The Role of the Free Fatty Acid, Lauric Acid, in Appetite Regulation and its Potential as an Appetite-Suppressant

A thesis submitted by
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<tr>
<td>$^{125}$I</td>
<td>125 Iodine</td>
</tr>
<tr>
<td>Apo A-IV</td>
<td>Apolipoprotein A-IV</td>
</tr>
<tr>
<td>APD</td>
<td>Antropyloroduodenal pressure waves</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>C4</td>
<td>Butyric acid, saturated fatty acid with 4 carbon atoms</td>
</tr>
<tr>
<td>C8</td>
<td>Caprylic acid, saturated fatty acid with 8 carbon atoms</td>
</tr>
<tr>
<td>C10</td>
<td>Decanoic acid, saturated fatty acid with 10 carbon atoms</td>
</tr>
<tr>
<td>C12</td>
<td>Lauric acid, saturated fatty acid with 12 carbon atoms</td>
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<tr>
<td>C18:1</td>
<td>Oleic acid, monounsaturated fatty acid with 18 carbon atoms</td>
</tr>
<tr>
<td>C18:2</td>
<td>Linoleic acid, polyunsaturated fatty acid with 18 carbon atoms</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
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<td>GLP-2</td>
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</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
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<tr>
<td>ID</td>
<td>Intruduodenal</td>
</tr>
<tr>
<td>IPPWs</td>
<td>Isolated pyloric pressure waves</td>
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<tr>
<td>L-81</td>
<td>Pluronic L-81</td>
</tr>
<tr>
<td>LOX</td>
<td>Loxiglumide</td>
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<tr>
<td>MMC</td>
<td>Migrating motor complex</td>
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<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
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<td>NPY</td>
<td>Neuropeptide Y</td>
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<td>PP</td>
<td>Pancreatic polypeptide</td>
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<td>PYY</td>
<td>Peptide YY</td>
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<td>PWs</td>
<td>Pressure waves</td>
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<td>Pressure wave sequences</td>
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<td>THL</td>
<td>Tetrahydrolipstatin</td>
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<td>VAS</td>
<td>Visual analogue scale questionnaire</td>
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Thesis summary

The presence of nutrients, particularly fat, in the small intestine modulates gastrointestinal function and subsequent energy intake, and it is well established that the digestion of fat into free fatty acids is required for these effects to occur. Furthermore, the effects of fatty acids are dependent on their chain length. The research presented in this thesis relates to the effects of fatty acids, particularly lauric acid, on the regulation of gastrointestinal function and the suppression of energy intake.

One of the first studies in humans to evaluate the effect of fatty acid chain length established that fatty acids with ≥ 12 carbon atoms slow gastric emptying, while fatty acids with ≤ 10 carbon atoms have no effect, indicating that there may be a separation in the effects of fatty acids occurring between those with ≤ 10 and ≥ 12 carbon atoms. More recent studies in humans have determined that there are marked differences in the effects of intraduodenal lauric acid, a saturated fatty acid with 12 carbon atoms (“C12”), and decanoic acid, a saturated fatty acid with 10 carbon atoms (“C10), on the modulation of gastrointestinal motility, hormone secretion and energy intake. For example, C12, but not C10, markedly suppresses energy intake, modulates pressure waves in the antropyloroduodenal (APD) region and stimulates glucagon-like peptide-1 (GLP-1) secretion, while both C12 and C10 stimulate cholecystokinin (CCK) secretion, however, the effect of C12 was much greater. A previous study in humans has also shown that intraduodenal administration of a long-chain fatty acid, such as oleic acid, a monounsaturated fatty acid with 18 carbon atoms (“C18:1”), suppresses energy intake when compared with a short-chain fatty acid, such as capric acid, a saturated fatty acid with 8 carbon atoms (“C8”). While there have been no direct comparisons between fatty acids with 12 or more carbon atoms (eg C12 vs C18:1) on gastrointestinal function
and energy intake, there is evidence in animals that C12 may be more potent in suppressing energy intake than C18:1.

The first study presented in this thesis (Chapter 4) assessed the effects of intraduodenal C12 and C10 in healthy men on the gastrointestinal hormones; ghrelin, peptide YY (PYY), glucagon-like peptide-2 (GLP-2) and pancreatic polypeptide (PP). C12, but not C10, markedly stimulated the secretion of PYY and GLP-2 and suppressed ghrelin secretion, while both C12 and C10 slightly increased PP secretion.

The effects of intraduodenal C12 and C18:1 delivered at the same energy load (0.4 kcal/min) on APD motility, secretion of CCK and PYY and energy intake were compared in healthy males (Chapter 5). Both C12 and C18:1 stimulated isolated pyloric pressure waves (IPPWs), suppressed the number of antral pressure waves (PWs) and increased plasma CCK concentrations, with no differences between the two fatty acids. In contrast, while both C12 and C18:1 increased basal pyloric pressure and plasma PYY concentrations, C12 had a greater effect on basal pyloric pressure than C18:1, while C18:1 had a greater effect on PYY than C12. Interestingly, C12, but not C18:1, suppressed energy intake.

While a previous study in humans has shown that C12 markedly suppressed energy intake, this was associated with nausea in some subjects, hence, confounding the interpretation of the results. In order to determine whether the effects of C12 on energy intake were physiological, or related to nausea, a dose-response study was performed using loads ranging from: 0.1 – 0.4 kcal/min, but this was also associated with varying C12 concentrations (Chapter 6). C12 potently modulated APD motility, increased
plasma CCK and GLP-1 concentrations and suppressed energy intake in a dose-dependent manner, in the absence of nausea. However, as both load and concentration of the C12 solutions were varied, it was unclear whether these effects were load- or concentration-dependent. Therefore, the study in Chapter 7 assessed the response to (i) increasing loads of C12 (0.2 – 0.4 kcal/min), at a fixed concentration (56 mM) and (ii) increasing concentrations of C12 (40 – 72 mM), at a fixed load (0.4 kcal/min), on gastrointestinal function and energy intake. Increasing load, but not concentration, of C12 modulated gastrointestinal motility, increased plasma CCK and PYY concentrations and suppressed energy intake.

As both CCK and GLP-1 are secreted in response to nutrient ingestion, the study in Chapter 8 assessed whether CCK-8 and GLP-1 interacted in their effects on gastrointestinal function and energy intake. Intravenous CCK-8 (1.8 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min) were administered alone and in combination. At the doses evaluated, CCK-8 suppressed energy intake, decreased the number of antral and duodenal PWs and increased IPPWs, while GLP-1 only decreased antral and duodenal PWs, but had no effect on energy intake and IPPWs. The combination of CCK-8 and GLP-1 only decreased the number of duodenal PWs to a greater extent than either infusion alone, but this did not exceed the sum of the individual effects of CCK-8 and GLP-1.

A previous study has demonstrated that following intragastric administration of C12, the effects of C12 on gastrointestinal function, including suppression of antral contractions, relaxation of the proximal stomach and stimulation CCK secretion, which are associated with the suppression of energy intake, are still maintained. Hence, C12
may have the potential to be utilised as an oral appetite-suppressant. The study in Chapter 9 investigated the effects of increasing doses of orally ingested C12 between 2 – 6 g on appetite and energy intake. While oral ingestion of C12 had no effect on appetite perceptions, subsequent energy intake was markedly suppressed, in the absence of adverse effects, following the ingestion of 2 g and 6 g of C12.

In conclusion, intraduodenal infusion of C12 in humans has marked effects on gastrointestinal function and energy intake, specifically, the modulation of APD motility, secretion of CCK, GLP-1, GLP-2, PYY and PP, and suppression of ghrelin secretion and energy intake, when compared with fatty acids with both shorter and longer chain lengths. The effect of C12 on gastrointestinal function and energy intake is also dependent on load, but not concentration, of C12 administration. Moreover, oral ingestion of C12 also has a marked effect on the suppression of energy intake, in the absence of any adverse effects.
Statement of originality

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

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

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder/s of those works.

Kate Feltrin
December 2007
Dedication

For my wonderful parents, Claude and Judy,

And

For my love, my best-friend, Troy

I am forever grateful
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The studies reported in this thesis were performed in the Discipline of Medicine University of Adelaide, Royal Adelaide Hospital and The Gastrointestinal Investigation Unit, Ward Q7, Royal Adelaide Hospital.

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Publications arising from thesis


Other publications


Chapter 1

Nutrient-regulation of appetite and gastrointestinal function

1.1 Introduction

There are a number of factors, including genetic, environmental and physiological, that contribute together to the acute and chronic regulation of appetite and energy intake. The long-term regulation of appetite and energy intake is influenced by genetic factors, such 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). An example of the heritability of body weight is that siblings that have been adopted by different families, and are living in different environments, have similar body weights, particularly those that are obese (Sorensen et al., 1989).

Some of the environmental factors that affect acute energy intake include, the amount an individual eats at a meal, the palatability of food (Yeomans et al., 2001), the portion size and energy density of meals (Levitsky and Youn, 2004, Kral et al., 2004, Matthiessen et al., 2003) and the time spent eating (Redd and de Castro, 1992). Physiological factors following meal ingestion, particularly changes in gastrointestinal function, play an important role in the acute regulation of appetite and energy intake. The presence of nutrients in the small intestine decreases perceptions of hunger, increases fullness and suppresses subsequent energy intake (Chapman et al., 1999, Cook et al., 1997, Lavin et al., 1996, Welch et al., 1988b). The acute regulation of appetite and energy intake by nutrients appears to be mediated, in part, by the slowing of gastric emptying (Seppele and Read, 1989), the modulation of gastrointestinal motility
(Geliebter et al., 1988, Sturm et al., 2004, Xu et al., 2005, Feinle et al., 2003) and the secretion/suppression of gastrointestinal hormones (Abbott et al., 2005, Matzinger et al., 1999, Turton et al., 1996).

The studies presented in this thesis address the role of postprandial gastrointestinal function in the regulation of appetite and energy intake. Accordingly, this chapter reviews what is known about the effects of nutrients on the modulation of: (i) gastrointestinal motility, specifically pressures in the antrum, pylorus and duodenum, and gastric emptying, and (ii) gastrointestinal hormone secretion, including that of ghrelin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-1), peptide YY (PYY) and pancreatic polypeptide (PP). The impact of energy load and concentration and the region and length of small intestine exposed to nutrients are also discussed. Finally, the role of other peripheral signals, ie adipose tissue and leptin and insulin secretion, in the chronic regulation of appetite, and the mechanisms by which peripheral signals involved in the regulation of appetite and energy intake are integrated in the central nervous system, are reviewed briefly.

1.2 Role of nutrients in the regulation of gastrointestinal motility

The motor activity of the gastrointestinal tract alternates between two distinct patterns – the so-called interdigestive migrating motor complex (MMC) during fasting and a ‘fed motility pattern’ that is initiated by food ingestion (Code and Marlett, 1975, Rees et al., 1982). The following section describes the patterns of motility in specific regions of the gastrointestinal tract, including the stomach, the pyloric sphincter and the small intestine, during fasting, and the changes induced by a meal (Figure 1.1).
**Figure 1.1:** Schematic diagram of the gastrointestinal tract, specifically the regions of the stomach and small intestine

### 1.2.1 Fasting gastrointestinal motility

The MMC is present in the fasting gastrointestinal tract and is in constant cycle until the next meal (Sarna and Otterson, 1988). It consists of three phases, with a total cycle time of ~ 90 – 120 min: phase I is a period of quiescence, which has a duration between 45 – 60 min, phase II consists of irregular phasic contractions, which has a duration between 45 – 90 min and phase III is characterised by powerful coordinated contractions, and lasts for only 5 – 10 min (Kellow et al., 1986, Sarna and Otterson, 1988). The contractions occurring during phase III of the MMC are at the maximal frequency of the electric pacemaker, which is 3 per min in the stomach and 12 per minute in the proximal small intestine (Sarna and Otterson, 1988). The overall pattern of fasting motility is of a propulsive nature, with coordinated waves usually starting in the stomach or duodenum and continuing to the distal small intestine, ensuring that any undigested food remaining in the stomach or small intestine is propelled along the gastrointestinal tract into the colon to be expelled subsequently (Sarna and Otterson,
1988). Once nutrients are ingested fasting motility is altered into a ‘fed’, or postprandial, motility pattern, which aids the digestion and absorption of nutrients.

1.2.2 Postprandial gastrointestinal motility

Postprandial motility patterns include, proximal gastric relaxation (Azpiroz and Malagelada, 1985a, Feinle et al., 1996), the stimulation of tonic and phasic pyloric pressures (Heddle et al., 1989, Heddle et al., 1988a, Houghton et al., 1988, Kumar et al., 1987, Tougas et al., 1992) and a reduction in both antral and duodenal contractions (Heddle et al., 1988a). These changes in gastrointestinal motility, particularly the stimulation of pressure waves isolated to the pylorus (Kumar et al., 1987), underlie the slowing of gastric emptying by nutrients (Heddle et al., 1989). Gastric emptying is regulated so that the flow of nutrients into the duodenum is at a rate that optimises nutrient digestion and absorption. The rate of gastric emptying is highly variable, ranging between ~ 0.4 – 4 kcal/min in healthy humans, and is dependent on the nature (ie liquid or solid), and macronutrient composition, of the food ingested (Brener et al., 1983, Edelbroek et al., 1992, Meyer et al., 1996, Little et al., 2007, Horowitz and Dent, 1991). High nutrient liquids are emptied in an overall linear pattern; in contrast, there is an initial lag phase (usually 20 – 40 min) before the emptying of solids commences, during which solid food is ground into small particles (Horowitz and Dent, 1991).

The interaction of nutrients with chemoreceptors in the small intestine is responsible for the induction of postprandial motility – infusion of nutrients directly into the small intestine changes fasting motility into a postprandial pattern (Heddle et al., 1988a) and slows gastric emptying (Heddle et al., 1989), whereas, intravenous nutrient infusions do not stimulate postprandial motility patterns (Hebuterne et al., 1993). The effect of
intraduodenal nutrients on the modulation of gastrointestinal motility may also be dependent on the type of macronutrient; fat appears to have a more potent effect on proximal gastric relaxation (Azpiroz and Malagelada, 1985a) and slowing of gastric emptying (Kumar et al., 1987, Welch et al., 1988a) than both protein and carbohydrate, and intraduodenal fat stimulates both tonic and phasic pyloric pressures more than carbohydrate (Cook et al., 1997).

1.3 Functions of different regions of the stomach and small intestine

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

1.3.1 Function of the stomach

The proximal and distal regions of the stomach have different functions following meal ingestion. The proximal stomach receives, and stores, the ingested food (Heddle et al., 1989, Houghton et al., 1988), while the distal stomach is responsible for the grinding and mixing of solid food into smaller particles of partially digested food, commonly called ‘chyme’ (Holt et al., 1982). In order for the proximal stomach to receive and store ingested food, two motor responses occur: the first is termed ‘receptive relaxation’, which is initiated by swallowing and lasts for about 20 seconds, and is associated with a decrease in gastric pressure (Azpiroz and Malagelada, 1985b), and this is followed by a prolonged relaxation, known as ‘adaptive relaxation’, which accommodates the increase in intragastric volume following a meal, minimising the increase in intragastric pressure (Azpiroz and Malagelada, 1985b). As food is ground into chyme in the distal stomach, propulsive contractions propel chyme from the
stomach into the small intestine (Pröve and Ehrlein, 1982, Houghton et al., 1988). The propulsive contractions occurring in the antrum are coordinated with pyloric relaxation (Kumar et al., 1987).

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 it may, accordingly, be the most important motor mechanism involved in the regulation of gastric emptying (Anvari et al., 1995, Kumar et al., 1987). The pylorus exhibits both tonic and phasic contractile activity, occurring over a narrow zone (approximately 2 mm), either in isolation or in temporal association with antral contractions, and regulates transpyloric flow (Heddle et al., 1988b, Heddle et al., 1989). Transpyloric flow is predominantly pulsatile, rather than continuous, occurring in episodes that last between 2 – 5 seconds (King et al., 1984, Malbert and Ruckebusch, 1989). While the volume of transpyloric flow is dependent on the timing of pyloric contractions, in relation to the onset of antral contractions (Anvari et al., 1995, Kumar et al., 1987), it is also influenced by intragastric pressure (Anvari et al., 1995).

1.3.3 Function of the small intestine

The primary site of nutrient digestion and absorption 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 (Borgstrom et al., 1957). In order for these functions to occur there is a reduction in the number of small intestinal pressure waves (Heddle et al., 1988a). 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.

1.4 Effect of nutrients on gastrointestinal hormone secretion

Meal ingestion, and the subsequent interaction of nutrients with small intestinal receptors, stimulates the secretion of a number of gastrointestinal hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), peptide YY (PYY) and pancreatic polypeptide (PP) (Lilja et al., 1984, Xiao et al., 1999, Herrmann et al., 1995, Adrian et al., 1976, Adrian et al., 1985, Miazza et al., 1985), while ghrelin secretion is suppressed (Shiiya et al., 2002, Cummings et al., 2001). The following sections discuss how different types of macronutrients modulate the stimulation, or suppression, of gastrointestinal hormones.

1.4.1 Cholecystokinin

CCK is synthesised and secreted from epithelial “I” cells located in the proximal small intestine (Polak et al., 1975, Larsson and Rehfeld, 1978) and is released in response to the presence of nutrients in the small intestine (Liddle et al., 1985, Miazza et al., 1985, Parker et al., 2005). While, the digestive products of all three macronutrients stimulate CCK, fat and protein have a greater effect than carbohydrate (Miazza et al., 1985, Liddle et al., 1985).

1.4.2 Glucagon-like peptides

There are two glucagon-like peptides, GLP-1 and GLP-2, which are both synthesised and co-secreted from enteroendocrine “L” cells located predominantly in the distal
small intestine and large intestine (Eissele et al., 1992, Yusta et al., 2000). Both GLP-1 and GLP-2 are secreted in a biologically active form, but are rapidly degraded into ‘inactive forms’ by dipeptidyl peptidase IV (Kieffer et al., 1995, Brubaker et al., 1997). GLP-1 and GLP-2 are both secreted in response to the presence of nutrients in the small intestine (Orskov et al., 1986); predominantly by small intestinal carbohydrate and fat (Feinle et al., 2002, Lavin et al., 1998, Xiao et al., 1999). Interestingly, as demonstrated recently, ‘sub threshold’ glucose loads (ie 0.1 kcal/min) transiently stimulate GLP-1 within 15 min (Pilichiewicz et al., 2007a, Kuo et al., 2007), which is inconsistent with the concept that GLP-1 secretion is dependent on the direct stimulation of the predominantly distally located “L” cells. – There are a number of possibilities that could explain this early rise in GLP-1 secretion, GLP-1 may be released (i) through a neuroendocrine loop to the distal small intestine, (ii) directly from the limited number of proximally located “L” cells in the duodenum and/or proximal jejunum (Theodorakis et al., 2006), or (iii) due to an initial rapid transit of glucose into the distal small intestine, subsequently inhibited by the release of GLP-1. Further studies are required to evaluate the mechanisms of GLP-1 release.

1.4.3 Peptide YY

PYY is synthesised, and secreted, from “L” cells, which are partly co-localised with GLP-1 secreting cells (Eissele et al., 1992), in the distal small intestine and large intestine (Adrian et al., 1985, Taylor, 1985). PYY is secreted in response to the presence of lipid (Pappas et al., 1986) and protein (Fu-Cheng et al., 1997) in the small intestine, but not carbohydrate (Groger et al., 1997). While PYY is secreted in the form of PYY\(_{(1-36)}\), dipeptidyl peptidase IV rapidly degrades PYY\(_{(1-36)}\) into PYY\(_{(3-36)}\), hence,
there are two forms of circulating PYY, which are present in equal quantities (Grandt et al., 1994).

**1.4.4 Pancreatic polypeptide**

PP is synthesised, and secreted, from endocrine cells in the pancreas (Adrian et al., 1976) in response all three macronutrients; with fat and protein having a greater effect than carbohydrate (Miazza et al., 1985). In contrast to the other gastrointestinal hormones, the secretion of PP is not dependent on small intestinal nutrient stimulation; in humans, distending the stomach with a balloon at 300 and 600 ml, in the absence of nutrients, increases plasma PP concentrations by 17% and 74%, respectively (Koop et al., 1990), hence, gastric distension is probably the most potent stimulus for PP secretion. Moreover, vagal, cholinergic stimulation is also an important regulator of PP secretion in humans (Schwartz et al., 1978).

**1.4.5 Ghrelin**

Ghrelin is synthesised, and secreted, from cells located in the gastric mucosa (Kojima et al., 1999) and, in contrast to CCK, GLP-1 GLP-2, PYY and PP secretion, ghrelin is suppressed, rather than stimulated, by nutrients (Cummings et al., 2004, Cummings et al., 2001). In animals, all three macronutrients suppress ghrelin secretion when infused into the stomach or duodenum (Overduin et al., 2005, Gomez et al., 2004). In humans, oral carbohydrate may have greater effects than fat, which in turn, has a greater effect than protein (Monteleone et al., 2003, Greenman et al., 2004). Interestingly, intravenous glucose, but not lipid, has been shown to suppress ghrelin secretion (Gomez et al., 2004, Shiiya et al., 2002), suggesting that post-absorptive factors play a role in the nutrient-induced suppression of ghrelin.
Although ghrelin is secreted from the stomach, 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 (Parker et al., 2005, Williams et al., 2003, Overduin et al., 2005). For example, the magnitude and duration of ghrelin suppression is equivalent regardless of whether nutrients are infused into the stomach or small intestine (Overduin et al., 2005, Parker et al., 2005), and, in rats, when gastric emptying is prevented using a pyloric cuff, oral glucose has no effect (Williams et al., 2003).

In summary, one of the most important determinants of CCK, GLP-1, GLP-2, PYY, PP and ghrelin secretion, is the interaction of nutrients with chemoreceptors in the small intestine. This nutrient-dependent stimulation, or suppression, of gastrointestinal hormones is, in many cases, also dependent on the type of macronutrient.

1.5 Relationship between appetite and energy intake with gastrointestinal motility

Meal ingestion, and the subsequent interaction of nutrients with chemoreceptors in the small intestine, decreases hunger, increases fullness and reduces subsequent energy intake (Chapman et al., 1999, Cook et al., 1997, Lavin et al., 1996, MacIntosh et al., 2001a). Recent studies support the concept that the associated modulation of gastrointestinal mechanisms, specifically changes in stomach, pylorus and small intestinal motility, may contribute to the effects on appetite and energy intake.

1.5.1 Proximal stomach

Studies in humans indicate that distension of the proximal stomach contributes to the postprandial regulation of appetite and energy intake (Khan et al., 1993, Geliebter et al., 1988). Distension of the proximal stomach with an air-filled balloon at rates between
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20 – 200 ml/min, was associated with increasing perceptions of fullness (Khan et al., 1993). Moreover, the threshold volume for the induction of fullness is less when the rate of distension is slower, indicating that the type of receptors responsible for signalling gastric sensations are also dependent on the rate of meal ingestion (Khan et al., 1993). Distending the proximal stomach with a water-filled balloon at volumes of 400, 600 and 800 ml reduced energy intake in a volume-dependent manner (Geliebter et al., 1988). However, there were limitations associated with both of these studies; a barostat was not used, hence, it is unclear whether the observed effects are due to changes in pressure or volume. Moreover, the region of the stomach distended was not clearly defined, ie balloons were not positioned precisely. While these studies demonstrate 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.

1.5.2 Distal stomach

Studies in humans suggest that distension of the distal stomach following meal ingestion probably plays a more important role than the proximal stomach, in the regulation of appetite (Jones et al., 1997, Santangelo et al., 1998). For example, following the consumption of a nutrient drink, the perception of fullness is closely related to antral area in healthy subjects, such that the greater the antral area, the greater the perception of fullness, while there is no subsequent relationship between sensations of fullness with either total or proximal stomach content (Jones et al., 1997). Furthermore, in healthy young and older subjects, energy intake after a “yoghurt preload” is inversely related to antral area, ie a larger antral area is associated with a greater reduction in energy intake (Sturm et al., 2004) (Figure 1.2).
Figure 1.2: Relationship between energy intake (kcal) and antral area (cm$^2$) at a buffet meal at $t = 70$ min in young ($n = 12$) and older ($n = 12$) subjects who received preloads of water (0 kcal), 250 kcal or 750 kcal. $r = -0.90$, $P < 0.0001$ (from Sturm et al., 2004).

1.5.3 Pylorus

Studies in both animals and humans suggest that an increase in pyloric pressures may contribute to the regulation of energy intake. In dogs, electrical stimulation of the pylorus, increasing both tonic and phasic pyloric pressures, has been reported to be associated with a reduction in energy intake (Xu et al., 2005). Studies in humans also suggest that there is a functional association between the suppression of energy intake with the stimulation of tonic and phasic pyloric pressures (Feltrin et al., 2004). While the data are limited, there is a significant relationship between the magnitude of the suppression of energy intake and the increase in isolated pyloric pressure waves (IPPWs) (Brennan et al., 2007, unpublished observations, Pilichiewicz et al., 2007b) (Figure 1.3). However, a causal association remains to be established.
Figure 1.3: Relationship between energy intake (MJ) and isolated pyloric pressurewaves (IPPWs) following intravenous CCK (0.33, 0.66 and 2 ng/kg/min) in healthy males (n = 10) (from Brennan et al., 2007, unpublished observations).

1.5.4 Small intestine

As the interaction of nutrients with small intestinal chemoreceptors continues for hours after a meal, while gastric distension is relatively transient and diminishes rapidly, it is likely that the stimulation of chemoreceptors by nutrients in the small intestine makes a more important contribution to the regulation of appetite and energy intake than gastric distension (Sepple and Read, 1989, Read et al., 1994). It is well established in humans that infusion of nutrients directly into the duodenum, bypassing the influence of gastric emptying, decreases hunger, increases fullness and suppresses subsequent energy intake (Cook et al., 1997, Chapman et al., 1999, Feinle et al., 2000, Feinle et al., 2003). In contrast, intravenous nutrient administration has no effect on energy intake (Welch et al., 1985). Moreover, the combination of gastric distension with small intestinal nutrient stimulation has synergistic effects to increase fullness (Feinle et al., 1997), and reduce energy intake (Castiglione et al., 1998), which are substantially greater than
those of gastric distension alone, establishing that appetite perceptions and energy intake responses to gastric distension are modified by duodenal nutrients. The modulation of appetite and suppression of energy intake is also dependent on the type of macronutrients present in the small intestine. – While all three macronutrients suppress appetite and reduce subsequent energy intake in both animals and humans, there is evidence that fat is more potent than either carbohydrate or protein (Andrews et al., 1998, Burton-Freeman et al., 1997, Chapman et al., 1999, Cook et al., 1997, Feinle et al., 1997).

In summary, the presence of nutrients within the gastrointestinal tract, specifically the stomach and small intestine, has an important role in the regulation of appetite and energy intake. While distension of the proximal and distal stomach may reduce energy intake, the dominant factor appears to be the interaction of nutrients with chemoreceptors in the small intestine.

1.6 The role of gastrointestinal hormones in mediating the effects of nutrients on gastrointestinal motility and energy intake

There is substantial evidence that gastrointestinal hormones mediate, in part, the effects of nutrients to modulate gastrointestinal motility and suppress energy intake (Abbott et al., 2005, Fried et al., 1991a, Lieverse et al., 1994, Schirra et al., 2006). The following sections discuss the roles of CCK, GLP-1, GLP-2, PYY, PP and ghrelin in the regulation of gastrointestinal motor function and energy intake.
1.6.1 Cholecystokinin

It is well established that exogenous administration of CCK, usually administered as CCK-8, modulates gastrointestinal motility and energy intake (Liddle et al., 1989, Fried et al., 1991a, Rayner et al., 2000, Kissileff et al., 1981). Moreover, studies employing specific CCK₁ receptor antagonists, such as loxiglumide, have demonstrated that CCK plays a physiological role in the regulation of postprandial gastrointestinal motility and energy intake (Fried et al., 1991a, Fried et al., 1991b, Lieverse et al., 1994, Matzinger et al., 2000).

Effects of CCK on gastrointestinal function

Exogenous administration of CCK mimics the effects of nutrients on gastrointestinal function. For example, in humans intravenous CCK-8 stimulates gallbladder and pancreatic secretion (Liddle et al., 1989), slows gastric emptying (Valenzuela and Defilippi, 1981, Fried et al., 1991a), decreases antral and duodenal pressure waves and stimulates isolated pyloric pressure waves (Fraser et al., 1993, Rayner et al., 2000). Studies in both animals and humans, using the CCK₁ receptor antagonist, loxiglumide, have established that endogenous CCK has a physiological effect on gastrointestinal function. For example, the slowing of gastric emptying (Fried et al., 1991a, Fried et al., 1991b) and stimulation of gallbladder contraction (Meyer et al., 1989) induced by a meal are attenuated by concurrent intravenous administration of loxiglumide. Hence, it is clear that CCK mediates, at least in part, the effect of nutrients on gastrointestinal function.
Effects of CCK on appetite and energy intake

As is the case with gastrointestinal motor function, exogenous administration of CCK mimics the effects of nutrients on appetite and energy intake, so that subsequent energy intake and hunger are reduced, and fullness increased (Kissileff et al., 1981, Gutzwiller et al., 2000, MacIntosh et al., 2001b). Furthermore, in healthy subjects the suppression of energy intake (Lieverse et al., 1994, Matzinger et al., 2000, Matzinger et al., 1999) and the perception of fullness (Feinle et al., 1996), induced by intraduodenal lipid, are completely abolished by loxiglumide. Moreover, intravenous administration of loxiglumide increases both energy intake and sensations of hunger (Beglinger et al., 2001) (Figure 1.4). Hence, CCK is a physiological regulator of appetite, acting via CCK1-receptor mechanisms.

NOTE: This figure is included on page 16 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.4: Effect of intraduodenal (ID) saline or fat □ the specific CCK1 receptor antagonist, loxiglumide (LOX), on energy intake. ID fat + LOX attenuates the effect of lipid on the suppression of energy intake. * P < 0.05 vs ID fat + IV saline (from Matzinger et al, 1999).
1.6.2 Glucagon-like peptides (GLP-1 and GLP-2)

While exogenous administration of GLP-1 has been shown to modulate gastrointestinal function, appetite and energy intake, few studies have administered the specific GLP-1 receptor antagonist exendin(9–39) to determine its physiological role. There is currently no GLP-2 receptor antagonist available for use in humans, so the physiological role of GLP-2 in the modulation of gastrointestinal function and energy intake is unknown, although in animals exogenous GLP-2 has been shown to affect both gastrointestinal motility (Wojdemann et al., 1998) and energy intake (Tang-Christensen et al., 2000).

Effects of GLP-1 and GLP-2 on gastrointestinal function

Exogenous GLP-1 mimics the effects of nutrients on gastrointestinal function, indirectly suggesting that GLP-1, in part, mediates the effects of nutrients. Intravenous GLP-1 slows gastric emptying (Schirra et al., 1997, Nauck et al., 1997, Little et al., 2006b, Delgado-Aros et al., 2002), relaxes the proximal stomach (Schirra et al., 2000), increases meal retention in the distal stomach (Little et al., 2006b), suppresses antral and duodenal pressure waves (Schirra et al., 2000) and stimulates tonic and phasic pyloric pressures (Schirra et al., 2000). A recent study in humans suggests that endogenous GLP-1 plays a physiological role in mediating the effects of nutrients on APD motility (Schirra et al., 2006). – The administration of exendin (9-39) attenuated the effects of intraduodenal glucose to stimulate pancreatic secretion, stimulate tonic and phasic pyloric motility and suppress antral and duodenal pressure waves (Schirra et al., 2006).
Studies in humans have shown that intravenous GLP-2 has the capacity to enhance nutrient absorption (Jeppesen et al., 2001) and, in animals, GLP-2 promotes intestinal cell growth (Drucker et al., 1996) and mimics the effects of nutrients to inhibit antral motility (Wojdemann et al., 1998). In contrast, exogenous GLP-2 in “physiological” doses appears to have no effect on gastrointestinal motility in humans (Schmidt et al., 2003), suggesting that there may be species differences in the effects of GLP-2 on gastrointestinal function.

**Effects of GLP-1 and GLP-2 on appetite and energy intake**

Exogenous GLP-1 has been reported to suppress energy intake and modulate appetite sensations in humans (Flint et al., 2001, Näslund et al., 1999, Flint et al., 1998, Gutzwiller et al., 1999), although observations are inconsistent (Long et al., 1999), and accordingly the anorexigenic effect of exogenous GLP-1, even in pharmacological doses is somewhat controversial (Verdich et al., 2001). In rats, exendin(9-39) more than doubled food intake (Turton et al., 1996), strongly suggesting that, at least in animals, GLP-1 plays a physiological role in the regulation of appetite and energy intake. However, as no studies in humans have assessed the effect of GLP-1 receptor antagonism on energy intake, the role of endogenous GLP-1 in the regulation of energy intake in humans remains uncertain.

The effect of exogenous GLP-2 on energy intake is also unclear; in rats intracerebroventricular (Tang-Christensen et al., 2000), but not peripheral (Scott et al., 1998), administration of GLP-2 has been reported to suppress energy intake, however, in humans intravenous GLP-2 appears to have no effect on energy intake (Schmidt et al., 2003, Sorensen et al., 2003). Until studies have assessed the effects of a GLP-2
receptor antagonist, the effect of endogenous GLP-2 on energy intake will remain uncertain.

1.6.3 Peptide YY

Exogenous PYY\textsubscript{(3-36)} has been shown to modulate gastrointestinal function and energy intake in both animals and humans, and studies in animals indicate that endogenous PYY is involved in the regulation of energy intake.

