ORAL AND SMALL INTESTINAL SENSITIVITY TO FATS IN LEAN AND OBESE HUMANS: IMPLICATIONS FOR ENERGY INTAKE REGULATION IN OBESITY

A thesis submitted by

Radhika Seimon

For the degree of

Doctor of Philosophy

Discipline of Medicine

School of Medicine

The University of Adelaide

March 2012

Contents

List of Figures

List of Tables

Abstract

The research presented in this thesis focuses on the complex and interrelated oral and gastrointestinal mechanisms involved in the regulation of appetite and energy intake in lean and obese individuals. The three broad areas of research that have been investigated in the thesis include: i) the gastrointestinal motor and hormonal functions involved in the regulation of energy intake in healthy individuals; ii) the effects of oral and intraduodenal nutrients on gastrointestinal motility and hormone release, appetite and energy intake in obese compared with lean individuals; and iii) the effects of acute and prolonged energy restriction on gastrointestinal function, appetite and energy intake.

Following ingestion of a meal, the interaction of nutrients with receptors in the small intestinal lumen modulates gastropyloroduodenal motility, stimulates the release of gastrointestinal hormones, and suppresses appetite and energy intake. It appears that modulation of gastrointestinal functions, that is, gastrointestinal motility and hormone release/suppression, mediate the regulation of appetite and acute energy intake in humans, at least in part. Changes in motility and hormone secretion occur concurrently with changes in appetite; however, there is little information regarding which, if any, of these factors are independent determinants of energy intake. In the study presented in **Chapter 5**, we determined independent predictors of energy intake and identified specific changes in gastrointestinal motor and hormone functions (i.e. stimulation of

pyloric pressures and plasma cholecystokinin) that are associated with the suppression of acute energy intake in healthy lean males.

The incidence of obesity is rapidly increasing and, currently, the therapies used for the prevention and management of obesity have limited long-term benefits. In addition, the available therapies have largely ignored the pivotal role of the gastrointestinal tract in the regulation of appetite. There is evidence that gastrointestinal function in obesity is modified, which may be the result of the eating habits of obese individuals and, in turn, may also contribute to the maintenance of obesity by causing insufficient suppression of energy intake. However, much of the literature relating to gastrointestinal function in the obese is inconclusive and controversial. A better understanding of any adaptations that occur in obesity is important, particularly in regards to treatment approaches for weight loss.

There is also evidence that previous patterns of energy intake, in excess or in restriction, even when sustained for short periods, have the capacity to modify gastrointestinal function and energy intake. For example, in humans following a high fat diet for two weeks, gastric emptying and mouth-to-caecum transit in response to a high fat test meal were faster. In contrast, fasting had the opposite effect and a four-day fast slowed gastric emptying of a glucose drink in both lean and obese subjects, suggesting that a reduction in nutrient exposure may increase the sensitivity of gastrointestinal responses to nutrients in the obese.

Although many studies have addressed aspects of gastrointestinal function in the obese, there is a lack of studies that have evaluated gastric emptying and gastrointestinal hormone release specifically GLP-1 and GIP, given the risk of diabetes in obesity, as well as previous patterns of nutrient intake concurrently. In the study presented in **Chapter 6**, we evaluated the effects of oral ingestion of a nutrient liquid on gastric emptying, oro-caecal transit, plasma GLP-1 and GIP, appetite and energy intake, as well as, habitual energy and fat intake in lean, overweight and obese individuals. We reported no differences in gastric emptying, intragastric distribution or oro-caecal transit between the lean, overweight and obese groups. After the drink, blood glucose and plasma insulin were greater in the obese, when compared with both the lean and overweight groups, however, there were no differences in plasma GLP-1 or GIP concentrations, appetite and energy intake at the buffet meal or habitual energy intake between the groups. In the obese, the magnitude of the rise in blood glucose was inversely related to the gastric emptying, suggesting that obesity per se, in the absence of differences in habitual energy intake, has no effect on gastric emptying or incretin hormone release and that gastric emptying influences postprandial blood glucose in the obese.

In **Chapter 7**, we investigated the hypothesis that gastrointestinal and oral sensitivity to fat is compromised in the obese and directly related to their high fat/energy consumption. For this purpose, we investigated the effects of an intraduodenal infusion (to bypass gastric emptying), of a fatty acid (oleic acid) on gastrointestinal function, appetite and energy intake, and relationships with habitual energy intake and oral fatty acid detection threshold in lean and obese individuals. We report that pyloric pressure,

which plays a major role in the regulation of gastric emptying, was lower in response to intraduodenal oleic acid infusion, with trends for reduced cholecystokinin stimulation and energy intake responses in the obese compared with lean. Oral fatty acid detection thresholds were higher in obese compared with lean subjects, and obese subjects also had greater habitual energy and fat intakes than lean subjects. The results suggest that the ability to detect fats both orally and within the gastrointestinal tract is compromised in obese males, probably due to their increased fat consumption.

In the study presented in **Chapter 8**, we evaluated the hypothesis that in obese individuals, the effects of duodenal fat on gastrointestinal motor and hormone function, and appetite would be enhanced by a short period on a very low calorie diet. We demonstrated that following a 70% four-day very low calorie diet there was a significant increase in pyloric pressure and the stimulation of PYY and suppression of ghrelin was greater during an intraduodenal lipid infusion. In addition, following the four-day very low calorie diet, appetite perceptions and energy intake in response to intraduodenal lipid were reduced, indicating that gastrointestinal function, appetite and energy intake in obese can be enhanced over a short period.

Given that gastrointestinal function is sensitive to changes even over short periods of dietary restriction, it is important to determine whether these changes are maintained in the long term in order to determine the efficacy of energy restriction therapies for obesity. To maintain dietary restriction and weight loss in the longer term, we used a 30%, as opposed to 70%, energy-restricted diet. In the study presented in **Chapter 9**, we evaluated the effects of an acute (in lean and obese) and prolonged (in obese only)

30% energy restriction on gastrointestinal function and appetite in response to an intraduodenal lipid infusion. In contrast to the previous 70% very low calorie diet study, there were no differences in gastrointestinal motor or hormonal function in the obese following the acute or prolonged 30% dietary restriction period, although there was a trend for energy intake to be reduced. However, in the lean, there was a decrease in plasma CCK and an increase in ghrelin concentrations following the acute period of dietary restriction with no differences in gastrointestinal motility or energy intake, suggesting that a 30% energy-restricted diet diminishes gastrointestinal hormone responses in lean, but not obese, which may suggest that obese are less sensitive to this caloric restriction.

These observations will contribute to the advances in basic appetite physiology and will have clinical implications for further development of dietary interventions for the treatment of obesity.

