ± 0.6 kg/m²) were studied on two separate occasions before, and immediately after, intake of a very-low calorie diet (VLCD) for 4 days. On both study days, APD motility and appetite perceptions were measured during a 120 min intraduodenal infusion of 10 % Intralipid® at 2.86 kcal/min. Immediately
after the infusion, energy intake at a buffet meal was quantified. Following the VLCD there was an increase in basal pyloric pressure (mean values between $t = 0$ – 120 min: visit 1: $3 \pm 1$ mmHg, visit 2: $6 \pm 1$ mmHg) ($P < 0.05$) and the number (visit 1: $1078 \pm 17$, visit 2: $1402 \pm 20$) and amplitude (visit 1: $39 \pm 3$ mmHg, visit 2: $51 \pm 5$ mmHg) of IPPWs ($P < 0.05$ for both), and a decrease in the number of antral (visit 1: $694 \pm 29$, visit 2: $212 \pm 11$) and duodenal (visit 1: $2989 \pm 91$, visit 2: $1910 \pm 66$) PWs ($P < 0.05$ for both), during the infusion period. Following the VLCD, hunger was less ($P < 0.05$ for both), and energy intake was reduced (visit 1: $4378 \pm 691$, visit 2: $3634 \pm 701$ kJ) ($P < 0.05$). These observations indicate that ‘sensitivity’ to the effects of small intestinal lipid on gastrointestinal motility and appetite is enhanced by exposure to a four-day VLCD.

### 10.2 INTRODUCTION

There are now more than 250 million obese people worldwide, representing ~ 7% of the adult population (Bray 2003). In Australia, the incidence of obesity has more than doubled in the past 20 years, with 54% of the population classified as either overweight or obese in 2005 (Access Economics for Diabetes Australia, 2006). Conversely, malnutrition is also common, particularly in the elderly where the age-associated physiologic reduction in appetite and energy intake, which has been termed ‘the anorexia of aging’ probably contributes to its development (Donini et al. 2003, Chapman 2007). Current therapeutic interventions for the treatment of obesity and malnutrition are of limited efficacy (Morley 1997, Weigle 2003, Kaplan 2005), and have largely ignored the pivotal role of the
gastrointestinal tract in the regulation of appetite and the relationships between appetite and energy intake with gastrointestinal function.

In health, the interaction of nutrients, particularly fat, with the small intestine has potent effects to modulate gastrointestinal motor function, including the slowing of gastric emptying, resulting, at least in part, from the suppression of antral and duodenal PWs and the stimulation of phasic and tonic pyloric motility (Cunningham et al. 1991, Cook et al. 1997, Feltrin et al. 2004), and the suppression of appetite and energy intake (Chapman et al. 1999, Feinle et al. 2003, Pilichiewicz et al. 2007). The release of a number of gastrointestinal hormones including CCK (Lilja et al. 1984), GLP-1 (Herrmann et al. 1995) and PYY (Pilichiewicz et al. 2005), and the suppression of ghrelin (Cummings et al. 2001), appear to mediate, at least in part, these effects of nutrients. There is also evidence that changes in upper gastrointestinal motility, particularly that of the pylorus, may, per se, affect appetite (Chapter 6) (Feltrin et al. 2004, Pilichiewicz et al. 2007).

Studies evaluating gastrointestinal function and appetite in the obese are limited and have yielded conflicting information. For example, gastric emptying in the obese has been reported to be either faster (Tosetti et al. 1996), similar (French et al. 1993) or slower (Maddox et al. 1989), when compared with lean subjects. In another study, gastric emptying was shown to be slower following ingestion of meals with increasing caloric content (Horowitz et al. 1986), suggesting that small intestinal feedback to slow gastric emptying is preserved in the obese.
Gastrointestinal hormone secretion, both fasting and postprandially, may also be modified in the obese, with higher fasting (Baranowska et al. 2000), and postprandial (French et al. 1993), plasma CCK concentrations. Since in lean subjects, changes in gastrointestinal function occur concurrently with modifications in appetite and energy intake, any disturbances in the sensitivity to nutrients following meal ingestion, are likely to impact on energy intake in obesity.

There is evidence that previous patterns of energy intake, both in excess and restriction, have the capacity to modify gastrointestinal function even when sustained for short periods of time (Cunningham et al. 1991, Cunningham et al. 1991, Corvilain et al. 1995), such that excess nutrients decrease, and energy restriction, increases the effects of nutrients on gastrointestinal function. Chronic exposure to a two week high-fat diet (Cunningham et al. 1991), or 400 g glucose/day for 3 days (Cunningham et al. 1991), both accelerated gastric emptying of a high-fat meal and a glucose drink respectively in healthy subjects. In contrast, fasting has the opposite effect. Following a 4-day fast, gastric emptying of glucose was slower in both lean and obese subjects (T_{50} following overnight fast: 78 ± 6 min and 4-day fast: 95 ± 5 min) (Corvilain et al. 1995). It is of interest that, ~ 50 % of patients with anorexia nervosa, a condition usually associated with dramatic dietary restriction, have delayed gastric emptying which is reversed by reintroduction of oral nutrition and before significant weight gain (Rigaud et al. 1988). In addition, in critically ill patients nutrient deprivation is associated with delayed gastric emptying and increased plasma CCK and PYY.
(Nguyen et al. 2007). In this group there is evidence that increased small intestinal feedback, i.e. enhanced stimulation of pyloric and suppression of antral pressures, contributes to this slowing of gastric emptying (Chapman et al. 2005).

We have now evaluated the effects of short-term energy restriction on APD motility, and the relationships between these effects with those on hunger and energy intake in the obese. The broad hypothesis was that acute energy restriction would increase the sensitivity of APD motor, appetite and energy intake responses to fat in the obese.