**Effects of PYY on gastrointestinal function**

In humans, exogenous PYY\textsubscript{(3-36)} slows gastric emptying (Allen et al., 1984, Savage et al., 1987) and mouth-to-caecum transit time in a dose-related manner (Savage et al., 1987). Similarly, in rats exogenous PYY\textsubscript{(3-36)} slows the transit of lipid in the distal small intestine (Lin et al., 1996). These data suggest that PYY\textsubscript{(3-36)} may be involved in mediating the effects of nutrients on gastrointestinal function. Furthermore, in animals, exogenous PYY\textsubscript{(3-36)} stimulates the secretion of lymphatic apolipoprotein A-IV (Kalogeris et al., 1998), which as discussed (Chapter 2.5) is secreted in response to lipid (Kalogeris et al., 1996), suggesting that PYY may be an endocrine mediator of the effects of lipid present in the distal gut on the production and release of apolipoprotein A-IV. Moreover, the presence of lipid in the proximal small intestine stimulates PYY secretion, in both animals and humans, and concurrent administration of a CCK\textsubscript{1} receptor antagonist abolishes this effect (Degen et al., 2007, Lin et al., 2000). This indicates that CCK serves as a foregut signal, linking fat in the proximal gut with the release of PYY from the distal small intestine. While PYY is a known agonist of the Y2 receptor (Keire et al., 2000), the effect of endogenous PYY on gastrointestinal function has hitherto not been evaluated.
Effects of PYY on appetite and energy intake

While studies in humans have reported that exogenous PYY\textsubscript{(3-36)} has the capacity to suppress energy intake (Batterham et al., 2002, Batterham et al., 2003a, Neary et al., 2005), a physiological role remains uncertain. Other studies have either failed to demonstrate any effect of PYY on energy intake in rats (Tschop et al., 2004), or that pharmacological, but not ‘physiological’, concentrations of PYY\textsubscript{(3-36)} suppress energy intake in humans (Degen et al., 2005). Moreover, in the study conducted by Degen et al (Degen et al., 2005), the pharmacological dose of PYY\textsubscript{(3-36)} also induced nausea, suggesting that the suppression of energy intake by PYY\textsubscript{(3-36)} may represent an adverse effect due to the induction of nausea. However, a recent study in rats has effectively shown that the anorexigenic effect of PYY\textsubscript{(3-36)} is inhibited by a PYY receptor antagonist, and that the central administration of a PYY receptor antagonist increases energy intake (Abbott et al., 2005), indicating that PYY does have a physiological role to suppress energy intake. Until studies in humans assess the effect of a PYY receptor antagonist on energy intake following nutrient ingestion, the physiological role of PYY in the regulation of energy intake in humans will remain uncertain.

1.6.4 Pancreatic polypeptide

Exogenous administration of PP in both animals and humans has been reported to modify gastrointestinal function and suppress energy intake. Currently there is no PP receptor antagonist available for use in humans.

Effects of PP on gastrointestinal function

Exogenous PP has a number of effects on gastrointestinal function in both animals and humans; pancreatic enzyme and bicarbonate secretion and biliary output is inhibited
(Adrian et al., 1979, Funakoshi et al., 1988, Greenberg et al., 1979, Beglinger et al., 1984) and gallbladder filling is increased (Conter et al., 1985). Moreover, in dogs, exogenous PP converts fasting motility into postprandial motility (Hall et al., 1983), and in rodents and humans exogenous PP slows gastric emptying in a dose-related manner (Asakawa et al., 2003, Schmidt et al., 2005). Therefore, exogenous PP mimics the effects of nutrients on gastrointestinal motility and gastric emptying. No studies have, hitherto, assessed the effect of a PP receptor antagonist on gastrointestinal motor function, hence, the role of endogenous PP in mediating the effects of nutrients on gastrointestinal function remains uncertain.

**Effects of PP on appetite and energy intake**

While there are only a limited number of studies that have investigated the effect of exogenous PP on appetite and energy intake, in both animals and humans, it is clear that PP has an anorexigenic effect. For example, in rats, intraperitoneal infusion of PP is a potent suppressant of subsequent energy intake (Asakawa et al., 2003); similarly, in humans, exogenous PP suppresses both hunger and energy intake at a subsequent meal (Jesudason et al., 2007, Batterham et al., 2003b). An indirect comparison of two human studies suggests that the effect of PP on energy intake are dose-related (Batterham et al., 2003b, Jesudason et al., 2007), however, in both studies the plasma PP concentrations were supraphysiological, possibly inducing nausea. Furthermore, no studies have assessed the effect of a PP receptor antagonist on energy intake, hence, a role for endogenous PP in the regulation of energy intake remains to be established.
1.6.5 Ghrelin

Exogenous administration of ghrelin affects gastrointestinal function and energy intake, and in contrast to the other gastrointestinal hormones, ghrelin probably plays a dominant role in the regulation of fasting gastrointestinal motor function and meal initiation.

Effects of ghrelin on gastrointestinal function

In both animals and humans, exogenous ghrelin stimulates gastric motility in a dose-dependent manner (Masuda et al., 2000, Tack et al., 2006) and induces phase III activity in the stomach (Tack et al., 2006). In both humans and mice, gastric emptying is accelerated in response to exogenous ghrelin administration (Levin et al., 2006, Dornonville de la Cour et al., 2004). However, as discussed (section 1.4.5), ghrelin secretion is suppressed following a meal (Monteleone et al., 2003, Greenman et al., 2004), the relevance of the effects of ghrelin on gastric emptying is unclear. Until the effects of a ghrelin receptor antagonist has been evaluated, the role of endogenous ghrelin in the regulation of gastrointestinal function will remain uncertain.

Effects of ghrelin on appetite and energy intake

In contrast to the other fore-mentioned gastrointestinal hormones, ghrelin has an orexigenic effect, such that, in humans exogenous, acute, administration of ghrelin decreases fullness and increases hunger and energy intake at a subsequent meal (Levin et al., 2006, Wren et al., 2001a). In rats, chronic ghrelin administration (7 days) has been shown to increase body weight (Wren et al., 2001b). Again, the effects of a ghrelin antagonist have not been evaluated.
In summary, exogenous administration of gastrointestinal hormones, including CCK, GLP-1, GLP-2, PYY and PP mimic the effects of small intestinal nutrients on gastrointestinal function and energy intake, while exogenous ghrelin affects fasting motility and meal initiation. Currently, a physiological role on gastrointestinal function has only been clearly established in humans for CCK, although there is strong evidence from one study that endogenous GLP-1 affects gastric motility. Further studies are required to determine the physiological role of GLP-1, GLP-2, PYY, PP and ghrelin in the regulation of gastrointestinal function and energy intake.

1.7 Role of region and length of small intestinal nutrient exposure on gastrointestinal function and energy intake

As discussed, the interaction of nutrients with chemoreceptors in the small intestine is essential for the regulation of gastrointestinal function (sections 1.2.2) and energy intake (sections 1.5.4). Studies in both animals and humans have shown that both the region and length of small intestinal nutrient exposure are important in mediating these effects (Aponte et al., 1985, Chaikomin, et al., unpublished observations, Lin et al., 1989, Lin et al., 1990, Meyer et al., 1998b, Meyer et al., 1998c, Little et al., 2006a).

1.7.1 Effects of the length of small intestinal nutrient exposure

The length of small intestinal nutrient contact modifies the effects of nutrients on gastrointestinal function and energy intake, presumably by influencing the number of receptors exposed to nutrients. In dogs, the slowing of gastric emptying is greater as the length of small intestine exposed to fat or glucose is increased (Lin et al., 1990, Lin et al., 1989). For example, exposure of the entire small intestine to nutrients had a greater effect than when exposure was limited to the first 65 cm of the small intestine, which in
turn, had a greater effect than exposure to only the first 15 cm of small intestine (Lin et al., 1990, Lin et al., 1989) (Figure 1.5). Similarly, in humans, when glucose was allowed access to the entire small intestine antral pressure waves were suppressed more than when exposure was limited to the first 60 cm of the small intestine (Little et al., 2006a).

NOTE: This figure is included on page 24 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.5: The effect of 1.0 M glucose infused into the first 15 cm, 65 cm, or the entire, small intestine or a control saline solution on the rate of gastric emptying of a saline control meal in dogs (n = 17). The slowing of gastric emptying is greater as the length of small intestine exposed to glucose is increased (from Lin et al, 1989).

In dogs it has been reported that, PYY secretion is stimulated when fat was exposed to the entire length of the small intestine, but not the proximal small intestine alone (Aponte et al., 1985). Similarly, in humans, when glucose was allowed access to the entire small intestine plasma concentrations of GLP-1 were increased and plasma ghrelin concentrations were suppressed more than when glucose exposure was restricted to the first 60 cm (Little et al., 2006a). Moreover, in dogs, the magnitude of CCK and
PYY response is greater when fat is exposed to both the proximal, and distal, small intestine simultaneously, as opposed to each region alone (Lin and Chey, 2003).

As with gastrointestinal motor function, energy intake is also dependent on the length of the small intestine exposed to nutrients. In rats, when the entire small intestine is exposed to fat or carbohydrate, there is a greater suppression of subsequent energy intake, than when shorter segments of the small intestine are exposed (Meyer et al., 1998c, Meyer et al., 1998b). Moreover, when both the proximal, and distal, regions of the small intestine were exposed to nutrients, the suppression of energy intake was greater than with either region alone (Meyer et al., 1998c).

### 1.7.2 Effects of the region of small intestinal nutrient exposure

The region of small intestine exposed to nutrients has been shown to be an important determinant of effects on gastrointestinal motility, hormone secretion and energy intake (Chaikomin et al., unpublished observations, Lin et al., 1990, Lin and Chey, 2003, Aponte et al., 1985, Meyer et al., 1998b, Welch et al., 1988b). Infusion of nutrients into the proximal small intestine may induce gastric relaxation (Azpiroz and Malagelada, 1985a) and slow gastric emptying (Lin et al., 1990) to a greater extent than when the distal small intestine is exposed to nutrients, suggesting that exposure of the proximal small intestine to nutrients may be more important for the regulation of gastric tone and emptying, than that of the distal small intestine. The region of small intestinal nutrient exposure also modifies gastrointestinal hormone secretion (Aponte et al., 1985, Chaikomin et al., unpublished observations), so that in humans glucose infused into the duodenum increases CCK secretion more than infusion into the mid-jejunum (Chaikomin et al., unpublished observations). In rats PYY is only secreted when fat is
present in the distal, but not the proximal, small intestine (Aponte et al., 1985). This region-dependent effect of small intestinal nutrient exposure is, at least in part, a reflection of the location of hormone secreting cells, as CCK secreting cells are located predominantly in the proximal small intestine (Polak et al., 1975) (section 1.4.1), while PYY secreting cells are located in the distal small intestine and colon (Adrian et al., 1985) (section 1.4.3).

The region of small intestinal nutrient exposure also affects subsequent energy intake. In animals and humans, nutrients infused into the duodenum inhibit energy intake to a greater extent than when infused into the mid-jejunum (Chaikomin et al., unpublished, Meyer et al., 1998b). Similarly, in humans, fat infused directly into the jejunum has been shown to suppress energy intake to a greater extent than direct infusion of fat into the ileum (Welch et al., 1988b).

1.8 Role of energy load and concentration of nutrients delivered into the small intestine

In both animals and humans the effects of nutrients on gastrointestinal function and energy intake are modified by the energy load and concentration of nutrients delivered into the small intestine. The load of nutrients, independent of concentration, modifies some gastrointestinal responses, while other responses are modified by the concentration, independent of load, and some responses are modified by both load and concentration.

1.8.1 Physiological responses dependent on load

Increasing the load of nutrients in the small intestine increases the length of small intestine exposed to nutrients, which, as discussed previously, leads to the stimulation
of a greater number of small intestinal receptors (Meyer et al., 1998c). In animals, the secretion of pancreatic enzymes stimulated by hydrogen ions (Meyer et al., 1970b), L-phenylalanine (Meyer et al., 1976) and oleic acid (Meyer and Jones, 1974), have all been shown to be dependent on the small intestinal nutrient load, independent of concentration. In dogs, the slowing of gastric emptying induced by the presence of glucose in the small intestine appears to be dependent only on the load of glucose, independent of concentration, when infused at concentrations between 250 – 1000 mM (Lin et al., 1989). Studies in humans have also demonstrated that the rate of gastric emptying of glucose is dependent on the load of glucose administered, independent of concentration, such that the higher the energy load the greater the slowing of gastric emptying (Brener et al., 1983, Hunt et al., 1985). Similarly, in humans, increasing loads of glucose, independent of concentration, have a load-dependent effect on gastrointestinal function and energy intake, such that the greater the load, the greater the stimulation of basal pyloric pressure, secretion of CCK and GLP-1, reduction of duodenal pressure waves and suppression of energy intake. Studies in humans have also reported that increasing loads of intraduodenal lipid between 1.33 – 4 kcal/min, at a constant concentration have a load-dependent effect on the stimulation of pyloric pressures and the secretion of CCK and PYY, such that the greater the load, the greater the stimulation of pyloric pressures and the secretion of both CCK and PYY (Pilichiewicz et al., 2005). Accordingly, it is clear that in both animals and humans the nutrient load modifies the effects of nutrients on a number of gastrointestinal responses.

1.8.2 Physiological responses dependent on concentration

Animal studies have demonstrated that some physiological responses are modified by the nutrient concentration, independent of load. The stimulation of pancreatic
bicarbonate secretion by fatty acids is greater with increasing concentrations, at a constant load, while there is no effect of increasing loads, at a constant concentration (Meyer and Jones, 1974). While it has been established that the slowing of gastric emptying by intraduodenal glucose in animals and humans is primarily dependent on the load (Brener et al., 1983, Hunt et al., 1985, Lin et al., 1989), in rats, gastric emptying is slowed when fatty acids are infused into a segment of small intestine at 27 mM, but not when infused at 3 mM (Lin et al., 1990). In contrast to the effects of glucose, this study indicates that a relationship between the load of fatty acids, particularly on gastric emptying, is only evident when fatty acids are delivered into the small intestine above a certain concentration threshold.

1.8.3 Physiological responses dependent on both load and concentration

While some physiological responses are modified by the load or concentration of nutrients, independent of one another, the effect of fatty acids to suppress energy intake in rats has been shown to be dependent on both the load and concentration (Meyer et al., 1998b). Increasing the load of fatty acids infused into the small intestine (3, 6 and 12 ml/h), at a constant concentration (80 mM), produced a load-dependent suppression of energy intake (Meyer et al., 1998b). Similarly, increasing concentrations of fatty acids (20, 40 and 80 mM), at a constant load (12 ml/h), produced a concentration-dependent suppression of energy intake (Meyer et al., 1998b).

These studies establish that the load and concentration of nutrient delivery in the small intestine modifies the effect of nutrients on gastrointestinal function and energy intake.
1.9 Interactions between stimuli within the gastrointestinal tract on appetite and energy intake

As the ingestion of a meal triggers a number of gastrointestinal mechanisms involved in energy intake, including gastric distension, stimulation of small intestinal receptors and the release, or suppression, of gastrointestinal hormones, within approximately 15 min from meal ingestion (Feltrin et al., 2004, Jansen et al., 1994), there is the potential for these factors to interact, potentially enhancing their effects on appetite and energy intake (Feinle et al., 1997, Castiglione et al., 1998, Pappas et al., 1989, Kissileff et al., 2003, Gutzwiller et al., 2000).

1.9.1 Interactions between gastric distension with small intestinal nutrients

While the stimulus of gastric distension alone, as achieved by inflating a balloon in the stomach, causes a sensation of epigastric pressure, the concurrent infusion of lipid, or carbohydrate, into the small intestine, at a rate similar to normal gastric emptying (~ 2 kcal/min), results in a more ‘meal-like’ fullness (Feinle et al., 1997). The combination of gastric distension and intraduodenal nutrients also suppresses energy intake more than gastric distension alone in both animals and humans (Castiglione et al., 1998, Pappas et al., 1989). 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 Interaction between gastric distension and CCK

In humans, the combination of gastric distension (300 ml distilled water in a balloon) and intravenous CCK-8 (112 ng/ml for 23 min) reduced subsequent energy intake by 200 g, which is substantially greater than the sum of the effects of either CCK (96 g), or
distension (3 g), alone (Kissileff et al., 2003), indicating that gastric distension and intravenous CCK interact synergistically to suppress energy intake.

1.9.3 Interaction between small intestinal nutrients and CCK

In healthy males, the combination of a 400 ml banana shake ‘pre-load’ and intravenous CCK-8 increased sensations of fullness and decreased hunger, and suppressed subsequent energy intake (421 g) to a greater extent, than when the ‘pre-load’ was administered alone (645 g) (Gutzwiller et al., 2000). Here, 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 Interaction between CCK and ghrelin

When an intraperitoneal injection of CCK is administered simultaneously with ghrelin, the orexigenic effect of ghrelin is attenuated (Kobelt et al., 2005), suggesting that CCK may modify the action of ghrelin.

1.9.5 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). 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.
A recent study investigated the effects of combined intravenous infusions of CCK (0.2 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min) on appetite sensations and energy intake in healthy subjects (Gutzwiller et al., 2004) and demonstrated that the combined infusions of CCK and GLP-1 had a synergistic effect to decrease hunger. That infusion of CCK and GLP-1 in combination did not have a synergistic, or additive, effect to decrease energy intake, was, accordingly, unexpected (Figure 1.6). This study did not evaluate the effects of the combination of CCK and GLP-1 on gastroduodenal motility, which remains to be determined (Chapter 8).

**Figure 1.6:** Scores for hunger (A) and energy intake (B) following intravenous CCK-33, GLP-1, GLP-1 + CCK-33 and placebo (saline) in 24 healthy males. There was a synergistic effect of GLP-1 + CCK-33 to decrease hunger, but not energy intake; all three infusions decreased energy intake to the same extent. * vs placebo (P < 0.05) (from Gutzwiller et al, 2004).

**1.10 Role of adipose tissue in the chronic regulation of appetite and energy intake**

While appetite and energy intake are affected acutely by signals arising from the gastrointestinal tract, the longer-term regulation of energy intake and, hence, body weight, appear to be regulated predominantly by two hormones that are secreted in proportion to the mass of adipose tissue: (i) insulin, secreted by pancreatic beta cells
and (ii) leptin, secreted by white adipose tissue (Woods et al., 1974, Woods et al., 2003). There is evidence that both hormones play fundamental roles in long-term energy balance and body weight maintenance (Zhang et al., 1994, Woods et al., 1979). For example, chronic, central insulin administration suppresses energy intake and body weight in baboons in a dose-dependent manner, such that the higher the dose of insulin, the greater the suppression of energy intake and body weight (Woods et al., 1979). Similarly, animals and humans with mutations causing leptin deficiency have marked hyperphagia (compulsive overeating) and severe obesity (Montague et al., 1997, Zhang et al., 1994). Treatment with leptin injections induces weight loss, such that in ob/ob mice ~ 40% of their body weight is lost after 33 days of treatment (Halaas et al., 1995) and in a human patient, ~ 17% of their initial body weight was lost following 12 months of treatment (Farooqi et al., 1999).

1.11 Role of the central nervous system in the regulation of appetite and energy intake

Peripheral signals involved in the regulation of appetite and energy intake, including those arising from the gastrointestinal tract and those related to adipose tissue, are all integrated in the central nervous system (Figure 1.7). Distension of the stomach following a meal 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, and the stimulation of these receptors activates vagal afferents, which signal information to the brain, specifically the hypothalamus and brainstem (Andrews et al., 1980, Barber and Burks, 1983, Melone, 1986, Davison, 1972). Gastrointestinal hormones stimulate appetite centres of the hypothalamus, particularly the arcuate nucleus, either directly (Turton et al., 1996, Blevins et al.,
2000), or via vagal afferents (Blackshaw and Grundy, 1990, Garlicki et al., 1990).
Similarly, leptin (Elias et al., 1999) and insulin (Corp et al., 1986) activate the accurate nucleus of the hypothalamus directly. These peripheral signals modify the activity of two populations of neurons within the arcuate nucleus. One population co-expresses cocaine- and amphetamine-related transcript and pro-opiomelanocortin, which act to inhibit energy intake (Batterham et al., 2002, Fan et al., 2004) and the second population of neurons increases energy intake, and co-expresses neuropeptide Y (Asakawa et al., 2001). Accordingly, the acute and chronic regulation of appetite and energy intake involves the integration of signals arising from the gastrointestinal tract and adipose tissue in the central nervous system, particularly the hypothalamus.

NOTE: This figure is included on page 33 of the print copy of the thesis held in the University of Adelaide Library.

**Figure 1.7:** Overview of the peripheral and central sites involved in the regulation of appetite and energy intake (from Badman and Flier 2005).
1.12 Summary

This chapter has reviewed the role of postprandial gastrointestinal function, specifically the modulation of gastrointestinal motility and hormone secretion, and their interactions, in the acute regulation of appetite and energy intake. This chapter has also briefly discussed the involvement of other peripheral factors, ie insulin and leptin, in the long-term regulation of appetite and how these peripheral signals are integrated in the central nervous system for the acute and chronic regulation of appetite and energy intake. The study described in Chapter 8 has addressed the following hypothesis arising from this review: the effects of intravenous CCK-8 and GLP-1 on energy intake and antropyloroduodenal motility would be greater when the two peptides are administered together than either peptide alone.
Chapter 2

The effects of fatty acids on gastrointestinal function and energy intake

2.1 Introduction

The effects of fat on gastrointestinal function and energy intake are dependent on the digestion of triglycerides to free fatty acids. For example, in both humans and animals fat digestion is required for the slowing of gastric emptying (Pilichiewicz et al., 2003), the modulation of gastrointestinal motility (Feinle et al., 2003), secretion of pancreatic enzymes, secretin, CCK, GLP-1, PYY and PP (Meyer and Jones, 1974, Feinle et al., 2003, Feinle-Bisset et al., 2005), suppression of ghrelin secretion (Feinle-Bisset et al., 2005), and the suppression of appetite and energy intake (Feinle et al., 2003). Studies in both animals and humans have demonstrated that gastrointestinal responses to fatty acids are dependent on their chain length, such that fatty acids with a chain length of $\geq$ 12 carbon atoms have a greater effect on gastrointestinal function and energy intake than fatty acids with $\leq$ 10 carbon atoms (Matzinger et al., 2000, McLaughlin et al., 1999, Hunt and Knox, 1968, Feltrin et al., 2004).

This chapter reviews current knowledge relating to the importance of fat digestion and the effects of fatty acid chain length on gastrointestinal function and energy intake, and the impact of load and concentration of fatty acids on these effects. The role of absorptive pathways, post-absorptive secretions and vagal afferent stimulation, in mediating the effects of fatty acids on gastrointestinal function and energy intake are
discussed. Non-physiological/non-specific factors, including putative cytotoxic effects of fatty acids on small intestinal mucosa, which have the potential to suppress energy intake are also reviewed.

2.2 The importance of fat digestion

While dietary lipids are commonly ingested in the form of triglycerides, there is compelling evidence that their digestive products, free fatty acids, are primarily responsible for the gastrointestinal effects of lipid. This has been determined by using lipase inhibitors (tetrahydrolipstatin (THL) or orlistat), which block the digestion of fat into fatty acids, and thereby abolish small intestinal fat absorption. A high-fat meal normally empties from the stomach slowly; following the oral administration of a lipase inhibitor, resulting in ~ 30% fat malabsorption, gastric emptying is accelerated significantly (Borovicka et al., 2000, Pilichiewicz et al., 2003). Similarly, the effects of intraduodenal triglyceride to induce proximal gastric relaxation (Feinle et al., 2001) stimulate isolated pyloric pressure waves and suppress antral and duodenal pressure waves (Feinle et al., 2003), are essentially abolished when fat digestion is inhibited by concomitant administration of the lipase inhibitor, THL, which results in ~ 80% lipase inhibition, and apparently fasting motility is observed (Feinle et al., 2003) (Figure 2.1). Lipase inhibition also abolishes the effects of intraduodenal triglycerides to stimulate plasma CCK, GLP-1, PP and PYY, and suppress plasma ghrelin, concentrations (Feinle et al., 2003, Feinle et al., 2001, Feinle-Bisset et al., 2005). Moreover, considering that the suppression of energy intake by enteral fat is mediated, at least in part, by changes in gastrointestinal function, it is not surprising that subsequent energy intake is increased when fat digestion is inhibited (Feinle et al., 2003, O'Donovan et al., 2003).
Figure 2.1: Example of antropyloroduodenal pressure patterns during duodenal infusion of a triglyceride emulsion without (FAT; A), and with, 120 mg of the lipase inhibitor THL (FAT-THL; B). A: infusion of FAT results in a “fed” motor pattern, characterised by isolated pyloric (P) pressure waves and inhibition of antral (A) and duodenal (D) phasic pressures. B: in contrast, when fat digestion was inhibited, there was pronounced propulsive antropyloroduodenal pressure activity (from Feinle et al., 2003).

For example, in healthy subjects, the addition of the lipase inhibitor, orlistat, to a highfat yoghurt (O'Donovan et al., 2003), or a duodenal lipid infusion (Feinle et al., 2003), increases subsequent energy intake. In the latter study, energy intake after a 90 min intraduodenal triglyceride infusion was 1237 ± 104 kcal without THL and 1433 ± 86 kcal with THL (120 mg). Hence, there is unequivocal evidence that fatty acids play an important role in mediating the effects of dietary fat on gastrointestinal function, including gastric emptying, motility in the antropyloroduodenal (APD) region and gastrointestinal hormone secretion, as well as energy intake.
2.3 Effect of fatty acids on gastrointestinal motility, hormone secretion and energy intake

Gastrointestinal responses to fat are not only dependent on its digestion into fatty acids, but also the chain length of fatty acids. Studies, in both animals and humans, have demonstrated that fatty acids with a chain length of ≥ 12 carbon atoms have a greater effect on gastrointestinal function and to suppress energy intake when compared with fatty acids with ≤ 10 carbon atoms. The following discussion focuses on current information relating to the effects of fatty acids with different chain lengths on gastrointestinal function (motility, pancreas, gallbladder and hormone secretion) and energy intake.

2.3.1 Effect of fatty acids on gastroduodenal motility

One of the first studies in humans to demonstrate that the slowing of gastric emptying by fat was dependent on fatty acid chain length, was reported by Hunt and Knox in 1968 (Hunt and Knox, 1968). Intragastric fatty acids with ≥ 12 carbon atoms emptied from the stomach more slowly than fatty acids with ≤ 10 carbon atoms, indicating that there is a ‘separation’ in the effects of fatty acids of 10 and 12 carbon atoms (Figure 2.2). Many years following these observations, McLaughlin and colleagues (McLaughlin et al., 1999) determined in humans that lauric acid, a saturated fatty acid with 12 carbon atoms (“C12”), when infused into the stomach, relaxed the fundus and reduced the amplitude of antral contractions, as measured by ultrasonography, to a greater extent than fatty acids with ≤ 11 carbon atoms. Similarly, a more recent study in healthy men demonstrated that intraduodenal administration of C12 stimulates pyloric motility and suppresses both antral and duodenal pressure waves, to a greater extent than decanoic acid, a saturated fatty acid with 10 carbon atoms (“C10”) (Feltrin et al.,
2004) which, as discussed in Chapter 1.2, are motor mechanisms that underlie the normal slowing of gastric emptying (Heddle et al., 1989). These studies have, thus, established in humans that fatty acid chain length is an important determinant of the effects of fatty acids in the regulation of gastrointestinal motility, specifically that fatty acids with ≥ 12 carbon atoms modulate antropyloroduodenal motility more than fatty acids with ≤ 10 carbon atoms.

![Figure 2.2:](image)

Figure 2.2: The relation between chain length of fatty acids and their effectiveness in slowing gastric emptying. ● number of subjects (adapted from Hunt & Knox, 1968).

### 2.3.2 Effect of fatty acids on pancreatic and gallbladder function

Fatty acid chain length also influences pancreatic and gallbladder function in humans (Malagelada et al., 1976, Isaacs et al., 1987). For example, intraduodenal infusion of octanoic acid, a saturated fatty acid with 8 carbon atoms (“C8”), C12 and oleic acid, a monounsaturated fatty acid with 18 carbon atoms (“C18:1”), all stimulate pancreatic enzyme secretion, with C18:1 being more potent than C12, and both C12 and C18:1 having a greater effect than C8 (Malagelada et al., 1976). Intraduodenal infusion of
C18:1 and C12 also both increased bilirubin output when compared with C8, which had no effect (Malagelada et al., 1976). Similarly, oral ingestion of a triglyceride emulsion, consisting mostly (79 %) of C18:1, stimulated gallbladder contractility, thereby decreasing gallbladder volume, when compared with a triglyceride emulsion containing mainly (81 %) C8 (Isaacs et al., 1987). Accordingly, the effect of fatty acids on pancreatic and gallbladder secretion are dependent on their chain length.

2.3.3 Effect of fatty acids on gastrointestinal hormone secretion

The secretion of gastrointestinal hormones, including CCK, GLP-1 and PP, has also been shown to be dependent on the chain length of fatty acids (Barbera et al., 2000, Isaacs et al., 1987, Matzinger et al., 2000, McLaughlin et al., 1999, Feltrin et al., 2004). An early study in humans reported that CCK secretion following oral ingestion of triglyceride emulsions, containing fatty acids of different chain lengths, was dependent on fatty acid chain length (Isaacs et al., 1987). A triglyceride emulsion containing mainly C18:1 (79 %) markedly increased plasma CCK to peak concentrations of ~ 30 pmol/L, compared with a triglyceride emulsion composed mainly of C8 (81 %), which increased plasma CCK to ~ 4 pmol/L (Isaacs et al., 1987). In more recent studies, intraduodenal infusion of C18:1 and C12, but not C8 or C10, markedly increased plasma CCK concentrations (Matzinger et al., 2000, Feltrin et al., 2004). Similarly, intragastric infusion of C12, but not C10 or C11, has been shown to stimulate CCK (McLaughlin et al., 1999), however, interpretation of these results is confounded by the addition of the non-ionic surfactant Tween 80, used to solubilise the fatty acids. Because there was a transient increase in plasma CCK concentration in response to the control solution, probably due to the addition of the surfactant, McLaughlin and colleagues concluded that both C10 and C11 had no effect on CCK secretion, even
though both fatty acids increased plasma CCK concentrations from baseline. In contrast, a more recent study established that while intraduodenal C10 and C12 both stimulate CCK secretion (Feltrin et al., 2004), the effect of C10 (peak concentration: 9.0 pmol/L) is substantially less than that of C12 (peak concentration: 13.4 pmol/L). Therefore, fatty acid chain length does modulate the effect of fatty acids on CCK secretion, so that fatty acids with ≥ 12 carbon atoms have a greater effect on plasma CCK secretion than fatty acids with ≤ 10 carbon atoms.

Few studies have evaluated the effect of fatty acids on the secretion of other gastrointestinal hormones. Only one study has to date in humans (Feltrin et al., 2004) evaluated the effect of fatty acid chain length on GLP-1 secretion, and in contrast to CCK secretion, in the dose evaluated, only intraduodenal C12, but not C10, stimulated GLP-1 secretion. Studies in humans have also established that both intragastric and intraduodenal triglyceride emulsions, consisting mainly of C18, increase plasma PP concentrations when compared with triglyceride emulsions consisting mainly of C8, which apparently have no effect (Barbera et al., 2000, Isaacs et al., 1987). The effects of free fatty acids on PP secretion in humans have hitherto not been evaluated, hence it is not known whether there is marked discrepancy in the effects of C10 and C12, as is also the case with GLP-1 secretion (Chapter 4). In rats, the presence of C18:1 in both the proximal, and distal, small intestine stimulates the secretion of PYY (Pappas et al., 1986, Lin et al., 2000). Currently no studies in humans have assessed the effect of fatty acid chain length on the stimulation of PYY or GLP-2 secretion, or the suppression of ghrelin (Chapter 4).
2.3.4 Effect of fatty acid chain length on appetite and energy intake

The effects of fatty acids on appetite and energy intake are also dependent on their chain length. In rats, small intestinal infusions of fatty acids with 12 or 18 carbon atoms, ie lauric or oleic acid, suppress energy intake, while fatty acids with 8 or 10 carbon atoms, ie octanoic or decanoic acid, have no effect (Meyer et al., 1998b). Similarly, in humans, duodenal infusion of C18:1, but not C8, inhibits energy intake, however, in this particular study the C18:1 infusion delivered almost twice as much energy, at almost twice the concentration, as the C8 infusion; therefore, it remains uncertain whether the observed differences in the effects of C18:1 and C8 on energy intake were related to the chain length, energy load and/or concentration, of fatty acids (Matzinger et al., 2000). In a recent study in humans, duodenal infusion of C12 over 90 min at 0.38 kcal/min (total energy delivered: 142 kJ) markedly attenuated feelings of hunger and desire to eat during the period of infusion and reduced energy intake at a subsequent test meal by ~ 2860 kJ, whereas, C10, when infused at the same load and concentration, failed to inhibit energy intake (Figure 2.3), clearly establishing that when administered directly into the small intestine fatty acids with 12 carbon atoms have a greater effect to suppress energy intake than those with 10 carbon atoms. In this study, the C12 infusion was associated with nausea in 5 out of 8 subjects, hence, potentially confounding interpretation of the effect of C12 to suppress energy intake, ie the reduction in energy intake reflect nausea, as opposed to a true effect of C12 alone. Although, energy intake was still less in 3 subjects who did not experience nausea following the C12 infusion (~ 1800 kJ), the magnitude of the reduction was greater in those that did (~ 3515 kJ) (Feltrin et al., 2004). Accordingly, further studies are required to determine whether C12 has the capacity to suppress energy intake without inducing nausea in humans (Chapter 6).
Figure 2.3: Energy intake (in kJ) at a buffet meal in response to 90 min duodenal infusions of C12, C10, or control in healthy young subjects (total energy delivered by C12 and C10: 142 kJ). C12 markedly reduced energy intake, whereas there was no difference between C10 and control. Treatment effect, P < 0.001; * vs control and C10, P < 0.001 (from Feltrin et al, 2004).

While differences in the effects of fatty acids on energy intake between fatty acids with \( \geq 12 \) carbon atoms with those with \( \leq 10 \) carbon atoms have clearly been established, there is little information about the comparative effects of fatty acids with more than 12 carbon atoms. Currently only one study, in rats, has directly compared the effects of C12 and C18 on the suppression of energy intake (Meyer et al., 1998b).

– Infusion of C12 into the colon suppressed energy intake more than an equicaloric infusion of C18:1, while intraduodenal infusion of equimolar concentrations of C12 and C18:1 suppressed energy intake to the same extent (Meyer et al., 1998b). However, at equimolar concentrations the C18:1 would have delivered twice the amount of energy than the C12 infusion, due to the longer chain length, suggesting that if the two fatty acids were administered at equicaloric loads, C12 may be more effective than C18:1 in reducing energy intake. An indirect comparison of results across different studies in humans suggests that there may be differences in the efficiencies amongst fatty acids.
with ≥ 12 carbon atoms. For example, intraduodenal C12 (142 kJ) reduced energy intake by ~ 2860 kJ (Feltrin et al., 2004), while intraduodenal C18 (288 kJ) only reduced energy intake by ~ 1580 kJ (Matzinger et al., 2000), despite delivering twice the energy of the C12 infusion. Currently, no studies in humans have directly compared the effects of equicaloric intraduodenal infusions of C12 and C18:1 on energy intake (Chapter 5).