Declaration of Originality

I, Radhika Seimon, certify that 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 made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Radhika Seimon

March 2012

Publications Arising from This Thesis

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

Seimon RV, Lange K, Little TJ, Brennan IM, Pilichiewicz AN, Feltrin KL, Smeets AJ, Horowitz M, Feinle-Bisset C; 2010 'Pooled-data analysis identifies pyloric pressures and plasma cholecystokinin concentrations as major determinants of acute energy intake in healthy, lean men', Am J Clin Nutr, vol. 92, no. 1, pp. 61–68.

Brennan IM, **Seimon RV**, Luscombe-Marsh ND, Otto B, Horowitz M, Feinle-Bisset C; 2010 'Effects of acute dietary restriction on gut motor, hormone and energy intake responses to duodenal fat in obese men', International Journal of Obesity, vol. 153, pp. 1–9.

Stewart JE, **Seimon RV**, Otto B, Keast RS, Clifton PM, Feinle-Bisset C; 2011, 'Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men', Am J Clin Nutr, vol. 93, no. 4, pp. 703–711.

Dedication

To my late mother and to my father.

For your unconditional love and sacrifices,

For your continual support and encouragement, and

For your selflessness,

I am forever grateful.

Acknowledgements

The studies reported in this thesis were performed in the Discipline of Medicine, University of Adelaide and the Department of Nuclear Medicine, PET and Bone Densitometry at the Royal Adelaide Hospital. While conducting this research, I was financially supported by a Dawes Postgraduate Research Scholarship (2009–2012), jointly provided by the Royal Adelaide Hospital and the University of Adelaide.

First and foremost, I would like to thank my principle supervisor, Professor Christine Feinle-Bisset. I am ever so grateful for your continual support, encouragement, wisdom and friendship. Thank you for the time you spent on this thesis and for your patience with it all. I am so privileged to have had the opportunity to work and be mentored by you. Thank you also for the opportunities to travel overseas and attend conferences to present my work.

To the Christine Feinle-Bisset group, thank you for your help and support - it has been a pleasure working with you all. Special thank you to Tanya Little and Natalie Luscombe-Marsh for all the advice, encouragement and support. Briohny Bartlett and Asimina Kallas, thank you so much for your friendship and for all the laughs. Thank you for the invaluable contributions to the studies presented within this thesis and for giving up your weekends to come in to help.

To Diana Gentilcore, Amy Ryan, Gabrielle Heruc, Tongzhi Wu and Laurence Trahair, it has been great sharing an office with you all. Thank you for the laughs, chats and the many welcomed distractions.

To all other staff and students in the Discipline of Medicine and the Department of Nuclear Medicine, PET and Bone Densitometry, thank you for being so supportive throughout my candidature and thank you for all your help with the studies presented in this thesis. To Antonietta Russo, Professor Karen Jones and Max Bellon, thank you for your help with the gastric emptying study. Thank you to Professor Michael Horowitz for the support and encouragement through the years. Thank you to Judith Wishart, Scott Standfield and Bärbel Otto for the hormone analysis and Kylie Lange for help with the statistical analysis.

A special thank you to all the individuals who volunteered their time to participate in the studies presented in this thesis.

Thank you to all my friends for your love and encouragement. A special thank you to my friends Nadine Matti and Priya Jeyakumar, I am so blessed to have you in my life. Thank you for everything - your friendship and continuous love and support. Lisa Lines and Nick Brandenburg, thank you so much for your friendship and advice and a huge thank you for taking the time to proof read my thesis.

Last, but not least, my beautiful family, my father, and my brothers, Yohan and Yolan. Thank you so much for all that you have done for me, your unconditional love, support and encouragement throughout the years. I am truly blessed and forever grateful.

List of Abbreviations

ANOVA	analysis of variance
APD	antropyloroduodenal
AUC	area under the cure
BMI	body mass index
CCK	cholecystokinin
CHO	carbohydrates
CV	coefficient of variation
DMNV	dorsal motor nucleus of the vagus
EDTA	ethylenediaminetetraacetic acid
GIP	glucose-dependent insulinotropic polypeptide
HOMA	homeostasis model assessment
IPPW	isolated pyloric pressure waves
LHA	lateral hypothalamic areas
MI	motility index
MMC	migrating motor complex
MSG	monosodium glutamate
PVN	paraventricular nuclei
PW	pressure waves
PWS	pressure wave sequences
PYY	peptide tyrosine tyrosine
TEI	total energy intake

TMPD	transmucosal potential difference
VAS	visual analogue scale
VLCD	very low calorie diet

Chapter 1: Thesis Overview

Human eating behaviour is complex and a number of genetic, environmental and physiological factors contribute to the short- and long-term regulation of appetite and energy intake. Physiological factors that influence when and how much we eat play an important role in the acute regulation of energy intake. The human gastrointestinal tract is a highly specialised organ system that allows us to extract nutrients from complex mixtures of food matrices and expel or eliminate non-nutrient and potentially toxic compounds. Following ingestion of a meal, there are a number of changes that occur within the oral cavity and gastrointestinal tract, of which the primary function is to achieve optimal nutrition as well as efficient nutrient digestion and absorption.

The oral cavity is the first site of nutrient detection. The interaction of nutrients with taste receptor cells on the tongue induce a signalling cascade that activates gustatory nerves and transmits sensory information to the hypothalamus and brainstem. The human gustatory system detects the taste qualities of sweet, sour, bitter, umami and salty, and more recently, the existence of a taste modality responsive to oral fatty acids has become evident. Oral taste perception is important as it prevents the ingestion of toxic substances, recognised often by the lack of palatability and/or bitter taste. In addition, oral taste perception contributes to determining individual food preference and dietary habits, in that the ability to detect nutrients, particularly fat, may affect food choice since dietary fat enhances the palatability of foods.

The stomach acts primarily as a reservoir for food, but also as a principal organ in the initial stages of mixing and grinding of nutrients. As food moves into the stomach after swallowing, the distension of the stomach activates gastric mechanoreceptors located in the wall of the proximal stomach that are responsive to stretch. As food passes into the small intestine, segmentation allows mixing with digestive juices in the intestine and peristaltic motor activity propels the nutrients along the length of the intestine, allowing interaction of nutrients with the small intestine to activate luminal chemoreceptors.