10.3 SUBJECTS AND METHODS

10.3.1 Subjects

Ten obese males (aged 50 ± 1 years (range 45 – 55 years); BMI 34 ± 0.6 kg/m² (range 32 – 36 kg/m²)) were recruited according to guidelines described in Chapter 4.2. Data derived from a pilot study conducted in four subjects was used to estimate the required sample size for the current study. Assuming a within-subject standard deviation in energy intake of 820 kJ, a mean difference in energy intake between visits of 820 kJ would be detectable with a sample size of 10 subjects at 80 % power and a Bonferroni adjusted significance level of 5 %.
10.3.2 Study design

Each subject attended the laboratory on two occasions, once after an overnight fast (visit 1) and once following four-days of a very low calorie diet (VLCD) (visit 2). On both visits, the effects of a 120-min intraduodenal infusion of a lipid emulsion (10 % Intralipid, 300mOsmol/kg, delivery rate: 1.1 kcal/min, Baxter Healthcare, Old Toongabbie, NSW, Australia; infusion rate: 2.86 kcal/min) on APD motility, plasma CCK, appetite, and energy intake were quantified.

10.3.3 Very-low calorie diet (VLCD)

The four-day VLCD involved a 70 % reduction of a subject’s daily energy requirements (estimated using the Harris Benedict equation and a physical activity factor between 1.4 – 1.5 based on an individual’s daily activity) (Harris and Benedict 1918). To achieve this, subjects were provided with a detailed meal plan diary (Appendix IV) and the majority of food items required for the diet period. The VLCD consisted of both liquid meal replacements and regular food items, as described in Chapter 4.9.4. Throughout the VLCD, subjects were required to maintain a food diary by detailing the quantity of food ingested at each meal. In addition, subjects were required to list the detail and quantity of all non-caloric beverages consumed per day. Subjects were asked to maintain their normal physical activity over the course of the study. Subjects were phoned during day two of the four-day VLCD to assess their progress.
10.3.4 Protocol

On the morning of each study day, the subject attended the laboratory at 0830 h when they were intubated, via an anaesthetised nostril, with a 17-channel manometric catheter, as described in Chapter 4.5. An intravenous cannula was placed in a forearm vein for blood sampling.

Once the catheter was positioned correctly, a baseline ($t = -15$ min) blood sample was taken and a VAS, assessing perceptions of appetite (Parker et al. 2004), administered. At $t = 0$ min, infusion of the lipid emulsion commenced and continued for 120 min. During the infusion, blood samples were obtained and VAS completed at regular intervals, i.e. every 15 min between $t = 0 – 90$ min and every 30 min until $t = 150$ min. At $t = 120$ min, the subject was extubated and immediately offered a standardised, cold, buffet-style meal. The types of food, as well as the macronutrient composition and energy content of the meal, are described in detail in Appendix III. After the meal, $t = 150$ min, the intravenous cannula was removed and subjects were allowed to leave the laboratory.

10.3.5 Measurements

10.3.5.1 Antropyloroduodenal pressures

APD pressures were analysed for (i) the number and amplitude of PWs in the antrum and duodenum, (ii) basal pyloric pressure (pyloric ‘tone’), (iii) the number and amplitude of IPPWs and (iv) pressure wave sequences (PWSs), as described in Chapter 4.5.
10.3.5.2 Plasma hormone concentrations

Blood samples were collected for the measurement of plasma CCK, PYY and ghrelin, as described in Chapters 4.7.1.1, 4.7.1.3 and 4.7.1.2. The results of these analyses are not yet available.

10.3.5.3 Appetite

Appetite ratings (hunger) were assessed using validated VAS (Parker et al. 2004) as described in Chapter 4.8.1. Nausea and bloating were also assessed.

10.3.5.4 Energy intake

Energy intake was assessed, as described in Chapter 4.8.2.

10.3.6 Statistical analysis

Baseline values (‘0’) were calculated as the mean of values obtained at \( t = -15 \) and 0 min for VAS, and between \( t = -15 \) to 0 min for the total number and mean amplitude of antral and duodenal PWs, IPPWs, basal pyloric pressures and total number of PWSs. The number and amplitude of antral and duodenal PWs were expressed as total, and mean, values respectively during the 120 min of the infusion period. IPPWs, basal pyloric pressure and PWSs were expressed as mean values of 15 min intervals between 0 – 120 min (i.e. 0 - 15, 15 - 30, … , 105 - 120
min). To evaluate the temporal responses during the infusion period, data were divided into two periods, i.e. from $t = 0 – 60$ min and $t = 60 – 120$ min. All data were expressed as changes from baseline. IPPWs, basal pyloric pressures, PWSs and VAS were analyzed by repeated-measures ANOVA, with time and treatment as factors. AUCs for basal pyloric pressure, the number and amplitude of IPPWs and VAS data were determined using the trapezoidal rule. The number and amplitude of antral and duodenal PWs, and energy intake were analyzed by one-way ANOVA. Post-hoc paired comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed when ANOVAs revealed significant effects. Correlations, corrected for repeated-measures, were assessed for the change between visit 1 and visit 2 in the total number of antral and duodenal PWs and PWSs, and AUCs (between $t = 0 – 120$ min) for basal pyloric pressures, number of IPPWs and hunger with changes in the percentage reduction in energy intake between visit 1 and visit 2, using the method described by Bland and Altman (Bland and Altman 1995). Only $r$ values $> 0.5$ were considered relevant. Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

10.4 RESULTS

Ten subjects were enrolled into the study, however, one subject failed to comply with the VLCD and was excluded from the study, and one withdrew for personal reasons. The remaining eight subjects completed the two study days and tolerated the experimental conditions well. The average 30 % daily energy requirement for
subjects was $3938 \pm 63$ kJ. Based on the dietary records maintained by the subjects, 100% compliance with the VLCD was achieved.