In summary, studies in both humans and animals have demonstrated that the modulation of gastrointestinal motility, hormone secretion and energy intake by fatty acids is dependent on their chain length, such that, fatty acids with ≥ 12 carbon atoms have a substantially greater effect when compared with fatty acids with ≤ 10 carbon atoms. While there is much less known about the effects of different fatty acids with 12 or more carbon atoms on energy intake, animal studies suggest that the suppression of energy intake may not be altered by increasing chain length.

### 2.4 Impact of load and concentration in the effects of fatty acids on gastrointestinal function and energy intake

As discussed in Chapter 1.8, the effects of nutrients on gastrointestinal function and energy intake are modified by the load and concentration of nutrient administered, in animals, and this has also been demonstrated specifically in the case of fatty acids ≥ 12 carbon atoms. In dogs, the secretion of pancreatic enzymes in response to C18:1 is load-dependent, but concentration-independent while, in contrast, the stimulation of pancreatic bicarbonate and slowing of gastric emptying appear to be concentration-dependent, but load-independent (Meyer and Jones, 1974). Interestingly, the effect of C12 and C18:1 on energy intake are dependent on the both fatty acid load and
concentration (Meyer et al., 1998b). Increasing the load of small intestinal C12 and C18:1 (3, 6 and 12 ml/h), at a constant concentration, resulted in a load-dependent suppression of energy intake, such that the greater the fatty acid load, the greater the reduction of energy intake (Meyer et al., 1998b). Similarly, increasing concentrations of C12 and C18:1 (20, 40 and 80 mM), at a constant load, was associated with a concentration-dependent suppression of energy intake (Meyer et al., 1998b).

No studies in humans have hitherto determined whether the effects of fatty acids on gastrointestinal function and energy intake are dependent on load and/or concentration, hence, further studies in humans are required to determine whether the suppression of energy intake and the modulation of gastrointestinal function by fatty acids are dependent on load and/or concentration (Chapter 7).

2.5 Role of post-absorptive factors in the regulation of gastrointestinal function and energy intake by fatty acids

Currently, factors which mediate the effects fatty acids on gastrointestinal function and energy intake are poorly defined, however, studies in animals have demonstrated that physiological factors, including absorption pathways and vagal afferent stimulation, may, at least in part, mediate the effect of fatty acids on gastrointestinal function and energy intake. The following discussion summarises evidence that differences in (i) absorption pathways, (ii) the secretion of chylomicrons and apolipoprotein A-IV and (iii) the activation of vagal afferents, may contribute to the regulation of gastrointestinal function and energy intake by fatty acids.
2.5.1 Fatty acid absorption pathways

Following fat digestion there are two different pathways of fatty acid absorption, involving the portal vein and the lymphatic system. Early studies in rats established that more than 80 % of fatty acids with ≤ 10 carbon atoms are transported via the portal vein and more than 80 % of fatty acids with > 12 carbon atoms are transported via the lymphatic system, however, C12 is transported via both pathways in approximately equal amounts (Bloom et al., 1951). While for all fatty acids, a small percentage is absorbed using the alternative pathway, as chain length is increased progressively above 12 carbon atoms, relatively less fatty acid is absorbed via the portal vein, and as chain length is decreased below 12 carbon atoms, relatively less is absorbed via the lymphatic system (Bloom et al., 1951, Kiyasu et al., 1952). The absorption of fatty acids with ≥ 12 carbon atoms via the lymphatic pathway is associated with the activation of post-absorptive factors, including the secretion of chylomicrons and apolipoprotein A-IV (apo A-IV) (Green et al., 1980, Tso et al., 2001), and studies in animals suggest strongly that these post-absorptive factors are responsible for mediating the effects of these fatty acids on gastrointestinal function and energy intake (Raybould et al., 1998, Meyer et al., 1998a, Glatzle et al., 2002, Fujimoto et al., 1993, Okumura et al., 1996).

2.5.2 Synthesis and secretion of chylomicrons and apolipoprotein A-IV

Both chylomicrons and apo A-IV are secreted into the circulation following the absorption of fatty acids with ≥ 12 carbon atoms (Green et al., 1980, Tso et al., 2001), however, while chylomicron formation is stimulated directly by the absorption of fatty acids into enterocytes (Tso et al., 2001), apo A-IV synthesis and secretion are dependent on chylomicron formation (Hayashi et al., 1990). For example, while the administration of the “detergent”, Pluronic L-81 (L-81), still allows lipid to enter the enterocyte, it
blocks chylomicron formation within the enterocyte (Halpern et al., 1988), and leads to an inhibition of apo A-IV synthesis and secretion (Hayashi et al., 1990). Moreover, when administration of L-81 is ceased the accumulated lipid in the enterocyte is assembled rapidly into chylomicrons and released into the lymph, with a subsequent marked increase in apo A-IV secretion (Hayashi et al., 1990).

**Role of chylomicrons and apolipoprotein A-IV in the regulation of gastrointestinal function and energy intake**

There is evidence that both chylomicrons and apo A-IV modulate gastrointestinal function and suppress energy intake in animals (Raybould et al., 1998, Meyer et al., 1998a, Glatzle et al., 2002, Fujimoto et al., 1993, Okumura et al., 1996). Blocking the transport of chylomicrons, and hence apo A-IV secretion, using L-81, abolishes the slowing of gastric emptying (Raybould et al., 1998) and suppression of energy intake (Meyer et al., 1998a) by fat, providing indirect evidence that chylomicron and apo A-IV secretion are responsible for these effects. Moreover, studies in rats have established that the central (via the jugular vein), and intravenous, administration of chylomicrons inhibits gastric motility (Glatzle et al., 2002) and that central administration of apo A-IV slows gastric emptying and decreases energy intake in a dose-dependent manner (Fujimoto et al., 1993, Okumura et al., 1996). Hence there is persuasive evidence that chylomicrons and apo A-IV mediate, at least in part, the effects of fatty acids with ≥ 12 carbon atoms on gastrointestinal function and energy intake.

**2.5.3 Role of vagal afferent activation**

Animal studies indicate that vagus nerve activation is an important post-absorptive factor in mediating the effects of fatty acids on gastrointestinal motility (Glatzle et al.,
2003) and energy intake (Greenberg and Smith, 1996, Yox et al., 1991). The vagus nerve conveys information from the gut to the brainstem, which has a profound influence on the effects of nutrients on gastrointestinal function (Grundy and Scratcherd, 1989) and energy intake (Randich et al., 2000, Cox et al., 2004). When vagal nerve endings are severed or damaged, the effect of nutrients on energy intake (Yox and Ritter, 1988, Yox et al., 1991) and gastrointestinal function (Yamagishi and Debas, 1978, Raybould, 1991) are markedly attenuated. Studies in animals have also demonstrated that the extent of vagal activation is dependent on the type of lipid, i.e., triglycerides or fatty acids, as well as the chain length of fatty acids.

Exposure of the ileum and jejunum to triglycerides and fatty acids increases vagal activity, however, fatty acids produce a much larger response (Randich et al., 2000). Polyunsaturated linoleic (“C18:2”), and oleic (C18:1), acid increased vagal afferent activity more than a triglyceride emulsion (~79 % C18:1) and corn oil (~95 % C18:2) (Randich et al., 2000), indicating that fatty acids, rather than triglycerides, are responsible for the stimulation of vagal afferents. Studies in animals have also demonstrated that the pathways involved in the stimulation of vagal afferents by fatty acids are dependent on their chain length (Lal et al., 2001, Melone, 1986). For example, two groups of vagal receptors in the cat intestine have been identified, – those responsive to C18:1 and C18:2 and those that respond to C8 (Melone, 1986). A study in rats investigated the mechanisms involved in the activation of vagal afferents by fatty acids (Lal et al., 2001). – Using a specific CCK₁ receptor antagonist, it was determined that C18:1 activates vagal afferents indirectly, via a CCK-dependent mechanism, whereas sodium butyrate, a saturated fatty acid with 4 carbon atoms (“C4”) acts directly on vagal fibres (Lal et al., 2001). However, these studies only assessed a limited
number of fatty acids, those with 18, 8 or 4 carbon atoms, and it is, therefore, unknown whether there is a separation in the effects of fatty acids between those with \( \leq 10 \) and \( \geq 12 \) carbon atoms. These studies in animals have, therefore, established, that there are differences in the pathways, and mechanisms, involved in the stimulation of vagal afferents between fatty acids of different chain lengths, which may, in part, mediate the effects of fatty acids with \( \geq 12 \) carbon atoms on gastrointestinal function and energy intake. Studies of this type cannot be performed in humans, hence the effect of fatty acids of different chain lengths on vagal afferent activation, and whether these are associated with changes in gastrointestinal function and/or energy intake, are unknown.

In addition to the activation of vagal afferents by fatty acids, the administration of lymph containing both chylomicrons (Glatzle et al., 2003) and apo A-IV (Glatzle et al., 2004) activates vagal afferents. However, the stimulation of vagal afferents by both chylomicrons and apo A-IV is abolished by the administration of a CCK\(_1\) receptor antagonist, indicating that chylomicrons and apo A-IV both activate vagal afferents via a CCK-dependent mechanism. Hence, the secretion of chylomicrons and apo A-IV by fatty acids with \( \geq 12 \) carbon atoms probably stimulate the secretion of CCK from enteroendocrine cells, which then stimulates vagal afferents via CCK\(_1\) receptors. Therefore, based on animal data, the slowing of gastric emptying, modulation of antropyloroduodenal motility and suppression of energy intake by fatty acids with \( \geq 12 \) carbon atoms in humans is likely to be closely related to stimulation of vagal afferents, following the synthesis and secretion of chylomicrons and apo A-IV, mediated by CCK\(_1\) receptors.
2.6 Cytotoxic properties of fatty acids on intestinal mucosa – potential effects on energy intake

The presence of fatty acids in the small intestine has been reported to cause transient damage to intestinal mucosa, as defined by the disruption of intestinal villi membranes and increased permeability of the intestine to large molecules (Velasquez et al., 1993b, Velasquez et al., 1993a, Ramirez et al., 1997, Kvietys et al., 1991). Although, this type of damage is restored rapidly when the exposure of the small intestine to fatty acids ceases (Kvietys et al., 1991). In vitro studies of epithelial cell monolayers, and in vivo studies in rats, indicate that injury to epithelial cells is nutrient-specific, such that the digestive products of carbohydrate and protein do not cause detectable injury to epithelial cells in vivo or in vitro, while, in contrast, fatty acids markedly disrupt the epithelial monolayer in both conditions (Kvietys et al., 1991). Furthermore, these cytotoxic effects of fatty acids are greater with increasing chain length, concentration and degree of saturation (Velasquez et al., 1993a, Velasquez et al., 1993b).

Interestingly, in animals, the cytotoxic effects of fatty acids may possibly induce nausea, which, as discussed, may contribute to the suppression of energy intake by fatty acids with ≥ 12 carbon atoms. In rats, increased injury to small intestinal mucosa by C18:1 is associated with a reduction in energy intake (Ramirez et al., 1997), which may represent an adverse effect due to nausea. As discussed (section 2.3.4), in humans, an intraduodenal C12 infusion induced nausea in 5 of 8 subjects, which may have been responsible for the marked reduction of energy intake (Feltrin et al., 2004). The reasons for the induction of nausea by the C12 infusion are unclear, but may represent a cytotoxic effect of fatty acids on small intestinal mucosa, although, an inhibition of energy intake was also evident in 3 subjects that did not experience nausea (Feltrin et
al., 2004). Moreover, in the study conducted by Feltrin and colleagues (Feltrin et al., 2004), C10 was also infused intraduodenally, and this has been shown to have comparable cytotoxic effects on the small intestinal mucosa to C12 (Velasquez et al., 1993b), but was not associated with nausea or a reduction in energy intake. Therefore, it is not likely that the suppression of energy intake by fatty acids, particularly C12, is the result of cytotoxicity, although this possibility cannot be discounted.

2.7 Effects of oral ingestion of fatty acids

It is apparent from the above discussion that acute intraduodenal administration of fatty acids is very effective in suppressing energy intake in animals, and possibly, in humans. However, studies are required to establish whether intraduodenal fatty acids, particularly C12, suppress energy intake in the absence of nausea in humans (Chapters 5, 6 and 7). C12 could potentially be utilised as an oral, non-pharmacological appetite-suppressant in the management and prevention of obesity, however, there is no information about the effect of oral administration of C12 on energy intake in humans (Chapter 9).

2.7.1 Comparative effects of intraduodenal, intragastric and oral nutrient delivery on energy intake

There is evidence in humans that the modulation of gastrointestinal function and energy intake by intraduodenal nutrients are maintained when nutrients are infused into the stomach or ingested orally. This is perhaps intuitively surprising, given that a number of gastrointestinal functions, including gastric emptying, are regulated by small intestinal feedback. While there is limited information about the comparative effects of oral versus intraduodenal nutrient administration, one study reported that oral ‘mixed
nutrient’ soup ingestion suppressed hunger and desire to eat and increased fullness, to a greater extent than intraduodenal soup administration (Cecil et al., 1998), which may reflect the effects of gastric distension and/or slower exposure of the small intestine to nutrients. However, the comparative effects of oral nutrient ingestion and intraduodenal nutrient administration on subsequent energy intake have not been evaluated. Intragastric administration of C12 in humans is known to reduce antral contractions, relax the proximal stomach, increase gallbladder contractions and increase CCK secretion (McLaughlin et al., 1999), demonstrating that the effects of C12 on gastrointestinal function are evident following intragastric administration. Accordingly, it would be anticipated that orally administered C12 would also produce changes in gastrointestinal function that are associated with the reduction of appetite and energy intake, but this has not been formally studied (Chapter 9).

2.8 Summary

This chapter has reviewed current knowledge relating to the effects of fatty acids, particularly their chain length, on the regulation of gastrointestinal function and energy intake. There is persuasive evidence, in both animals and humans, that there is a marked distinction in the effects of fatty acids, with $\leq 10$ carbon atoms from those with $\geq 12$ carbon atoms, on gastrointestinal motor and hormonal function and energy intake. While C12 has a marked stimulatory effect on the secretion of CCK and GLP-1, and C10 appears to only increase CCK slightly, and not GLP-1, it is not known how C12 and C10 affect the suppression of ghrelin and the secretion of PYY, GLP-2 and PP (Chapter 4). Although there is information about the comparative effects of fatty acids with $\leq 10$ carbon atoms and those that have $\geq 12$ carbon atoms, no studies in humans have hitherto directly compared the effects of fatty acids with 12 or 18 carbon atoms (eg
C12 vs C18) on energy intake and gastrointestinal function when infused into the duodenum at the same energy load (Chapter 5). Moreover, while intraduodenal C12 had been shown to be a potent suppressant of energy intake, this was associated with nausea, confounding interpretation of the effect of C12 and dictating the need for studies to determine whether C12 has the capacity to suppress energy intake without inducing nausea (Chapter 6) and, if so, whether this effect is dependent on the load and/or concentration of C12 (Chapter 7). Finally, while the effects of C12 to suppress antral contractions, relax the proximal stomach and stimulate CCK are maintained when C12 is administered intragastrically, there is no information as to whether oral administration of C12 also suppresses energy intake (Chapter 9).
Chapter 3

Common methodologies

3.1 Introduction
This chapter describes all the methods and techniques that are common to the studies reported in Chapters 4 – 9. All of these techniques are established and have been used extensively in the laboratory in which the author performed her studies. These techniques include the assessment of gastrointestinal motility, gastrointestinal hormone concentrations, appetite and energy intake.

3.2 Subjects
Healthy young male subjects, aged 18 – 50 years old, and of normal body weight for their height (body mass index (BMI) 19 – 25 kg/m²), were recruited through the combined use of flyers located within the Royal Adelaide Hospital and local universities (The University of Adelaide, The University of South Australia and Flinders University) and, if necessary, advertisements were placed in “The Messenger” newspaper.

3.2.1 Inclusion/Exclusion criteria
Each subject was questioned prior to their inclusion into a study to exclude:

(i) restrained eaters (score > 12 using ‘factor 1’ of the Three Factor Eating Questionnaire) (Stunkard and Messick, 1985)
(ii) significant gastrointestinal symptoms, disease or surgery
(iii) diabetes mellitus, cardiovascular or respiratory disease or other significant illnesses, as assessed by the author

(iv) current use of medications known to have the potential to affect gastrointestinal motor function, body weight or appetite (eg orlistat, buscopan)

(v) smoking > 10 cigarettes per day

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

(vii) allergy to local anaesthetic

Subjects in the study described in Chapter 8 were also excluded if their haemoglobin concentrations were outside the normal range: 135 – 175 g/l or biomedical measurements of liver/renal function were in excess of the following ranges:

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

### 3.3 Ethics approval

All study protocols were approved by the Royal Adelaide Hospital Ethics Committee; approvals from the Royal Adelaide Hospital Investigational Drug Sub-Committee and Therapeutic Goods Administration were obtained for the intravenous administration of the hormones, CCK and GLP-1 (Chapter 8). All subjects provided written, informed consent prior to their inclusion into a study and were offered an honorarium for their participation.
3.4 Study environment

All studies were conducted within clinical study rooms in the University of Adelaide Discipline of Medicine, Royal Adelaide Hospital and attempts were made to exclude any environmental factors, specifically social interaction or influence by any other individual. In studies that assessed APD motility (Chapters 4 – 8) subjects were positioned supine on a hospital bed (Heddle et al., 1988a). In studies quantifying the effect of oral nutrient ingestion on appetite and energy intake (Chapter 9), subjects were seated at a table. The latter subjects were allowed to read (material unrelated to food) or listen to music, except when blood samples were taken and/or questionnaires assessing appetite were completed, however, in manometric studies (Chapters 4 – 8), subjects were only allowed to read (material unrelated to food) or listen to music whilst the catheter was being positioned, and these activities were ceased upon commencement of intraduodenal infusion. During the consumption of the buffet-style meal to assess energy intake, subjects were seated at a table in all studies and were not permitted to read, listen to music, or converse.

3.5 Measurement of antropyloroduodenal motility

3.5.1 Nasoduodenal intubation and manometry

A manometric catheter allows measurement of pressure waves in specific regions of the gastrointestinal tract, including the antrum, pylorus and duodenum. Pressures in the APD region were measured using a 4 mm (outer diameter), 17-channel silicone manometric catheter (originally manufactured by Dentsleeve Pty Ltd, Adelaide, Australia (Chapters 4 – 6 and 8), and subsequently by Dentsleeve International Ltd, Ontario, Canada (Chapter 7)). Subjects were intubated at ~ 0830h after fasting from at
least 2200h the night before. The catheter was inserted through an anaesthetised nostril into the stomach and allowed to pass into the duodenum by peristalsis (Heddle et al., 1989). The channels, or side-holes, of the manometric catheter were spaced at 1.5 cm intervals; six side-holes (channels 1 – 6) were positioned in the antrum, a 4.5 cm sleeve sensor (channel 7) present on the back of the sleeve (channels 8 and 9), to measure pressure waves (PWs) occurring over the entire pyloric region, was positioned across the pylorus, and 7 side-holes (channels 10 – 16) were positioned in the duodenum. An additional channel, used for intraduodenal infusions, was positioned 11.75 cm distal to the end of the sleeve sensor. The correct positioning of the catheter, so the sleeve sensor straddled the pylorus, was maintained by continuous measurement of the transmucosal potential difference (TMPD) between the most distal antral channel (channel 6) (~-40 mV) and the most proximal duodenal channel (channel 10) (~0 mV) (Heddle et al., 1989). For this purpose a saline-filled cannula (20 G cannula filled with 0.9 % sterile saline) was inserted subcutaneously in the left forearm and used as a reference electrode (Heddle et al., 1989). All manometric channels were perfused with degassed, distilled water, except for the two TMPD channels, which were perfused with degassed 0.9 % saline, at 0.15 ml/min (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 manipulations began 10 – 15 min after the phase III activity had passed, and during a period of motor quiescence (phase I of the MMC).

### 3.5.2 Data acquisition and analyses

Manometric pressures were digitised and recorded on two different, computer-based, systems; (i) PowerMac 7100/75; Apple Computer, Cupertino, CA, USA, running commercially available software (HAD, Associate Professor Geoff Hebbard,
Melbourne, Australia) (Chapters 6 – 8) and (ii) Dimension 2400; Dell Computer Corporation, Round Rock, TX, USA, running commercially available software (Oakdale, Flexisoft®, Associate Professor Geoff Hebbard, Melbourne, Australia) (Chapter 5), both written in Labview 3.1.1 (National Instruments). The data obtained were stored for subsequent analysis. APD pressures were analysed for the number and amplitude of IPPWs and PWs in the antrum and duodenum using custom-written software (Gastrointestinal Motility Unit, Utrecht, The Netherlands (Samsom et al., 1998)), modified to the author’s requirements. Basal pyloric pressure (“tone”) was also calculated for each minute by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side hole from the mean basal pressure recorded at the sleeve (Heddle et al., 1988b), using custom-written software (MAD, Prof Charles-Henri Malbert, INRA, Rennes, France). Phasic PWs in the antrum, and IPPWs, were defined by an amplitude $\geq 10$ mmHg, with a minimum interval of 15 s between peaks. Phasic duodenal PWs were defined by an amplitude $\geq 10$ mmHg, with a minimum interval of 3 s between peaks (Heddle et al., 1988a). In studies described in Chapters 5, 6 and 8, APD pressure wave sequences (PWSs) were also analysed and defined as two or more temporally related pressure waves with onsets within $\pm 5$ s in the antrum, or $\pm 3$ s in the duodenum, of each other (Samsom et al., 1998).

### 3.6 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 aprotinin per ml blood (Trasylol;
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.

### 3.6.1 Plasma ghrelin

In Chapter 4, ghrelin-like immunoreactivity was measured with a specific and sensitive radioimmunoassay, as described (Patterson et al., 2005). This assay cross-reacted fully (100 %) with both octanoyl and desoctanoyl ghrelin and did not cross-react with any other known gastrointestinal or pancreatic hormone. The antiserum, SC-10368 (Santa Cruz Biotechnology), was used at a final dilution of 1:50,000. 125 iodine ($^{125}$I) ghrelin was prepared with Bolton & Hunter reagent (Amersham International, UK) and purified by high pressure liquid chromatography (HPLC) using a linear gradient from 10 to 40 % acetonitrile, 0.05 % trifluoroacetic acid over 90 min. The specific activity of ghrelin label was 48 Bq/fmol. The assay was performed in a total volume of 0.7 ml of 0.06 M phosphate buffer (pH 7.2) containing 0.3 % bovine serum albumin (BSA) and was incubated for 3 days at 4°C before separation of free and bound antibody label by charcoal absorption. The assay detected changes of 20 pmol/L of plasma ghrelin with a 95 % confidence limit. The intra-assay coefficient of variation (CV) was 5.5 %.

### 3.6.2 Plasma cholecystokinin

Plasma CCK concentrations (Chapters 5 – 8) were determined following ethanol extraction, using an established radioimmunoassay (MacIntosh et al., 2001b). A commercially-available antibody raised in rabbits against synthetic sulphated CCK-8 was employed (C258, Lot 105H4852, Sigma Chemical, St Louis, MO, USA). This antibody binds to all CCK analogues with the sulphated tyrosine residue in position 7,
has a cross-reactivity of 26% with unsulphated CCK-8, less than 2% cross-reactivity with human gastrin (0.2% with gastrin I and 1% with Big gastrin) and does not bind to structurally unrelated peptides. The intra-assay CV was 9% and the inter-assay CV was 27%. The assay had a minimum detection limit of 2.5 pmol/L.

### 3.6.3 Plasma glucagon-like peptide-1

Plasma GLP-1 concentrations (Chapters 6 and 8) were measured by radioimmunoassay (Wishart et al., 1998). Antibody, provided by Professor SR Bloom (Hammersmith Hospital, London), did not cross-react with glucagon, gastric inhibitory peptide, or other gut or pancreatic peptides and has been demonstrated by chromatography to measure intact GLP-1 (7-36) amide. It is likely that this antibody also reacts with the degraded form of GLP-1 (9-36) amide. The intra-assay CV was 17% and inter-assay CV was 18%, and had a minimum detection limit of 1.5 pmol/L.

### 3.6.4 Plasma glucagon-like peptide-2

Plasma GLP-2 concentrations (Chapter 4) were measured by radioimmunoassay using antibody FT-17 raised in a rabbit immunised with synthetic human GLP-2 (Bachem UK Ltd.) conjugated to BSA by glutaraldehyde and used at a final dilution of 1:350,000. The antibody cross-reacted 100% with human and rat GLP-2, less than 0.1% with GLP-1, and did not cross-react with any other known gastrointestinal or pancreatic hormone. The $^{125}$I (Tyr$^0$)GLP-2 label was prepared using iodogen and purified by HPLC. The specific activity of GLP-2 label was 54 Bq/fmol. The assay was performed in duplicate using 100 µl of neat plasma in 0.7 ml of 0.06 M phosphate buffer (pH 7.2) containing 0.3% BSA and incubated for 3 days at 4°C and free and bound antibody
label separated by charcoal absorption. The intra-assay CV was 6.2%. The assay had a
minimum detection limit of 5 pmol/L of plasma GLP-2 with 95 % confidence.

3.6.5 Plasma peptide YY

Three different radioimmunoassays were utilised for the determination of plasma PYY
centrations. In Chapter 5 the antiserum (kindly donated by Dr B Otto, Medizinische
Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) used was raised
in rabbits against human PYY-(1-36) (Sigma-Aldrich, St. Louis, MO, USA). This
antiserum showed < 0.001% cross-reactivity with human pancreatic polypeptide or
sulphated CCK-8 and 0.0025% cross-reactivity with human neuropeptide Y (NPY).
Tracer (NEX3410) was purchased from Perkin Elmer (Boston, MA, USA). Standards
(1.6 – 50 fmol/tube) or samples (200µl plasma) were incubated in assay buffer (0.05 M
phosphate containing 0.5 % bovine serum albumin and 0.02 % azide, pH 7.4) with 100
µl antiserum at a final dilution of 1:10,000 for 24 hours at 4°C, then 100 µl iodinated
PYY (10,000 cpm) was added and the incubation continued for another 24 hours.
Separation of the antibody-bound tracer from free tracer was achieved by addition of
200 µl of dextran-coated charcoal containing gelatin (0.015 g gelatin, 0.09 g dextran,
0.15 g charcoal/30 ml assay buffer), incubation at 4°C for 20 minutes then
centrifugation at 2000 x g and 4°C for 25 min. Radioactivity of the bound fraction was
determined by counting the supernatants in a gamma counter. The intra-assay CV was
12.3 %, and the inter-assay CV was 16.6 %, with a minimum detection limit of 1.5
pmol/L.
In Chapter 7 the antiserum (also kindly donated by Dr B Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) used was raised in rabbits against human PYY-(1-36) (Sigma-Aldrich, St. Louis, MO, USA), as described (Pilichiewicz et al., 2005). This antiserum showed < 0.001% cross-reactivity with human PP and sulfated CCK-8 and 0.0025% cross-reactivity with human NPY. Tracer (purchased from Prosearch International, Victoria, Australia) was prepared by radiolabeling synthetic human PYY(1–36) (Auspep; Parkville, VIC, Australia) using the lactoperoxidase method. Monoiodo-tyrosine-PYY was separated from free $^{125}$I, diiodo-PYY, and unlabeled PYY by reverse-phase HPLC. Standards (1.6–50 fmol/tube) or samples (200 µl plasma) were incubated in assay buffer (pH 7.4) with 100 µl antiserum at a final dilution of 1:10,000 for 20–24 h at 4°C, 100 µl iodinated PYY (10,000 cpm) was then added, and the incubation continued for another 20 – 24h. Separation of the antibody-bound tracer from free tracer was achieved by the addition of 200 µl dextran-coated charcoal containing gelatin, was incubated at 4°C for 20 min, and then centrifuged at 4°C for 25 min. Radioactivity of the bound fraction was determined by counting the supernatants in a gamma counter. The intra-assay CV was 12.3 %, and the inter-assay CV was 16.6 %, with a minimum detection limit of 4 pmol/L.

In Chapter 4, the assay measured both the hormone fragment (PYY-(3-36)) and the full length hormone (PYY-(1-36)), both of which are biologically active, as described previously (Adrian et al., 1985). The antiserum (Y21) was produced in rabbits against synthetic porcine PYY coupled to bovine serum albumin by glutaraldehyde and used at a final dilution of 1:50,000. This antibody cross-reacts fully with the biologically active circulating forms of PYY, but not with PP, NPY, or other known gastrointestinal hormones. $^{125}$I PYY was prepared by the iodogen method and purified by HPLC. The
specific activity of the $^{125}$I PYY label was 54 Bq/fmol. The assay was performed in a total volume of 0.7 ml of 0.06 M phosphate buffer (pH 7.2) containing 0.3 % BSA. The assay was incubated for three days at 4°C before separation of the free and antibody bound label by sheep anti-rabbit antibody. The minimum detection limit of the assay was 2.5 pmol/L, with an intra-assay CV of 5.8 %.

### 3.6.6 Plasma pancreatic polypeptide

Plasma PP concentrations (Chapter 4) were measured using a specific and sensitive radioimmunoassay (Adrian et al., 1976). The assay cross-reacted fully (100 %) with human PP and did not cross-react with any other member of the pancreatic polypeptide family, or gastrointestinal hormone. Antiserum against human PP was produced in rabbits and used at a final dilution of 1:560,000. $^{125}$I PP was prepared by the iodogen method and purified by HPLC. The specific activity of the $^{125}$I PP label was 54 Bq/fmol. The assay was performed in a total volume of 0.7 ml of 0.06 M phosphate buffer (pH 7.2) containing 0.3 % BSA, and was incubated for three days at 4°C before separation of the free and antibody-bound label by charcoal absorption. The minimum detection limit of the assay was 3.5 pmol/L, and the intra-assay CV was 5.7 %.

### 3.7 Measurement of appetite perceptions and energy intake

#### 3.7.1 Three Factor Eating Questionnaire

All studies assessed appetite perceptions during, and acute energy intake following, an intervention (ie administration of intraduodenal or oral free fatty acids, or intravenous
hormones). Accordingly, it was important to exclude subjects that were restrained eaters, ie those who restrict their food intake to control their weight (Herman and Mack, 1975). Subjects’ eating habits were determined by the Three Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985) (see Appendix I). The TFEQ assesses three dimensions of eating habits, including Factor 1: cognitive restraint of eating (‘restrained eating’), Factor 2: disinhibited eating (‘disinhibition’) and Factor 3: susceptibility of hunger (‘hunger’). The questionnaire composes 51 questions; 21 items are associated with Factor 1, 16 with Factor 2 and 14 with Factor 3. The factor relevant to the studies in this thesis was Factor 1 – ‘eating restraint’.

Stunkard and Messick, 1985 (Stunkard and Messick, 1985) determined the reliability of the questionnaire by examining two subject groups; unrestrained eaters (individuals that ate freely and were of normal weight) (n = 45) and restrained eaters (individuals who closely monitored their eating and were overweight) (n = 53). The study demonstrated a high reliability coefficient for each of the three factors; 0.93 for Factor 1 – restraint, 0.91 for Factor 2 – disinhibition and 0.85 for Factor 3 – hunger. The final mean score ± SEM for Factor 1 was 6.0 ± 5.5 for unrestrained eaters and 14.3 ± 3.6 for restrained eaters, therefore, all subjects included in studies outline in Chapters 4 – 9 were screened prior to their inclusion and those that scored > 12 for ‘dietary restraint’ (ie those that scored outside the range of ‘unrestrained’) were excluded.

3.7.2 Visual analogue scale questionnaires

Perceptions of appetite, including hunger, fullness, desire to eat and prospective consumption (“how much food do you think you could eat right now?”) were measured using validated visual analogue scale questionnaires (VAS) (Parker et al., 2004, Sepple
and Read, 1989). The gastrointestinal symptoms of nausea and bloating were also assessed. Each VAS evaluated a sensation on a 100 mm horizontal line, where 0 mm represented ‘sensation not felt at all’ and 100 mm ‘sensation felt very much’ (see Appendix II). Subjects were asked to place a vertical mark on the 100 mm line to indicate what they were feeling at that particular point in time. Other perceptions, such as drowsiness, anxiety, happiness, energy levels and how comfortable they felt, were also assessed to distract from the main purpose of the questionnaire, but were not quantified.

### 3.7.3 Energy intake

Energy intake at breakfast (Chapter 9) and/or lunch (Chapters 4 – 9) was determined to quantify the effect of treatments on energy intake. All meals were presented as an *ad libitum* buffet meal. – The meal at breakfast consisted of sliced toast, crumpets, a ‘breakfast bar’, muffin, yoghurt, fruit, flavoured milk, juice, margarine, jam and vegemite. The cold, buffet-style meal at lunch consisted of sliced bread, cold meats, cheese, sliced vegetables, an assortment of desserts and drinks, fruit, margarine and mayonnaise. The amount (g), energy content (kJ), and macronutrient distribution (g fat, carbohydrate and protein), of each item, in both the breakfast and lunch buffet meals, are detailed in Appendices III & IV, respectively. The amount of food provided at the buffet meals was in excess of what the subject was expected to eat (Lavin et al., 1996) and subjects were allowed up to 30 min to consume the buffet meal freely until comfortably full.

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

3.8 Preparation and administration of study interventions

3.8.1 Free fatty acids

The fatty acids administered in the studies were commercially available, food grade products, suitable for human consumption, that were administered either directly into the small intestine, or ingested orally. The fatty acids that were administered included:

- decanoic acid, a saturated fatty acid with 10 carbon atoms (“C10”), purchased from Sigma-Aldrich, Milwaukee, WI, USA (Chapter 4)
- dodecanoic acid (‘lauric acid’), a saturated fatty acid with 12 carbon atoms (“C12”), purchased from Sigma-Aldrich, Milwaukee, WI, USA (Chapters 4 – 7, 9).
- oleic acid, a monounsaturated fatty acid with 18 carbon atoms (“C18:1”), purchased from Pfaltz & Bauer, inc., Waterbury, CT, USA (Chapter 5)

Intraduodenal administration

C12 was dissolved in either distilled water (Chapters 4 and 5) or 0.9 % saline (Chapters 6 and 7) with sodium hydroxide (NaOH) (Sigma-Aldrich, Louis, MO, USA) to keep the fatty acid in solution, resulting in a pH between 8.2 – 8.4. C10 and C18:1 were dissolved easily in distilled water and had NaOH and hydrochloric acid (HCl) (Merck Pty. Ltd., Victoria, Australia) added, respectively, to adjust the pH to match that of the C12 solutions. Free fatty acids were administered directly into the duodenum via an infusion port incorporated in the 17-channel manometric catheter, described in section 3.5.1. Duodenal administration of free fatty acids ensured their delivery at the location
of small intestinal receptors in a standardised fashion without the potentially confounding effects of gastric emptying. The energy loads of the fatty acid solutions were based on the rate that fatty acids empty from the stomach, which is between 0.2 – 0.4 kcal/min (Hunt and Knox, 1968). The concentrations of the solutions were within the range of fatty acid concentrations observed in the small intestine after triglyceride digestion (Borgstrom et al., 1957, Porter et al., 1971, Ament et al., 1972). The energy load, concentration, delivery rate and amount of fatty acids, as well as control solutions, are detailed in each of the relevant chapters (Chapters 4 – 7).