Gastric motility is controlled predominantly by the vagus nerve. Vagal afferents project to the nucleus tractus solitaries, where they form synapses with interneurones that project to the dorsal motor nucleus of the vagus (DMNV) and to higher brain centres. From the DMNV, efferent projections return to the stomach modulating the activity of the muscle cells through activation of either inhibitor or excitatory motor neurons. Signals from chemical stimulation of the small intestine during food ingestion are triggered by the detection of food molecules by specialised cells in the mucosa of the small intestine. These cells then release gut hormones, messenger molecules, which interact with receptors located on vagal afferents and signal to the brain. The hormones may also act in a classical endocrine way by travelling in the bloodstream to interact with receptors located centrally in the brain. These subconscious signals are then integrated by the brain centres that control food intake, most importantly in areas known as the medulla and hypothalamus, particularly the arcuate nucleus, either directly or via nerve fibres that project to the other hypothalamic areas, including the paraventricular nuclei (PVN) and the lateral hypothalamic areas (LHA). The net output of the PVN enhances the potency of satiation signals in the hindbrain, thereby acting to inhibit

energy intake. In contrast, the net output of the LHA suppresses the activity of satiation signals, thereby increasing energy intake.

Together, the activation of mechanoreceptors and chemoreceptors induce feedback inhibition of gastric emptying, which serves to prolong gastric distension and regulate the rate at which nutrients enter the small intestine, allowing for their optimal digestion and absorption. It is well known that signals arising from the gastrointestinal tract play a fundamental role in the regulation of appetite and that these are potentially modifiable to facilitate an increase or a reduction in energy intake.

Previous patterns of dietary exposure have the capacity to modify gastrointestinal function, which may be associated with changes in appetite and energy intake. Gastrointestinal, motor and hormonal, appetite responses, and oral sensitivity to fat are attenuated following high fat diet exposure. This may be an important mechanism underlying changes in energy intake, subsequent weight gain, and the development of obesity, as it is known that obese individuals display an increased preference for the consumption of fatty foods and that the proportion of dietary fat consumed in the diet is higher in obese individuals than in lean. It is conceivable that obesity may, at least in part, be a disorder of compromised nutrient sensing due to increased energy and fat intake. The mechanisms behind these abnormalities remain unclear, but may involve the desensitisation of gastrointestinal enteroendocrine cells, which may adapt to dietary conditions, including consumption of a high fat diet. Although it appears that gastrointestinal hormone function is disturbed in the obese, the effects on gastrointestinal motility have not been widely investigated, while studies that have

evaluated the effects of nutrients on gastric emptying in obese humans are inconclusive and controversial.

Conversely, dietary restriction may enhance the sensitivity of the small intestine to the presence of nutrients and thus facilitate appetite suppression. There has been growing interest in understanding the effects of energy restriction on gastrointestinal function and energy intake that leads towards the design of better diets that are effective for weight loss, yet are not associated with adaptions counter-productive to weight loss. It will be important to also determine whether these changes are maintained in the long term in order to determine the efficacy of energy restriction therapies for obesity. Any potential modification in gastrointestinal function, including gastric emptying, gastrointestinal motility and hormone release, during energy restriction, and the relationship with subsequent changes in appetite and energy intake, may have implications for the obese, particularly concerning treatment approaches for weight loss.

As the worldwide prevalence of obesity continues to increase, understanding the modulation of factors, including gastrointestinal motor and hormonal function, that play important roles in appetite regulation will enable us to understand the factors that contribute to their increased appetite and energy intake, and thus to develop strategies to counteract these changes, for better treatment outcomes.

Chapter 2: Oral and Gastrointestinal Factors Involved in the Regulation of Appetite and Energy Intake

2.1 Introduction

It is well established that the interaction of nutrients with small intestinal receptors induces effects on gastrointestinal function, including motility and hormone secretion, that contribute to the suppression of appetite and energy intake. Recently, evidence has been emerging for a sensory system that detects the presence of fatty acids within the oral cavity, and thus may also play an important role in the regulation of energy intake.

This chapter provides an overview of the gastrointestinal and orosensory factors involved in the regulation of appetite and energy intake. Accordingly, literature relating to the acute effects of nutrients on gastric and small intestinal motor function, gastrointestinal hormone release, appetite, energy intake and oral fat perception, is reviewed.

2.2 Anatomy and function of the oral cavity and the gastrointestinal tract

The first site of nutrient detection occurs in the oral cavity where ingested material, including food and toxins, is sensed. From an evolutionary point of view, this is vital for the health and survival of humans, with sweet and savoury sensations promoting the intake of energy-rich foods of nutritional benefit, while bitterness is closely associated

with the presence of toxins leading to its avoidance. The term 'taste' generally refers to the flavour of food, which is generated from the inputs from all of the anatomically and functionally distinct sensory systems; however, from a biological perspective, taste refers only to sensations received from stimulated taste receptor cells in the oral cavity (Kare and Mattes 1990). Taste begins on the tongue, where epithelial-derived taste receptor cells detect chemical cues (Travers et al. 1987; Lindemann 1996; Smith and Margolskee 2001). Taste buds are onion-shaped structures of between 50 and 150 clustered taste receptor cells that are distributed on the surface of the tongue and soft palate. Each taste bud has projections at the apical tip of the taste bud called a taste pore through which tastants make contact with the taste cell receptors (see **Figure 2.1**).

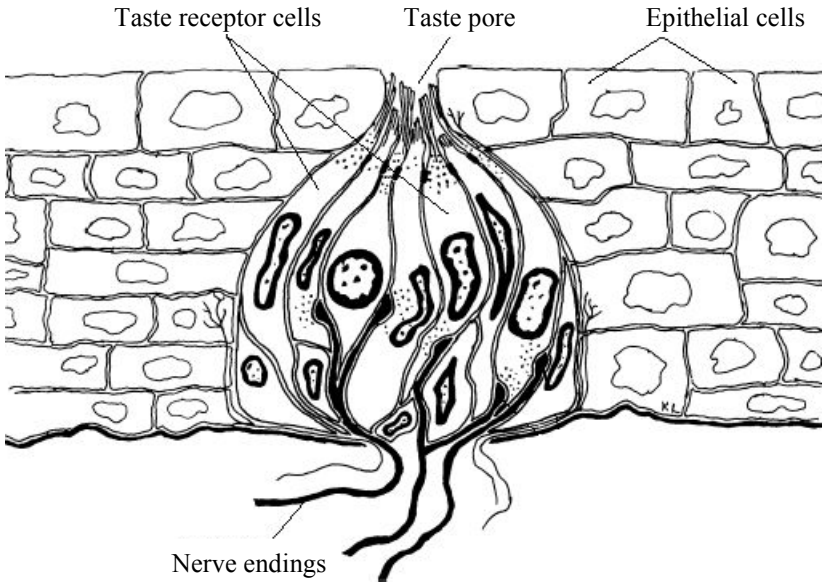


Figure 2.1: Basic anatomy of a taste bud

These signals are transmitted from the basal nerve endings to the nucleus tractus solitarius in the brainstem where taste is perceived. Human taste sensations are categorised into five distinguishable qualities: sweet, salty, sour, bitter and umami (the taste of monosodium glutamate [MSG]). Although less well established, there is accumulating evidence supporting a taste component for fatty acid.