### 10.4.1 Antropyloroduodenal pressures

#### 10.4.1.1 Antral pressures

During both visit 1 and visit 2 there was a gradual decline in the number of antral PWs throughout the infusion period (time effect: $P < 0.05$). There was a significant effect of treatment on the number, but not the amplitude, of antral PWs ($P < 0.05$) (Table 10.1). The number of antral PWs was less on visit 2, when compared with visit 1 throughout the infusion period ($t = 0 – 120$ min).

#### 10.4.1.2 Pyloric pressures

*Basal pressure (‘tone’)*

During visit 1, basal pyloric pressure increased in response to intraduodenal lipid administration until $t = 30$ min (time effect: $P < 0.05$), before decreasing to baseline levels by $t = 120$ min (Figure 10.1A). During visit 2, basal pyloric pressure increased markedly until $t = 45$ min (time effect: $P < 0.01$), after which levels declined. There was a significant effect of treatment on basal pyloric pressure ($P < 0.05$), which was greater during visit 2, when compared with visit 1. Between $t = 0 – 60$ min, basal pyloric pressure was greater during visit 2, when compared with visit 1 ($P < 0.01$), while there was no difference between $t = 60 –$
120 min. Peak basal pyloric pressure was also greater on visit 2 (13 ± 2 mmHg), when compared with visit 1 (8 ± 2 mmHg) (P < 0.05).

**Phasic pressures**

The number of IPPWs increased in response to lipid administration until \( t = 45 \) min during visit 1, and \( t = 30 \) min on visit 2 (time effect: P < 0.01 for both), after which the responses plateaued (Figure 10.1B). There was a significant effect of treatment on the number of IPPWs (P < 0.05), which was higher during visit 2, when compared with visit 1. Between \( t = 0 – 60 \) min, the number of IPPWs were greater during visit 2, when compared with visit 1 (P < 0.05), while there was no difference between \( t = 60 – 120 \) min. Peak number of IPPWs was greater on visit 2 (37 ± 3), when compared with visit 1 (28 ± 2) (P < 0.05).

Administration of lipid increased the amplitude of IPPWs until \( t = 30 \) min during both visit 1 and visit 2 (time effect: P < 0.01 for both), after which the responses plateaued (Figure 10.1C). There was an effect of treatment on the amplitude of IPPWs (P < 0.01), which was higher during visit 2 when compared with visit 1 (P < 0.01). Between \( t = 0 – 60 \) min, the amplitude of IPPWs were higher during visit 2, when compared with visit 1 (P < 0.05), while there was no difference between \( t = 60 – 120 \) min. The peak amplitude of IPPWs was also higher on visit 2 (63 ± 7 mmHg), when compared with visit 1 (51 ± 6 mmHg) (P < 0.05).
10.4.1.3 Duodenal pressures

During both visit 1 and visit 2 there was a gradual decline in the number of duodenal PWs throughout the infusion period (time effect: $P < 0.05$). There was a significant effect of treatment on the number, but not the amplitude, of duodenal PWs ($P < 0.05$) (Table 10.1). The number of duodenal PWs was less on visit 2, when compared with visit 1 throughout the infusion period ($t = 0 – 120$ min).

10.4.1.4 Pressure wave sequences

Only PWSs that spanned over 2 – 6 channels (1.5 – 10.5 cm) were analysed statistically, as PWSs spanning over 7 – 15 channels were infrequent (no/120 min: visit 1, 6 ± 1; visit 2, 2 ± 0.5). There was a significant effect of treatment on the number of PWSs travelling over two (i.e. 1.5 < 3 cm), three (i.e. 3 < 4.5 cm), four (i.e. 4.5 < 6 cm) and five (i.e. 6 < 7.5 cm) ($P < 0.05$ for all), channels which were less on visit 2, when compared with visit 1 (Figure 10.2).

10.4.2 Appetite

There was no difference in baseline scores for hunger between study days. There was a treatment by time interaction for hunger ($P < 0.001$) (Figure 10.3A). Hunger was less during visit 2 between $t = 30 – 120$ min ($P < 0.05$), when compared with visit 1.
There was no difference in scores for nausea or bloating at baseline between study days. There was no effect of treatment on nausea, which increased less than 5 % from baseline (Figure 10.3B). There was a significant effect of time, but not treatment, on bloating (time effect: $P < 0.05$) (Figure 10.3C). On visit 2, bloating was ~10 mm higher than baseline between $t = 90 – 120$ min when compared with baseline ($P < 0.05$).

### 10.4.3 Energy intake

There was a significant effect of treatment on both the amount eaten (g) ($P < 0.05$) and energy intake (kJ) ($P < 0.05$) at the buffet meal (Table 10.2). Both the amount eaten, and energy intake, were less at visit 2 when compared with visit 1 by 8 % and 17 %, respectively. There was no difference in macronutrient distribution, i.e. the percentage of energy from fat, carbohydrate and protein consumed at the buffet meal, between study days.

### 10.4.4 Relationships between energy intake with antropyloroduodenal motility and appetite

There were direct relationships between energy intake with the number of antral PWs ($r = 0.70, P < 0.05$) and hunger ($r = 0.80, P < 0.01$), and an inverse relationship between energy intake with basal pyloric pressure ($r = -0.70, P < 0.05$), but not IPPWs ($r = -0.60, P < 0.1$).
Table 10.1: Total number and mean amplitude of antral and duodenal pressure waves during intraduodenal infusion of 10% Intralipid® (rate: 2.86 kcal/min) for 120 min before (visit 1) and after (visit 2) a four-day VLCD.