**Oral administration**

C12 was packaged into hydroxypropylmethyl cellulose capsules (size 00, Capsuline Inc, Pompano Beach, FL, USA) and ingested orally with water (Chapter 9). The study reported in Chapter 9 consisted of two parts, a ‘pilot study’ and the ‘main study’ and placebo capsules were also administered in both parts; in the ‘pilot study’ capsules were initially filled with polyethyleneglycol (Ajax Chemicals, supplied by University of Adelaide, SA, Australia), however, in the ‘main study’, this was changed to ascorbic acid (Chem-Supply, Gillman, SA, Australia) because ascorbic acid is commonly used as a control (or placebo) substance in pharmaceutical trials and is absorbed in the gastrointestinal tract without affecting appetite or energy intake. Subjects were unable to discriminate between the placebo capsules and the C12 capsules, visually, or by weight. The amounts (g) of all substances described above are detailed in Chapter 9.

**3.8.2 Hormones for intravenous infusion**

**Cholecystokinin**
CCK-8 was purchased from Clinalfa®, Merck Biosciences, Laeufelfingen, Switzerland (Chapter 8), dissolved in 0.9 % sterile saline and infused at a rate of 1.8 pmol/kg/min (2 ng/kg/min) for 150 minutes. This dose was chosen as it has been shown to stimulate pyloric motility and suppress energy intake, while resulting in relatively physiological plasma concentrations (Rayner et al., 2000, MacIntosh et al., 2001b).

Glucagon-like peptide-1
GLP-1 was purchased from Clinalfa®, Merck Biosciences, Laeufelfingen, Switzerland (Chapter 8). GLP-1 was dissolved in 0.9 % sterile saline and infused at a rate of 0.9 pmol/kg/min for 150 minutes. This dose was chosen as it had been shown to relax the proximal stomach and suppress energy intake, while resulting in apparently physiological plasma concentrations (Flint et al., 1998, Schirra et al., 2002).

3.9 Data and statistical analyses
Data analyses in each study are described in detail in the relevant chapter. Data were analysed using commercially available statistical software Statview Version 5.0 (SAS Institute Inc., North Carolina, USA) and SuperANOVA Version 1.1 (Abacus Concepts Inc., Berkeley, California, USA). In accordance with correct statistical practice, significant effects are reported as time-by-treatment interactions, treatment-effects and/or time-effects, in this hierarchy, i.e. by definition when a treatment effect is reported, no time-by-treatment interaction is evident. Post-hoc paired comparisons, corrected for multiple comparisons by Bonferroni’s correction, were performed if analysis of variance (ANOVA) revealed significant effects. Statistical significance was accepted at P < 0.05 and data are presented as means ± SEM.
Chapter 4

Effect of fatty acid chain length on suppression of ghrelin and stimulation of PYY, GLP-2 and PP secretion in healthy men

4.1 Summary
This study evaluated the effects of fatty acid chain length on ghrelin, PYY, GLP-2 and PP secretion. It was hypothesised that intraduodenal administration C12, but not C10, would decrease plasma ghrelin and increase PYY, GLP-2 and PP concentrations. Plasma hormone concentrations were measured in 7 healthy men during 90 min intraduodenal infusions of (i) C12, (ii) C10 or (iii) control (rate: 2 ml/min, 0.375 kcal/min for C12 and C10) and after a buffet-meal that was consumed following the infusion. C12 markedly suppressed plasma ghrelin and increased both PYY and GLP-2 (all P < 0.05) compared with control and C10, while C10 had no effect. Both C10 and C12 increased PP concentrations slightly (P < 0.05). In conclusion, the effects of intraduodenal fatty acids on ghrelin, PYY and GLP-2, but not PP, secretion are dependent on their chain length.

4.2 Introduction
Subsequent to meal ingestion, the interaction of nutrients with receptors in the small intestine modulates a number of gastrointestinal functions, including gastric emptying (Heddle et al., 1989), gastrointestinal hormone secretion (Feltrin et al., 2004) and energy intake (Chapman et al., 1999). In response to a meal, a number of hormones
including CCK (Lilja et al., 1984), GLP-1 (Lavin et al., 1998), GLP-2 (Yusta et al., 2000) and the two forms of PYY, PYY (1–36) and PYY (3–36) (Lin and Chey, 2003), are released from cells located in the small intestine, and PP (Miazza et al., 1985) from the pancreas, while the release of ghrelin, which is synthesized predominantly in the stomach (Kojima et al., 1999), is suppressed (Cummings et al., 2001). Studies in animals and humans indicate that CCK, GLP-1, PYY (3-36) (the latter converted from PYY by the enzyme dipeptidyl peptidase IV (Mentlein et al., 1993)), and PP contribute to the suppressive effects of nutrients on energy intake (Batterham et al., 2002, Batterham et al., 2003b, Flint et al., 1998, Kissileff et al., 1981), while ghrelin is an appetite stimulant (Wren et al., 2001a). The effect of GLP-2 on energy intake is unclear; in rats intracerebroventricular (Tang-Christensen et al., 2000), but not peripheral (Scott et al., 1998), administration suppresses energy intake, while in healthy humans intravenous GLP-2 appears to have no effect (Sorensen et al., 2003).

The digestive products of fat, fatty acids, appear to be primarily responsible for the gastrointestinal effects of fat (Feinle et al., 2003, Feinle-Bisset et al., 2005). The effects of fat on gastrointestinal motor and hormonal function and appetite are also dependent on the chain length of fatty acids (Feltrin et al., 2004). It was recently reported that in healthy subjects intraduodenal C12, stimulated pyloric motility much more than C10 (Feltrin et al., 2004). While C12 and C10 both stimulated CCK, the effect of C12 was much greater, and C12, but not C10, stimulated GLP-1. Furthermore, energy intake at a meal consumed immediately after intraduodenal fatty acid infusion was reduced by C12, but not C10, and C12, but not C10, decreased hunger and desire to eat, and induced nausea in 4 of the 7 subjects. Furthermore, it was recently shown that fat digestion is also required for ghrelin suppression, as well as the stimulation of PYY and
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PP (Feinle-Bisset et al., 2005). It is currently not known whether fatty acid chain length influences the secretion of ghrelin, PYY, GLP-2 and PP in humans.

Samples from a previous study (Feltrin et al., 2004) have now been assayed to address the hypothesis that intraduodenally administered C12, but not C10, would decrease ghrelin, and increase PYY, GLP-2 and PP, concentrations.

4.3 Materials and methods

4.3.1 Subjects

Seven healthy, male subjects, with a mean age of 24 ± 4 years (range 19 – 47 years) and of normal body weight for their height (mean BMI 22.0 ± 0.6 kg/m²), were included in this study and recruited according to guidelines as outlined in Chapter 3.2.

4.3.2 Study design

Each subject was studied on three occasions, separated by 3 - 10 days, to evaluate, in double-blind, randomised fashion, the effects of intraduodenal infusion of (i) C12, (ii) C10 or (iii) control solution, for 90 min on plasma ghrelin, PYY, GLP-2 and PP concentrations.

4.3.3 Preparation of fatty acid solutions

For preparation of the fatty acid solutions, 5.3 g of C12 or C10 was dissolved in sodium hydroxide (0.89 g for C12, 1.21 g for C10) and distilled water, to a total volume of 250 ml, with a resulting pH of 8.2. The control solution (distilled water) was also adjusted to a pH of 8.2 by the addition of sodium hydroxide. Both fatty acid solutions were delivered at a load of 0.375 kcal/min and had a concentration of 106 mM (in the case of
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C12) and 123 mM (in the case of C10). The infusion rate was 2 ml/min, thus, the total volume infused in 90 min was 180 ml.

4.3.4 Protocol

Subjects were intubated, via an anaesthetised nostril, with a 17-channel manometric catheter (see Chapter 3.5.1). An intravenous cannula was placed into a right antecubital vein for blood sampling for the subsequent determination of plasma ghrelin, PYY, GLP-2 and PP.

Once the catheter was positioned correctly (Heddle et al., 1989) (see Chapter 3.5.1), at t = 0 min (during phase I of the interdigestive MMC), a baseline blood sample was taken, and duodenal infusion of (i) C12, (ii) C10 or (iii) control solution commenced and continued for 90 min. Blood samples were taken every 15 min until t = 90 min, when the infusion was terminated; the subject was then extubated and immediately offered a cold, buffet-style meal (Feltrin et al., 2004). After the meal, further blood samples were taken at t = 120 min and t = 150 min. The intravenous cannula was then removed and the subject allowed to leave the laboratory.

4.3.5 Measurement of plasma ghrelin, PYY, GLP-2 and PP concentrations

Venous blood samples (10 ml) were collected for the determination of plasma ghrelin, PYY, GLP-2 and PP concentrations (as described in Chapter 3.6).

4.3.6 Data and statistical analyses

Plasma ghrelin, PYY, GLP-2 and PP concentrations were analysed by repeated measures ANOVA with time and treatment as factors, for both the infusion period (t = 0
– 90 min) and postprandial period (t = 120 and 150 min, compared with t = 90 min), respectively. Areas under the curve (AUC) between 0 – 90 min were calculated, using the trapezoidal rule, and analysed by one-way ANOVA.

4.4 Results

4.4.1 Plasma ghrelin concentrations (Figure 4.1)

Effect of duodenal infusion. There was a significant treatment by time interaction (P < 0.05) for plasma ghrelin concentrations. C12 progressively reduced ghrelin, and this was significant at t = 75 min and t = 90 min (P < 0.01, for both). There was no change in ghrelin during control and C10 infusions. Infusion of C12 suppressed plasma ghrelin compared with control (P < 0.001) and C10 (P < 0.01) between t = 45 – 90 min. The AUC between 0 – 90 min for plasma ghrelin was greater with C12 compared with control (P < 0.05), and there was a trend for AUC to be greater with C12 compared with C10 (P = 0.07).

Effect of meal. There was a significant reduction in plasma ghrelin after the meal, at t = 120 min for C10 (P < 0.05) and at t = 150 min for both control and C10 (P < 0.01, for both) compared with premeal concentrations (ie t = 90 min). In contrast, following C12 infusion there was no further suppression of ghrelin. However, following C12 infusion, ghrelin concentrations remained lower when compared with control and C10 (P < 0.05, for both), with no difference between control and C10.

4.4.2 Plasma PYY concentrations (Figure 4.2)

Effect of duodenal infusion. There was a significant treatment by time interaction (P < 0.001) for plasma PYY concentrations. C12 progressively increased plasma PYY
compared with baseline, and this was significant between $t = 45 - 90$ min ($P < 0.001$); in contrast C10 and control had no effect. Infusion of C12 increased plasma PYY when compared with both control and C10 ($P < 0.01$, for both) from $t = 30 - 90$ min, with no difference between C10 and control. The AUC between $0 - 90$ min for plasma PYY was greater with C12 compared with both control and C10 ($P < 0.001$, for both).

**Effect of meal.** Plasma PYY increased following control at $t = 120$ min ($P < 0.01$) and following both control and C10 at $t = 150$ min ($P < 0.001$, for both) when compared with premeal concentrations ($t = 90$ min). In contrast, there was no further change in PYY following C12. However, following the C12 infusion ($t = 120$ and 150 min) PYY concentrations remained higher when compared with both control and C10 ($P < 0.001$, for both).

### 4.4.3 Plasma GLP-2 concentrations (Figure 4.3)

**Effect of duodenal infusion.** There was a significant treatment by time interaction ($P < 0.001$) for plasma GLP-2 concentrations. C12 progressively increased GLP-2 compared with baseline, and this was significant between $t = 45 - 90$ min ($P < 0.001$), while C10 and control had no effect. Infusion of C12 increased plasma GLP-2 when compared with both control ($P < 0.001$) and C10 ($P < 0.05$) from $t = 30 - 90$ min, with no difference between C10 and control. The AUC between $t = 0 - 90$ min for plasma GLP-2 was greater with C12 compared with both control and C10 ($P < 0.001$, for both).

**Effect of meal.** Following control there was a significant increase in GLP-2 concentrations when compared with the premeal concentrations ($t = 90$ min) ($P < 0.001$). In contrast, there was no change in GLP-2 following C12 or C10. However,
following C12 (t = 120 and 150 min) GLP-2 concentrations remained higher when compared with control and C10 (P < 0.001, for both).

### 4.4.4 Plasma PP concentrations (Figure 4.4)

**Effect of duodenal infusion.** There was a significant effect of time (P < 0.05) on plasma PP. Both C12 and C10 increased PP concentrations compared with baseline; C12 between t = 15 – 90 min (P < 0.05) and C10 between t = 45 – 75 min (P < 0.05), however, the magnitude of the response was small. There were no significant differences in the AUC of plasma PP between treatments between t = 0 – 90 min.

**Effect of meal.** PP concentrations were higher at t = 120 min following both control and C10 (P < 0.01, for both) and at t = 150 min following control (P < 0.01) when compared with premeal concentrations (t = 90 min). In contrast, there was no significant change in PP following C12. When compared with C12, PP concentrations were greater at t = 120 min following both control and C10 (P < 0.001, for both) and at t = 150 min following control (P < 0.05).
Figure 4.1: Plasma concentrations of ghrelin during 90 min intraduodenal infusions of C12, C10 and control (t = 0 – 90 min) and after ingestion of a buffet-style lunch (t = 120 and 150 min). § C12 significantly different from baseline (t = 0) from 75 - 90 min, P < 0.01; * C12 significantly different from C10 and control from 45 – 90 min, P < 0.01; Δ significantly different from respective values at t = 90 min, P < 0.05. Data are means ± SEM (n = 7).
Figure 4.2: Plasma concentrations of PYY during 90 min intraduodenal infusions of C12, C10 and control (t = 0 – 90 min) and after ingestion of a buffet-style lunch (t = 120 and 150 min). § C12 significantly different from baseline (t = 0) from 45 – 90 min, P < 0.001; * C12 significantly different from C10 and control from 30 – 90 min, P < 0.01; Δ significantly different from respective values at t = 90 min, P < 0.01. Data are means ± SEM (n = 7).
Figure 4.3: Plasma concentrations GLP-2 during 90 min intraduodenal infusions of C12, C10 and control (t = 0 – 90 min) and after ingestion of a buffet-style lunch (t = 120 and 150 min). § C12 significantly different from baseline (t = 0) from 45 – 90 min, P < 0.001; * C12 significantly different from C10 and control from 30 – 90 min, P < 0.05; Δ significantly different from respective values at t = 90 min, P < 0.001. Data are means ± SEM (n = 7).
Figure 4.4: Plasma concentrations of PP during 90 min intraduodenal infusions of C12, C10 and control (t = 0 – 90 min) and after ingestion of a buffet-style lunch (t = 120 and 150 min). § C12 significantly different from baseline (t = 0) from 15 – 90 min, P < 0.05; # C10 significantly different from baseline (t = 0) from t = 45 - 75 min, P < 0.05; Δ significantly different from respective values at t = 90 min, P < 0.05. Data are means ± SEM (n = 7). Please note: Y-axis scales in (D) vary due to the differences in the magnitude of PP secretion between the duodenal infusion period (t = 0 – 90 min) and after the meal (t = 120 and 150 min).
4.5 Discussion

A previous study has shown that there were major differences in the effects of C12 and C10, when administered intraduodenally, on energy intake, antropyloroduodenal motility and secretion of CCK and GLP-1 in healthy males (Feltrin et al., 2004). This current study demonstrated that C12 and C10 also have discrepant effects on the secretion of ghrelin, PYY and GLP-2, but apparently not PP. C12, but not C10, markedly suppressed ghrelin, and stimulated PYY and GLP-2, secretion, whereas C10 had no effect. In contrast, both C12 and C10 increased PP secretion slightly, with no difference between them, whereas meal ingestion was associated with a marked increase in PP following control and C10, but not C12.

It has previously been shown that fat digestion is required for the secretion of PYY (Feinle-Bisset et al., 2005), and the current study extends this finding by demonstrating that fatty acid chain length is also important. This study is the first to evaluate the effects of fatty acids of different chain length on GLP-2 secretion. The pattern of PYY and GLP-2 release in response to intraduodenal C12 was similar to that observed for GLP-1 (Feltrin et al., 2004); as GLP-1 and GLP-2 are co-released (Orskov et al., 1986) and GLP-1 and PYY are both secreted from L cells (Eissele et al., 1992), this is not surprising. During the C12 infusion plasma GLP-2 and PYY both increased within 45 min and continued to rise over the 90-minute infusion period. Thus, the pattern of PYY, GLP-2 and GLP-1 secretion during the C12 infusion differed from that of CCK, reported previously (Feltrin et al., 2004), which was maximal within 15 min and then plateaued. The initial stimulation of PYY is likely to represent a response to the rise in CCK, as in dogs (Lin et al., 2000), while it is not known whether the same mechanism(s) drive(s) initial GLP-1 and GLP-2 secretion. In contrast, the continuing
rise in both PYY and GLP-2 after 45 min may well relate to the direct contact of fatty acid with the more distal site of PYY (Adrian et al., 1985) and GLP-2 (Munroe et al., 1999) peptide-releasing cells in the ileum and colon.

Meal ingestion, particularly meals containing fat and carbohydrate, but not protein (Erdmann et al., 2003, Greenman et al., 2004), is well known to suppress ghrelin (Cummings et al., 2001), and the underlying mechanisms apparently differ between fat and carbohydrate (Mohlig et al., 2002, Feinle-Bisset et al., 2005, Parker et al., 2005). In humans, both intraduodenal and intravenous glucose administration (Parker et al., 2005, Mohlig et al., 2002) decrease plasma ghrelin and increase plasma insulin (Erdmann et al., 2005), suggesting a role for post-absorptive factors. In contrast, exposure of the small intestinal lumen to lipid is required for ghrelin suppression (Feinle-Bisset et al., 2005), as intravenous administration of lipid is ineffective (Mohlig et al., 2002). The current study establishes that the chain length of fatty acids in the small intestine has the capacity to modulate ghrelin secretion. Interestingly, the rise in both PYY and GLP-2 during the C12 infusion was evident from 45 min, which was much earlier than the suppression of ghrelin (~ 75 min), suggesting that PYY and GLP-2 may play a role in the suppression of ghrelin. Indeed, intravenous administration of PYY suppresses ghrelin secretion in humans (Batterham et al., 2003a), and GLP-1 has been reported to decrease ghrelin secretion in the isolated rat stomach (Lippl et al., 2004). The time delay of this response may be indicative of the requirement for a critical threshold for plasma PYY (and/or other hormones) to be achieved for effective ghrelin suppression.

In contrast to ghrelin, PYY and GLP-2, the secretion of PP was minimal throughout the intraduodenal fatty acid infusion, but increased markedly after the meal, suggesting that
gastric distension represents a more important stimulus for PP secretion than small intestinal exposure to fatty acids. Indeed, as reported (Feltrin et al., 2004), subjects ate more following the C10 and control infusion, compared with C12. Efferent, vagal stimulation in animals (Schwartz et al., 1978) and gastric distension in humans (Koop et al., 1990) are known to stimulate PP alone, and vagal, cholinergic stimulation is an important regulator of PP secretion in humans (Schwartz et al., 1978).

A number of mechanisms may account for the discrepant effects of intraduodenal C12 and C10 on ghrelin, PYY and GLP-2, but not PP, secretion. Fatty acids with ≥ 12 carbon atoms are transported from the gut predominantly through the lymphatic system and packaged in chylomicrons, while fatty acids with ≤ 11 carbon atoms are absorbed predominantly directly into the portal vein (McDonald and Weidman, 1987). There is evidence that fatty acids with > 10 carbon atoms can act on CCK–secreting cells directly (McLaughlin et al., 1998), indicating that pre-absorptive factors may contribute to the differences between C10 and C12.

The hormones stimulated by C12, including PYY, GLP-2, PP, GLP-1 and CCK, are anorexigenic peptides (Kissileff et al., 1981, Flint et al., 1998, Batterham et al., 2002, Batterham et al., 2003b, Tang-Christensen et al., 2000), whereas ghrelin, which was suppressed by C12, increases energy intake (Wren et al., 2001a). Although this study does not establish whether the suppression of ghrelin and stimulation of PYY and GLP-2 (as well as CCK and GLP-1, reported previously (Feltrin et al., 2004)) are responsible for the suppression of energy intake following infusion of C12, these results are consistent with the concept that these hormones mediate the effect of C12 on energy intake.
Some limitations of this study should be recognized. As only 7 subjects were included, there is the risk of type 2 errors, however, the outcome of the statistical analyses was clear-cut. As only one dose of each fatty acid solution was evaluated, further studies are required to examine the potentially independent effects of different energy loads and concentrations of both C12 and C10 on hormone secretion, gastrointestinal motility and energy intake (see Chapters 6 and 7).

In conclusion, this study has demonstrated that in healthy humans the effects of intraduodenal fatty acids on ghrelin, PYY and GLP-2, but apparently not PP, secretion are dependent on their chain length. Accordingly, the role of these peptides, together with CCK and GLP-1, in the mediation of energy intake suppression, as well as the mechanisms underlying the modulation of the secretion of these peptides, by C12 warrant further evaluation.
Chapter 5

Comparative effects of intraduodenal administration of lauric and oleic acids on antropyloroduodenal motility, plasma cholecystokinin and peptide YY, appetite and energy intake in healthy male

5.1 Summary

The regulation of gastrointestinal function and energy intake by fatty acids is dependent on their chain length. Animal studies suggest that C12 may have more potent suppressive effects on energy intake than C18:1. The aims of this study were to compare the effects of equicaloric loads of C12 and C18:1 on APD motility, plasma CCK and PYY concentrations, appetite and energy intake. Thirteen healthy males (aged 20 – 46 years) were studied on three occasions in double-blind, randomised fashion. APD PWs, plasma hormones and appetite perceptions were measured during 60 min intraduodenal infusions of (i) C12, (ii) C18:1 or (iii) control (rate: 4 ml/min, energy load for C12 and C18:1: 0.4 kcal/min); between 60 – 90 min, energy intake at a buffet meal was quantified. C12 and C18:1 both reduced antral (P < 0.001) and duodenal (P < 0.01) PWs and stimulated IPPWs (P < 0.01) and plasma CCK (P < 0.001), with no differences between them. While C12 and C18:1 both increased basal pyloric pressure (P < 0.05), C12 had a greater effect than C18:1 (P < 0.01). In contrast, while both C12 and C18:1 increased plasma PYY (P < 0.001), C18:1 had a greater effect than C12. C12, but not C18:1, suppressed energy intake (P < 0.05). At the load
administered, C12, but not C18:1, suppressed energy intake and C12 was a more potent stimulant of pyloric tone. These discrepant effects are not apparently accounted for by changes in CCK or PYY secretion.

5.2 Introduction

While dietary fat is ingested in the form of triglycerides, its effects on gastrointestinal function and energy intake are mediated by the digestive products, fatty acids. Accordingly, the effects of lipid to slow gastric emptying (Raybould et al., 1998), stimulate phasic and tonic pyloric pressures, decrease antral and duodenal pressures (Heddle et al., 1988a), stimulate the secretion of CCK, GLP-1, PYY, and suppress ghrelin secretion (Feinle et al., 2003, Feinle-Bisset et al., 2005) and energy intake (Chapman et al., 1999, Feinle et al., 2003), are abolished by lipase inhibition, which prevents fat digestion (Meyer et al., 1994, Meyer and Jones, 1974, Feinle et al., 2003, Feinle et al., 2001, Feinle-Bisset et al., 2005). The gastrointestinal effects of fat are also dependent on the acyl chain length of fatty acids. Fatty acids with ≥12 carbon atoms slow gastric emptying (Hunt and Knox, 1968), modulate APD motility (Feltrin et al., 2004, McLaughlin et al., 1999) and gastrointestinal hormone secretion (Chapter 4, Feltrin et al., 2004, McLaughlin et al., 1999), and suppress energy intake (Matzinger et al., 2000, Feltrin et al., 2004) more than fatty acids with ≤10 carbon atoms.

Indirect comparison of results from different studies (Feinle et al., 2003, Feltrin et al., 2004) suggests that the effects on gastrointestinal function and energy intake may vary amongst fatty acids with ≥12 carbon atoms. For example, it has previously been reported that intraduodenal infusion of C12, at a load of 0.4 kcal/min, resulted in a peak plasma CCK concentration of ~ 12 pmol/L (Feltrin et al., 2004), while a long-chain
triglyceride emulsion consisting mainly of C18:1, only resulted in a peak plasma CCK concentration of ~ 6 pmol/L, despite being infused into the duodenum at a much higher load (2.8 kcal/min) (Feinle et al., 2003). In rodents, Meyer et al (Meyer et al., 1998b) reported that small intestinal infusions of C12 or C18:1, in equimolar concentrations (0.96 mmol/h), have comparable effects on energy intake, however, at this concentration C18:1 delivered twice the amount of energy compared with C12. Given that fatty acids are emptied from the stomach at loads of ~ 0.2 – 0.4 kcal/min (Hunt and Knox, 1968, Little et al., 2007), it is probable that if the two fatty acids were administered in equicaloric amounts, C12 would be more effective in reducing energy intake than C18:1. Currently no studies have determined the comparative effects of C12 and C18:1 on gastrointestinal function and energy intake in humans.

The aims of this study were to evaluate the hypotheses that, (i) at equicaloric loads intraduodenal C12 would suppress appetite and energy intake more than C18:1 and (ii) that these effects would be associated with specific changes in gastrointestinal function, such that C12 would inhibit antral and duodenal pressures, stimulate pyloric pressures and increase plasma CCK and PYY concentrations more than C18:1.

5.3 Materials and methods

5.3.1 Subjects

Thirteen healthy males were included in the study. The number of subjects was derived from power calculations based on a previous study (Chapman et al., 1999); it was calculated that with 13 subjects there would be a 10 % decrease in energy intake at $\alpha = 0.05$, with a power of 80 %. Subjects had a mean age of 26 ± 2 (range 20 – 46) years
and were required to have a normal body weight for their height (mean BMI 22.9 ± 0.6 kg/m²) and were recruited according to guidelines as outlined in Chapter 3.2.

5.3.2 Study design

Each subject was studied on three occasions, separated by 3 – 10 days, to evaluate, in double-blind, randomised fashion, the effects of intraduodenal infusion of the fatty acids, (i) C12 or (ii) oleic acid C18:1, or (iii) control (0.9 % saline) for 60 min, on APD motility, plasma CCK and PYY concentrations, appetite and energy intake.

5.3.3 Preparation of C12 and C18:1 solutions

The C12 solution was prepared by dissolving 4.52 g of C12 with 0.6 g of NaOH in 0.9 % saline, to a total volume of 400 ml, with a resulting pH of 8.2. The C18:1 solution was prepared by dissolving 4.88 g of C18:1 in distilled water to a volume of 400 ml. The pH of the C18:1 and control (0.9 % saline) solutions was adjusted to 8.2 by the addition of hydrochloric acid and NaOH, respectively. Both fatty acid solutions delivered the same energy content, ie 0.4 kcal/min (total: 24 kcal); the concentration of the C12 solution was 56 mM and that of the C18:1 solution was 43 mM. All solutions were infused at a rate of 4 ml/min, thus the total volume infused was 240 ml.

5.3.4 Protocol

Subjects were intubated, via an anaesthetised nostril, with a 17-channel manometric catheter (see Chapter 3.5.1). An intravenous cannula was placed into a right forearm vein to obtain blood samples for subsequent determination of plasma CCK and PYY concentrations.
Following correct positioning of the catheter, a baseline blood sample was taken (ie at t = -10 min), and the subject completed a VAS for the assessment of appetite-related sensations, as well as nausea and bloating (see Chapter 3.7.2). At t = 0 min, duodenal infusions were commenced at a rate of 4 ml/min for 60 min (i.e. t = 0 – 60 min). APD pressures were recorded throughout the infusion; blood samples were collected and VAS completed every 10 min between t = 0 – 60 min. At t = 60 min, the infusion was terminated and the subject was extubated and provided with a standardised, cold buffet-style meal (as described in Chapter 3.7.3). The types of food, as well as the macronutrient composition and energy content, of the meal are described in detail in Appendix IV. Further blood samples were collected, and VAS completed by the subject, at t = 90 and 120 min, the intravenous cannula was then removed and the subject was allowed to leave the laboratory.

5.3.5 Measurements

Antropyloroduodenal pressures

APD pressures were analysed for (i) number and amplitude of antral PWs (ii) basal pyloric pressure (tone), (iii) number and amplitude of IPPWs, (iv) number and amplitude of duodenal PWs, and (v) number and length of PWSs involving the antrum, pylorus and duodenum, as described in Chapter 3.5.2.

Plasma CCK and GLP-1 concentrations

Venous blood samples (10 ml) were collected for the determination of plasma CCK and PYY concentrations, as described in Chapter 3.6.
Appetite perceptions and energy intake

Hunger, fullness, desire to eat, prospective consumption, nausea and bloating were assessed by VAS, as described in Chapter 3.7.2.

Energy intake (kJ), the amount of food consumed (g) and the macronutrient distribution (% of energy from fat, carbohydrate and protein) was assessed (see Chapter 3.7.3).

5.3.6 Data and statistical analysis

Baseline (‘0’) values were calculated as the means of values obtained at t = -10 and 0 min for VAS scores and hormones, and between t = -10 to 0 min for basal pyloric pressures and total numbers and mean amplitudes of IPPWs, and antral and duodenal PWs and the number of APD PWSs. During the infusion period basal pyloric pressures were expressed as means over 10 min periods, while numbers and amplitudes of isolated pyloric pressure waves, antral and duodenal pressure waves were expressed as total numbers and mean values, respectively. APD PWSs were expressed as the total number of PWs spanning over 2 (ie 1.5 - < 3 cm), 3 (i.e. 3 - < 4.5 cm), …, 15 (ie 21 - < 22.5 cm) channels during the 60 minute infusion period.

VAS scores, basal pyloric pressures and plasma hormone concentrations were analysed by repeated-measures ANOVA, with time and treatment as within-subject factors. The number of APD PWSs was analysed by repeated measures ANOVA with length of propagation (1.5 - < 3, 3 - < 4.5, …, 21 - < 22.5 cm) and treatment as factors. One-way ANOVA was used to assess the effect of treatment on total numbers and mean amplitudes of IPPWs, antral and duodenal PWs, energy intake and macronutrient distribution.
5.4 Results
All subjects completed the three study days and tolerated the experimental conditions well.

5.4.1 Antropyloroduodenal motility
Antral pressure waves
There was a significant effect of treatment on both the number and amplitude of antral PWs (P < 0.001) (Table 5.1). C12 and C18:1 both reduced the number and amplitude of antral PWs substantially when compared with control (P < 0.01), with no difference between them.

Pyloric pressures
Basal pyloric pressure. There was a significant treatment x time interaction for basal pyloric pressure (P < 0.05) (Figure 5.1). C12 and C18:1 both increased basal pyloric pressure when compared with control, C12 between t = 0 – 60 min (P < 0.01) and C18:1 between t = 10 – 40 min (P < 0.05). The effect of C12 was greater than that of C18:1 between t = 20 – 60 min (P < 0.01).

Isolated pyloric pressure waves. There was a significant effect of treatment on the number and amplitude of IPPWs (P < 0.01) (Table 5.1). C12 and C18:1 caused an approximately three-fold increase in the number of IPPWs when compared with control (P < 0.01), with no difference between them. C12 and C18:1 both also increased the amplitude of IPPWs when compared with control (P < 0.05), with no difference between them.
**Duodenal pressure waves**

There was a significant effect of treatment on the amplitude, but not the number, of duodenal PWs (P < 0.01) (**Table 5.1**). C12 and C18:1 both reduced the amplitude of duodenal PWs modestly when compared with control (P < 0.05), with no difference between them.

**Antropyloroduodenal pressure wave sequences**

There was no effect of treatment on the number of APD PWSs spanning 2 – 15 channels (data not shown).

### 5.4.2 Plasma hormone concentrations

**Plasma CCK**

Baseline plasma CCK concentrations did not differ among study days. There was a significant treatment x time interaction for plasma CCK concentrations (P < 0.001) (**Figure 5.2A**). C12 and C18:1 both increased plasma CCK between t = 10 – 60 min compared with control (P < 0.001), with a slightly greater effect of C18:1 compared with C12 between t = 10 – 20 min (P < 0.05). The rise in plasma CCK in response to both C12 and C18:1 occurred rapidly, ie in the first 10 min, and thereafter concentrations plateaued until the end of the infusion. Plasma CCK immediately before the meal, ie at t = 60 min, did not differ between C12 and C18:1.

**Plasma PYY**

Baseline plasma PYY concentrations were (inexplicably) slightly higher on the control day compared with both C12 and C18:1 (P < 0.001), with no difference between C12 and C18:1. There was a significant treatment x time interaction for plasma PYY (P <
0.001) (Figure 5.2B). C12 and C18:1 both increased plasma PYY, between t = 40 – 60 min and t = 30 – 60 min, respectively, compared with control (P < 0.001). The effect of C18:1 was greater than that of C12 between t = 30 – 60 min (P < 0.001). The increase in plasma PYY in response to both C12 and C18:1 occurred progressively and was evident from ~ 30 min. Immediately before the meal, ie at t = 60 min, plasma PYY was slightly higher following C18:1 compared with C12.

5.4.3 Appetite perceptions and energy intake

There was no effect of treatment on perceptions of hunger, fullness, desire to eat, prospective consumption, nausea or bloating (data not shown). Scores did not change significantly from baseline over the 60 min infusion period.