Once food is swallowed, the ingested nutrients pass through the oesophagus, which is a muscular tube lined with stratified squamous epithelium, into the stomach, by peristalsis. The stomach is a J-shaped sac-like organ, with elaborate neural and hormonal control mechanisms. It is divided anatomically into the fundus, corpus and antrum (see **Figure 2.2**).

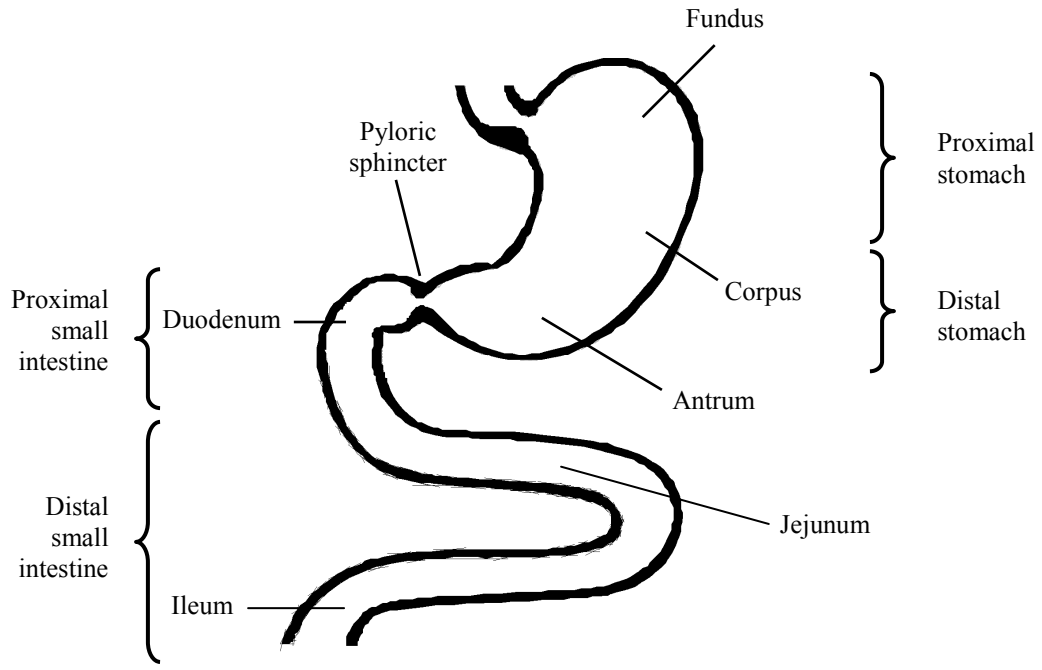


Figure 2.2: Basic anatomy of the stomach and small intestine

Functionally, the stomach can be divided into two regions: i) the proximal compartment, which includes the fundus and the proximal corpus and predominantly acts as a temporary 'store' for ingested food; and ii) the distal compartment, which includes the antrum and is responsible for grinding solid food into smaller particles and mixing it with gastric secretions into a so-called chyme (Holt et al. 1982). Connecting the antrum to the duodenum is the pylorus, which is a short 2 cm region. The pylorus functions primarily to regulate the outflow of gastric contents into the small intestine, and this is the most important motor mechanism involved in the regulation of gastric emptying (Anvari et al. 1995). The rate of gastric emptying is determined by the pressure generated in the proximal stomach through tonic contractions and by the size of the pyloric opening; that is, the higher the pressure and the larger the opening, the faster the rate of emptying. Following meal ingestion (i.e. when the stomach contains food), the pylorus acts as a 'sieve' that impairs the emptying of particles greater than 1 mm in diameter (Meyer et al. 1979). Antral grinding of the larger particles reduces them to a size that can pass through the pylorus into the small intestine. The small intestine is a muscular tube approximately 5 m in length and can be divided into three regions: the most proximal region, the duodenum (~ 25 cm long), the jejunum (~ 2 m long) and the distal region, the ileum (~ 3 m long). The majority of digestion and absorption occurs in the small intestine once the chyme enters the duodenum. Here, it is further mixed with bile, pancreatic juice from the pancreas and intestinal enzymes. 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.

2.3 Fasting and nutrient-induced, postprandial gastrointestinal motility

The motor activity in the upper gastrointestinal tract alternates between two distinct patterns: i) the interdigestive migrating motor complex (MMC) in the fasted state; and ii) the fed motility pattern that is initiated in the postprandial state following food ingestion. The following section describes the motility patterns in specific regions of the upper gastrointestinal tract, including the stomach, pylorus and small intestine, during the fasting and the postprandial states.

2.3.1 Fasting motor patterns

During the fasting state, the gastrointestinal tract exhibits a distinct cyclical pattern of motility, termed the MMC. The MMC comprises of three phases with a cycle time of approximately 120 min (but that can vary greatly): i) phase I, a period of motor quiescence lasting for about 40–60 min; ii) phase II, a period of irregular phasic contractions with a progressively increasing frequency lasting for about 45–90 min; and iii) phase III, a period of regular contractions, which is characterised by intense, rhythmic contractions that occur at maximal frequency and amplitude of the electrical pacemaker, which in the stomach is three and in the duodenum 12 contractions per minute, lasting for about 5–10 min. Approximately 50% of the phase III episodes commence in the stomach, with the remainder originating in the small intestine, ensuring that any undigested food in the upper gastrointestinal lumen is propelled distally.