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Δ(Visit 1-Visit 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antral waves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>87 ± 29</td>
<td>27 ± 11*</td>
<td>60 ± 23</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>26 ± 2</td>
<td>20 ± 4</td>
<td>6 ± 4</td>
</tr>
<tr>
<td><strong>Duodenal waves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>374 ± 91</td>
<td>238 ± 66*</td>
<td>135 ± 52</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>25 ± 2</td>
<td>25 ± 2</td>
<td>0.5 ± 2</td>
</tr>
</tbody>
</table>

Data are mean ± SEM (n = 8). * P < 0.05 vs visit 1.
Table 10.2: Energy intake at a buffet meal immediately following intraduodenal infusion of 10% Intralipid® (rate: 2.86 kcal/min) for 120 min before (visit 1) and after (visit 2) a four-day VLCD.

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Δ(Visit 1-Visit 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amount eaten (g)</strong></td>
<td>1022 ± 114</td>
<td>943 ± 115*</td>
<td>69 ± 64</td>
</tr>
<tr>
<td><strong>Energy intake (kJ)</strong></td>
<td>4377 ± 691</td>
<td>3634 ± 700*</td>
<td>744 ± 302</td>
</tr>
<tr>
<td><strong>Energy (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>35 ± 2</td>
<td>33 ± 4</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>44 ± 2</td>
<td>44 ± 5</td>
<td>1 ± 4</td>
</tr>
<tr>
<td>Protein</td>
<td>22 ± 2</td>
<td>24 ± 2</td>
<td>-2 ± 1</td>
</tr>
</tbody>
</table>

Data are mean ± SEM (n = 8). * P < 0.05 vs visit 1.
Figure 10.1: Basal pyloric pressure (A) and number (B), and amplitude (C), of isolated pyloric pressure waves (IPPWs) in 15 min intervals, during intraduodenal infusion of 10% Intralipid® (rate: 2.86 kcal/min) for 120 min before (visit 1) and after (visit 2) a four-day VLCD. * P < 0.05 vs visit 1. Data are mean ± SEM (n = 8).
Figure 10.2: Number of pressure wave sequences (PWSs) during intraduodenal infusion of 10% Intralipid® (rate: 2.86 kcal/min) for 120 min before (visit 1) and after (visit 2) a four-day VLCD. * P < 0.05 vs visit 1. Data are mean ± SEM (n = 8).
**Figure 10.3:** Scores for *hunger* (A), *nausea* (B) and *bloating* (C) during intraduodenal infusion of 10% Intralipid® (rate: 2.86 kcal/min) for 120 min before (visit 1) and after (visit 2) a four-day VLCD. *P < 0.05 vs visit 1. Data are mean ± SEM (n = 8).
10.5 DISCUSSION

This study has evaluated the effects of a four-day VLCD on APD motility, appetite and energy intake responses to a intraduodenal lipid infusion in obese males. The results indicate that, in the short-term, a VLCD increases the ‘sensitivity’ to small intestinal fat on motility, appetite and energy intake in this group, such that there was a decrease in the number of antral and duodenal PWs and an increase in basal pyloric pressure and the number and amplitude of IPPWs and a reduction in hunger and energy intake. The magnitude of these differences was substantial and these observations have implications for the dietary management of obesity.

Following acute energy restriction, gastrointestinal motility was modified in response to an intraduodenal lipid infusion so that there was a greater stimulation of basal pyloric pressure and the number and amplitude of IPPWs, and a greater suppression of antral and duodenal PWs at visit 2, when compared with visit 1. These results strongly indicate that the ‘sensitivity’ of the upper gastrointestinal region to the effects of small intestinal lipid was enhanced. Short-term fasting has been reported to slow gastric emptying of glucose in both lean and obese subjects (Corvilain et al. 1995), and the slowing of gastric emptying in anorexia nervosa, and the critically ill, is reversed following the reintroduction of oral nutrition (Rigaud et al. 1988, Nguyen et al. 2007), suggesting that acute dietary restriction is associated with adaptive changes in the mechanisms responsible for feedback of gastric emptying. A previous study has demonstrated that the slowing of gastric emptying induced by small intestinal lipid is associated with an increase in the
number of IPPWs, and the suppression of antral and duodenal PWs (Heddle et al. 1989). Therefore, the observed slowing of gastric emptying following a four-day fast (Corvilain et al. 1995), is consistent with the observations of the current study.

To the author’s knowledge, this is the first study to assess the effect of acute energy restriction in the obese on appetite and energy intake responses to small intestinal nutrient. The results indicate that over a very short period of time, perceptions of hunger are modified and there is a ∼ 17% reduction in energy intake. While in our current study energy intake was only assessed acutely, if sustained over a longer period of time it could lead to substantial weight loss. For example, subjects who complete comprehensive VLCD programs generally lose 15 – 25% of their initial weight within 3 – 4 months (Wadden et al. 1992, Anderson et al. 1994, Anderson et al. 1994, Mustajoki and Pekkarinen 2001). That, these subjects regain 40 – 50% of the weight lost within 1 – 2 years (Wadden et al. 1992, Anderson et al. 1994, Anderson et al. 1994), which has generally been attributed, in part, to a lack of dietary compliance, but it may be that during a longer period of energy restriction the adaptive mechanisms to preserve energy intake develop. This should be evaluated.

The mechanisms underlying the observed effects on energy intake are uncertain. Given the evidence that following the VLCD, small intestinal receptors are more sensitive to the presence of fat, i.e. profound changes in motility parameters within the first 60 min of the infusion period, nutrient-mediated signals sent to the brain may translate to substantial changes in hunger and subsequent energy intake.
(despite no differences in motility responses in the latter 60 min of the infusion period). Changes in the release and/or suppression of gastrointestinal hormones, including CCK, PYY and ghrelin, by small intestinal fat may also be important, however, these plasma hormone analyses are awaited. Previous work in healthy lean subjects, including that by the author, has demonstrated an association between energy intake and pyloric motility, i.e. energy intake is inversely related to pyloric stimulation (Chapter 6) (Brennan et al. 2005, Pilichiewicz et al. 2007).