There was a significant effect of treatment on energy intake (P < 0.05) (Table 5.2). C12 decreased energy intake when compared with control and C18:1 by ~10 % (P < 0.05), with no difference between control and C18:1. There was a trend (P = 0.06) for C12 to reduce the amount (g) of food ingested when compared with both control and C18:1 (Table 5.2). There was no difference in the percentage of fat, carbohydrate or protein ingested between treatments (Table 5.2).
Table 5.1: Total numbers and mean amplitudes of antral, pyloric and duodenal PWs during 60 min intraduodenal infusion of C12, C18:1 or control (0.9% saline).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C12</th>
<th>C18:1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antral pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>50 ± 13</td>
<td>0 ± 2*</td>
<td>5 ± 2*</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>62 ± 13</td>
<td>19 ± 8*</td>
<td>22 ± 6*</td>
</tr>
<tr>
<td><strong>Isolated pyloric pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>38 ± 7</td>
<td>121 ± 17*</td>
<td>120 ± 18*</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>23 ± 3</td>
<td>30 ± 4*</td>
<td>32 ± 4*</td>
</tr>
<tr>
<td><strong>Duodenal pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>451 ± 83</td>
<td>339 ± 53</td>
<td>300 ± 55</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>29 ± 2</td>
<td>25 ± 2*</td>
<td>24 ± 1*</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 13). * vs control, P < 0.05.
Table 5.2: Energy intake from the buffet meal, and macronutrient distribution, following 60 min intraduodenal infusion of C12, C18:1 or control (0.9% saline).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>C12</th>
<th>C18:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal)</td>
<td>1265 ± 92</td>
<td>1134 ± 80*</td>
<td>1249 ± 72</td>
</tr>
<tr>
<td>Amount consumed (g)</td>
<td>1206 ± 127</td>
<td>1062 ± 120</td>
<td>1200 ± 113</td>
</tr>
<tr>
<td>Energy (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>34 ± 2</td>
<td>35 ± 3</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>43 ± 3</td>
<td>43 ± 3</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Protein</td>
<td>22 ± 1</td>
<td>23 ± 1</td>
<td>22 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 13). * vs control and C18:1, P < 0.05.
Figure 5.1: Basal pyloric pressure during 60 min intraduodenal infusions of C12, C18:1 or control. * C12 vs control, P < 0.01; § C12 vs C18:1, P < 0.01; # C18:1 vs control, P < 0.05. Data are means ± SEM (n = 13).
Figure 2: Plasma CCK (A) and PYY (B) concentrations during 60 min intraduodenal infusions of C12, C18:1 or control. Data are means ± SEM (n = 13). * C12 vs control, P < 0.01; § C18:1 vs C12, P < 0.01; # C18:1 vs control, P < 0.05. Data are means ± SEM (n = 13).
5.5 Discussion

This study compared the effects of equicaloric intraduodenal infusions of C12 and C18:1 on APD motility, gastrointestinal hormone secretion and energy intake and established that, at the load given, C12 and C18:1 both (i) reduced the number and amplitude of antral, and reduced the amplitude of duodenal, PWs, (ii) increased basal pyloric pressure and the number and amplitude of phasic pyloric PWs and (iii) stimulated plasma CCK and PYY concentrations. Arguably of most interest is that the stimulation of basal pyloric pressure by C12 was substantially greater than that of C18:1, and that C12, but not C18:1, reduced energy intake. These differential effects were not apparently attributable to effects on CCK and PYY secretion; in fact the stimulation of PYY by C18 was clearly greater than that induced by C12.

In rats, infusion of C12 into the colon suppressed energy intake more than an equicaloric infusion of C18:1, while equimolar intraduodenal infusions of C12 and C18:1 suppressed energy intake to the same extent (Meyer et al., 1998b), suggesting that, when given at equicaloric loads, C12 might suppress energy intake more than C18:1. Therefore, we hypothesised in our study that in humans intraduodenal C12 would suppress energy intake to a greater extent than C18:1, when administered at the same energy load. Indeed, C12 suppressed energy intake by ~ 550 kJ, or ~10 %, when compared with control, while C18:1 was ineffective.

In contrast, the hypothesis that specific changes in gastrointestinal function would underlie the greater suppression of energy intake by C12 proved incorrect. The suppression of energy intake was expected to be associated with a greater modulation of APD motility, particularly the stimulation of IPPWs (Brennan et al., 2007, unpublished
observations, Xu et al., 2005) and hormone secretion (Abbott et al., 2005, Lieverse et al., 1994), however, C12 and C18:1 both stimulated IPPWs and plasma CCK comparably, and while C12 increased basal pyloric pressure more than C18:1, C18:1 increased PYY concentrations more than C12. In dogs, electrical stimulation of the pylorus, increasing both tonic and phasic pressures, is associated with suppression of energy intake (Xu et al., 2005), and a recent study has established that there is an inverse relationship between energy intake and the number of isolated pyloric pressure waves in humans, ie the stimulation of pyloric motility is associated with the suppression of energy intake (Brennan et al., 2007, unpublished observations). The administration of a CCK receptor antagonist in humans (Lieverse et al., 1994, Matzinger et al., 1999), and PYY receptor antagonist in animals (Abbott et al., 2005), abolishes the suppressive effect of fat on energy intake, demonstrating that CCK and PYY, at least in part, mediate the effects of fat on energy intake. Hence, it was postulated that a greater suppression of energy intake by C12 would be associated with greater stimulation of isolated pyloric pressure waves and/or greater hormone secretion compared with C18:1, neither of which proved to be the case.

There are several other possibilities to explain the differences, or discrepancies, in effects between C12 and C18:1 on energy intake. (a) Interestingly, basal pyloric pressures were markedly greater during the C12 infusion, despite comparable plasma CCK concentrations between C12 and C18:1. Similarly, equimolar amounts of C12 have been reported to stimulate much higher outputs of pancreatic bicarbonate per output of pancreatic protein secretion than C18:1 (Meyer and Jones, 1974). These two observations suggest important, qualitative differences in how the gastrointestinal tract senses and responds to C12 vs C18:1, the reasons for which are currently undefined.
(b) Shorter-chain fatty acids (including C12) are absorbed ten times more rapidly than longer-chain fatty acids (including C18:1) (Westergaard and Dietschy, 1976). In rats, when maltose or lactose is confined to a fixed segment of proximal small intestine, so that the slowly absorbed lactose can not activate feedback from ileum, maltose suppresses energy intake more than lactose, which is known to be absorbed about one tenth as fast as maltose (Meyer et al., 1998c). This observation with carbohydrates suggests that the rate of flux across enterocytes within a fixed length of small intestine determines the magnitude of the effect on energy intake that is generated by that intestinal segment. No comparable experiments have been performed with C12 vs C18:1 to confirm or refute this idea with fatty acids. (c) Shorter-chain fatty acids are oxidized more extensively in the liver to anorexogenic ketone bodies than longer-chain fatty acids (Bach et al., 1996) and undergo a more extensive conversion to malonyl-coA. Malonyl-coA is believed by some to be an important intracellular signal of energy stores in the hypothalamus that modulates feeding behaviour (Bouchard, 2000). Thus, a third possibility to explain the greater inhibition of energy intake by C12 than by C18:1 is a significant difference in post-absorptive metabolism that leads acutely to a cascade of anorexigenic signals. Differences in saturation between C12 (saturated) and C18:1 (monounsaturated) are unlikely to have been responsible for the observed effects on energy intake, considering that increasing un-saturation of fatty acids has been reported to be associated with greater suppression of energy intake in humans (French et al., 2000, Lawton et al., 2000).

As discussed, fatty acids empty from the stomach into the small intestine at rates between 0.2 – 0.4 kcal/min (Hunt and Knox, 1968), and it has previously been established that C12 that suppresses energy intake at ~ 0.4 kcal/min (Feltrin et al.,
2004), therefore, in this study C18:1 was also infused at 0.4 kcal/min, to match this maximal load of C12, which was probably insufficient for an effect on energy intake, despite effects on gastrointestinal function. The latter is supported by the findings from one study, in which C18:1 suppressed subsequent energy intake, when infused at the reported rate of 0.77 kcal/min for 60 min (Matzinger et al., 2000). Nevertheless, it is also possible that in our study both C12 and C18:1 may have maximally suppressed antral, and stimulated isolated pyloric, pressures (eg the mean numbers of isolated pyloric pressure waves during C12 and C18:1 infusions was ~2/min where the maximum is ~ 3/min (Heddle et al., 1988a) and there were virtually no antral waves), precluding quantification of potential differences between C12 and C18:1. Accordingly, further studies determining the effects of increasing doses of C18:1 on energy intake and their relationship with gastrointestinal function in humans would be of interest.

In conclusion, this study has demonstrated, that at the load administered, C12, but not C18:1, suppresses energy intake and C12 is a more potent stimulant of pyloric tone. This suppression of energy intake could not be related directly to changes in gastrointestinal function, considering both C12 and C18:1 suppressed antral pressure waves, stimulated isolated pyloric pressures, and increased plasma CCK concentrations, to the same extent, while C18:1 increased PYY concentrations more than C12. Taken together, these data confirm the potent appetite-suppressant effects of intraduodenal C12, but also suggest that this may not be accounted for entirely by the actions of C12 on gastrointestinal function.
Chapter 6

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

6.1 Summary

It was recently reported that intraduodenal infusion of C12 (at 0.375 kcal/min, 106 mM) stimulates IPPWs, inhibits antral and duodenal PWs, stimulates the release of CCK and GLP-1, and suppresses energy intake, and that these effects are much greater than those seen in response to an isocaloric C10 infusion. Administration of C12 was, however, associated with nausea, confounding interpretation of the results. The aim of this study was to evaluate the effects of increasing intraduodenal doses of C12 on APD motility, plasma CCK and GLP-1 concentrations, appetite and energy intake. Thirteen healthy males were studied on four days in double-blind, randomised, fashion. APD pressures, plasma CCK and GLP-1 concentrations and appetite perceptions were measured during 90 minute intraduodenal infusion of C12 at either (i) 0.1 (14 mM), (ii) 0.2 (28 mM) or (iii) 0.4 (56 mM) kcal/min, or (iv) saline (control) (rate: 4 ml/min). Energy intake was determined at a buffet meal immediately following the infusion. C12 dose-dependently stimulated IPPWs, decreased antral and duodenal motility, and stimulated secretion of CCK and GLP-1 ($r > 0.4$, $P < 0.05$ for all). C12 at 0.4 kcal/min suppressed energy intake compared with control and C12 at 0.1 and 0.2 kcal/min ($P < 0.05$). These effects were observed in the absence of nausea. In conclusion, intraduodenal C12 dose-dependently modulated APD motility and gastrointestinal hormone release in healthy
male subjects, while effects on energy intake were only apparent with the highest dose infused (0.4 kcal/min), possibly because only at this dose modulation of APD motility and gastrointestinal hormone secretion was sufficient for a suppressant effect on energy intake.

6.2 Introduction

Studies utilising pharmacological agents, such as THL, to inhibit fat digestion have provided evidence that the effects of fat on gastric emptying, gastrointestinal motility, gastrointestinal hormone secretion and appetite are dependent on the presence of free fatty acids in the small intestine (Feinle et al., 2003, O'Donovan et al., 2003, Matzinger et al., 2000, Feinle et al., 2001, Pilichiewicz et al., 2003, Borovicka et al., 2000, Schwizer et al., 1997). The effects of free fatty acids on gastrointestinal function, including motility, hormone release and energy intake are also dependent on their acyl chain length (Feltrin et al., 2004, Hunt and Knox, 1968, McLaughlin et al., 1999, Matzinger et al., 2000). Hunt and Knox (Hunt and Knox, 1968) in 1968, were the first to demonstrate that fatty acids with a chain length of 12 or more carbon atoms empty from the stomach much more slowly than fatty acids containing 10 or less carbon atoms.

In a recent study intraduodenal administration of C12, at a rate of 0.375 kcal/min and a concentration of 106 mM, was shown to stimulate pyloric motility and suppress antral and duodenal motility in healthy subjects much more than C10 (Feltrin et al., 2004). Intraduodenal C12 also suppresses ghrelin secretion (Chapter 4), stimulates the release of CCK (Feltrin et al., 2004, McLaughlin et al., 1999), GLP-1 (Feltrin et al., 2004), PYY, GLP-2, and PP (Chapter 4), whilst C10, in the dose evaluated, stimulated PP and
CCK, albeit to a lesser extent than C12, and had no effect on plasma concentrations of GLP-1 (Feltrin et al., 2004), PYY, GLP-2 and ghrelin (Chapter 4). Intraduodenal infusion of C18:1, but not C8, has been shown to inhibit energy intake in humans (Matzinger et al., 2000), and an inhibitory effect of intraduodenal infusion of C12, but not C10, on appetite and energy intake (Feltrin et al., 2004) was recently reported in healthy subjects. In this latter study, infusion of C12 potently attenuated ratings of hunger and desire to eat and suppressed energy intake at a subsequent meal (Feltrin et al., 2004). However, in some subjects C12 also induced nausea, and the suppression of energy intake was greater in those subjects (~ 3515 kJ) when compared with those that did not experience nausea (~ 1800 kJ), confounding interpretation of the observations (Feltrin et al., 2004). It is also possible, albeit less likely, that the observed effects on gastrointestinal motility and hormone secretion may also have been attributable to nausea. Therefore, it remains unclear whether the modulation of APD motility, gastrointestinal hormone secretion, appetite, and energy intake during intraduodenal infusion of C12 represents a physiological effect of C12, or is secondary to the induction of nausea.

The mechanisms by which C12 inhibits subsequent energy intake are unclear. There is some evidence that the effects of C12 are dependent on the release of CCK (Lal et al., 2004), for example, the inhibitory effects of C12 on gastric emptying and the perception of intragastric volume are attenuated by the CCK₁ receptor antagonist, loxiglumide (Lal et al., 2004). The effects of fatty acids also appear to involve the activation of vagal afferents, either directly, or via CCK (Lal et al., 2001, Cox et al., 2004). The effects of C12 on energy intake may also be mediated through the actions of GLP-1 (Feltrin et al., 2004), PYY and GLP-2 (Chapter 4) and the changes in gastrointestinal motility,
particularly the stimulation of pyloric motility (Xu et al., 2005). In animals, the effects of small intestinal C12 on gastric emptying and energy intake may be influenced by both the concentration, and/or energy load (Meyer et al., 1998b, Lin et al., 1990), although the energy load may be relatively more important (Lin et al., 1990). It is, therefore, possible that either the concentration and/or the energy load of C12 used in a previous study by Feltrin et al (Feltrin et al., 2004), may have contributed to the observed effects of C12 on appetite, energy intake and nausea by modulating gastrointestinal motility and hormone secretion. The concentration of the C12 solution (106 mM) employed in this previous study was based upon that which had been infused intragastrically (100 mM) in humans without inducing nausea (McLaughlin et al., 1999). The observation that infusion of C10 at a slightly higher (123 mM) concentration was not associated with adverse effects also argues against the concept that the concentration of the C12 solution was responsible for the observed nausea. However, under physiological conditions, ie following ingestion of a meal, fatty acids are present within the small intestine at much lower concentrations, ranging from approximately 25 – 65 mM (Ament et al., 1972, Borgstrom et al., 1957, Porter et al., 1971). Therefore, infusion of C12 at these concentrations may have more physiological effects on gastrointestinal function and energy intake. Likewise, it is possible that the energy load of C12 delivered to the small intestine may play a role in mediating the observed effects on APD motility, gastrointestinal hormone release, perceptions of appetite, and energy intake. The data of Hunt and Knox (Hunt and Knox, 1968) suggest that C12 empties from the stomach at rates ranging from approximately 0.1 to 0.4 kcal/min, however, the load-dependency has not been investigated.
This study evaluated the effects of increasing intraduodenal doses of C12, at (i) 0.1 (14 mM), (ii) 0.2 (28 mM) or (iii) 0.4 (56 mM) kcal/min, or (iv) saline (control), on APD motility, plasma CCK and GLP-1 concentrations, appetite and energy intake, to test the hypothesis that C12 would dose-dependently stimulate phasic and tonic pyloric motility, suppress antral and duodenal PWs and stimulate the release of CCK and GLP-1, in the absence of nausea, and that these effects of C12 would be associated with a dose-dependent suppression of energy intake.

6.3 Materials and methods

6.3.1 Subjects

Thirteen healthy males were included in the study; the number of subjects was based on power calculations derived from a previous study (Chapman et al., 1999); it was calculated that with 13 subjects there would be a 10 % decrease in energy intake at $\alpha = 0.05$, with a power of 80 %. Subjects had a mean age of $23 \pm 2$ years (range 19 – 30 years) and were required to have a normal body weight for their height (mean BMI $23.6 \pm 0.5$ kg/m$^2$) and were recruited according to guidelines as outlined in Chapter 3.2.

6.3.2 Study design

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

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

For the preparation of C12 solutions, 1.13, 2.26 or 4.52 g of C12 were dissolved with 0.18, 0.36 or 0.75 g of NaOH, respectively, in 0.9 % saline to a total volume of 400 ml, with a resulting pH of 8.4. The pH of the control solution (0.9 % saline) was adjusted to 8.4 by the addition of NaOH. All solutions were infused at a rate of 4 ml/min, so that the total volume infused in 90 min was 360 ml.

6.3.4 Protocol

Subjects were intubated, via an anaesthetised nostril, with a 17-channel manometric catheter (see Chapter 3.5.1). An intravenous cannula was placed into the right antecubital vein for blood sampling for the subsequent determination of plasma CCK and GLP-1 concentrations.

Following correct positioning of the catheter, a baseline blood sample was taken (ie at t = -15 min), and the subject completed a VAS for the assessment of appetite-related
Increasing doses of C12 on gastrointestinal function, hormones and appetite  Chapter 6

sensations, as well as nausea and bloating (see Chapter 3.7.2). At $t = 0$ min, intraduodenal infusion of C12 commenced at a rate of 4 ml/min for 90 min (ie $t = 0 – 90$ min). APD pressures were recorded throughout the infusion; blood samples were collected and VAS completed every 15 min. At $t = 90$ min, the infusion was terminated and the subject was extubated and provided with a standardised, cold, buffet-style meal (as described in Chapter 3.7.3). The types of food, as well as the macronutrient composition and energy content, of the meal are described in detail in Appendix IV. Further blood samples were collected and VAS completed by the subject at $t = 120$ and 150 min, the intravenous cannula was then removed and the subject was allowed to leave the laboratory.

6.3.5 Measurements

Antropyloroduodenal pressures
APD pressures were analysed for (i) number and amplitude of antral PWs (ii) basal pyloric pressure (tone), (iii) number and amplitude of IPPWs, (iv) number and amplitude of duodenal PWs, and (v) number and length of PWSs involving the antrum, pylorus and duodenum, as described in Chapter 3.5.2.

Plasma CCK and GLP-1 concentrations
Venous blood samples (10 ml) were collected for the determination of plasma CCK and GLP-1 concentrations, as described in Chapter 3.6.

Appetite perceptions and energy intake
Hunger, fullness, desire to eat, prospective consumption, nausea and bloating were assessed by VAS, as described in Chapter 3.7.2.
Energy intake (kJ), the amount of food consumed (g) and the macronutrient distribution (% of energy from fat, carbohydrate and protein) was assessed (see Chapter 3.7.3).

6.3.6 Data and statistical analyses

For the number and amplitude of antral and duodenal PWs, number and amplitude of IPPWs, basal pyloric pressures and number of APD PWSs, baseline values (0) were calculated as the mean of values obtained between \( t = -15 \) to 0 min. For VAS and plasma CCK and GLP-1 concentrations, baseline values (0) were calculated as the mean of values obtained at \( t = -15 \) and 0 min. The number and amplitude of IPPWs and basal pyloric pressures were expressed as mean values over 15 min periods during the 90 min infusion period (ie 0 – 15, 15 – 30,…, 75 – 90 min), while the number and amplitude of antral and duodenal PWs were expressed as mean values for the entire 90 min infusion period. APD PWSs were expressed as the total number of PWs spanning over 2 (ie 1.5 - < 3 cm), 3 (ie 3 - < 4.5 cm), …, 15 (ie 21 - < 22.5 cm) channels during the 90 minute infusion period. All data were expressed as changes from baseline values.

The number and amplitude of IPPWs, basal pyloric pressures, VAS scores and plasma hormone concentrations were analysed by repeated measures ANOVA with time (\( t = 0 \) – 15, 15 – 30,…, 75 – 90 min for IPPWs and basal pyloric pressures, and \( t = 0, 15, 30,…, 90 \) min for VAS scores and plasma hormone concentrations) and treatment as factors. The number of APD PWSs was analysed by repeated measures ANOVA with length of propagation (1.5 - < 3 cm, 3 - < 4.5 cm, …, 21 - < 22.5 cm) and treatment as factors. One-way ANOVA was used to analyse the effects of treatment on the number and amplitude of antral and duodenal PWs, energy intake (kJ), macronutrient distribution and the amount (g) of food consumed at the buffet meal. Dose-response
relationships were determined using linear associations between the dose of C12 administered (ie 0, 0.1, 0.2 or 0.4 kcal/min) and the mean values over 90 min of the number and amplitude of antral and duodenal PWs, the number of IPPWs, basal pyloric pressure, APD PWSs, energy intake, as well as the plasma CCK and GLP-1 concentrations at 90 min, by calculating correlation coefficients adjusted for repeated measures (Bland and Altman, 1995).

### 6.4 Results

All subjects completed the four randomised study days, and the study protocol was tolerated well by these subjects.

#### 6.4.1 Antropyloroduodenal pressures

**Antral pressures**

There was no statistically significant effect of treatment on the number of antral PWs (P = 0.08) (Table 6.1). C12(0.1) appeared to stimulate antral PWs compared with control, while C12(0.2) and C12(0.4) appeared to decrease the number of antral PWs compared with control and C12(0.1). There was a significant effect of treatment on the amplitude of antral PWs (P < 0.01). C12(0.4) reduced the amplitude of antral PWs compared with control (P < 0.01) and C12(0.1) (P < 0.01). C12(0.2) reduced the amplitude of antral PWs compared with control (P < 0.01) and C12(0.1) (P < 0.01). There was an inverse relationship between the number and amplitude of antral PWs with the dose of C12 administered, such that the greater the dose of C12, the lower the number (r = -0.3, P < 0.05) and amplitude (r = -0.41, P < 0.01) of antral PWs.
Pyloric pressures

Basal pyloric pressure (tone). There was no significant effect of treatment, or time, on basal pyloric pressure, although the mean values for C12(0.4) were higher compared with control, C12(0.1) and C12(0.2) (Figure 6.1A). There was no relationship between the dose of C12 administered and basal pyloric pressure.

Phasic pressures. There was a significant treatment x time interaction for the number of IPPWs (P < 0.01) (Figure 6.1B). C12(0.4) increased the number of IPPWs between t = 0 – 60 min compared with control (P < 0.05), and between t = 0 – 45 min compared with C12(0.1) (P < 0.05) and decreased the number IPPWs between t = 60 – 75 min compared with C12(0.2) (P < 0.01). C12(0.2) increased the number of IPPWs between t = 0 – 90 min compared with control (P < 0.05) and between t = 15 – 90 min compared with C12(0.1) (P < 0.05 for both). C12(0.1) increased the number between t = 0 – 15 min compared with control (P < 0.01). There was no effect of treatment on the amplitude of IPPWs. There was a relationship between the number, but not the amplitude, of IPPWs with the dose of C12 administered, such that the greater the dose of C12, the greater the number of IPPWs (r = 0.35, P < 0.05).

Duodenal pressures

There was a significant effect of treatment on the number, but not the amplitude, of duodenal PWs (P < 0.01) (Table 6.1). Infusion of C12(0.4) decreased the number of duodenal PWs compared with control (P < 0.001), C12(0.1) (P < 0.001) and C12(0.2) (P < 0.05). C12(0.2) decreased the number of duodenal PWs compared with C12(0.1) (P < 0.05). There was an inverse relationship between the number, but not amplitude,
of duodenal PWs with the dose of C12 administered, such that the greater the dose of C12, the lower the number of duodenal PWs ($r = -0.46$, $P < 0.001$).

**Antropyloroduodenal pressure waves sequences**

There was a significant effect of treatment on the number of PWSs over 2 (ie $1.5 < 3$ cm), 3 (ie $3 < 4.5$ cm), 4 (ie $4.5 < 6$ cm), 5 (ie $6 < 7.5$ cm), 6 (ie $7.5 < 9$ cm) and 7 (ie $9 < 10.5$ cm) channels ($P < 0.001$) (Figure 6.2). Infusion of C12(0.4) decreased the number of PWSs spanning 2, 3, 4 and 5 channels compared with control, C12(0.1) and C12(0.2) ($P < 0.001$), the number of PWSs spanning 6 channels compared with control ($P < 0.05$), and the number of PWSs spanning 7 channels compared with C12(0.1) ($P < 0.05$). Infusion of C12(0.2) decreased the number of PWSs spanning 2 channels compared with C12(0.1) ($P < 0.001$). Infusion of C12(0.1) increased the number of PWSs spanning 2, 3 and 4 channels compared with control ($P < 0.01$). PWSs spanning 8 and more channels (ie $\geq 10.5$ cm) were not analysed statistically, as they were very infrequent (a total of 26 waves spanning 8 – 15 channels, 9 during the control infusion, 14 during C12(0.1), 2 during C12(0.2) and < 1 during C12(0.4)).

**6.4.2 Plasma hormone concentration**

**Plasma CCK concentrations**

Baseline plasma CCK concentrations did not differ between study days (Control: $4.0 \pm 0.4$ pmol/L, C12(0.1): $3.9 \pm 0.3$ pmol/L, C12(0.2): $3.8 \pm 0.3$ pmol/L and C12(0.4): $3.9 \pm 0.3$ pmol/L). There was a significant treatment x time interaction for plasma CCK concentrations ($P < 0.001$) (Figure 6.3A). Infusion of C12(0.4) increased plasma CCK concentrations between $t = 15 – 90$ min compared with control ($P < 0.001$) and C12(0.1) ($P < 0.001$), and between $t = 30 – 90$ min compared with C12(0.2) ($P < 0.01$).
Infusion of C12(0.2) increased plasma CCK concentrations between t = 15 – 90 min compared with control (P < 0.001), and at t = 15, 30, 60 and 90 min compared with C12(0.1) (P < 0.05). Infusion of C12(0.1) increased plasma CCK concentrations at t = 15 and between 45 – 90 min compared with control (P < 0.05). Plasma concentrations of CCK peaked at approximately 15 min. There was a relationship between plasma concentrations of CCK at t = 90 min with the dose of C12 administered, such that the greater the dose of C12, the greater the concentration of CCK at 90 min (r = 0.74, P < 0.001).

**Plasma GLP-1 concentrations**

Baseline plasma GLP-1 concentrations were slightly variable over study days (Control: 13.9 ± 1.8 pmol/L, C12(0.1): 17.6 ± 2.9 pmol/L, C12(0.2): 16.7 ± 3 pmol/L, C12(0.4): 14.9 ± 1.8 pmol/L). There was a significant treatment x time interaction for plasma GLP-1 concentrations (P < 0.01) (Figure 6.3B). Infusion of C12(0.4) increased plasma GLP-1 concentrations from t = 30 – 90 min compared with control (P < 0.001), at t = 30 and 60 – 90 min compared with C12(0.1) (P < 0.05), and at t = 45 and 75 min compared with C12(0.2) (P < 0.05). Infusion of C12(0.2) increased plasma GLP-1 concentrations at t = 30, 60 and 90 min compared with control (P < 0.01), and at t = 30 min compared with C12(0.1) (P = 0.001). Infusion of C12(0.1) increased plasma GLP-1 concentrations at t = 45 and 90 min compared with control (P < 0.05). There was a relationship between the plasma concentrations of GLP-1 at 90 min with the dose of C12 administered, such that the greater the dose of C12, the greater the concentration of GLP-1 at t = 90 min (r = 0.46, P < 0.001).
6.4.3 Appetite perceptions and energy intake

There was no effect of treatment on hunger, fullness, desire to eat, prospective consumption, bloating or nausea (data not shown). Scores did not change significantly from baseline over the 90 min infusion period.

There was a significant effect of treatment on energy intake ($P < 0.05$). C12(0.4) decreased energy intake compared with control ($P < 0.01$), C12(0.1) ($P < 0.05$) and C12(0.2) ($P < 0.05$). There was no significant effect of treatment on either the amount (g) or the macronutrient distribution of food consumed at the buffet-meal (Table 6.2). There was no relationship between energy intake and the amount of C12 administered.
Table 6.1: Total number and amplitude of antral and duodenal pressure waves during 90 min intraduodenal infusions of C12 at 0.1, 0.2 and 0.4 kcal/min, or control.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C12(0.1)</th>
<th>C12(0.2)</th>
<th>C12(0.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antral pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>106 ± 20</td>
<td>166 ± 82</td>
<td>24 ± 10</td>
<td>1 ± 3</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>39 ± 6</td>
<td>40 ± 7</td>
<td>21 ± 4*#</td>
<td>19 ± 3*#</td>
</tr>
<tr>
<td><strong>Duodenal pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>778 ± 110</td>
<td>851 ± 110</td>
<td>508 ± 96#</td>
<td>275 ± 79*#†</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>32 ± 3</td>
<td>30 ± 2</td>
<td>30 ± 2</td>
<td>31 ± 4</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 13). * vs. control, P < 0.05; # vs C12(0.1), P < 0.05; † vs C12(0.2), P < 0.05.
Table 6.2: Energy intake from the buffet meal, and macronutrient distribution, in response to 90 min intraduodenal infusions of C12 at 0.1, 0.2 and 0.4 kcal/min, or control.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C12(0.1)</th>
<th>C12(0.2)</th>
<th>C1 (0.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy intake (kJ)</strong></td>
<td>5932 ± 495</td>
<td>5902 ± 475</td>
<td>5815 ± 520</td>
<td>5101 ± 521*</td>
</tr>
<tr>
<td><strong>Amount consumed (g)</strong></td>
<td>1369 ± 118</td>
<td>1367 ± 99</td>
<td>1399 ± 118</td>
<td>1305 ± 108</td>
</tr>
<tr>
<td><strong>Energy (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>33 ± 1</td>
<td>34 ± 2</td>
<td>32 ± 2</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>43 ± 2</td>
<td>43 ± 2</td>
<td>46 ± 2</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>Protein</td>
<td>22 ± 1</td>
<td>22 ± 1</td>
<td>22 ± 1</td>
<td>21 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 13). * vs. control, C12(0.1) and C12(0.2), P < 0.05.
Figure 6.1: Basal pyloric pressure (A) and number of IPPWs (B) during 90 min intraduodenal infusion of C12 at 0.1, 0.2 and 0.4 kcal/min, and control. * vs control, P < 0.05; # vs 0.1, P < 0.05; § vs. C12(0.4), P < 0.01. Data are means ± SEM (n = 13).
Figure 6.2: APD PWSs during 90 min intraduodenal infusion of C12 at 0.1, 0.2 and 0.4 kcal/min, and control. * vs control, P < 0.05; # vs C12(0.1), P < 0.05; § vs C12(0.2), P < 0.05. Data are means ± SEM (n = 13).
Figure 6.3: Plasma concentrations of CCK (A) and GLP-1 (B) during 90 min intraduodenal infusion of C12 at 0.1, 0.2 and 0.4 kcal/min, and control. * vs control, P < 0.05; # vs C12(0.1), P < 0.05; § vs C12(0.2), P < 0.05. Data are means ± SEM (n = 13).
6.5 Discussion

This study establishes that intraduodenal administration of C12 modulates APD motility and gastrointestinal hormone release in a dose-dependent fashion, such that the greater the dose of C12 administered, the greater the stimulation of IPPWs, suppression of antral and duodenal PWs and APD PWSs, and stimulation of the gastrointestinal hormones, CCK and GLP-1. In contrast, at the doses used, appetite perceptions were not affected and suppression of energy intake was only apparent with the 0.4 kcal/min dose, occurring in the absence of nausea.

Previous studies have established that intraduodenal infusion of C12 slows gastric emptying (Hunt and Knox, 1968, Lal et al., 2004), increases proximal gastric relaxation (Lal et al., 2004), suppresses antral (Feltrin et al., 2004, McLaughlin et al., 1999) and duodenal motility (Feltrin et al., 2004), stimulates isolated pyloric pressure waves (Feltrin et al., 2004) and stimulates the release of CCK (McLaughlin et al., 1999, Feltrin et al., 2004), GLP-1 (Feltrin et al., 2004), PYY, GLP-2 and PP (Chapter 4), and suppresses ghrelin (Chapter 4). This study extends these observations by demonstrating that the responses are dependent upon the dose of C12 administered to the small intestine, and that even very low doses of C12 have potent effects. For example, infusion of C12 at doses as low as 0.1 and 0.2 kcal/min, resulting in a total energy delivery of only 9 and 14 kcal, respectively, over the 90 min infusion period, produced these effects. In previous studies using intraduodenal lipid infusion at a rate of 2.8 kcal/min (Feinle et al., 2003), the stimulation of pyloric motility and plasma CCK concentrations were maximal at about ~30 – 45 min when approximately 84 – 126 kcal would have been delivered to the small intestine. This contrasts with the current study in which maximal effects of the C12 infusion on pyloric motility and plasma hormone
secretion were observed after 15 min, when only 1.5, 3 or 6 kcal had been delivered to
the small intestine during infusion of C12 at 0.1, 0.2 and 0.4 kcal/min, respectively.
This provides persuasive evidence that the effects of C12 on pyloric motility and plasma
concentrations of CCK and GLP-1 are much more potent than those of long-chain
triglycerides. The reasons for this are currently unknown, but may perhaps be explained
by the fact that C12 represents only approximately 6 % of dietary fatty acids, therefore,
under normal conditions exposure of the small intestine to C12 is likely to be limited.

The demonstrated dose-dependent effects of C12 on plasma concentrations of CCK and
GLP-1 suggest that the release of CCK and GLP-1 in response to C12 is also dependent
upon the amount of C12 present in the small intestinal lumen, ie infusion of C12 at 0.4
kcal/min resulted in a greater secretion of CCK than during infusion of C12 at 0.1 or 0.2
kcal/min. The secretion profiles of CCK and GLP-1 also varied, CCK was secreted
almost immediately after the start of the C12 infusions, and concentrations plateaued
after 30 mi. In contrast, there was a 30 min delay before plasma GLP-1 concentrations
were increased, and GLP-1 progressively increased during the entire 90 minutes of
infusion of C12. It is possible, that during infusion of C12, the absorption capacity of
the proximal small intestine was exceeded, resulting in progressively greater amounts of
C12 reaching the distal small intestine, the site of GLP-1 release (Eissele et al., 1992),
thereby explaining the gradual increase in GLP-1 secretion.