2.3.2 Postprandial motor patterns

The interaction of nutrients with chemoreceptors within the small intestinal lumen following meal ingestion is responsible for the conversion of fasting motility into a 'fed' or postprandial motility pattern. After a meal, two motor responses occur in the proximal stomach. The first, termed 'receptive relaxation', is initiated by swallowing, which lasts for approximately 20 seconds, and is associated with a decrease in intragastric pressure. Secondly, there is a prolonged period of relaxation of the proximal stomach termed 'adaptive relaxation', which occurs so that the meal can be accommodated, that is, the increase in intragastric volume is not usually associated with a substantial increase in intragastric pressure (Azpiroz and Malagelada 1987). The distal stomach, or the antrum, is involved in grinding the food into small particles, as well as mixing the food with gastric secretions to initiate digestion. The propulsive contractions move the chyme from the stomach into the duodenum in between phasic and tonic pyloric contractions, that is, when the pylorus is opened. When chyme enters the duodenum from the stomach, there is further mixing with digestive secretions including pancreatic and gallbladder secretions and brush boarder enzymes, which further aid digestion and thereby making it available for absorption. These postprandial motility patterns in the upper gastrointestinal tract including proximal gastric relaxation (Feinle et al. 1996), suppression of both antral and duodenal contractility (Heddle et al. 1988b) and regular tonic and phasic pyloric contraction (Heddle et al. 1988b; Kumar et al. 1987; Heddle et al. 1989) lead to the slowing of gastric emptying and ensure that chyme is delivered from the stomach into the small intestine at an overall rate of approximately 1–3 kcal/min (Hunt et al. 1985) (see **Figure 2.3**).

Figure 2.3: Motor patterns associated with normal gastric emptying (Rayner and Horowitz 2005).

2.3.2.1 Gastric emptying

Gastric emptying is predominantly a pulsatile, rather than continuous, process and patterns of transpyloric flow reflect the integration of motor activity in the proximal stomach, antrum, pylorus and proximal small intestine (Horowitz et al. 1994). The coordinated activity of the stomach and pylorus results in the delivery of chyme into the small intestine at a rate allowing for optimal digestion and absorption of ingested food.

The patterns of gastric emptying are dependent upon the state, that is, liquid or solid, and the macronutrient composition of the ingested meal (Horowitz and Dent 1991; Edelbroek et al. 1992). Gastric emptying of nutrients containing liquids and liquefied solids approximates an overall linear fashion (Horowitz and Dent 1991). In contrast, gastric emptying of solids approximates an overall non-linear, monoexponential fashion

(Horowitz and Dent 1991; Collins et al. 1991). The emptying of solids is characterised by an initial lag phase, usually 10–30 min before emptying commences, during which solids move from the proximal into the distal stomach and are ground into small particles followed by an emptying phase that approximates a linear pattern (Collins et al. 1991), at least until the stomach is close to empty.

2.4 Effects of nutrients on gastrointestinal hormones

The presence of nutrients within the gastrointestinal lumen stimulates the release of a number of gastrointestinal hormones. These include cholecystokinin (CCK) (Liddle et al. 1985; Lieverse et al. 1994a) and glucose-dependent insulinotropic polypeptide (GIP), secreted from the proximal small intestine (Rehfeld 1978; Fehmann et al. 1995), glucagon-like peptide-1 (GLP-1) (Flint et al. 1998a; Gutzwiller et al. 1999), and peptide tyrosine tyrosine (PYY) (Pappas et al. 1986), secreted from the distal small intestine (Eissele et al. 1992; Adrian et al. 1985a), while the release of ghrelin, predominantly secreted from the stomach (Kojima et al. 1999), is suppressed (Cummings et al. 2001; Wren et al. 2001a). For the purpose of this chapter, insulin, which is secreted from the pancreas (Anderson and Long 1947), although not a gastrointestinal hormone, is discussed here because of its effects on blood glucose regulation. The following section summarises the current knowledge of the mechanisms underlying the release or suppression of these hormones. Their role in the regulation of gastrointestinal motility, blood glucose and energy intake is discussed later in the chapter.

2.4.1 Cholecystokinin

CCK, one of the first discovered gastrointestinal hormones, was first identified and characterised to regulate the control of gall bladder contractions and pancreatic secretion and then also gastrointestinal motility. Over the last 25–30 years, numerous and extensive studies demonstrated the biologically active CCK hormone to have a major role in the regulation of feeding. CCK is synthesised predominantly in the 'I' cells of the duodenal and jejunal mucosa. CCK peaks after 15–30 min of meal ingestion in response to the presence of nutrients, in particular, fat and protein (Liddle et al. 1985; Lieverse et al. 1994a; Larsson and Rehfeld 1978) and to a lesser extent, carbohydrate (Parker et al. 2005), in the small intestinal lumen. CCK-8, CCK-58 and CCK-33/39 are the main biologically active forms of CCK found in the human brain, intestine and circulation, with CCK-58 and CCK-33/39 the most abundant forms (Eysselein et al. 1990). CCK elicits a number of physiological effects within the gastrointestinal system, including the regulation of gastrointestinal motility (Brennan et al. 2005; Brennan et al. 2008), stimulation of gallbladder contraction (Liddle et al. 1985), pancreatic enzyme secretion (Harper and Raper 1943), slowing of gastric emptying (Liddle et al. 1986), and suppression of energy intake (Kissileff et al. 1981). Two receptors have been described for CCK, CCK_1 and CCK_2 (Moran and Kinzig 2004). The CCK_1 receptor is found in peripheral tissues, including pancreatic acini, gallbladder, pyloric smooth muscle and enteric vagal afferent nerves (Smith et al. 1984) and also in the central nervous system, particularly in regions involved in the regulation of food intake, including the nucleus tractus solitarius, the area postrema and the dorsal medial hypothalamus (Moran et al. 1986; Hill et al. 1987). The CCK_2 receptor is also present in the central nervous system, including the cerebral cortex, the hypothalamus,

and on vagal afferents (Gaudreau et al. 1983) and in the gastric mucosa (Wank et al. 1994). The physiological actions of CCK have been established by studies using the specific CCK_1 receptor antagonist, dexloxiglumide (Beglinger et al. 2001; Fried et al. 1991a; Lal et al. 2004; Meyer et al. 1989).

2.4.2 Peptide tyrosine tyrosine

PYY is a 36 amino acid peptide synthesised by endocrine 'L' cells, located predominantly in the ileum and the large intestine (Adrian et al. 1985a). It is secreted as $PYY_{(1-36)}$, and is rapidly degraded to $PYY_{(3-36)}$ by the enzyme dipeptitidyl peptidase IV (DPP-IV) (Grandt et al. 1994). The secretion of PYY is stimulated within 30 min of meal ingestion, plateaus within approximately 90 min, and can remain elevated for up to six hours (Ueno et al. 2008). Its release is proportional to the caloric load of the ingested nutrients (Ekblad and Sundler 2002). Despite being secreted predominantly in the distal small intestine, the release of PYY does not depend solely on direct nutrient exposure of the distal small intestine; studies in dogs have demonstrated that PYY may also be released indirectly by fat present in the proximal small intestine (Lin et al. 2000), secondary to the stimulation of CCK secretion (Lin et al. 2000; Kuvshinoff et al. 1990; McFadden et al. 1992). PYY is one of the mediators of the 'ileal brake'. It slows gastric emptying and intestinal transit of a meal, in order to increase efficacy of digestion and nutrient absorption by the small intestine (Grudell and Camilleri 2007). While some studies have reported that administration of exogenous $PYY_{(3-36)}$ decreases energy intake in humans (Batterham et al. 2002; Neary et al. 2005), observations are inconsistent. PYY also acts to inhibit pancreatic and gastric secretions and gall bladder contraction (Adrian et al. 1985b).