Therefore, in the current study the author anticipated that any changes in energy intake would be related to APD motility, particularly pyloric pressures. Consistent with this concept, there was a relationship between energy intake with basal pyloric pressure, i.e. following the VLCD the reduction in energy intake was associated with the stimulation of pyloric motility, although it should be recognised that this does not establish a causal association.

Some limitations of the study need to be recognised. Firstly, no lean, control subjects were included and this limits our interpretations of the findings. In addition, the order of the study days was not randomised for logistical reasons. Only one dose of intraduodenal fat infusion, and one type of caloric restriction was employed. Hence, the effects of higher, or lower, fat infusion, administration of macronutrients, and less marked energy restriction, are uncertain. It is known that the effects of small intestinal lipid on APD motility, gut hormones, appetite and energy intake are load-dependent (Pilichiewicz et al. 2007). Only male volunteers were included and, accordingly, our observations may not be applicable to females. Moreover, as all subjects were obese (the target group for therapeutic
energy restriction), it is unknown if our findings apply to individuals who are overweight or morbidly obese, or patients with malnutrition. Finally, as discussed the chronic effects of the VLCD were not evaluated. It will be important to determine whether the effects on appetite and energy intake observed in the current study are sustained during periods of longer-term energy restriction.

10.6 CONCLUSIONS

In summary, this study has demonstrated that in obese males, following a VLCD there was a significant increase in pyloric pressures, and a decrease in antral and duodenal pressure waves and pressure wave sequences, during a 120 minute intraduodenal lipid infusion. Furthermore, following the VLCD, hunger and energy intake were less. These observations indicate that the sensitivity of gastrointestinal responses to small intestinal fat is increased by a short period of energy restriction.
CHAPTER 11

Conclusions

The studies reported in this thesis have evaluated aspects of the complex and interrelated postprandial gastrointestinal mechanisms involved in the regulation of appetite and energy intake. The three broad areas that have been investigated include: (i) the effect of gastrointestinal hormones on gastric motility, gastrointestinal hormone release/suppression, appetite and energy intake in healthy lean subjects, (ii) the effect of oral macronutrients on appetite and energy intake in both lean and obese subjects, and (iii) the effects of acute energy restriction on gastric motility, gastrointestinal hormone release, appetite and energy intake in obese subjects.

The study in Chapter 5 assessed possible interactions between intravenous CCK-8 (1.8 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min) that may modulate ghrelin and PYY release. At the doses evaluated, exogenous CCK-8 and GLP-1 had discrepant effects on the secretion of ghrelin and PYY; CCK-8 markedly suppressed ghrelin, whereas GLP-1 had no effect and the stimulation of PYY by CCK-8 was attenuated markedly by GLP-1. These observations have provided insights into the potential mechanisms through which gastrointestinal hormones
Conclusions

interact to modulate gut function, appetite and energy intake. Future studies, potentially using specific CCK and GLP-1 antagonists, are required to clarify the physiological relevance of our observations.

The effects of increasing doses of CCK-8 on gastrointestinal motility, gut hormone release and the relationships between these effects with those on hunger and energy intake were evaluated in Chapter 6. Exogenous CCK-8 stimulated pressures in the pylorus, increased plasma PYY concentrations and suppressed desire-to-eat and energy intake in a dose-dependent manner, while all CCK-8 doses equally suppressed ghrelin. The observation that CCK-8 affects other gastrointestinal hormones provides additional insight into potential mechanisms through which CCK-8 modulates gut motility, appetite and energy intake. There were relationships between plasma CCK-8 with basal pyloric pressure and isolated pyloric pressure waves, and energy intake with isolated pyloric pressure waves. In view of this, the relationship between energy intake and pyloric motility, as well as the combination of CCK-8 with other gut peptides to reduce appetite and potentially induce weight loss, warrants further investigation.

The study in Chapter 7 assessed the acute effects of test meals either high in fat, protein or carbohydrate, and increasing amounts of protein, on appetite and energy intake in lean and obese subjects, and compared these responses between lean and obese. In lean subjects, hunger was less, and fullness greater, following ingestion of the HF and HP meals, whereas there was no difference between the macronutrients in the obese. In lean subjects, energy intake was reduced following
the HF and HP meals when compared with the HC meal. In obese subjects, the HP and AP reduced energy intake when compared with the HF and HC, and HC meal, respectively. The percentage change in energy intake between the HF and AP test meals was significantly different between lean and obese, suggesting that obese subjects may be less sensitive to the satiating effects of fat. A successful weight loss diet should result in a sustained suppression of appetite and food intake. In the context of obesity, chronic studies are now required to investigate if the acute effects of a moderate protein intake, i.e. ~ 0.8 – 1.6 g/kg, are maintained over prolonged periods of time.

The studies presented in the subsequent two chapters (Chapters 8 and 9) investigated the contribution of factors that may influence the effects of oral macronutrients on gastrointestinal function, appetite and energy intake. The study in Chapter 8 demonstrated that, in a laboratory setting, appetite perceptions and energy intake in response to a nutrient preload in healthy lean men were highly reproducible, and that this consistency in energy intake was associated with reproducible patterns of gastric emptying and insulin and CCK secretion. The study in Chapter 9 demonstrated in females that gastric emptying was slower and glycaemic, plasma GLP-1 and insulin responses were lower. Hunger and energy intake were less during the follicular, when compared with the luteal, phase. Furthermore, energy intake and glucose, plasma GLP-1 and insulin responses were related to gastric emptying. In addition, these parameters were reproducible when assessed twice within one phase of the menstrual cycle i.e. the follicular phase. Hence, our observations strongly suggest that the menstrual cycle should be
controlled for in research studies investigating gastrointestinal function, appetite and energy intake, glycaemia and gastrointestinal hormone concentrations. Specifically, female subjects included in multiple visit research studies should ideally be assessed during the same phase of the menstrual cycle.