The effects of C12 on appetite and energy intake were not dose-dependent at the doses
utilised in this study. Infusion of C12 at 0.4 kcal/min, but not 0.1 and 0.2 kcal/min,
decreased energy intake by ∼ 830 kJ compared with control, without inducing nausea.
While the highest dose of C12 suppressed energy intake, this occurred in the absence of
changes in appetite perceptions. This suggests that perceptions of appetite may be regulated by mechanisms differing from those involved in the control of acute energy intake, and may perhaps require higher energy loads. The effects of C12 on energy intake were much more marked in a previous study (Feltrin et al., 2004), in which C12 suppressed subsequent energy intake by ~2780 kJ compared with control, however, this was associated with a marked increase in nausea. – Although the decrease in hunger and energy intake could not altogether be attributed to nausea, as the subjects who did not experience nausea still significantly decreased their energy intake (Feltrin et al., 2004). The observations in the current study provide support for a physiological role of C12 in appetite suppression.

It has been suggested that the effects of nutrients on subsequent energy intake are mediated by changes in gastrointestinal motility and gastrointestinal hormone release, however, this study has revealed discordance between these effects. The motor patterns associated with infusion of C12 at 0.2 and 0.4 kcal/min are known to be associated with the slowing of gastric emptying (Heddle et al., 1989), which is thought to play a role in suppressing energy intake (Sepple and Read, 1989). Recent evidence has suggested that electrical stimulation of the pylorus is able to suppress energy intake in dogs (Xu et al., 2005), thus implying an important role for the pylorus in the regulation of energy intake. It is, however, interesting to note that despite C12 at 0.2 kcal/min having a more prolonged stimulatory effect on IPPWs than C12 at 0.4 kcal/min, there was no effect on energy intake. Furthermore, by the time of the meal, the effect of C12 at 0.4 kcal/min had returned to baseline yet energy intake was suppressed only following this infusion, suggesting that its inhibitory effects on energy intake did not depend on the motility effects. This suggests that factors other than the stimulation of IPPWs are required to
inhibit energy intake. The discrepant effects seen in this study may be explained by the different patterns of secretion of the gastrointestinal hormones CCK and GLP-1, as intravenous infusion of both CCK and GLP-1 suppresses energy intake in humans (Kissileff et al., 1981, Flint et al., 1998). C12 at 0.4 kcal/min stimulated the secretion of CCK and GLP-1 more than C12 at 0.2 kcal/min. It is interesting to note that while C12 at 0.2 kcal/min stimulated the release of CCK to a similar concentration to that previously observed in studies using intraduodenal infusion of a long-chain triglyceride emulsion (Feinle et al., 2003), in which there was a significant suppression of energy intake, there was no suppression of energy intake with C12 at 0.2 kcal/min. This may have been due to the number of subjects studied, however, it seems unlikely that increasing the number of subjects would show an effect of the lower doses on energy intake, as there was no trend at all towards decreased energy intake using these doses. It is likely that there are different threshold requirements for the effects observed on motility, gastrointestinal hormone release, appetite and energy intake, ie while the lower doses were sufficient to stimulate motility and hormone release, only the 0.4 kcal/min infusion was sufficient to suppress energy intake. Likewise, while none of the doses were sufficient to have an effect on perceptions of appetite, ie hunger, fullness, desire to eat and prospective consumption, C12 0.4 kcal/min suppressed subsequent energy intake.

While this study has demonstrated a clear dose-responsive effect of C12 on APD motility and gastrointestinal hormone release, it is unclear whether the concentration, or the energy load, of C12 administered mediated these effects of C12, as in order to keep the volume of the infusion identical on all study days, the concentration of the solutions varied. Animal studies have suggested that the inhibition of gastric emptying by
intestinal C18:1 is dependent upon the concentration, but not the load, of C18:1 (Lin et al., 1990). Conversely, the effects of C18:1 and C12 on energy intake, at concentrations ranging from 20 to 80 mM, were shown to be load, but not concentration, dependent in rats (Meyer et al., 1998b). In a previous study, there was a more marked increase in the number of IPPWs, basal pyloric pressure, secretion of CCK and GLP-1, as well as decreased perceptions of hunger and desire to eat, and energy intake during infusion of C12 at 0.375 kcal/min, with a concentration of 106 mM (Feltrin et al., 2004), compared with the current observations for C12 at 0.4 kcal/min and 56 mM, suggesting that concentration may be important. However, further studies are required to examine the independent effects of energy load and concentration of C12 (see Chapter 7).

In conclusion, this study has demonstrated a dose-dependent effect of acute intraduodenal C12 on APD motility and gastrointestinal hormone secretion, and at the highest dose used, an inhibitory effect on energy intake, in the absence of nausea.

**Acknowledgement:** KL Feltrin and TJ Little conducted the study described in this chapter with equal contributions, hence, it was also submitted as part of a Doctor of Philosophy degree by TJ Little, University of Adelaide, 2007.
Chapter 7

Effects of lauric acid on upper gut motility, plasma cholecystokinin and peptide YY and energy intake are load, but not concentration, dependent in humans

7.1 Summary
Animal studies suggest that the effects of fatty acids on gastric emptying and pancreatic secretion are both concentration-, and load-, dependent, while their suppressive effect on energy intake is only load-dependent. It was postulated that, in humans, the modulation of APD pressure waves, plasma CCK and PYY concentrations and energy intake by intraduodenal C12 would be load-, but not concentration-, dependent. Two groups of 12 healthy males were each studied on three separate occasions in double-blind, randomised fashion. APD pressure waves, plasma CCK and PYY and appetite perceptions were measured during intraduodenal infusions of C12 at (1) different loads of (i) 0.2, (ii) 0.3 or (iii) 0.4 kcal/min (all 56 mM) for 90 min or (2) different concentrations of (i) 40, (ii) 56 or (iii) 72 mM (all 0.4 kcal/min) for 60 min. Energy intake at a buffet meal consumed immediately following each infusion was quantified. Suppression of antral and duodenal pressure waves, stimulation of pyloric pressure waves, stimulation of plasma CCK and PYY, and suppression of energy intake, were related to the load of C12 administered ($r > 0.65$, $P < 0.05$). In contrast, there were no concentration-dependent effects of C12 on any of these parameters. In conclusion, in humans the effects of intraduodenal C12 on APD motility, plasma CCK and PYY and
energy intake appear to be related to load, but not concentration, at least at the loads and concentrations evaluated.

### 7.2 Introduction

It is well established that fatty acids are responsible for mediating the effects of fat on gastrointestinal motility, gastrointestinal hormone secretion, including CCK from the proximal, and PYY from the distal, small intestine (Lin and Chey, 2003, Lieverse et al., 1994), appetite and energy intake in humans (Chapter 6, Feinle et al., 2003, Feltrin et al., 2004, Hunt and Knox, 1968, McLaughlin et al., 1999, Matzinger et al., 2000). As demonstrated in Chapter 6, intraduodenal infusion of C12 inhibits antral and duodenal PWs, stimulates pyloric motility, increases plasma CCK concentrations and suppresses energy intake in a dose-related fashion. In this previous study, C12 was infused at loads of 0.1, 0.2, and 0.4 kcal/min, as 14, 28, and 56 mM solutions, respectively, and, while a concentration as low as 14 mM (at 0.1 kcal/min) was shown to stimulate IPPWs and release CCK, as the load and concentration were varied in parallel, it was not possible to determine whether the load, or concentration, of C12 (or both) was responsible for the observed effects. In this context animal studies have demonstrated that increasing the load of C12 (or other nutrients) is associated with an increased length of the small intestine in contact with C12 (Meyer et al., 1998c), because, as the absorptive capacity is reached for a certain segment of the small intestine, C12 travels further along the small intestine until absorbed. While such studies have not been performed in humans, it is very likely that this is also the case in humans. In contrast, increasing the concentration of C12 is unlikely to modify the length of small intestinal contact, particularly if the amount of C12 is constant.
In an earlier human study (Feltrin et al., 2004), C12 infused intraduodenally as a 106 mM solution at 0.4 kcal/min suppressed energy intake about twice as much, and released CCK some three times as much, as a 0.4 kcal/min load of lauric acid at 56 mM in Chapter 6. Because (a) 106 mM is more than twice the concentration of luminal fatty acids observed after a fatty meal (Borgstrom et al., 1957, Ament et al., 1972, Porter et al., 1971, Porter and Saunders, 1971), (b) cytotoxicity of fatty acids on gut mucosa is known to be concentration-dependent (Velasquez et al., 1993b) and (c) the 106 mM, but not the 56 mM, C12 solution induced nausea, it is unclear whether the responses observed were toxic, or physiological and, thus, whether the differential effects of a 0.4 kcal/min infusion at 106 and 56 mM reflected a physiological importance of concentration, independent of load, or not.

It is well established that nutrient-stimulated signals from the small intestine of experimental animals regulate pancreatic secretion (Meyer et al., 1970a, Meyer et al., 1970b, Meyer and Jones, 1974, Meyer et al., 1976), gastric emptying (Lin et al., 1989, Lin et al., 1990) and energy intake (Meyer et al., 1998b) in a load-dependent fashion, independent of nutrient concentrations, over the ranges tested. Humans also sense caloric loads to regulate gastric emptying (Hunt et al., 1985, Brener et al., 1983) or energy intake (Rolls et al., 1991), and while it is clear that humans modulate gastric emptying in response to glucose loads, but not concentrations (Brener et al., 1983, Hunt et al., 1985), whether loads, or concentrations, of nutrients regulate human energy intake is controversial (Porikos et al., 1982, Rolls et al., 1998). The ability of dietary fats in the gastrointestinal lumen to inhibit subsequent energy intake with some, albeit varying, degrees of accuracy (Rolls et al., 1991, Shide et al., 1995) suggests that fat, specifically lipolytic products, must be sensed in a load-dependent, concentration-
independent, fashion. Determination of whether load and/or concentration of fatty acids modulates gastrointestinal function, appetite and energy intake in humans, is important to an understanding of the mechanisms of action of fatty acids in the gut, which may be of relevance to the pathogenesis of obesity.

This study examined in healthy humans the responses to intraduodenal loads of C12 of 0.2, 0.3 and 0.4 kcal/min at a fixed concentration (56 mM) and contrasted them to concentrations of 40, 56, and 72 mM at a fixed load (0.4 kcal/min), to evaluate the effects of load, and concentration, of C12 on APD motility, plasma CCK and PYY concentrations, appetite and energy intake. It was hypothesised that if fatty acid concentration (above 40 mM) was the dominant stimulus, there should be no dose-response in the ‘load’ study, but a definite dose-response in the ‘concentration’ study, whereas, if load dominated there would be no dose-response to concentration, but a definite load-dependent effect.

7.3 Materials and methods

7.3.1 Subjects

A total of 24 healthy males were studied, 12 subjects in each of the two protocols described below, with 3 subjects participating in both protocols. The number of subjects was based on power calculations derived from Chapter 6. It was calculated that with 12 subjects there would be a 15% decrease in energy intake at $\alpha = 0.05$, with a power of 80%. Subjects had a mean age of $23 \pm 1$ (range 18 – 36) years and were of normal body weight for their height (body mass index $22.3 \pm 0.3 \text{ kg/m}^2$) and were recruited according to guidelines as outlined in Chapter 3.2.
7.3.2 Study design

Each subject was studied on three occasions, separated by 3 – 10 days, to evaluate, in double-blind, randomised fashion the effects of intraduodenal infusion of C12 at varying loads or concentrations on APD motility, plasma CCK and PYY, appetite and energy intake. For this purpose, solutions were designed to deliver (i) different loads (0.2 – 0.4 kcal/min) of C12 at a constant concentration (56 mM) (“C12 load”) or (ii) different concentrations (40 – 72 mM) of C12 at a constant load (0.4 kcal/min) (“C12 concentration”) to the small intestine. Load was expressed as kcal/min, since it is well known that gastric emptying of nutrients is regulated on a kcal/min basis (Horowitz et al., 1996, Brener et al., 1983). It was a natural progression to initiate the “C12 load” protocol first, as this followed on directly from the study detailed in Chapter 6, and the “C12 concentration” protocol was initiated subsequently.

7.3.3 Preparation of C12 solutions

The loads of the solutions were selected on the basis of previous studies in humans, indicating that fatty acids empty from the stomach into the small intestine at ~ 0.1 - 0.4 kcal/min (Hunt and Knox, 1968) and that intraduodenal loads of C12 at 0.1 - 0.4 kcal/min for 90 min are well tolerated and do not induce nausea (Chapter 6). While the concentrations of the solutions were selected to be within the range of fatty acid concentrations that occur in the small intestine after triglyceride digestion (Borgstrom et al., 1957), it should be acknowledged that the range of fatty acid concentrations (40 – 72 mM) in this study is quite narrow, however, as explained below, only these concentrations could be tolerated by the subjects in the absence of adverse effects.
Experimental protocol 1: Different loads of C12 at 56 mM (“C12 load”)

C12 was delivered to the small intestine at loads of (i) 0.2 kcal/min (“C12(0.2)”; total energy: 18 kcal (75 kJ)), (ii) 0.3 kcal/min (“C12(0.3)”; total energy: 27 kcal (112.5 kJ)) or (iii) 0.4 kcal/min (“C12(0.4)”; total energy: 36 kcal (150 kJ)). The solution was prepared using 4.52 g of C12 dissolved with 0.75 g of NaOH in 0.9 % saline, to a total volume of 400 ml, with a concentration of 56 mM. To achieve the different loads, the solution was infused at rates of (i) 2 ml/min, (ii) 3 ml/min or (iii) 4 ml/min for 90 minutes, thus, the total volumes infused were 180 ml, 270 ml and 360 ml, respectively.

Experimental protocol 2: Different concentrations of C12 at 0.4 kcal/min (“C12 concentration”)

C12 was delivered to the small intestine at concentrations of (i) 40 mM (“C12(40)”), (ii) 56 mM (“C12(56)”; or (iii) 72 mM (“C12(72)”). For this purpose 4.8 g, 4.52 g and 3.6 g of C12 were dissolved with 0.67 g, 0.75 g and 0.45 g of NaOH, respectively, in 0.9 % saline, to total volumes of 600 ml, 400 ml and 250 ml, respectively. Solutions were infused at rates of (i) 5.7 ml/min (40 mM), (ii) 4.0 ml/min (56 mM) or (iii) 3.1 ml/min (72 mM) for 60 min, thus, the total volumes infused were 342 ml, 240 ml and 186 ml, respectively. Each infusion delivered a load of 0.4 kcal/min, thus, the total amount of C12 infused was 24 kcal (100 kJ) with all three solutions.

Initially this study was planned to evaluate a wider range of concentrations, however, in pilot studies it became apparent that concentrations less than 40 mM (eg 24 mM) induced adverse effects, including severe abdominal cramps and diarrhoea, most likely due to the large volume (9.4 ml/min) required to deliver a load of 0.4 kcal/min. Furthermore, the solutions in this protocol had also been planned to be infused for 90
min, as was the case with the “C12 load” protocol, however, pilot studies demonstrated that the 40 mM C12 solution (5.7 ml/min), when infused for 90 min, resulted in abdominal cramps or diarrhoea in some subjects (probably also because of the volume infused), but could be tolerated without adverse effects for 60 min, hence, the difference in the duration of the infusions between the two study protocols. None of the subjects who participated in the pilot studies was included in the final protocol.

7.3.4 Protocol

Subjects were intubated, via an anaesthetised nostril, with a 17-channel manometric catheter (see Chapter 3.5.1). An intravenous cannula was placed into the right antecubital vein for blood sampling for the determination of plasma CCK and PYY concentrations.

Following correct positioning of the catheter a baseline venous blood sample was taken (t = -15 min), and a VAS, assessing appetite-related sensations and nausea and bloating, was administered (see Chapter 3.7.2). At t = 0 min duodenal infusion of C12 was commenced. APD pressures were monitored throughout the infusion period, and blood samples were taken, and VAS administered, every 15 min between t = 0 – 90 min for “C12 load”, and between t = 0 – 60 min for “C12 concentration”. At t = 90 min (“C12 load”), or t = 60 min (“C12 concentration”), the infusion was terminated, the subject immediately extubated and provided with a cold buffet-style meal (as described in Chapter 3.7.3). The types of food, as well as the macronutrient composition and energy content, of the meal are described in detail in Appendix IV. After ingestion of the meal the intravenous cannula was removed and the subject was allowed to leave the laboratory.
7.3.5 Measurements

Antropyloroduodenal pressures

APD pressures were analysed for: (i) number and amplitude of antral PWs, (ii) basal pyloric pressure (tone), (iii) number and amplitude of IPPWs and (iv) number and amplitude of duodenal PWs, as described in 3.5.2.

Plasma CCK and PYY concentrations

Venous blood samples (10 ml) were collected for the determination of plasma CCK and PYY concentrations, as described in Chapter 3.6.

Appetite perceptions and energy intake

Hunger, fullness, desire to eat, prospective consumption, nausea and bloating were assessed by VAS, as described in Chapter 3.7.2.

Energy intake (kJ), the amount of food consumed (g) and the macronutrient distribution (% of energy from carbohydrate, fat and protein) were assessed (see Chapter 3.7.3).

7.3.6 Data and statistical analyses

Baseline (‘0’) values were calculated as the means of values obtained at t = -15 and 0 min for VAS scores and hormones, and between t = -15 to 0 min for basal pyloric pressures, number and amplitude of IPPWs, and antral and duodenal PWs. Basal pyloric pressures and the number and amplitude of IPPWs were expressed as means over 15 min segments during the infusion period. Numbers and amplitudes of antral and duodenal PWs were expressed as total number and mean values, respectively, during the infusion period. To enable a direct comparison between the “C12 load” and
“C12 concentration” protocols, the area under the curve for basal pyloric pressure and the total number of IPPWs and antral and duodenal PWs, were calculated between $t = 0 - 60 \text{ min}$ for C12(0.4) and compared with C12(56), as both infusions delivered the same load (0.4 kcal/min) and concentration (56 mM).

VAS scores, basal pyloric pressures, number and amplitude of IPPWs and plasma hormone concentrations were analysed by repeated-measures ANOVA, with time and treatment as within-subject factors. One-way ANOVA was used to assess the effect of treatment on energy intake, macronutrient distribution, total numbers and mean amplitudes of antral and duodenal PWs. One-way ANOVA was also used to compare areas under the curves for basal pyloric pressures, the total number of IPPWs and antral and duodenal PWs (between $t = 0 - 60 \text{ min}$), plasma CCK and PYY (at $t = 60 \text{ min}$) and energy intake, between C12(0.4) and C12(56). To evaluate load- or concentration-dependent responses, correlations were performed between numbers and amplitudes of antral and duodenal PWs, basal pyloric pressure, number and amplitude of IPPWs, plasma CCK and PYY concentrations and energy intake, with the natural logarithm (ln) of each load and concentration. The slopes of the linear regressions were then tested to see if they were greater, or less, than zero (Elashoff, 1981).

7.4 Results

All subjects completed the three randomised study days, and both experimental conditions were well tolerated.
### 7.4.1 Effects of load of C12

#### Antropyloroduodenal pressures

##### Antral pressures

There was a significant effect of treatment on the number, but not the amplitude, of antral pressure waves ($P < 0.01$) (Table 7.1). Both C12(0.3) and C12(0.4) reduced the number of antral PWs compared with C12(0.2) ($P < 0.01$), with no difference between C12(0.3) and C12(0.4). There was an inverse relationship between the number ($r = -0.76$, $P < 0.05$), but not the amplitude, of antral PWs with the load of C12 administered.

##### Pyloric pressures

**Basal pyloric pressure.** There was an effect of time ($P < 0.001$), but not of treatment on basal pyloric pressure (Figure 7.1A). C12(0.2) increased basal pyloric pressure in the first 15 min compared with baseline ($P < 0.05$), and both C12(0.3) and C12(0.4) increased basal pyloric pressure over the first 30 min of the infusion compared with baseline ($P < 0.05$). There was no relationship between basal pyloric pressure and the load of C12 administered.

**Isolated pyloric pressure waves.** All treatments increased both the number and amplitude of IPPWs throughout the 90 min infusion period compared with baseline ($P < 0.05$, for all). There was a significant treatment x time interaction for the number of IPPWs ($P < 0.01$) (Figure 7.1C). Both C12(0.3) and C12(0.4) increased the number of IPPWs compared with C12(0.2) between $t = 0 – 30$ min ($P < 0.01$), with no difference between C12(0.3) and C12(0.4). There was no effect of treatment on the amplitude of IPPWs (data not shown), however, there was a significant effect of time ($P < 0.001$).
There was a direct relationship between the number ($r = 0.70, P < 0.05$) and amplitude ($r = 0.66, P < 0.05$) of IPPWs with the load of C12 administered.

**Duodenal pressures**

There was a significant effect of treatment on the number, but not the amplitude, of duodenal PWs ($P < 0.001$) (Table 7.1). C12(0.3) decreased the number of duodenal PWs compared with C12(0.2) ($P < 0.01$), and C12(0.4) decreased the number of duodenal PWs compared with C12(0.2) ($P < 0.01$) and C12(0.3) ($P < 0.05$). There was an inverse relationship between the number ($r = -0.70, P < 0.001$), but not the amplitude, of duodenal PWs with the load of C12 administered.

**Plasma hormone concentrations**

**Plasma CCK**

Baseline plasma CCK concentrations did not differ among study days. There was a significant treatment x time interaction for plasma CCK ($P < 0.001$) (Figure 7.2A). Both C12(0.3) and C12(0.4) increased plasma CCK compared with C12(0.2) between $t = 15 – 90$ min ($P < 0.05$), and C12(0.4) when compared with C12(0.3) at $t = 45$ min ($P < 0.01$). There was also a significant effect of time for plasma CCK ($P < 0.001$). All treatments increased plasma CCK between $t = 15 – 90$ min compared with baseline ($P < 0.001$, for all). There was a direct relationship between plasma CCK concentrations with the load of C12 administered ($r = 0.91, P < 0.001$).

**Plasma PYY**

Baseline plasma PYY concentrations did not differ among study days. There was a significant treatment x time interaction for plasma PYY ($P < 0.001$) (Figure 7.2C).
Both C12(0.3) and C12(0.4) increased plasma PYY compared with C12(0.2) between \( t = 30 - 90 \) min (\( P < 0.01 \)), and C12(0.4) compared with C12(0.3) at \( t = 30, 45 \) and 90 min (\( P < 0.05 \)). There was also a significant effect of time for plasma PYY (\( P < 0.001 \)). C12(0.2) increased plasma PYY between \( t = 45 - 90 \) min (\( P < 0.001 \)), and both C12(0.3) and C12(0.4) between \( t = 30 - 90 \) min, compared with baseline (\( P < 0.01 \)). There was a direct relationship between plasma PYY concentrations with the load of C12 administered (\( r = 0.79, P < 0.001 \)).

**Appetite perceptions and energy intake**

There was no effect of treatment on perceptions of hunger, fullness, desire to eat, prospective consumption nausea or bloating (data not shown). There was a significant effect of treatment on energy intake (kJ) (\( P < 0.01 \)) ([Table 7.2](#)). C12(0.4) decreased energy intake compared with C12(0.3) and C12(0.2) (\( P < 0.01 \)), with no difference between C12(0.2) and C12(0.3). There was an inverse relationship between energy intake with the load of C12 administered (\( r = -0.70, P < 0.05 \)). The percentage of energy from fat, carbohydrate or protein did not differ between treatments ([Table 7.2](#)), hence, the decrease in energy intake reflected a comparable reduction in all three macronutrients.

### 7.4.2 Effects of concentration of C12

**Antropyloroduodenal pressures**

**Antral pressures**

There was no effect of treatment on the number or amplitude of antral PWs ([Table 7.1](#)).


**Pyloric pressures**

Basal pyloric pressure. There was an effect of time ($P < 0.001$), but not of treatment on basal pyloric pressure (Figure 7.1B). Both C12(40) and C12(56) increased basal pyloric pressure between $t = 15 – 60$ min ($P < 0.05$ for all) and C12(72) between $t = 15 – 45$ min ($P < 0.01$) compared with baseline.

Isolated pyloric pressure waves. There was an effect of time ($P < 0.001$, for both), but not of treatment on the number or amplitude of IPPWs (Figure 7.1D). All three treatments increased the number and amplitude of IPPWs ($P < 0.001$, for all) between $t = 15 – 60$ min compared with baseline.

**Duodenal pressures**

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

**Plasma hormone concentrations**

*Plasma CCK*

Baseline plasma CCK concentrations did not differ among study days. There was an effect of time ($P < 0.001$), but not treatment on plasma CCK (Figure 7.2B). All treatments increased plasma CCK between $t = 15 – 60$ min ($P < 0.001$, for all) compared with baseline.

*Plasma PYY*

Baseline plasma PYY concentrations did not differ among study days. There was an effect of time ($P < 0.001$, for all), but not treatment on plasma PYY (Figure 7.2D).
C12(56) increased plasma PYY between t = 30 – 60 min (P < 0.001), and both C12(40) (P < 0.001) and C12(72) (P < 0.001) between t = 45 – 60 min, compared with baseline.

**Appetite perceptions and energy intake**

There was no effect of treatment on perceptions of hunger, fullness, desire to eat, prospective consumption, nausea and bloating (data not shown). There was an effect of time on scores for bloating (P < 0.05). C12(40) increased bloating slightly between t = 15 – 30 min (P < 0.01) compared with baseline, while C12(56) and C12(72) had no effect. There was no effect of treatment on energy intake or percentage of fat, carbohydrate or protein consumed (Table 7.2).

There were no relationships between any parameter with the concentration of C12 administered.

**7.4.3 Comparison between C12(0.4) (“C12 load”) and C12(56) (“C12 concentration”)**

There were no statistically significant differences in basal pyloric pressures, numbers of IPPWs, antral and duodenal PWs (between t = 0 – 60 min) or plasma CCK or PYY concentrations (at t = 60 min) between C12(0.4) and C12(56), although mean values for basal pyloric pressure and the number of IPPWs tended to be greater during C12(56) compared with C12(0.4). There was also no difference in energy intake between the two conditions.
Table 7.1: Total number and mean amplitude of antral and duodenal PWs during intraduodenal infusion of C12 at different loads and concentrations.

<table>
<thead>
<tr>
<th></th>
<th>C12 load (kcal/min)†</th>
<th>C12 concentration (mM)§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C12(0.2)</td>
<td>C12(0.3)</td>
</tr>
<tr>
<td><strong>Antral pressure waves</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>61 ± 20</td>
<td>11 ± 4*</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>18 ± 4</td>
<td>12 ± 2</td>
</tr>
<tr>
<td><strong>Duodenal pressure wave</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>734 ± 71</td>
<td>531 ± 78*</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>25 ± 1</td>
<td>26 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 12). † C12 infused at 0.2 (C12(0.2)), 0.3 (C12(0.3)) and 0.4 (C12(0.4)) kcal/min (all at 56 mM) or 90 min. § C12 infused at 40 (C12(40)), 56 (C12(56)), 72 (C12(72)) mM (all at 0.4 kcal/min) for 60 min. * vs C12(0.2), P < 0.05; # vs C12(0.2)/C12(0.3), P < 0.05.
Table 7.2: Energy intake and macronutrient distribution from buffet meal after intraduodenal infusion of C12 at different loads and concentrations.

<table>
<thead>
<tr>
<th></th>
<th>C12 load (kcal/min)†</th>
<th>C12 concentration (mM)§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C12(0.2)</td>
<td>C12(0.3)</td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>5610 ± 545</td>
<td>5488 ± 271</td>
</tr>
<tr>
<td>Energy (%)</td>
<td>31 ± 1</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>Fat</td>
<td>48 ± 2</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>22 ± 1</td>
<td>21 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 12). † C12 infused at 0.2 (C12(0.2)), 0.3(C12(0.3)) and 0.4 (C12(0.4)) kcal/min (all at 56 mM) for 90 min. § C12 infused at 40 (C12(40)), 56 (C12(56)), 72 (C12(72)) mM (all at 0.4 kcal/min) for 60 min. * vs. C12(0.2), P < 0.05.
Figure 7.1: Basal pyloric pressure during intraduodenal infusion of C12 at (A) different loads (0.2, 0.3 and 0.4 kcal/min, all at 56 mM) for 90 min or (B) different concentrations (40, 56 and 72 mM, all at 0.4 kcal/min) for 60 min. Number of IPPWs during intraduodenal infusion of C12 at (C) different loads (0.2, 0.3 and 0.4 kcal/min, all at 56 mM) for 90 min or (D) different concentrations (40, 56 and 72 mM, all at 0.4 kcal/min) for 60 min. * C12(0.4) and C12(0.3) vs C12(0.2), P < 0.001; † C12(0.3) vs C12(0.2), P < 0.01; § C12(0.2) vs C12(0.4), P < 0.01. Data are means ± SEM (n = 12).
Figure 7.2: Plasma concentrations of CCK during intraduodenal infusion of C12 at (A) different loads (0.2, 0.3 and 0.4 kcal/min, all at 56 mM) for 90 min or (B) different concentrations (40, 56 and 72 mM, all at 0.4 kcal/min) for 60 min. * C12(0.4) and C12(0.3) vs C12(0.2), P < 0.01; # C12(0.4) vs C12(0.3), P < 0.05. Plasma concentrations of PYY during intraduodenal infusion of C12 at (C) different loads (0.2, 0.3 and 0.4 kcal/min, all at 56 mM) for 90 min or (D) different concentrations (40, 56 and 72 mM, all at 0.4 kcal/min) for 60 min. * C12(0.4) and C12(0.3) vs C12(0.2), P < 0.01; # C12(0.4) vs C12(0.3), P < 0.05. Data are means ± SEM (n = 12).
7.5 Discussion

This study has demonstrated that, in healthy humans, the load, but not the concentration, of C12 modulates APD motility, plasma CCK and PYY concentrations and energy intake, at least at the loads and concentrations administered. Specifically, the greater the load of C12, the greater the (i) suppression of antral and duodenal PWs, (ii) increase in the number and amplitude of IPPWs, (iii) secretion of CCK and PYY and (iv) suppression of energy intake. In contrast, there was no significant difference in these responses when the concentration was altered from 40 – 72 mM, at a fixed load of 0.4 kcal/min.

This study is the first to directly compare the effects of variations in load and concentration of intraduodenal fatty acids, specifically C12, in humans. It has been well established that although nutrient receptors in the small intestine do not detect calories per se, these receptors are tuned in such a way as to optimise caloric delivery, ie gastric emptying of nutrients is regulated on a kcal/min basis (Horowitz et al., 1996). Load-dependence, with concentration-independence, of responses evoked by luminal stimuli in the small intestine has been demonstrated in a variety of situations in animal experiments, including stimulation of pancreatic secretion by hydrogen ions infused at concentrations ≥ 1 mM (Meyer et al., 1970b), stimulation of pancreatic enzyme secretion, by luminal L-phenylalanine infused at concentrations between 8 – 128 mM (Meyer et al., 1976) and luminal C18:1 infused between 5 – 80 mM (Meyer and Jones, 1974), inhibition of gastric emptying by luminal glucose at concentrations between 250 – 1000 mM (Lin et al., 1989) and inhibition of energy intake by either luminal C18:1 or C12 infused at concentrations between 20 – 80 mM (Meyer et al., 1998b). In each case, load-dependence was ultimately shown in these animal models to be related to the
length of small intestine exposed to each stimulus and, thus, presumably to the number of sensors excited. A previous dose-response study of C12 in humans (Chapter 6) suggested that the threshold for concentration-dependence, at least for inhibition of gastrointestinal pressures and hormone release, was ≥ 14 mM. Accordingly, it is not altogether surprising that, at considerably higher concentrations (40 – 72 mM), load-dependence, but concentration-independence, was observed for these responses. In the current study, the effect of ‘load’ of C12 could not be discriminated from that of ‘length’ of small intestinal contact, however, a previous study in humans supports the concept that ‘load’ and ‘length’ are synonymous (Little et al., 2006a), as is the case in animals (Meyer et al., 1998c).

There are only a few reports in humans from which the concentrations of free fatty acids in the postcibal duodenum or jejunum can be calculated and the majority of these were conducted more than 30 years ago (Ament et al., 1972, Borgstrom et al., 1957, Hofmann and Borgstroem, 1964, Porter and Saunders, 1971, Porter et al., 1971). Observations derived from these studies indicate that fatty acid concentrations range from ~ 29 – 67 mM in the duodenum after fatty meals. More recent observations in animals and humans (Meyer et al., 1996) have demonstrated that the rate of duodenal entry of dietary fat (and thus, the rate of release and luminal concentrations of free fatty acids) varies with the amount of fat ingested, as well as the state of emulsification of fat in the meal, so the above estimates were likely conditioned by the circumstances of the test meals. The range of fatty acid concentrations utilised in the present study (40 – 72 mM) overlaps physiological postprandial concentrations in the human duodenum. It is appropriate to note that because C12 is a less prevalent dietary fatty acid, its use herein in these concentrations is atypical of luminal conditions. Nevertheless, these results
clearly demonstrate load-dependent, but concentration-independent, responses to this particular fatty acid. Because C12 and the most prevalent dietary fatty acid, C12, exhibited similar load-dependent, concentration-independent, effects on satiety in rats (Meyer et al., 1998b), it could be speculated that the present results with C12 in humans can be generalised to all fatty acids with chain lengths of 12 or more carbon atoms.