2.4.3 Ghrelin

Ghrelin is a 28-amino acid peptide (Kojima et al. 1999) secreted predominantly from the fundic region of the stomach and from pancreatic islet cells and the distal small intestine (Tritos and Kokkotou 2006) by oxyntic cells. It has been identified as the endogenous ligand for the growth hormone secretagogue receptor (Kojima et al. 1999; Date et al. 2000). In contrast to most other gastrointestinal hormones, ghrelin concentrations are increased, rather than suppressed, in the fasting state (Cummings et al. 2001) and concentrations are reduced, following nutrient ingestion, with the magnitude of suppression depending on the meal composition (Erdmann et al. 2003). Studies in both animals and humans have demonstrated that ghrelin suppression is dependent upon exposure of nutrients to the small intestine, not the stomach (Parker et al. 2005; Williams et al. 2003a; Overduin et al. 2005). It is thought that ghrelin may be involved in meal initiation (Cummings et al. 2001; Tschop et al. 2001a).

2.4.4 Glucagon-like peptide-1 (GLP-1)

GLP-1 is a 33-amino acid peptide hormone, which is a product of the glucagon gene. It is released from 'L' cells, located predominantly in the distal small intestinal mucosa (Eissele et al. 1992), peaking after 15–30 min of meal ingestion. GLP-1 is released in response to the presence of nutrients in the small intestine, predominantly by carbohydrate and fat (Näslund et al. 1998a; Feinle et al. 2003; Feinle et al. 2002), but also protein (Bowen et al. 2006) and is rapidly biodegraded into a biological inactive form in human serum by the enzyme DPP-IV (Mentlein et al. 1993). GLP-1 is the major incretin hormone enhancing insulin secretion and suppressing glucagon release to regulate blood glucose control. GLP-1 also slows gastric emptying (Nauck et al. 1997)

thereby reducing nutrient delivery to the small intestine, contributing to blood glucose control, and has also been shown to reduce energy intake (Turton et al. 1996); however, this effect is controversial.

2.4.5 Glucose-dependent insulinotropic polypeptide

GIP is a 42-amino acid peptide, synthesised and released from intestinal 'K' cells. It is located predominantly in the duodenum and proximal jejunum (Fehmann et al. 1995) and, in humans, is secreted in response to carbohydrate (Kreymann et al. 1987) and fat (Falko et al. 1975). GIP was originally isolated from porcine intestine on the basis of its ability to inhibit gastric acid secretion and was termed 'gastric inhibitory peptide' (Brown et al. 1970). Later, it was found that intravenous administration of GIP simulates insulin secretion during hyperglycaemia (Dupre et al. 1973; Morgan 1996). The primary physiological role of GIP is that of an incretin hormone and, in addition, GIP also inhibits gluconeogenesis in the liver, enhances glucose uptake in muscles and promotes proliferation, survival and differentiation of pancreatic beta-cells (Meier and Nauck 2004).

2.4.6 Insulin

Insulin is released by β-cells of the islet of Langerhans in the pancreas. Insulin is transported through the blood–brain barrier and accesses neurons in the hypothalamus to maintain energy homeostasis. The magnitude of insulin secretion in response to carbohydrates is dependent on the release of the incretin hormones, GLP-1 and GIP (Lavin et al. 1998; Pilichiewicz et al. 2007a). The primary role of insulin relates to

glucose homeostasis and it is released in response to an increase in blood glucose concentration.

2.5 Effect of gastrointestinal hormones on gastrointestinal motility and blood glucose control

Gastrointestinal hormones released in response to nutrient ingestion have a variety of functions in relation to gastrointestinal motility and blood glucose regulation. In the following section, we discuss the role of CCK, GLP-1, PYY and ghrelin in gastrointestinal motility and the role of GLP-1 together with GIP in blood glucose homeostasis. While many of the studies described have employed exogenous administration of hormones to demonstrate effects on gastrointestinal motor function, in order to establish a physiological role of these hormones, specific receptors antagonists need to be employed.

2.5.1 CCK

In both animals (Moran and McHugh 1988; McHugh and Moran 1986) and humans (Liddle et al. 1986), exogenous administration of CCK slows gastric emptying (Liddle et al. 1986; McHugh and Moran 1986). The slowing of gastric emptying by CCK may be due to a reduction in antral and duodenal contractions and stimulation of tonic and phasic pressure waves (Brennan et al. 2005; Liddle et al. 1986; Rayner et al. 2000). Using specific antagonists to the CCK_1 receptor (e.g. loxiglumide), research has established that in both animals and humans the effects of CCK on gastrointestinal function are mediated by CCK_1 receptors (Feinle et al. 1996; Fried et al. 1991b; Katschinski et al. 1996). For example, in rats, dexloxiglumide (the active enantiomer of

loxiglumide) blocks CCK-induced delays in gastric emptying (Scarpignato et al. 1996). In humans, the inhibitory effects of fat on gastric emptying and gastroduodenal motility are attenuated by administration of loxiglumide (Feinle et al. 1996; Katschinski et al. 1996). For example, in one study, gastric emptying of a liquid fat meal in humans reported that an intravenous infusion of loxiglumide stimulated antral contractions and decreased gastric half emptying time (Schwizer et al. 1997). In another study, during duodenal perfusion of a mixed liquid meal for 150 min, loxiglumide administration decreased antral, pyloric and duodenal contractions (Katschinski et al. 1996), indicating that the inhibitory effects of fat on gastric emptying and gastrointestinal motility are mediated by CCK, at least in part.

2.5.2 PYY

Administration of $PYY_{(3-36)}$ modulates gastrointestinal motor function in both animals and humans. Intramuscular infusion of $PYY_{(3-36)}$ in rhesus monkeys slows gastric emptying of saline in a dose-dependent fashion (Moran et al. 2005) and in humans, intravenous administration of $PYY_{(3-36)}$ slows gastric emptying and mouth-to-caecum transit (Savage et al. 1987). Further, as PYY secreting cells are located predominantly in the distal small intestine and the secretion of PYY is related to the fat-induced inhibition of distal gastrointestinal motility, PYY acts as the primary mediator of the fat-induced 'ileal brake', that is, the slowing of gastric emptying and intestinal transit of the meal, in order to increase nutrient absorption, induced by distal small intestinal feedback (Read et al. 1984; Spiller et al. 1984).