There is evidence that both previous patterns of macronutrient intake and fasting affect gastrointestinal function. The study described in Chapter 10 demonstrated that following a four-day very-low calorie diet (VLCD) there was a significant increase in basal pyloric pressure and the number and amplitude of isolated pyloric pressure waves, and a decrease in the number of antral and duodenal pressure waves and pressure wave sequences, during a 120 minute intraduodenal lipid infusion. In addition, following the four-day VLCD, hunger and prospective consumption scores were lower, and energy intake was reduced, indicating that gastrointestinal function, appetite and energy intake in the obese can be modified over a short period of time. The chronic effects of a period of energy restriction were not evaluated. Hence, it would be important to determine whether the effects on appetite and energy intake observed in Chapter 10 are sustained during periods of longer-term energy restriction.

The studies reported in this thesis provide novel insights relating to the regulation of appetite and energy intake by gastrointestinal motor function and hormone release and/or suppression in healthy lean and obese subjects. These observations will contribute to advances in knowledge regarding basic appetite physiology. Furthermore, the data presented in this thesis have clinical implications for the
successful management of obesity and support dietary intervention, including the manipulation of both the approach, and composition of the diet, as potential treatments for obesity.
Appendix I

THREE-FACTOR EATING QUESTIONNAIRE

Name:  Date:

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

1. When I smell a freshly baked pizza, I find it very difficult to keep from eating, even if I have just finished a meal.
   (T)   (F)

2. I usually eat too much at social occasions, like parties and picnics.
   (T)   (F)

3. I am usually so hungry that I eat more than three times a day.
   (T)   (F)

4. When I have eaten my quota of calories/fat, I am usually good about not eating any more.
   (T)   (F)

5. Dieting is so hard for me because I just get too hungry.
   (T)   (F)

6. I deliberately take small helpings as a means of controlling my weight.
   (T)   (F)

7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.
   (T)   (F)

8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.
   (T)   (F)

9. When I feel anxious, I find myself eating.
   (T)   (F)

10. Life is too short to worry about dieting.
    (T)   (F)

11. Since my weight goes up and down, I have gone on reducing diets more than once.
    (T)   (F)

12. I often feel so hungry that I just have to eat something.
    (T)   (F)

13. When I am with someone who is overeating, I usually overeat too.
    (T)   (F)
14. I have a pretty good idea of the number of calories/grams of fat in common foods. 
   (T)    (F)

15. Sometimes when I start eating, I just can’t seem to stop. 
   (T)    (F)

16. It is not difficult for me to leave something on my plate. 
   (T)    (F)

17. At certain times of the day, I get hungry because I have got used to eating then. 
   (T)    (F)

18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to 
   make up for it. 
   (T)    (F)

19. Being with someone who is eating often makes me hungry enough to eat also. 
   (T)    (F)

20. When I feel blue, I often overeat. 
   (T)    (F)

21. I enjoy eating too much to spoil it by counting calories, counting grams of fat or watching my 
   weight. 
   (T)    (F)

22. When I see a real delicacy, I often get so hungry that I have to eat right away. 
   (T)    (F)

23. I often stop eating when I am not really full as a conscious means of limiting the amount I eat. 
   (T)    (F)

24. I get so hungry that my stomach often seems like a bottomless pit. 
   (T)    (F)

25. My weight has hardly changed at all in the last ten years. 
   (T)    (F)

26. I am always hungry, so it is hard for me to stop eating before I finish the food on my plate. 
   (T)    (F)

27. When I feel lonely, I console myself by eating. 
   (T)    (F)

28. I consciously hold back at meals in order not to gain weight. 
   (T)    (F)

29. I sometimes get very hungry late in the evening or at night. 
   (T)    (F)

30. I eat anything I want any time I want. 
   (T)    (F)

31. Without even thinking about it, I take a long time to eat. 
   (T)    (F)
Appendix I

32. I count calories/grams of fat as a conscious means of controlling my weight.
   (T)   (F)

33. I do not eat some foods because they make me fat.
   (T)   (F)

34. I am always hungry enough to eat at any time.
   (T)   (F)

35. I pay a great deal of attention to changes in my figure.
   (T)   (F)

36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.
   (T)   (F)

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

37. How often are you dieting in a conscious effort to control your weight?
   1 2 3 4
   rarely sometimes usually always

38. Would a weight fluctuation of 3 kg affect the way you live your life?
   1 2 3 4
   not at all slightly moderately very much

39. How often do you feel hungry?
   1 2 3 4
   only at sometimes often almost
   meal times between between always
   meals meals

40. Do your feelings of guilt about overeating help you to control your food intake?
   1 2 3 4
   never rarely often always

41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?
   1 2 3 4
   easy slightly moderately very
difficult difficult difficult

42. How conscious are you of what you are eating?
   1 2 3 4
   not at all slightly moderately extremely

43. How frequently do you avoid “buying large” on tempting foods?
   1 2 3 4
   almost seldom usually almost
   never always

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Appendix I

44. How likely are you to shop for low calorie or low fat foods?
   1  2  3  4
   unlikely slightly moderately very
   likely likely likely

45. Do you eat sensibly in front of others and splurge alone?
   1  2  3  4
   never rarely often always

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?
   1  2  3  4
   unlikely slightly moderately very

47. How frequently do you skip dessert because you are no longer hungry
   1  2  3  4
   almost seldom at least almost
   never once a week every day

48. How likely are you to consciously eat less than you want?
   1  2  3  4
   unlikely slightly moderately very
   likely likely likely

49. Do you go on eating binges even though you are not hungry?
   1  2  3  4
   never rarely sometimes at least
   once a week