There are a number of limitations in the study design that need to be recognised. Even in previously reported animal experiments (Meyer et al., 1976, Meyer et al., 1998c, Meyer et al., 1970b, Meyer et al., 1970a), there were restrictions as to how much loads could be manipulated by altering volume rates at low concentrations, because as volume rates were increased, the animals frequently developed diarrhoea and, eventually, vomiting. This operational problem makes it difficult, if not impossible, except in unusual circumstances (Meyer et al., 1970a, Lin et al., 1990), to examine sensor responsiveness of luminal stimuli at lower concentrations. The same problem compromised the present design, where the pilot study aimed to assess C12 at concentrations of 24, 40 and 56 mM, for 90 min. However, the rate required to deliver 0.4 kcal/min for the 24 mM solution was 9.4 ml/min and frequently resulted in severe abdominal cramps, and then diarrhoea, before the end of the infusion. Consequently, the study design that had to be accepted was C12 solutions infused at 40, 56 and 72 mM, and at the shorter duration of 60 min, which decreased the total volume of C12 solution received by each subject. The variations in the infusion periods (90 min for “C12 load” and 60 min for “C12 concentration”) make it somewhat difficult to directly compare the two protocols, and it may be argued that the reason why only a load-, but not a concentration-dependent, effect was seen, was as a result of the longer infusion period. However, both studies had a common infusion, which delivered C12 at 0.4
Fatty acid load vs concentration on gastrointestinal function and energy

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When areas under the curves for basal pyloric pressure, numbers of IPPWs and antral and duodenal PWs, as well as CCK and PYY, were determined for the first 60 min of the C12(0.4) infusion and compared with the C12(56) infusion, no differences were found in these parameters, although mean values for the number of IPPWs and basal pyloric pressure were greater during the C12(56) infusion compared with the C12(0.4) infusion. This most likely reflects inter-subject variability, as only three subjects participated in both study parts, and it is well recognised that there is substantial inter-individual variability for measures of gastrointestinal motility, including gastric emptying and intestinal transit (Collins et al., 1983, Degen and Phillips, 1996). Indeed when data from the two days were compared informally in these three subjects, they were found to be very similar (data not shown). Furthermore, there were no differences in energy intake, despite the C12(0.4) infusion delivering ~30% more energy (12 kcal), when compared with the C12(56) infusion. These comparisons demonstrate that both infusions of C12 at 0.4 kcal/min and 56 mM, in the different conditions, overall had comparable effects on motility, plasma hormone secretion and energy intake, also making it doubtful that an additional 30 min of the C12 infusion in the “C12 concentration” protocol would have revealed an effect of concentration. In addition, as all of the C12 infusions in the “C12 load” protocol were at 56 mM, a ‘load-dependent’ effect would not have been demonstrated if this concentration had evoked a maximal effect on gastrointestinal function and energy intake, which was clearly not the case.

While these data indicate clear load-dependent, but concentration-independent, effects on motility, hormones, appetite and energy intake, the results may have potentially also been confounded by the fact that both limbs of the study employed varying rates of...
volume inflows, from 2 – 4 ml/min in the “C12 load” part and from 3.1 – 5.7 ml/min in the “C12 concentration” part. However, if increasing volume rates of flow would have increased responses, there should have been a systematic effect in the C12 concentration study, but this did not occur over the range of 3.1 – 5.7 ml/min. Nevertheless, it cannot be concluded with entire certainty, as this study, due to the number of study conditions, did not allow the inclusion of a volume control for each nutrient infusion, which would almost certainly not have been logistically feasible, particularly in relation to subject recruitment. Previous intraduodenal studies, including those investigating the effects of fatty acid chain length (Feltrin et al., 2004) and increasing doses of C12 (Chapter 6), have included control infusions and demonstrated that changes in APD motility and gut hormones, as well as appetite, over time are minimal.

In conclusion, this study demonstrated that, at the loads and concentrations administered and using the example of C12, load, but not concentration, modulates APD motility patterns, plasma CCK and PYY concentrations and energy intake in response to intraduodenal fatty acids.

**Acknowledgement:** KL Feltrin and TJ Little conducted the study described in this chapter with equal contributions, hence, it was also submitted as part of a Doctor of Philosophy degree by TJ Little, University of Adelaide, 2007.
Chapter 8

Evaluation of the interactions between cholecystokinin and glucagon-like peptide-1 in their effects on appetite, energy intake and antropyloroduodenal motility in healthy men

8.1 Summary

There is evidence that CCK and GLP-1 mediate the effects of nutrients on appetite and gastrointestinal function and that their interaction may be synergistic. The hypothesis was that intravenous CCK-8 and GLP-1 would have synergistic effects on appetite, energy intake and APD motility. Nine healthy males (age 22 ± 1 years) were studied on four separate days in double-blind, randomised fashion. Appetite perceptions and APD pressures were measured during 150 min intravenous infusions of (i) isotonic saline (control), (ii) CCK-8 (1.8 pmol/kg/min), (iii) GLP-1 (0.9 pmol/kg/min) or (iv) both (ii) and (iii) (CCK-8/GLP-1). At 120 min energy intake at a buffet meal was quantified. CCK-8, but not GLP-1, increased fullness, decreased desire to eat and subsequent energy intake and increased the number and amplitude of IPPWs and basal pyloric pressure (P < 0.05). Both CCK-8 and GLP-1 decreased the number of antral and duodenal PWs (P < 0.05), and CCK-8/GLP-1 decreased the number of duodenal PWs more than either CCK-8 or GLP-1 alone (P < 0.02). In conclusion, at the doses evaluated, exogenously administered CCK-8 and GLP-1 had discrepant effects on appetite, energy intake and APD pressures, and the effects of CCK-8/GLP-1, in
combination, did not exceed the sum of the effects of CCK-8 and GLP-1, providing no evidence of synergism.

8.2 Introduction

Meal ingestion triggers a number of stimuli within the gastrointestinal tract that modulate APD motility, gastrointestinal hormone secretion and appetite, including gastric distension (Kissileff et al., 2003), the presence of nutrients in the small intestine (Feinle et al., 1997, Feltrin et al., 2004, Cook et al., 1997) and the release of gastrointestinal hormones, including CCK and GLP-1 (Lilja et al., 1984, Herrmann et al., 1995). While some inconsistencies exist in regards to the roles of CCK and GLP-1 in appetite regulation, there is persuasive evidence that both these hormones modulate the effects of nutrients on gastrointestinal function and appetite (Flint et al., 1998, Schirra et al., 2000, Fraser et al., 1993, MacIntosh et al., 2001b, Verdich et al., 2001). Both CCK and GLP-1, when administered intravenously to healthy subjects, appear to have comparable effects on appetite and energy intake, increasing the perception of fullness and decreasing hunger and energy intake (Kissileff et al., 1981, Flint et al., 1998, Verdich et al., 2001). CCK and GLP-1 also modulate gastroduodenal contractile activity and slow gastric emptying (Anvari et al., 1998, Fraser et al., 1993, Schirra et al., 2000); the latter may contribute to the suppression of energy intake (Hellstrom and Naslund, 2001, Verdich et al., 2001). Studies using the CCK₁ antagonist, loxiglumide, have established that the effects of CCK on gastric emptying and appetite, in humans, are mediated through the CCK₁ receptor (Fried et al., 1991a, Beglinger et al., 2001); the effects of GLP-1 antagonists on gastric emptying and appetite have not been evaluated in humans.
While ingestion of a meal is known to increase plasma concentrations of CCK and GLP-1 within ~ 15 min (Lilja et al., 1984, Herrmann et al., 1995), there is little information relating to any possible interaction in the effects of these two hormones (Gutzwiller et al., 2004). This knowledge is potentially important for an understanding of the mechanisms underlying energy intake regulation, with evidence that the development of obesity is associated with different patterns of gastrointestinal hormone release (Ranganath et al., 1996, Wisen et al., 1992). A number of studies have investigated possible interactions between other gastrointestinal stimuli (Gutzwiller et al., 2000, Kissileff et al., 2003, Geary et al., 1992, Feinle et al., 1997). For example, gastric distension and CCK may have synergistic (ie the combined effect of the two stimuli is greater than the sum of their individual effects), rather than additive (ie the combined effect of the two stimuli equals the sum of their individual effects), effects (Kissileff et al., 2003). In a previous study the combination of gastric distension (with 300 ml water in a balloon) and intravenous CCK-8 (dose: 112 ng/ml (102 pmol/min) for 23 min), reduced energy intake (g) in healthy male and female subjects to a greater extent (by 200 g) than either CCK-8 (96 g) or distension (3 g) alone (Kissileff et al., 2003). Observations derived from a recent study are consistent with the concept of an interaction in the effects of CCK and GLP-1 (Feltrin et al., 2004). This study investigated the effects of duodenal infusion of the fatty acids, C10 and C12, on appetite, APD motility and CCK and GLP-1 release in healthy subjects. C12 was shown to decrease energy intake and stimulate IPPWs when compared with both C10 and control. C10 and C12 also differed in their effects on CCK and GLP-1 release, in that C12 increased plasma CCK and GLP-1, while C10 increased plasma CCK, albeit to a lesser extent than C12, and had no effect on GLP-1. It is, therefore, plausible that the
combined actions of CCK and GLP-1 (possibly with other gut peptides) were responsible for the more potent effects of C12 on appetite and APD motility.

A study investigating the effects of intravenous CCK-33 and GLP-1, alone and in combination, on energy intake and appetite, has recently been published (Gutzwiller et al., 2004). In this study, intravenous infusion of both CCK-33 and GLP-1 resulted in a non-significant decrease in the perception of hunger before a meal. In contrast, when CCK-33 and GLP-1 were infused concurrently, there was a reduction in hunger when compared with CCK-33 or GLP-1 alone. However, surprisingly, the combination of CCK-33 and GLP-1 did not reduce energy intake any more than either CCK-33 or GLP-1 alone, thus, the effect was infra-additive. Currently, the effect of the combination of CCK and GLP-1 on APD motility is unknown.

The aims of the current study were, therefore, to determine the effects of intravenous CCK-8 and GLP-1, given alone or in combination, on appetite, energy intake and APD motility in healthy male subjects. The hypothesis was that intravenous infusions of CCK and GLP-1 would have synergistic effects on these parameters; specifically that the suppression of appetite and energy intake and modulation of APD motility would be greater when the two peptides are administered together compared with either peptide alone.

### 8.3 Materials and methods

#### 8.3.1 Subjects

Nine healthy males were included in the study; the number of subjects was based on power calculations derived from a previous study (MacIntosh et al., 2001b); it was
calculated that with 9 subjects there would be a 10% decrease in energy intake at $\alpha = 0.05$, with a power of 80%. Subjects had a mean age of $22 \pm 1$ years (range 18 – 27 years) and were required to have a normal body weight for their height (mean BMI $23 \pm 0.5 \text{ kg/m}^2$) and were recruited according to guidelines as outlined in Chapter 3.2.

8.3.2 Study design

Each subject was studied on four occasions, each separated by 3 – 10 days, to evaluate in randomised, double-blind fashion, the effects of 150 min intravenous infusion of (i) isotonic saline (control), (ii) CCK-8 at 2 ng/kg/min (CCK-8), (iii) GLP-1 at 0.9 pmol/kg/min (GLP-1) or (iv) both (ii) and (iii) (CCK-8/GLP-1) on APD motility, appetite and energy intake.

8.3.3 Protocol

Subjects were intubated, via an anaesthetised nostril, with a 17-channel manometric catheter, as described in Chapter 3.5.1. Intravenous cannulae were placed in an antecubital vein in each arm for the intravenous infusion and blood sampling for the subsequent determination of plasma CCK and GLP-1 concentrations.

Once the catheter was positioned correctly (Heddie et al., 1989), a ‘baseline’ ($t = -15$ min) blood sample was taken and a VAS administered to assess appetite-related perceptions and nausea and bloating (see Chapter 3.7.2). At $t = 0$ min, the infusion of either (i) control, (ii) CCK-8, (iii) GLP-1 or (iv) CCK-8/GLP-1 was commenced and continued for 150 min. During the infusion blood samples were taken and VAS completed at regular intervals. At $t = 120$ min, subjects were extubated and immediately provided 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 IV. At $t = 150$ min the infusion was terminated, a final blood sample was taken and a VAS administered. Subjects were then monitored for a further 30 min and, after removal of the intravenous cannulae, allowed to leave the laboratory.

8.3.4 Measurements

Appetite perceptions and energy intake
Hunger, fullness, desire to eat, prospective consumption, nausea and bloating were assessed by VAS, as described in Chapter 3.7.2.

Energy intake (kJ) and the amount of food consumed (g) and the macronutrient distribution (% of energy from fat, carbohydrate and protein) was assessed (see Chapter 3.7.3).

Antropyloroduodenal motility
APD pressures were analysed for (i) number and amplitude of antral PWs, (ii) basal pyloric pressure (tone), (iii) number and amplitude of IPPWs, (iv) number and amplitude of duodenal PWs, and (v) number and length of pressure wave sequences involving the antrum, pylorus and duodenum, as described in Chapter 3.5.2.

Plasma CCK and GLP-1 concentrations
Venous blood samples (10 ml) were collected for the determination of plasma CCK and GLP-1 concentrations, as described in Chapter 3.6.
8.3.5 Data and statistical analyses

Baseline (‘0’) was 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 amplitude of antral and duodenal PWs, IPPWs, mean basal pyloric pressures and total number of APD PWSs. The number and amplitude of antral and duodenal PWs were expressed as mean values during the first 120 min of the infusion period. IPPWs, basal pyloric pressure and PWSs were expressed as mean values for 15 min intervals between $t = -15 – 120$ min (i.e. $t = 0 – 15$, $15 – 30$, …, $105 – 120$ min). APD PWSs were expressed as the total number of waves travelling over 2, 3, …, 15 channels during the first 120 min of the infusion period. All data, with the exception of plasma CCK and GLP-1 concentrations, were expressed as changes from baseline.

VAS, plasma hormone concentrations, IPPWs and basal pyloric pressures were analysed by repeated measures ANOVA with time ($t = 0, 10, 20, 30, …, 120$ min, or $t = 0 – 15, 15 – 30, …, 105 – 120$, see above) and treatment as factors. The number of APD PWSs was analysed by repeated measures ANOVA with length of propagation ($1.5 – < 3$ cm, $3 – < 4.5$ cm, …, $21 – < 22.5$ cm) and treatment as factors. One-way ANOVA was used to analyse the effect of treatment on the number and amplitude of antral and duodenal PWs, as well as energy intake. Plasma CCK-8 and GLP-1 concentrations at 120, 150 and 180 min were compared using Student’s paired t-test. Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.
8.4 Results

All subjects completed the four randomised study days, and the study protocol was well tolerated.

8.4.1 Appetite perceptions and energy intake

Desire to eat

There was a significant effect of treatment on scores for desire to eat (P < 0.05) (Figure 8.1A). CCK-8 decreased desire to eat when compared with both control (P < 0.05) and GLP-1 (P < 0.01), while there was no difference between GLP-1 and control. There was no difference between CCK-8/GLP-1 and CCK-8 or GLP-1. There was a significant effect of time on scores for desire to eat (P < 0.01). Desire to eat decreased during the first 10 min of the CCK-8 infusion and did not change over the subsequent 110 min.

Fullness

There was a treatment by time interaction for scores for fullness (P < 0.05) (Figure 8.1B). CCK-8 increased fullness between t = 10 – 60 min when compared with control (P < 0.001), GLP-1 (P < 0.001) and CCK-8/GLP-1 (P < 0.001). At t = 90 min there was a trend for CCK-8 to increase fullness when compared with control and GLP-1 (P = 0.09), and CCK-8 increased fullness when compared with CCK-8/GLP-1 (P < 0.01).

Nausea

There was a significant effect of treatment on nausea (P < 0.05) (Figure 8.1C). CCK-8 increased nausea when compared with control (P < 0.05), whereas there was no difference between GLP-1 and control, or between CCK-8 and GLP-1. However, there
There was a trend for CCK-8/GLP-1 to increase nausea to a greater extent than GLP-1 (P = 0.07).

There was no treatment or time effect on hunger, prospective consumption or bloating (data not shown).

**Energy intake**

There was a significant effect of treatment on energy intake (P < 0.01) (Table 8.1). CCK-8 decreased energy intake when compared with both control (P < 0.01) and GLP-1 (P < 0.001), while there was no difference between GLP-1 and control. CCK-8/GLP-1 decreased energy intake when compared with GLP-1 (P < 0.001), but not CCK-8. Similarly, there was a treatment effect on the amount (g) consumed at the buffet meal (P < 0.01) (Table 8.1). CCK-8 decreased the amount eaten when compared with both control (P < 0.001) and GLP-1 (P < 0.01), however, there was no difference between GLP-1 and control. CCK-8/GLP-1 decreased the amount consumed when compared with GLP-1 (P < 0.05) and control (P < 0.01), but the effect did not differ from that of CCK-8 (data not shown). There was no difference in macronutrient distribution, i.e. the percentage of energy from fat, carbohydrate and protein consumed at the buffet meal, between treatments (Table 8.1)

### 8.4.2 Antropyloroduodenal pressures

**Antral pressures**

There was a significant effect of treatment on the number and amplitude of antral PWs (P < 0.01) (Table 8.2). CCK-8, GLP-1 and CCK-8/GLP-1 decreased the number and amplitude of antral PWs when compared with control (P < 0.01), with no difference...
between CCK-8, GLP-1 and CCK-8/GLP-1. While the mean number and amplitude of antral PWs was less with CCK-8/GLP-1 compared with CCK-8 or GLP-1, this difference was not significant.

### Pyloric pressures

**Basal pressure (‘tone’)**

There was a significant effect of treatment on basal pyloric pressure (P < 0.01) (Figure 8.2A). CCK-8 increased basal pyloric pressure when compared with both control (P < 0.001) and GLP-1 (P < 0.01), with no difference between GLP-1 and control. CCK-8/GLP-1 also increased basal pyloric pressure when compared with control (P < 0.05), with no difference between CCK-8/GLP-1 and CCK-8. There was a significant effect of time on basal pyloric pressure (P < 0.05). Both CCK-8 and CCK-8/GLP-1 markedly increased basal pyloric pressure during the first 15 min of infusion before gradually decreasing to baseline pressures by t = 120 min.

**Isolated pyloric pressure waves (‘phasic pressures’)**

There was a treatment by time interaction for the number of IPPWs (P < 0.01) (Figure 8.2B). CCK-8 increased the number of IPPWs when compared with both control (P < 0.01) and GLP-1 (P < 0.01) over the entire infusion period (t = 0 – 120 min), while there was no difference between GLP-1 and control. The combination of CCK-8/GLP-1 increased the number of IPPWs when compared with control between t = 0 – 30 min and t = 60 – 120 min (P < 0.05). In contrast, the number of IPPWs was less during CCK-8/GLP-1 compared with CCK-8 between t = 15 – 60 min and t = 75 – 90 min (P < 0.05), but greater when compared with GLP-1 between t = 0 – 45 min and t = 60 – 75 min (P < 0.05). There was an effect of treatment on the amplitude of IPPWs (P < 0.01)
CCK and GLP-1 interactions, appetite and gut motility

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(Figure 8.2C). CCK-8 increased the amplitude of IPPWs when compared with control (P < 0.01), but not GLP-1; there was no difference between GLP-1 and control. CCK-8/GLP-1 also increased the amplitude of IPPWs compared with control (P < 0.01), while there was no difference between CCK-8/GLP-1 and CCK-8.

Duodenal pressures

There was a significant effect of treatment on the number of duodenal PWs (P < 0.001) (Table 8.2). CCK-8, GLP-1 and the combination of CCK-8/GLP-1 decreased the number of duodenal PWs when compared with control (P < 0.001 for all), with no difference between CCK-8 and GLP-1. CCK-8/GLP-1 reduced duodenal PWs more than either CCK-8 (P < 0.01) or GLP-1 (P < 0.001) alone. There was a trend for the amplitude of duodenal PWs to differ between study conditions (P = 0.07); the amplitude tended to be lower after CCK-8, GLP-1 and CCK-8/GLP-1 when compared with control (Table 8.2).

8.4.3 Antropyloroduodenal pressure wave sequences

There was a significant effect of treatment on the number of APD PWSs spanning 2 (ie 1.5 < 3 cm) (P < 0.05), 3 (ie 3 < 4.5 cm) (P < 0.05), 4 (ie 4.5 < 6 cm) (P < 0.01), 5 (ie 6 < 7.5 cm) (P < 0.05), 6 (ie 7.5 < 9 cm) (P < 0.05), 7 (ie 9 < 10.5 cm) (P < 0.01), 8 (ie 10.5 < 12 cm) (P < 0.001) and 9 (ie 12 < 13.5 cm) (P < 0.001) channels (Figure 8.3). GLP-1, CCK-8/GLP-1, but not CCK-8, decreased the number of waves that spanned 2 channels compared with control (P < 0.05), although there was no difference between CCK-8, GLP-1 and CCK-8/GLP-1. CCK-8, GLP-1 and CCK-8/GLP-1 decreased the number of waves spanning 3, 4, 5, 7, 8 and 9 channels when compared to control (P < 0.05 for both), with no difference between them. CCK-8 and CCK-8/GLP-1 decreased
the number of waves spanning 6 channels (P < 0.05), whereas GLP-1 had no effect. PWSs spanning 10 or more channels were not analysed statistically, as they were very infrequent (a total of 18 waves spanning 10 – 15 channels, 7 during the control infusion, 3 during CCK-8, 5 during GLP-1 and 3 during CCK-8/GLP-1).

### 8.4.4 Plasma CCK and GLP-1 concentrations

There was a treatment by time interaction for plasma CCK-8 concentrations (P < 0.001) (Figure 8.4A). Infusion of CCK-8 and CCK-8/GLP-1 both elevated plasma CCK concentrations when compared with control (P < 0.001) and GLP-1 (P < 0.001) between t = 10 – 120 min. There was a significant rise in plasma CCK following meal ingestion during control and GLP-1 infusions between t = 150 and t = 180 min (P < 0.001), while plasma CCK decreased during infusion of CCK-8 and CCK-8/GLP-1 (P < 0.01). There was no difference between treatments at either t = 150 or 180 min.

There was a treatment by time interaction for plasma GLP-1 concentrations (P < 0.05) (Figure 8.4B). GLP-1 and CCK-8/GLP-1 increased plasma GLP-1 concentrations when compared with control (P < 0.001) and CCK-8 (P < 0.05) between t = 0 – 150 min. There was no difference in plasma GLP-1 between CCK-8 and control, or CCK-8/GLP-1 and GLP-1. Following ingestion of the buffet meal (ie between t = 120 and t = 150 min), there was a tendency for plasma GLP-1 to increase during infusion of control (P = 0.06), CCK-8 (P = 0.05) and GLP-1 (P = 0.07), while infusion of CCK-8/GLP-1 caused no further rise. There was no significant difference in plasma GLP-1 concentrations between t = 120 and 180 min or between treatments at t = 180 min.
Table 8.1: Energy intake and macronutrient distribution, in response to intravenous infusions of control, CCK-8, GLP-1 and CCK-8/GLP-1.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CCK-8</th>
<th>GLP-1</th>
<th>CCK-8/GLP-1</th>
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<tbody>
<tr>
<td><strong>Energy intake (kJ)</strong></td>
<td>5666 ± 461</td>
<td>4327 ± 459*</td>
<td>5672 ± 589</td>
<td>4378 ± 484*</td>
</tr>
<tr>
<td><strong>Amount consumed (g)</strong></td>
<td>1350 ± 118</td>
<td>943 ± 107*</td>
<td>1286 ± 117</td>
<td>1049 ± 134*</td>
</tr>
<tr>
<td><strong>Energy (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>31 ± 2</td>
<td>31 ± 3</td>
<td>35 ± 2</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>45 ± 2</td>
<td>46 ± 3</td>
<td>43 ± 2</td>
<td>45 ± 2</td>
</tr>
<tr>
<td>Protein</td>
<td>24 ± 1</td>
<td>23 ± 1</td>
<td>23 ± 1</td>
<td>23 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 9). * vs control and GLP-1, P < 0.05.
Table 8.2: Total number and amplitude of antral and duodenal PWs in response to intravenous infusions of control, CCK-8, GLP-1 and CCK-8/GLP-1.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CCK-8</th>
<th>GLP-1</th>
<th>CCK-8/GLP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antral pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>125 ± 23</td>
<td>36 ± 16*</td>
<td>43 ± 11*</td>
<td>18 ± 6*</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>70 ± 11</td>
<td>30 ± 5*</td>
<td>23 ± 3*</td>
<td>19 ± 3*</td>
</tr>
<tr>
<td><strong>Duodenal pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>844 ± 105</td>
<td>347 ± 43*</td>
<td>481 ± 58*</td>
<td>121 ± 27*#</td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>29 ± 2</td>
<td>22 ± 1</td>
<td>25 ± 2</td>
<td>25 ± 3</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 9). * vs control: P < 0.001; # vs CCK-8 and GLP-1: P < 0.05. Data are means ± SEM (n = 9).
Figure 8.1: Scores for (A) desire to eat, (B) fullness and (C) nausea during intravenous infusion of control, CCK-8, GLP-1 and CCK-8/GLP-1. * CCK-8 vs control/GLP-1: P < 0.05; § CCK-8 vs CCK-8/GLP-1: (P < 0.01); †CCK-8 vs control, GLP-1 and CCK-8/GLP-1: P < 0.001; # CCK-8 vs control: P < 0.05. Data are means ± SEM (n = 9).
**Figure 8.2:** Basal pyloric pressure (A) and number (B) and amplitude (C) of IPPWs, occurring during 15 min intervals, during intravenous infusion of control, CCK-8, GLP-1 and CCK-8/GLP-1. * vs control: P = 0.05; # vs GLP-1: P < 0.05; § vs CCK-8/GLP-1: P = 0.05. Data are means ± SEM (n = 9).
Figure 8.3: APD PWSs during intravenous infusion of control, CCK-8, GLP-1 and CCK-8/GLP-1. * CCK-8 vs control: P < 0.05; # GLP-1 vs control: P < 0.05; § CCK and GLP-1 vs control: P < 0.05). Data are means ± SEM (n = 9).
**Figure 8.4:** Plasma concentrations of CCK (A) and GLP-1 (B) during intravenous infusion and control, CCK-8, GLP-1 or CCK-8/GLP-1. * vs control: $P < 0.001$; # vs GLP-1: $P < 0.001$; § vs CCK-8: $P < 0.05$. Data are means ± SEM ($n = 9$).
8.5 Discussion

The observations derived from this study indicate that intravenous administration of CCK-8 and GLP-1, in the doses that were evaluated, have discrepant effects on appetite, energy intake and APD motility in healthy, young males. Infusion of CCK-8 decreased perceptions of appetite, energy intake, the number of antral and duodenal PWs and the number of APD PWSs and increased the number and amplitude of IPPWs, when compared with both control and GLP-1 infusions. In contrast, infusion of GLP-1 did not suppress appetite or energy intake or stimulate pyloric pressures, but decreased antral and duodenal PWs to the same extent as CCK-8. While the combination of CCK-8/GLP-1 decreased the number of duodenal PWs more than CCK-8 and GLP-1 alone, this was not the case for the effects on appetite or other motility parameters. Infusion of the combination of CCK-8/GLP-1 also had a comparable effect to CCK-8 alone on energy intake. For all parameters measured the effects of CCK-8/GLP-1 infusion did not exceed the sum of the individual effects of CCK-8 and GLP-1. Accordingly, there was no evidence to support the concept that CCK-8 and GLP-1 have synergistic effects on either perceptions of appetite or APD motility.

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. Intravenous infusion of CCK-8 has been reported in the majority of studies to increase fullness, suppress hunger and inhibit energy intake in healthy humans (Kissileff et al., 1981, MacIntosh et al., 2001b), consistent with the observations of this study. While it could be argued that the plasma CCK concentrations resulting from the infusion in this study may be moderately supraphysiological (Santangelo et al., 1998, Gomez Cerezo et al., 1991), they are
comparable with the concentrations observed following a meal in previous studies where plasma CCK-8 was measured using an identical assay (Sturm et al., 2003, MacIntosh et al., 1999). While the majority of studies have reported that intravenous infusion of GLP-1 decreased perceptions of appetite and energy intake (Flint et al., 1998, Gutzwiller et al., 1999, Verdich et al., 2001), some have failed to demonstrate any appetite-suppressant effect (Long et al., 1999, Näslund et al., 1998). A meta-analysis suggests that the effects of exogenous administration of GLP-1 on appetite and energy intake, while significant, are also modest (Verdich et al., 2001). Furthermore in some studies nausea and vomiting occurred following administration of relatively high doses of GLP-1 (~ 4.5 nmol/kg body weight) (Ritzel et al., 1995, Naslund et al., 2004). The dose of GLP-1 (0.9 pmol/kg/min) in this study was comparable to that in previous studies (Gutzwiller et al., 2004, Flint et al., 1998, Long et al., 1999) and resulted in slightly supraphysiological plasma levels, when compared with those observed in response to a meal or duodenal nutrient infusion (Pilichiewicz et al., 2003, Feinle et al., 2002). Furthermore, plasma GLP-1 concentrations at t = 180 min (ie following the meal and 30 min after the cessation of the GLP-1 infusion) were not significantly different from those at t = 120 min, and no nausea occurred in any subjects. Clearly, studies using specific GLP-1 antagonists (Schirra et al., 1998) are required to further establish, or refute, a physiological role for GLP-1 in the regulation of energy intake in humans.

When compared with individual infusions of CCK-8 and GLP-1, the combination of CCK-8/GLP-1 did not suppress desire to eat or enhance fullness any further. This observation is apparently at odds with the outcome of a recent study reporting that concurrent intravenous administration of CCK-33 and GLP-1 reduced hunger more than
either CCK-33 or GLP-1 alone (Gutzwiller et al., 2004). The reason for this discrepancy is unclear, however, it should be recognised that the type and dose of CCK used in the study by Gutzwiller et al (Gutzwiller et al., 2004) (CCK-33, 0.2 pmol/kg/min) are different from this study. Moreover, in that study, appetite perceptions were measured using a category scale and not, as in our investigation, a true VAS. An apparent anomaly in the study by Gutzwiller et al (Gutzwiller et al., 2004) was that the combination of CCK-33/GLP-1 did not suppress energy intake more than infusion of CCK-33, or GLP-1, alone, rather, there was an infra-additive reduction in energy intake. In the current study, while the combination of CCK-8/GLP-1 decreased energy intake, the magnitude of this reduction was comparable to that induced by CCK-8 alone.

Intravenous administration of both CCK and GLP-1 have been reported, in animals and humans, to suppress antral and duodenal PWs and stimulate pyloric pressures (Anvari et al., 1998, Fraser et al., 1993, Schirra et al., 2000), which are associated with the slowing of gastric emptying (Heddle et al., 1989). In this study, intravenous CCK-8 reduced the number of antral and duodenal PWs, the amplitude of antral PWs and PWSs and increased the number and amplitude of IPPWs. GLP-1 also reduced the number of antral and duodenal PWs and the amplitude of antral PWs and PWSs, but, perhaps surprisingly, had no effect on IPPWs. Hence, a dose of GLP-1, which did not affect appetite, energy intake or stimulate IPPWs, had comparable effects on antral and duodenal PWs to a dose of CCK-8 that did suppress appetite and energy intake and stimulated phasic and tonic pyloric pressures. It could, therefore, be argued that discrepant effects on pyloric motility may account for the observed differences in the effects of CCK-8 and GLP-1 on energy intake. While these data do not suggest that
there is a close relationship between changes in APD pressures and appetite perceptions, the relationship between pyloric motility and energy intake clearly warrants evaluation, particularly as a recent study has reported that electrical stimulation of the pylorus decreases energy intake in dogs (Xu et al., 2005). Certainly, the data from this study indicate that there are different thresholds for effects of GLP-1, compared with CCK-8, on gastrointestinal motility and energy intake.

This study represents the first evaluation of the combined effects of CCK-8 and GLP-1 on APD motility. CCK-8/GLP-1 significantly reduced the number of duodenal PWs, when compared with CCK-8 or GLP-1 alone, and mean values for antral PWs were also less. However, while it may be considered that the effects of CCK-8/GLP-1 on APD motility are greater than those of either CCK-8 or GLP-1 alone, there was no evidence of synergism. It should be recognised that the doses of CCK-8 and GLP-1 used may have exerted near maximal effects on APD motility and if this were to be 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 given concurrently. CCK-8 also stimulated the number of IPPWs slightly more than CCK-8/GLP-1, suggesting that infusion of GLP-1 may have attenuated the effect of CCK-8. While this has not been described previously, there is evidence of that effect from other gut hormones (Geary et al., 1992), for example, concurrent intravenous infusion of glucagon and CCK-8 reduced meal size less than the sum of the effects of glucagon and CCK-8 alone (Geary et al., 1992).

Some limitations of this study need to be recognised. – Only male volunteers were included and, accordingly, these observations may not be applicable to females. As
only 9 subjects were studied, the possibility of a type 2 error should be considered, although, as stated, the number of subjects recruited was based on power calculations from our previous studies (see section 8.3.1) (Feinle et al., 2003, MacIntosh et al., 2001b).

In summary, this study indicates that, at the doses evaluated, CCK-8 and GLP-1 have discrepant effects on appetite, energy intake and APD motility, while infusion of CCK-8, but not GLP-1, decreased perceptions of appetite and energy intake. Furthermore, while both CCK-8 and GLP-1 had significant effects on antral and duodenal PWs, the effect of the combination of CCK-8/GLP-1 did not exceed the sum of the effects of CCK-8 and GLP-1 alone, providing no evidence of synergism. Finally, infusion of CCK-8, but not GLP-1, suppressed energy intake and stimulated phasic and tonic pyloric pressures. In view of this, and other work (Xu et al., 2005), the relationship between energy intake and pyloric motility requires further exploration.

Acknowledgement: KL Feltrin and IM Brennan conducted the study described in this chapter with equal contributions, hence, it was also submitted as part of an Honours degree by IM Brennan, University of Adelaide, 2005.
Chapter 9

Acute effects of oral lauric acid on appetite and energy intake in healthy males

9.1 Summary
Currently available therapies for obesity not only have limited efficacy and a high prevalence of adverse effects, but have also largely ignored the role of the gastrointestinal tract in the regulation of appetite. The studies reported in Chapters 5 – 7 have established that intraduodenal infusion of the fatty acid, C12, reduces subsequent energy intake. The aims of this study were to investigate the hypothesis that increasing doses of orally ingested C12 would result in a dose-related suppression of appetite and subsequent energy intake at breakfast and lunch. Fourteen healthy men were studied on three separate occasions in double-blind, randomised fashion. Following ingestion of C12 (2 g, 4 g, or 6 g) or control; (i) perceptions of appetite, nausea and bloating (for 3 hours following breakfast ingestion), (ii) energy intake at breakfast (provided 30 min after C12 ingestion) and (iii) energy intake at lunch (provided 3 hours after breakfast ingestion), were measured. While there was no effect of C12 on energy intake at breakfast, energy intake at lunch was reduced after ingestion of both 2 g (P < 0.05) and 6 g (P < 0.01) of C12 compared with control. Total energy intake (breakfast + lunch) was less following ingestion of 6 g of C12 compared with control (P < 0.05) and 4 g (P < 0.01). In conclusion, acute oral administration of C12 reduces energy intake. Studies are now required to determine whether this effect is maintained during chronic administration of C12.
9.2 Introduction
The incidence of obesity (BMI > 30 kg/m²) is increasing, and more than 50% of Australian adults are overweight, of which some 20% are obese (Cameron et al., 2003, Booth et al., 2001). Obesity represents a major risk factor for a number of disorders, including diabetes, and cardiovascular, gall bladder and musculoskeletal diseases (Must et al., 1999). 2 – 7% of the total health care costs in Western countries are attributable to obesity (Birmingham et al., 1999). Numerous pharmacological treatments for obesity have been developed, however, most of these therapies only result in modest weight loss of 5 – 10% (Bray, 1995, James et al., 2000, McNulty et al., 2003), and several drugs have a high prevalence of adverse effects (Bray, 1995, Halpern and Mancini, 2003). The available therapies for obesity have largely ignored the role of the gastrointestinal tract in the regulation of appetite, as well as the important relationship between energy intake and gastrointestinal function.