2.5.3 Ghrelin

In the fasted state, in both animals and humans, exogenous administration of ghrelin, induces phase III of the MMC in the antrum and duodenum (Fujino et al. 2003; Tack et al. 2006). Following feeding, ghrelin stimulates antral contractions in animals (Fujino et al. 2003; Tanaka et al. 2009), and in humans, increases proximal gastric tone, promotes stomach emptying, and dose-dependently stimulates gastric motility (Tack et al. 2006; Masuda et al. 2000; Levin et al. 2006). However, as previously discussed, ghrelin release is suppressed following meal ingestion (Monteleone et al. 2003; Greenman et al. 2004); hence, the relevance of the effect of ghrelin on gastric emptying is unclear. In rats, intracerebroventricular or intravenous administration of a ghrelin agonist, Tranzyme Pharma (TZP-101), accelerates the rate of gastric emptying of a liquid meal (Dornonville de la Cour et al. 2004). However, until the effects of a ghrelin receptor antagonist have been evaluated in humans, the role of endogenous ghrelin in the regulation of gastrointestinal motility remain uncertain.

2.5.4 GLP-1

Exogenous administration of GLP-1, administered at doses between 0.3 and 1.2 pmol/kg/min, mimics the effects of nutrients and is consistent with the gastrointestinal motor effects underlying the slowing of gastric emptying in humans (Nauck et al. 1997; Meier et al. 2003; Schirra and Goke 2005; Delgado-Aros et al. 2002; Little et al. 2006a). This is associated with the relaxation of the proximal stomach (Delgado-Aros et al. 2002; Schirra et al. 2002), suppression of antral and duodenal motility (Brennan et al. 2005; Schirra et al. 2000), and stimulation of pyloric pressures (Schirra et al. 2000). The effect of endogenous GLP-1 on gastrointestinal motor function has been evaluated

using its specific receptor antagonist, exendin$_{(9\text{-}39)}$. Exendin$_{(9\text{-}39)}$ has been reported to attenuate the effects of intraduodenal glucose on antropyloroduodenal (APD) motility in humans (Schirra et al. 2006), and block the effects of GLP-1 on gastric emptying in rats (Tolessa et al. 1998), suggesting that endogenous GLP-1 plays a physiological role in mediating the effects of nutrients on gastrointestinal motility.

GLP-1 also plays an important role as an incretin hormone involved in the regulation of postprandial blood glucose concentrations (Kreymann et al. 1987). GLP-1 enhances insulin secretion, suppresses glucagon release and stimulates insulin-dependent glucose disposal in peripheral tissues (Gutniak et al. 1992; D'Alessio et al. 1994), thereby decreasing blood glucose (Nauck et al. 1998). These actions of GLP-1 have stimulated substantial interest for its potential use (Schirra et al. 1998), and that of specific GLP-1 analogues and agonists (Nauck 1998), to improve blood glucose control in type 2 diabetes (Meier et al. 2003; Nauck 1998). While there is increasing evidence to suggest this is effective, the precise mechanisms by which GLP-1 improves blood glucose regulation remains poorly defined. For example, while exogenous GLP-1 stimulates insulin secretion in the fasted state (Kreymann et al. 1987), when administered with a meal, there is an apparently paradoxical reduction in postprandial insulin concentrations (Nauck et al. 1997a). Observations that postprandial insulin secretion is reduced, rather than increased, by exogenous GLP-1 in healthy subjects (Nauck et al. 1997) and type 2 diabetes (Meier et al. 2003) suggest that the dominant mechanism by which exogenous GLP-1 improves postprandial blood glucose relates to the slowing of gastric emptying, and that GLP-1 may not be a physiological incretin hormone (Nauck et al. 1997). This concept is supported by a recent study in which the 'reversal' of the inhibitory effect of

exogenous GLP-1 on gastric emptying by the gastrokinetic drug erythromycin was associated with a substantial attenuation of its glucose-lowering effect, despite augmentation of the insulin and GIP responses (Meier et al. 2005).

2.5.5 GIP

There is limited information available on the effects of GIP on gastrointestinal motility. The available literature indicates that GIP has no effect on gastric emptying or motility (Meier et al. 2004; Miki et al. 2005). For example, in healthy humans, there was no difference in gastric emptying rates or emptying half time of a solid meal after treatment with intravenous GIP at 2 pmol/kg/min or placebo (Meier et al. 2004). In mice, subcutaneous injection of 100 μg human GIP did not affect gastrointestinal transit of an orally ingested barium sulphate meal (Miki et al. 2005). The primary physiological role for GIP is that of an incretin hormone. GIP acts directly on pancreatic islets to stimulate insulin secretion (Adrian et al. 1978; Taminato et al. 1977), thereby regulating blood glucose concentrations.

2.6 Effect of nutrients on appetite and energy intake, and the interrelation with gastrointestinal function

The upper gastrointestinal tract is an important source of satiety signals, and it has been well established that the presence of nutrients in the small intestine suppress appetite and energy intake. This section will discuss the gastrointestinal motor and hormonal functions thought to be involved in the regulation of appetite and energy intake.

2.6.1 Effects of nutrients on appetite and energy intake

Powerful satiety signals arise from the gastrointestinal tract in response to food ingestion, through the interaction of nutrients with receptors in the small intestinal lumen. Direct nutrient infusion into the small intestine allows the role of specific areas of the gastrointestinal tract in the control of appetite and energy intake to be investigated. Subjects receive no cues regarding the taste or palatability of the nutrients infused and variations in gastric emptying are not a potentially confounding factor. In humans, studies investigating the effects of intraduodenal nutrient administration have demonstrated that the presence of nutrients within the small intestinal lumen is associated with a decrease in perceptions of hunger, increase in fullness and subsequently a decrease in energy intake (Chapman et al. 1999; Cook et al. 1997; Lavin et al. 1996; MacIntosh et al. 2001a). For example, intraduodenal infusion of lipid at 0.25, 1.5 or 4 kcal/min suppressed hunger and subsequent energy intake in a dose-dependent manner (Pilichiewicz et al. 2007b). When infused directly into the small intestine, lipid increases fullness and decreases hunger in humans to a greater extent than isocaloric carbohydrate (Chapman et al. 1999; Cook et al. 1997; Andrews et al. 1998; Seimon et al. 2009a). In addition, in humans, infusion of nutrients (lipids, carbohydrates) into the small intestine is associated with suppression of food intake to a much greater extent than when the same nutrients are given intravenously (Lavin et al. 1996; Welch et al. 1985). For example, in a study in healthy subjects, where the effect of 20% glucose at 4 mL/min for 90 min administered intraduodenal and intravenous were compared, glucose suppressed hunger, increased fullness and reduced subsequent energy intake while the intravenous administration of glucose had little, if any, effect on perceptions of fullness or hunger or energy intake (Lavin et al. 1996; Welch et al.