50. To what extent does this statement describe your eating behaviour?
   “I start dieting in the morning, but because of any number of things that happen during the
day, by evening I have given up and eat what I want, promising myself to start dieting again
tomorrow.”
   1  2  3  4
   not like little like pretty good describes
   me me description me
   of me perfectly

51. On a scale of 1 to 6, where 1 means no restraint in eating (eat whatever you want, whenever
    you want it) and 6 means total restraint (constantly limiting food intake and never “giving in”),
    what number would you give yourself?
   1  2  3  4  5  6
   eat whatever you want, whenever you want it
   usually eat whatever you want, whenever you want it
   often eat whatever you want, whenever you want it
   often limit food intake, but often “give in”
   usually limit food intake, rarely “give in”
   constantly limit food intake, never “give in”
Appendix II

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

Not at all  |  Very much

I feel drowsy

Not at all  |  Very much

I feel bloated

Not at all  |  Very much

I feel anxious

Not at all  |  Very much

I feel hungry

Not at all  |  Very much

I feel full

Not at all  |  Very much

I feel happy

Not at all  |  Very much

I feel energetic

Not at all  |  Very much

How strong is your desire to eat?

Non existent  |  Very strong

I feel comfortable

Not at all  |  Very much

How much food do you think you could eat?

None  |  A large amount
## Appendix III

### COMPOSITION OF THE BUFFET-STYLE MEAL

<table>
<thead>
<tr>
<th>Food items</th>
<th>Amount served (g)</th>
<th>Energy content (kJ)</th>
<th>Fat (g)</th>
<th>Carbohydrate (g)</th>
<th>Protein (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wholemeal bread, 4 slices$^1$</td>
<td>125</td>
<td>1304</td>
<td>3.6</td>
<td>50.0</td>
<td>12.6</td>
</tr>
<tr>
<td>White bread, 4 slices$^1$</td>
<td>125</td>
<td>1295</td>
<td>2.9</td>
<td>56.4</td>
<td>11.8</td>
</tr>
<tr>
<td>Ham, sliced$^2$</td>
<td>100</td>
<td>453</td>
<td>3.6</td>
<td>0</td>
<td>18.8</td>
</tr>
<tr>
<td>Chicken, sliced$^3$</td>
<td>100</td>
<td>677</td>
<td>7.0</td>
<td>0</td>
<td>24.6</td>
</tr>
<tr>
<td>Cheese, sliced$^4$</td>
<td>85</td>
<td>1436</td>
<td>28.3</td>
<td>0.9</td>
<td>21.9</td>
</tr>
<tr>
<td>Tomato, sliced</td>
<td>100</td>
<td>56</td>
<td>0.1</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Lettuce</td>
<td>100</td>
<td>27</td>
<td>0</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Cucumber, sliced</td>
<td>100</td>
<td>44</td>
<td>0.1</td>
<td>1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Strawberry yoghurt$^5$</td>
<td>200</td>
<td>966</td>
<td>6.2</td>
<td>33.8</td>
<td>9.4</td>
</tr>
<tr>
<td>Fruit salad$^6$</td>
<td>140</td>
<td>343</td>
<td>0.1</td>
<td>19.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Chocolate custard$^7$</td>
<td>150</td>
<td>662</td>
<td>5.3</td>
<td>22.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Apple</td>
<td>170</td>
<td>359</td>
<td>0.2</td>
<td>21.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Banana</td>
<td>190</td>
<td>680</td>
<td>0.2</td>
<td>37.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Orange juice, Unsweetened$^8$</td>
<td>300</td>
<td>480</td>
<td>0.5</td>
<td>25.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Iced coffee$^9$</td>
<td>375</td>
<td>1073</td>
<td>6.4</td>
<td>37.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Water</td>
<td>600</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Margarine$^{10}$</td>
<td>20</td>
<td>609</td>
<td>16.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mayonnaise$^{11}$</td>
<td>20</td>
<td>310</td>
<td>6.5</td>
<td>4.0</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3000</strong></td>
<td><strong>10774</strong></td>
<td><strong>87.4</strong></td>
<td><strong>313.1</strong></td>
<td><strong>124.7</strong></td>
</tr>
</tbody>
</table>

$^1$Sunblest, Tiptop, Australia; $^2$Deli leg ham, Woolworths, Australia; $^3$Virginian chicken, Woolworths, Australia; $^4$Coon Tasty Cheese slices, Australian Co-operative Foods Ltd, Australia; $^5$Yoplait, National Foods Ltd, Australia; $^6$Goulburn Valley, SPC, Ardmona Operations Ltd, Australia; $^7$Yogo, National Foods Ltd, Australia; $^8$Daily Juice Company, Australia; $^9$Farmers Union, Balemar Pty Ltd, Australia; $^{10}$Flora, Unilever Australiasia, Australia; $^{11}$Kraft, Kraft Foods Ltd, Australia.
Appendix IV

VERY-LOW CALORIE DIET (VLCD) MEAL PLAN

FOUR-DAY MEAL PLAN

Name: ________________________________
Address: ________________________________
Phone: ________________________________
Study code: ________________

**Please return to Ms Ixchel Brennan (Ph: 8222 5039) on Visit 2

______________
GUILDELINES

Food intake
1. This is meal plan for you to follow for the next 4 days.

2. You are required to follow **ALL** dietary intake instructions as they are detailed in the meal plan.

4. Please fill in the diary **IMMEDIATELY** after eating i.e. tick off the food items consumed at each meal.

5. Please record **ALL** drinks such as tea / coffee (with or without milk), and any non-caloric beverages consumed (water or diet soft drinks).