The presence of nutrients, especially fat, in the small intestine decreases hunger and subsequent energy intake (Chapman et al., 1999, MacIntosh et al., 1999) and modulates gastroduodenal motility (Feinle et al., 1996, Heddle et al., 1988a), leading to a slowing of gastric emptying (Heddle et al., 1989). In response to enteral fat, a number of gastrointestinal hormones, including CCK, GLP-1, GLP-2, PYY and PP (Gutzwiller et al., 1999, Matzinger et al., 1999, Lieverse et al., 1994, Feltrin et al., 2004), are released into the circulation, while ghrelin is suppressed (Erdmann et al., 2003, Feinle-Bisset et al., 2005). These hormones mediate, at least in part, the effects of fat on appetite and gastrointestinal function (Abbott et al., 2005, Lieverse et al., 1995, Turton et al., 1996). Most dietary fats are ingested in the form of triglycerides, however, the effects of fat are mediated by their digestive products, free fatty acids. Recent studies indicate that the
effects of fatty acids on appetite and energy intake in humans are dependent on the chain length of fatty acids; specifically, those that have a chain length of 12 or more carbon atoms are more potent (Hunt and Knox, 1968, Feltrin et al., 2004, McLaughlin et al., 1999, Matzinger et al., 2000).

The studies reported in this thesis (Chapters 5, 6 and 7) have demonstrated that C12, when administered intraduodenally at loads of 0.4 kcal/min, and concentrations between 40 – 70 mM, decreases energy intake without inducing nausea. The effects of intraduodenal C12 on energy intake are associated with changes in antropyloroduodenal motility and gastrointestinal hormone secretion, including the stimulation of CCK, GLP-1, GLP-2, PYY, PP, and suppression of ghrelin (Chapters 5, 6 and 7, Feltrin et al., 2004). Given that intravenous CCK, GLP-1, PYY and PP have been reported to suppress (Batterham et al., 2002, Batterham et al., 2003b, Flint et al., 1998, Kissileff et al., 1981), and ghrelin to increase (Wren et al., 2001a), energy intake, the changes in gastrointestinal hormone secretion induced by C12 are likely to mediate, at least in part, the effects of C12 on of energy intake.

It is currently not known whether the appetite-suppressant effect of C12 observed in response to acute intraduodenal administration is maintained when C12 is administered orally. A previous study (McLaughlin et al., 1999) demonstrated that direct intragastric administration of C12 suppresses antral contractions, relaxes the proximal stomach, and stimulates both gallbladder contractility and CCK secretion. Accordingly, it may be expected that orally administered C12 would also produce comparable changes in gastrointestinal function, which are associated with the reduction of appetite and energy intake. The aims of this study were to investigate the hypothesis that increasing doses
of orally ingested C12 would result in a dose-related suppression of appetite and subsequent energy intake both at breakfast (30 min after C12 ingestion) and lunch (3 hours after breakfast ingestion).

**9.3 Pilot study: Effect of C12 on gastrointestinal symptoms**

Prior to assessing the effects of oral C12 on appetite and energy intake, a pilot study was conducted to assess the effect of increasing doses of oral C12 on gastrointestinal symptoms. As the effects of oral C12 had not been evaluated previously, it was not known whether oral C12 would be tolerated well or include adverse effects, such as nausea or bloating.

**9.3.1 Methods and materials**

**Subjects**

5 healthy, males, with a mean age of 25 ± 4 years (range 19 – 45) and of normal body weight for their height (mean BMI 22.5 ± 0.6 kg/m²), were recruited according to guidelines as outlined in Chapter 3.2.

**Study design**

Each subject was studied on five occasions, separated by 3 – 10 days, to evaluate in single-blind fashion, the effects of increasing doses of oral C12 at 1 g (C12(1g)), 2 g (C12(2g)), 3 g (C12(3g)) and 4 g (C12(4g)), or control, on nausea and bloating. The doses of C12 were based on the studies using intraduodenal C12 infusion (Chapters 5 – 7) and delivering C12 at doses between ~ 1 – 5 g, and a study delivering C12 into the stomach at ~ 2 – 5 g (McLaughlin et al., 1999). These studies demonstrated that at these doses C12 had effects on gastrointestinal function and/or energy intake.
Preparation of C12 capsules

Hydroxypropylmethyl cellulose capsules (size 00) were filled with 0.5 g C12 or 0.5 g polyethyleneglycol. The order of control capsule ingestion was randomised (on any of the 5 visits), however, as this was the first time that C12 was ingested orally by humans, doses of C12 were increased with each visit (starting with 1 g of C12), hence, the subjects, but not the investigators, were blinded to the treatments.

Protocol

Upon arrival (t = -15 min) subjects were seated in a chair and completed a VAS for the assessment of appetite-related sensations, as well as nausea and bloating (see Chapter 3.7.2). At t = 0 min, subjects ingested the capsules with 250 ml of water and VAS were administered every 15 min between t = 0 – 90 min, and then every 30 min between t = 90 – 180 min. At t = 180 min subjects were given a light lunch, for example, mixed sandwiches, after which the subject was allowed to leave the laboratory.

Measurement of gastrointestinal symptoms

Nausea and bloating were assessed by VAS for 180 min after capsule ingestion, as described in Chapter 3.7.2. Subjects were also asked to report any adverse effects in the subsequent 24 hours.

9.3.2 Results

No nausea, bloating (Figure 9.1) or any other adverse effects were experienced by any volunteer following ingestion of C12 or control (and up to 24 hours later).
9.3.3 Conclusion

Oral C12 at 1 g, 2 g, 3 g and 4 g appears to be well tolerated.

Figure 9.1: Scores for nausea (A) and bloating (B) following oral ingestion of lauric acid (C12) at 1 g, 2 g, 3 g, 4 g, or control in healthy subjects. Data are means ± SEM; n = 5.
9.4 Effects of oral C12 ingestion on appetite and energy intake

9.4.1 Methods and materials

Subjects

14 healthy males were included in the study; the number of subjects was based on power calculations derived from a previous study (O'Donovan et al., 2003); it was calculated that with 14 subjects there would be a 15 % decrease in energy intake at $\alpha = 0.05$, with a power of 80 %. Subjects had a mean age of $24 \pm 1$ years (range 19 – 41 years) and of normal body weight for their height (mean BMI $23.2 \pm 0.4 \text{ kg/m}^2$), and were recruited according to guidelines as outlined in Chapter 3.2.

Study design

Each subject was studied on four occasions, separated by 3 – 10 days, in double-blind, randomised fashion to evaluate the effects of different doses of oral C12 at (i) 2 g (C12(2g)), (ii) 4 g (C12(4g)) or (iii) 6 g (C12(6g)), or (iv) control, on appetite perceptions, nausea and bloating (45 min before breakfast and 210 min following breakfast), energy intake at breakfast (30 min after C12 ingestion) and energy intake at lunch (180 min after breakfast) (Figure 9.2). As there were no adverse effects following 4 g of C12 in the pilot study, the maximum dose was increased to 6 g.
Figure 9.2: Schematic diagram of the study protocol

Preparation of C12 capsules

C12 was filled in size 00 hydroxypropylmethyl cellulose capsules. Since each capsule contained 0.5 g C12, 12 capsules were required (to deliver 6 g C12). In order for the subjects and primary investigator to remain blinded, the subjects ingested 12 capsules on each occasion, and these were prepared by a co-investigator. Control capsules were filled with 0.5 g of ascorbic acid (see Chapter 3.8.1). Capsules were expected to dissolve in the stomach within ~ 6 min from time of ingestion.

Protocol

Upon arrival (t = -45 min) subjects were seated in a chair and completed a VAS for the assessment of appetite-related sensations, as well as nausea and bloating (see Chapter 3.7.2), and then swallowed the 12 capsules with 250 ml of water. At t = -30 min subjects completed another VAS. At t = -15 min, subjects were presented with a standardised, buffet-style breakfast, as described in Chapter 3.7.3, and allowed to eat for 15 min. VAS were administered every 15 minutes between t = 0 – 90 min, and then
Effect of oral C12 ingestion on appetite and energy intake

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every 30 min between t = 90 – 180 min (ie from the end of breakfast until the start of lunch). At t = 180 min, subjects were presented with a standardised, cold, buffet-style lunch, as described in Chapter 3.7.3, and allowed to eat for 30 min. A VAS was administered immediately after the meal (t = 210 min), after which the subject was free to leave the laboratory. The types of food, as well as the macronutrient composition and energy content, of the breakfast and lunch meals are described in Appendix III and Appendix IV, respectively.

Assessment of appetite and energy intake

Hunger, fullness, desire to eat, prospective consumption, nausea and bloating were assessed by VAS, as described in Chapter 3.7.2.

Energy intake (kJ), the amount of food consumed (g) and the macronutrient distribution (% of energy from carbohydrate, fat and protein) were evaluated (see Chapter 3.7.3).

Data and statistical analyses

VAS scores at t = -45 min were taken as baseline values. VAS scores (0 – 180 min) were analysed by repeated-measures ANOVA with time and treatment as within-subject factors. One-way ANOVA was used to analyse the effect of treatment on energy intake, amount of food consumed and its macronutrient distribution. Hunger and fullness scores immediately following breakfast ingestion (t = 0 min) were compared with baseline scores (t = -45 min) using Student’s paired t-test. Dose-response relationships between energy intake and the amount of C12 administered (ie, 0, 2, 4 or 6 g) and correlations between energy intake and hunger and fullness, were determined.
using linear associations by calculating correlation coefficients adjusted for repeated measures (Bland and Altman, 1995).

9.4.2 Results

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

Appetite perceptions and gastrointestinal symptoms

Hunger
Following breakfast, scores for hunger decreased when compared with baseline with all treatments (Figure 9.3A). Subsequently, hunger increased slowly between t = 0 – 180 min. While there was no significant effect of treatment on scores for hunger, mean scores following C12(6g) were lower when compared with control. There was a significant effect of time for hunger (P < 0.001), such that hunger was greater compared with t = 0 min (ie end of breakfast) following control from t = 75 min (P < 0.001), C12(2g) from t = 90 min (P < 0.05), C12(4g) from t = 60 min (P < 0.05), and C12(6g) of C12 from t = 90 min (P < 0.01), until t = 180 min (ie start of lunch). Scores for hunger at t = 180 min were still lower than scores at baseline following C12(2g) and C12(6g) (P < 0.05), but not C12(4g) and control.

Fullness
Following breakfast, scores for fullness increased when compared with baseline with all treatments (Figure 9.3B). Subsequently, fullness slowly decreased between t = 0 – 180 min. While there was no significant effect of treatment on scores for fullness, mean scores following C12(6g) were higher between t = 0 – 180 min than after the other
treatments. There was a significant effect of time for fullness (P < 0.001), such that scores for fullness were less compared with t = 0 min (ie end of breakfast) following control and C12(2g) from t = 45 min (P < 0.05 for both), C12(4g) from t = 30 min (P < 0.05), and C12(6g) from t = 75 min (P < 0.05), until t = 180 min (ie start of lunch). Scores for fullness at t = 180 min were still greater than scores at baseline following all treatments (P < 0.01).

Nausea and bloating

There was no effect of treatment on nausea (Figure 9.4A) or bloating (Figure 9.4B). There was a significant effect of time for bloating (P < 0.001), but not nausea. Following breakfast, bloating increased compared with baseline following all treatments. Subsequently, scores for bloating decreased gradually between t = 0 – 180 min, and were lower compared with t = 0 min (ie end of breakfast) following control from t = 30 min (P < 0.05), C12(2g) and C12(4g) from t = 15 min (P < 0.001), and C12(6g) from t = 60 min, until t = 180 min (ie start of lunch).

There was no effect of treatment on either desire to eat or prospective consumption (data not shown).

Energy intake

Breakfast

There was no effect of treatment on energy intake, the weight of food consumed, or the macronutrient distribution (Table 9.1).
**Lunch**

There was a significant effect of treatment on energy intake ($P < 0.05$) (**Table 9.1**). C12(2g) and C12(6g) both decreased energy intake when compared with control ($P < 0.05$), while the mean difference between C12(4g) and control did not reach statistical significance ($P = 0.17$). There were no differences between C12(2g), C12(4g) and C12(6g). Energy intake was suppressed by a mean of 565 kJ (~ 13 %) following C12(2g), 357 kJ (~ 8 %) following C12(4g) and 758 kJ (~ 18 %) following C12(6g).

When the amount of energy ingested from C12 is taken into account (ie 2 g: 74 kJ, 4 g: 149 kJ, 6 g: 223 kJ), there was still a net mean reduction in energy intake by 491 kJ, 209 kJ and 535 kJ with C12(2g), C12(4g) and C12(6g), respectively. There was no significant relationship between energy intake with the amount of C12 administered, or with scores for hunger, fullness, nausea or bloating at $t = 180$ min.

There was no effect of treatment on the weight or macronutrient distribution of food consumed (**Table 9.1**).

**Total energy intake (breakfast + lunch)**

There was a significant effect of treatment on total energy intake ($P < 0.05$) (**Table 9.1**). C12(6g) decreased energy intake when compared with both control ($P < 0.05$) and C12(4g) ($P < 0.01$), while there was no difference between C12(2g) and C12(6g), nor between control, C12(2g) and C12(4g). There was no significant relationship between energy intake with the amount of C12 administered.

There was no effect of treatment on the weight or macronutrient distribution of food consumed (**Table 9.1**).
Table 9.1: Energy intake, weight of food consumed and its macronutrient distribution following oral ingestion of lauric acid (C12) at 2 g, 4 g and 6 g, or control (at breakfast, lunch and total food consumed (breakfast + lunch)).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C12(2g)</th>
<th>C12(4g)</th>
<th>C12(6g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>4024 ± 211</td>
<td>4162 ± 163</td>
<td>4558 ± 262</td>
<td>3979 ± 268</td>
</tr>
<tr>
<td>Weight of food (g)</td>
<td>826 ± 50</td>
<td>821 ± 27</td>
<td>903 ± 63</td>
<td>768 ± 56</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>24.1 ± 1.6</td>
<td>23.3 ± 1.5</td>
<td>25.8 ± 1.4</td>
<td>26.0 ± 2.0</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>63.9 ± 2.1</td>
<td>64.7 ± 2.0</td>
<td>62.2 ± 1.8</td>
<td>62.0 ± 2.3</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>12.0 ± 0.6</td>
<td>12.1 ± 0.6</td>
<td>12.0 ± 0.5</td>
<td>12.0 ± 0.5</td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>4232 ± 151</td>
<td>3667 ± 283*</td>
<td>3874 ± 315</td>
<td>3474 ± 237*</td>
</tr>
<tr>
<td>Weight of food (g)</td>
<td>1039 ± 96</td>
<td>918 ± 74</td>
<td>1034 ± 77</td>
<td>949 ± 99</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>30.8 ± 1.7</td>
<td>31.4 ± 1.3</td>
<td>32.1 ± 1.4</td>
<td>33.1 ± 1.6</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>48.5 ± 2.4</td>
<td>49.1 ± 1.7</td>
<td>47.6 ± 2.0</td>
<td>46.2 ± 2.6</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20.6 ± 1.2</td>
<td>19.5 ± 1.0</td>
<td>20.3 ± 1.0</td>
<td>20.7 ± 2.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ)</td>
<td>8256 ± 297</td>
<td>7828 ± 269</td>
<td>8433 ± 463</td>
<td>7453 ± 389#</td>
</tr>
<tr>
<td>Weight of food (g)</td>
<td>1866 ± 134</td>
<td>1793 ± 86</td>
<td>1937 ± 121</td>
<td>1717 ± 141</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>27.4 ± 1.4</td>
<td>27.4 ± 1.0</td>
<td>28.9 ± 1.0</td>
<td>29.6 ± 1.5</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>56.2 ± 1.8</td>
<td>56.9 ± 1.1</td>
<td>54.9 ± 1.2</td>
<td>54.1 ± 1.7</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>16.3 ± 0.7</td>
<td>15.8 ± 0.5</td>
<td>16.2 ± 0.5</td>
<td>16.4 ± 0.7</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 14). * vs control: P < 0.05, # vs control and 4 g of C12: P<0.05.
Figure 9.2: Scores for hunger (A) and fullness (B) following ingestion of control or C12 at 2, 4 and 6 g. Scores were significantly different from this point, # control, † C12(2g), § C12(4g) and * C12(6g), compared with t = 0 min (ie end of breakfast) until t = 180 min (ie start of lunch), P < 0.05 for all. Data are means ± SEM; n = 14.
Figure 9.3: Scores for nausea (A) and bloating (B) following the ingestion of control or C12 at 2, 4 and 6 g. Scores were significantly different from this point, # control, † C12(2g), § C12(4g) and * C12(6g), compared with t = 0 min (ie end of breakfast) until t = 180 min (ie start of lunch), P < 0.05 for all. Data are means ± SEM; n = 14.
9.4.3 Discussion

This was the first study to evaluate the effects of oral C12 on appetite, gastrointestinal symptoms and energy intake in healthy, lean males, and establishes that when administered orally C12 has the capacity to suppress energy intake, and this does not reflect an aversive effect. C12 did not affect energy intake at breakfast (consumed 30 min after C12 ingestion), but decreased energy intake at lunch (consumed 225 min after C12 ingestion) by ~13 %, 8 % and 18 %, following 2 g, 4 g or 6 g of C12, respectively, when compared with control. Furthermore, total energy intake (breakfast + lunch) was reduced following 6 g of C12 compared with control by ~10 %. Hence, the magnitude of suppression of energy intake resulting from acute administration of C12 is substantial and would be of clinical significance if maintained during chronic administration.

The current study did not assess the effects of oral C12 on gastrointestinal function, but it has been established that intraduodenal C12 modulates antropyloroduodenal motility and stimulates CCK, GLP-1, GLP-2 and PYY, and suppresses ghrelin, secretion (Chapters 4 – 7, Feltrin et al., 2004), and that intragastric C12 (at a dose of ~2.5 g) modulates gastric motility and stimulates both CCK secretion and gallbladder contractions (McLaughlin et al., 1999). These changes in gastrointestinal motility (Brennan et al., 2007, unpublished observations, Xu et al., 2005) and hormone secretion (Lieverse et al., 1994, Abbott et al., 2005, Turton et al., 1996) appear to mediate the effects of fat on energy intake, although further studies are required to confirm that the suppression of energy intake by oral C12 is associated with changes in gastrointestinal function, particularly slowing of gastric emptying and the secretion and/or suppression of gastrointestinal hormones.
Although the suppression of energy intake by C12 was not clearly dose-related, it is clear that C12 effectively reduces energy intake following oral ingestion, although it is uncertain why 4 g of C12 did not significantly suppress energy intake. It is of interest that this reduction in energy intake was not associated with a change in the amount (g) of food consumed, that is, subjects consumed a less energy-dense meal. Furthermore, while both 2 g and 6 g of C12 suppressed energy intake, this occurred in the absence of significant changes in appetite perceptions, which has been demonstrated to occur following intraduodenal C12 (Chapters 5 – 7). Therefore, a reduction in subjective appetite perceptions is not required for the effects of oral C12 on energy intake, indicating that the perception of appetite may be regulated by different mechanisms to those involved in the control of acute energy intake. However, this may be a type 2 error, considering there were trends for C12 to reduce hunger and increase fullness and the inclusion of more subjects may have resulted in significant differences.

The current study assessed whether energy intake would be reduced 30 min following C12 ingestion, as a previous study had demonstrated that intragastric C12 (at a dose of ~ 2.5 g) stimulated CCK secretion within ~ 15 min (McLaughlin et al., 1999), however, energy intake at breakfast was not reduced. – Recently it has been reported that following intragastric administration of a fatty acid emulsion containing 40 g of oleic acid (“C18”), gastric emptying is markedly slowed, such that only 10 g of C18 had emptied from the stomach after 240 min (Hunt and Knox, 1968, Little et al., 2007), hence, it is highly likely that very little C12 would have emptied within 30 min, possibly explaining the lack of effect of C12 on energy intake at breakfast. Accordingly, 225 min following oral C12, it would be expected that all of the C12, at all doses, and probably the majority of the breakfast meal, would have emptied from the
stomach, stimulating maximal CCK and PYY concentrations (Little et al., 2007), hence, the marked reduction in energy intake at lunch.

A number of limitations of the study design must be recognised. The *ad libitum* breakfast may have encouraged subjects to overeat and consequently blunted the suppressive effect of C12 on energy intake at lunch. Administering a smaller, standardised breakfast may have revealed greater suppression of energy intake at lunch. The rate of gastric emptying of C12 was not controlled for, or measured, hence, there may have been inconsistencies in the delivery of C12 from the stomach into the small intestine between subjects. C12 is solid at room temperature, and in any form (solid or liquid), does not dissolve or disperse well in water. Accordingly, this may have caused the C12 not to empty into the small intestine at a constant rate, and optimisation of the intragastric distribution, or dispersion, of C12 is likely to achieve a more predictable delivery of C12 into the small intestine and, thus, more reliable suppression of energy intake.

Acute exposure of the small intestine to fatty acids may cause mucosal damage, including the disruption of the membrane of intestinal villi and increased permeability of the intestine to large molecules (Kviety et al., 1991, Ramirez et al., 1997, Velasquez et al., 1993a, Velasquez et al., 1993b). Hence, chronic ingestion of fat in the form of free fatty acids has the potential to be associated with undesirable small intestinal effects. However, oral ingestion of C12 is arguably unlikely to produce any permanent cytotoxic effects to small intestinal mucosa, considering that damage to the intestinal epithelium by fatty acids is comparable to that observed following ‘normal’ meal
digestion and absorption (Kviety et al., 1991). The effects of the longer-term ingestion of C12 on the small intestinal mucosa warrant further evaluation.

In summary, this study has established that acute oral ingestion of C12 has the capacity to reduce energy intake, without inducing adverse effects, in a dose as low as 2 g. These observations indicate the potential for C12 as a nutrient-based treatment for weight loss in obesity. In view of these observations studies are required to establish whether the acute suppression of energy intake by oral C12 is sustained during chronic administration, and whether the suppressive effect of oral C12 is evident in obese subjects.
Chapter 10

Conclusion

The studies reported in this thesis have evaluated the effects of fatty acids, particularly lauric acid, on the regulation of gastrointestinal function and the suppression of energy intake in healthy humans. The main aims of the studies were to assess (i) the effects of intraduodenal C12 and C10 on the secretion of gastrointestinal hormones, including ghrelin, GLP-2, PYY and PP, (ii) the effects of intraduodenal C12 and C18:1 delivered at the same energy load (0.4 kcal/min) on APD motility, secretion of CCK and PYY and energy intake, (iii) the effects of increasing doses of intraduodenal C12 on APD motility, gastrointestinal hormone secretion and energy intake to determine whether the effects of C12 on energy intake were physiological, or related to nausea, (iv) whether the effects of intraduodenal C12 on APD motility, gastrointestinal hormone secretion and energy intake were dependent on the load, or the concentration, (v) whether CCK-8 and GLP-1 interacted in their effects on energy intake and gastrointestinal function and (vi) the effects of increasing doses of orally ingested C12 on appetite and energy intake.

The study described in Chapter 4 assessed the effects of intraduodenal C12 and C10 in healthy men on the secretion of PYY, GLP-2 and PP, and the suppression of ghrelin. C12, but not C10, markedly stimulated the secretion of PYY and GLP-2 and suppressed ghrelin secretion, while both C12 and C10 slightly increased PP secretion. This study demonstrated that in healthy humans the effects of intraduodenal fatty acids on ghrelin, PYY and GLP-2, but apparently not PP, secretion are dependent on their chain length.
Accordingly, the role of these peptides, together with CCK and GLP-1, in the mediation of the suppression of energy intake, as well as the mechanisms underlying the modulation of the secretion of these peptides by C12, warrant further evaluation.

The comparison of the effects of intraduodenal C12 and C18:1 on APD motility, plasma CCK and PYY concentrations and energy intake in healthy men is described in Chapter 5. Both C12 and C18:1 reduced antral and duodenal PWs and stimulated IPPWs and plasma CCK, with no differences between them. While both C12 and C18:1 increased basal pyloric pressure, C12 had a greater effect than C18:1. In contrast, while both C12 and C18:1 increased plasma PYY, C18:1 had a greater effect than C12. C12, but not C18:1, suppressed energy intake. This study demonstrated, that at the load administered, C12, but not C18:1, suppresses energy intake and C12 was a more potent stimulant of pyloric tone. The suppression of energy intake could not be related directly to changes in gastrointestinal function, considering both C12 and C18:1 suppressed antral PWs, stimulated IPPWs, and increased plasma CCK concentrations, to the same extent, while C18:1 increased PYY concentrations more than C12. Taken together, these data confirm the potent appetite-suppressant effects of intraduodenal C12, but suggest that these effects are not entirely accounted for by the actions of C12 on gastrointestinal function.

It was recently reported that in healthy humans intraduodenal infusion of C12 modulates gastrointestinal function and suppresses energy intake, however, this was associated with nausea, confounding interpretation of the results. In order to determine whether the effects of C12 on energy intake were physiological, or related to nausea, a dose-response study was performed using loads ranging from: 0.1 – 0.4 kcal/min in
healthy males, but this was also associated with varying C12 concentrations (Chapter 6). C12 potently modulated APD motility, increased plasma CCK and GLP-1 concentrations in a dose-dependent manner, and at the highest dose, there was an inhibitory effect on energy intake, in the absence of nausea. However, as both load and concentration of the C12 solutions were varied it was not possible to determine whether the load, or concentration, of C12 (or both) was responsible for the observed effects, hence, the studies described in Chapter 7 examined in healthy humans the responses to intraduodenal loads of C12 of 0.2, 0.3 and 0.4 kcal/min at a fixed concentration (56 mM) and contrasted them to concentrations of 40, 56, and 72 mM at a fixed load (0.4 kcal/min), to evaluate the effects of load, and concentration, of C12 on APD motility, plasma CCK and PYY concentrations, appetite and energy intake. This study demonstrated that, at the loads and concentrations administered, load, but not concentration, modulates APD motility patterns, plasma CCK and PYY concentrations and energy intake in response to intraduodenal C12.

There is evidence that CCK-8 and GLP-1 mediate the effects of nutrients on appetite and gastrointestinal function and that their interaction may be synergistic. The study in Chapter 8 assessed whether CCK-8 and GLP-1 interacted in their effects on energy intake and gastrointestinal function. Intravenous CCK-8 (1.8 pmol/kg/min) and GLP-1 (0.9 pmol/kg/min) were administered alone and in combination. At the doses evaluated, CCK-8 suppressed energy intake, decreased the number of antral and duodenal pressure waves and IPPWs, while GLP-1 only decreased antral and duodenal PWs, but had no effect on energy intake and IPPWs. The combination of CCK-8 and GLP-1 only decreased the number of duodenal PWs to a greater extent than either infusion alone, but this did not exceed the sum of the individual effects of CCK-8 and GLP-1. At the
doses evaluated, exogenously administered CCK-8 and GLP-1 had discrepant effects on appetite, energy intake and APD pressures, and the effects of CCK-8/GLP-1, in combination, did not exceed the sum of the effects of CCK-8 and GLP-1, providing no evidence of synergism. In view of this, the dose of CCK may have had a maximal effect on the modulation of APD motility and the suppression of energy intake, hence the effects of a range of doses of CCK-8 on gastrointestinal function and energy intake requires further exploration.

A previous study had demonstrated that following intragastric administration of C12, the effects of C12 on gastrointestinal function, including suppression of antral contractions, relaxation of the proximal stomach and stimulation CCK secretion, which are associated with the suppression of energy intake, are still maintained, therefore, the study in Chapter 9 assessed whether increasing doses of orally ingested C12 between 2 – 6 g would result in a dose-related suppression of appetite and subsequent energy intake at breakfast and lunch. Energy intake at lunch, but not breakfast, was decreased after ingestion of both 2 g and 6 g of C12 compared with control. Total energy intake (breakfast + lunch) was decreased following ingestion of 6 g of C12 compared with control and 4 g. Total weight of food eaten (breakfast + lunch) was also reduced following 6 g and 2 g of C12 compared with 4 g C12. This study, thus, demonstrated that C12 maintains its suppressant effect on energy intake when ingested orally.

The studies presented in this thesis have provided insight into the effects of the fatty acid, lauric acid, on gastrointestinal function and energy intake, specifically, the modulation of APD motility, secretion of CCK, GLP-1, PYY and PP, suppression of ghrelin secretion and the suppression of energy intake, in healthy young men. The
marked suppression of energy intake following oral C12 ingestion suggests that C12 has the potential to be developed into a nutrient-based treatment for weight loss in obesity, however, future studies first need to establish whether the acute suppression of energy intake by oral C12 is sustained during chronic administration and whether the suppressive effects of oral C12 is evident in obese subjects.
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.
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)

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)
Appendix I

Name:               Date:

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 meal times | sometimes between meals | often between meals | almost always |

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

   | 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 difficult | moderately difficult | very 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 always |

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

   | 1 | 2 | 3 | 4 |
   | unlikely | slightly | moderately | very 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 unlikely 2 slightly 3 moderately 4 very likely likely likely likely

47. How frequently do you skip dessert because you are no longer hungry

1 almost 2 seldom 3 at least 4 almost never once a week every day

48. How likely are you to consciously eat less than you want?

1 unlikely 2 slightly 3 moderately 4 very likely likely likely likely

49. Do you go on eating binges even though you are not hungry?

1 never 2 rarely 3 sometimes 4 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 not like 2 little like 3 pretty good 4 describes of me little little 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 eat whatever you want, whenever you want it 2 usually eat whatever you want, whenever you want it 3 often eat whatever you want, whenever you want it 4 often limit food intake, but often “give in” 5 usually limit food intake, rarely “give in” 6 constantly limit food intake, never “give in”
Appendix II

Visual Analogue 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 breakfast

<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, 2 slices(^1)</td>
<td>60</td>
<td>626</td>
<td>1.7</td>
<td>24.0</td>
<td>6.1</td>
</tr>
<tr>
<td>White bread, 2 slices(^1)</td>
<td>60</td>
<td>622</td>
<td>1.4</td>
<td>27.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Crumpets(^2)</td>
<td>100</td>
<td>639</td>
<td>0.7</td>
<td>30.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Blueberry muffin(^3)</td>
<td>45</td>
<td>713</td>
<td>4.0</td>
<td>31.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Breakfast bar(^4)</td>
<td>40</td>
<td>620</td>
<td>1.8</td>
<td>29.1</td>
<td>2.5</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>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>120</td>
<td>430</td>
<td>0.1</td>
<td>23.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Orange</td>
<td>180</td>
<td>288</td>
<td>0.2</td>
<td>14.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Apple juice(^6)</td>
<td>260</td>
<td>486</td>
<td>2.6</td>
<td>28.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Iced chocolate(^7)</td>
<td>630</td>
<td>2155</td>
<td>21.4</td>
<td>59.2</td>
<td>20.8</td>
</tr>
<tr>
<td>Margarine(^8)</td>
<td>20</td>
<td>516</td>
<td>13.9</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Vegemite(^9)</td>
<td>20</td>
<td>119</td>
<td>0.2</td>
<td>1.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Strawberry jam(^10)</td>
<td>30</td>
<td>327</td>
<td>0.3</td>
<td>19.5</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1885</strong></td>
<td><strong>8749</strong></td>
<td><strong>54.6</strong></td>
<td><strong>338.3</strong></td>
<td><strong>62.8</strong></td>
</tr>
</tbody>
</table>

\(^1\) Sunblest, Tiptop, Australia; \(^2\) Gibbs Crumpets, Gibbs Pty Ltd, Australia; \(^3\) K-time muffin bar, Kellogg (Aust) Pty Ltd, Australia; \(^4\) Uncle Toby’s Sports Plus Breakfast Bar, The Uncle Toby’s Company, Australia; \(^5\) Yoplait, National Foods Ltd, Australia; \(^6\) Poptop apple drink, P & N Beverages Australia Pty Ltd, Australia; \(^7\) Pura Classic chocolate, National Foods Ltd, Australia; \(^8\) Flora, Unilever Australasia, Australia; \(^9\) Kraft, Kraft Foods Ltd, Australia; \(^10\) Heinz, Heinz Wattie’s Ltd, Australia.
## Appendix IV

### Composition of the buffet-style lunch

<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 slices¹</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 slices¹</td>
<td>125</td>
<td>1295</td>
<td>2.9</td>
<td>56.4</td>
<td>11.8</td>
</tr>
<tr>
<td>Ham, sliced²</td>
<td>100</td>
<td>453</td>
<td>3.6</td>
<td>0</td>
<td>18.8</td>
</tr>
<tr>
<td>Chicken, sliced³</td>
<td>100</td>
<td>677</td>
<td>7.0</td>
<td>0</td>
<td>24.6</td>
</tr>
<tr>
<td>Cheese, sliced⁴</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 yoghurt⁵</td>
<td>200</td>
<td>966</td>
<td>6.2</td>
<td>33.8</td>
<td>9.4</td>
</tr>
<tr>
<td>Fruit salad⁶</td>
<td>140</td>
<td>343</td>
<td>0.1</td>
<td>19.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Chocolate custard⁷</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, unsweetened⁸</td>
<td>500</td>
<td>800</td>
<td>5.0</td>
<td>42.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Iced coffee⁹</td>
<td>600</td>
<td>1788</td>
<td>10.2</td>
<td>61.8</td>
<td>21.0</td>
</tr>
<tr>
<td>Water</td>
<td>600</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Margarine¹⁰</td>
<td>20</td>
<td>609</td>
<td>16.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mayonnaise¹¹</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>3425</strong></td>
<td><strong>11808</strong></td>
<td><strong>95.7</strong></td>
<td><strong>354.6</strong></td>
<td><strong>136.9</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, Balmar Pty Ltd, Australia; ¹⁰ Flora, Unilever Australasia, Australia; ¹¹ Kraft, Kraft Foods Ltd, Australia.
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