Appetite
Please indicate how satisfied you are following **EVERY** meal by placing a vertical mark along the scale:

I feel satisfied

Not at all  ____________________________ Very much

Energy expenditure
Please indicate your activity level throughout each day by circling one of the following:

SEDENTARY / EASY / MODERATE / HARD / VERY HARD

Morning ketone reading
Please record the ketone reading from your first urination of the morning on **DAYS 2, 3, 4 and on the MORNING OF VISIT 2**

Daily weight record
Please weigh yourself **EVERY DAY** immediately when you awake (before ingestion of any food or drinks)
## DAY 1

**DATE:** _______ _______  **DAY OF THE WEEK:** ______________

**WEIGHT:** ______________

<table>
<thead>
<tr>
<th>BREAKFAST</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kicstart drink</td>
<td>1 sachet + 200 ml skim milk</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all  

[ ] Not at all  

[ ] Very

<table>
<thead>
<tr>
<th>LUNCH</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mixed grain bread</td>
<td>2 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ham</td>
<td>2 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tomato</td>
<td>30 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lettuce</td>
<td>100 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carrot</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apple</td>
<td>1 medium</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all  

[ ] Not at all  

[ ] Very

249
## Appendix IV

<table>
<thead>
<tr>
<th>DINNER</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean Cuisine</td>
<td></td>
<td>Beef Lasagne</td>
<td>360 g</td>
<td>⊗</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tomato</td>
<td>90 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td>80 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lettuce</td>
<td>200 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carrot</td>
<td>80 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salad dressing</td>
<td>30 g</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all much

Very
Please record below any drinks consumed throughout the day

<table>
<thead>
<tr>
<th>DRINKS</th>
<th>TIME</th>
<th>TYPE</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Milk (daily allocation)</td>
<td>100 g</td>
<td></td>
</tr>
</tbody>
</table>

Please indicate your activity level throughout today by circling one of the following:

SEDENTARY / EASY / MODERATE / HARD / VERY HARD
**DAY 2**

**DATE:** _____ _____ **DAY OF THE WEEK:** ______________

**WEIGHT:** ______________

<table>
<thead>
<tr>
<th>BREAKFAST</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kicstart drink</td>
<td>1 sachet + 200 ml skim milk</td>
<td>⊗</td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all       Very
much

<table>
<thead>
<tr>
<th>LUNCH</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mixed grain bread</td>
<td>2 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ham</td>
<td>2 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tomato</td>
<td>30 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lettuce</td>
<td>100 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carrot</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pear</td>
<td>1 medium</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all       Very
much
### Appendix IV

**DINNER TIME FOOD ITEM AMOUNT Check**

<table>
<thead>
<tr>
<th>DINNERTIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lean Cuisine</td>
<td>350 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chicken meal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>90 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cucumber</td>
<td>80 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>200 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carrot</td>
<td>80 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salad dressing</td>
<td>30 g</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all much

![Scale]

Very
Please record below any drinks consumed throughout the day

<table>
<thead>
<tr>
<th>DRINKS</th>
<th>TIME</th>
<th>TYPE</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
<td></td>
<td>100 g</td>
<td>⊗</td>
</tr>
</tbody>
</table>

Please indicate your activity level throughout today by circling one of the following:

SEDENTARY / EASY / MODERATE / HARD / VERY HARD
### DAY 3

**DATE:** _______ _______ **DAY OF THE WEEK:** ______________

**WEIGHT:** ______________

<table>
<thead>
<tr>
<th>BREAKFAST</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kicstart drink</td>
<td>1 sachet + 200 ml skim milk</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all  
| Very |

<table>
<thead>
<tr>
<th>LUNCH</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mixed grain bread</td>
<td>2 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ham</td>
<td>2 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tomato</td>
<td>30 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lettuce</td>
<td>100 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carrot</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apple</td>
<td>1 medium</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all  
<p>| Very |</p>
<table>
<thead>
<tr>
<th>DINNER</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean Cuisine</td>
<td></td>
<td>Beef Lasagne</td>
<td>360 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tomato</td>
<td>90 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td>80 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lettuce</td>
<td>200 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carrot</td>
<td>80 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salad dressing</td>
<td>30 g</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all much

Very
Please record below any drinks consumed throughout the day

<table>
<thead>
<tr>
<th>DRINKS</th>
<th>TIME</th>
<th>TYPE</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
<td></td>
<td>100 g</td>
<td>☒</td>
</tr>
</tbody>
</table>

Please indicate your activity level throughout today by circling one of the following:

SEDENTARY / EASY / MODERATE / HARD / VERY HARD
## DAY 4

**DATE:** _______ _______  **DAY OF THE WEEK:** ________________  
**WEIGHT:** ________________

<table>
<thead>
<tr>
<th>BREAKFAST</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kicstart drink</td>
<td>1 sachet + 200 ml skim milk</td>
<td>☐</td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all ———— Very

<table>
<thead>
<tr>
<th>LUNCH</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mixed grain bread</td>
<td>2 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ham</td>
<td>2 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tomato</td>
<td>30 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lettuce</td>
<td>100 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carrot</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pear</td>
<td>1 medium</td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all ———— Very
<table>
<thead>
<tr>
<th>DINNER</th>
<th>TIME</th>
<th>FOOD ITEM</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean Cuisine Chicken meal</td>
<td>360 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>90 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>80 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>200 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>80 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salad dressing</td>
<td>30 g</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I feel satisfied

Not at all much

Very
Please record below any drinks consumed throughout the day

<table>
<thead>
<tr>
<th>DRINKS</th>
<th>TIME</th>
<th>TYPE</th>
<th>AMOUNT</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
<td>100 g</td>
<td></td>
<td>⬤</td>
</tr>
</tbody>
</table>

Please indicate your activity level throughout today by circling one of the following:

SEDENTARY / EASY / MODERATE / HARD / VERY HARD
DAY 5 (Morning of Visit 2)

DATE: _______ ____ DAY OF THE WEEK: ________________

**Morning Ketone Reading: _____________ WEIGHT: ___________
REFERENCES


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References


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