1985), indicating that the effects of nutrients on appetite and energy intake are mediated primarily by the stimulation of small intestinal receptors. The interaction of nutrients with specific receptors in the small intestine stimulates the release of gastrointestinal satiety hormones, some of which slow down gastric emptying, thereby prolonging postprandial gastric distension and in this way also contributing to satiety, and it is now increasingly recognised that the modulation of gastrointestinal motor and hormonal function by nutrients contributes to their suppression of appetite and acute energy intake.

2.6.2 Role of gastrointestinal hormones in the regulation of appetite and energy intake

It is estimated that more than 50 hormones and regulatory peptides are synthesised in the gastrointestinal tract, primarily in response to food ingestion. Among the most studied gastrointestinal hormones are CCK, GLP-1, PYY and ghrelin. The following sections focus on the roles of these hormones in the regulation of appetite and energy intake.

2.6.2.1 CCK

It has been well established that acute administration of exogenous CCK suppresses appetite and energy intake. In rats, intraperitoneal administration of the biologically active, sulphated octapeptide of CCK, CCK-8, suppressed energy intake in a dose-dependent fashion and also sham feeding (Gibbs et al. 1973). In healthy young (Brennan et al. 2005; Kissileff et al. 1981) and older (MacIntosh et al. 2001b) humans, intravenous administration of CCK-8 and CCK-33 increased the perceptions of fullness,

decreased hunger and reduced subsequent energy intake although for definitive evidence of an involvement of endogenous CCK, receptor antagonist studies are required. Only a limited number of studies have evaluated the role of endogenous CCK in the regulation of appetite and energy intake using loxiglumide, a CCK_1 receptor antagonist (Beglinger et al. 2001; Lieverse et al. 1994b; Matzinger et al. 1999; Matzinger et al. 2000). In healthy humans, loxiglumide completely abolished the inhibitory effect of the concurrent administration of intraduodenal fat on appetite and energy intake (Matzinger et al. 1999; Feinle et al. 2001). Further, intravenous administration of dexloxiglumide for one hour prior to and during ingestion of a meal, increased energy intake and perceptions of hunger when compared with a saline infusion (Beglinger et al. 2001), providing evidence that CCK is an endogenous physiological satiety signal acting through CCK_1 receptor-mediated mechanisms.

2.6.2.2 PYY

A number of studies have evaluated the effects of PYY on appetite and energy intake, with conflicting results. It was originally reported that peripheral injection of PYY inhibits food intake in rats (Batterham et al. 2002); however, attempts to replicate these results have been unsuccessful, reporting no effect on energy intake (Tschop et al. 2004). In humans, intravenous infusion of 'physiological' concentration of PYY (2 $nmol/m^2$ of body-surface area) has been reported to inhibit energy intake for up to 12 hours (Batterham et al. 2003). However, a later study reported that only large doses of $PYY_{(3-36)}$, that is, 0.4–0.8 pmol/kg/min, suppressed energy intake in humans (Degen et al. 2005), which resulted in nausea, vomiting and abdominal pain in some subjects (Degen et al. 2005). Accordingly, it remains uncertain whether the reduction in energy

intake was a specific or adverse effect of PYY. It has been reported that pre-treatment of the arcuate nucleus with an antagonist specific for the Y2 receptor, BIIE0246, attenuated the inhibitory effect of an intraperitoneal dose of $PYY_{(3-36)}$ on food intake in rats (Abbott et al. 2005). Further, when BIIE0246 was administered into the arcuate nucleus alone, food intake was increased (Abbott et al. 2005), providing a role of endogenous PYY in the regulation of energy intake. However, this has not been evaluated in humans.

2.6.2.3 GLP-1

While GLP-1 is generally portrayed to have suppressant effects on energy intake, its effects in published studies are inconsistent. While a number of studies have demonstrated that exogenous administration of GLP-1 increases the perception of fullness and decreases hunger in animals (Turton et al. 1996) and inhibits energy intake in lean individuals (Neary et al. 2005; Flint et al. 1998b), a few studies demonstrated no effect (Brennan et al. 2005; Long et al. 1999). $Exendin_{(9-39)}$, a specific GLP-1 receptor antagonist, has been reported to attenuate the inhibitory effects of GLP-1 on energy intake in rats (Turton et al. 1996), suggesting that GLP-1 plays a physiological role in the regulation of appetite and energy intake at least in animals,. No studies to date have evaluated the effect of $exendin_{(9-39)}$ on energy intake in humans.

2.6.2.4 Ghrelin

During fasting, plasma ghrelin concentrations are high and suppressed following food ingestion (Cummings et al. 2001), supporting the concept that ghrelin has a role in meal initiation. Studies in both humans and rats have demonstrated that intravenous

administration of ghrelin stimulates food intake (Wren et al. 2001a; Wren et al. 2001b). In healthy subjects, intravenous administration of ghrelin (5 pmol/kg/min) increased energy intake by 28% from a buffet meal and increased hunger, when compared with saline (Wren et al. 2001b). No studies have investigated the role of endogenous ghrelin on energy intake using a specific ghrelin receptor antagonist; hence, the physiological role of ghrelin in feeding behaviour has not been determined.

2.6.3 Role of gastrointestinal motility on appetite and energy intake

The modulation of gastric motor function by nutrients, specifically changes in pyloric motility, may contribute to the effects on appetite and energy intake. For example, in dogs, electrical stimulation of the pylorus was associated with a suppression of energy intake (Xu et al. 2005). A study in our laboratory found an inverse relationship between the stimulation of pyloric pressures and subsequent energy intake (Brennan et al. 2007), providing the first indication for a link between specific changes in gastrointestinal motor function and energy intake suppression in humans. This suggests that an individual in whom there is greater stimulation of pyloric pressures may eat less, potentially because small intestinal feedback is greater.

It appears that modulation of these gastrointestinal functions, that is, gastrointestinal motility and hormone release/suppression, mediate the regulation of appetite and acute energy intake in humans, at least in part, although it is important to recognise that the discussed relationships do not provide evidence for a causal association between gastrointestinal function and energy intake. Changes in motility and hormone secretion occur concurrently with changes in appetite, and, therefore, it is not surprising that there

is little information regarding which, if any, of these factors are independent determinants of energy intake. For example, although CCK does play a role in the process, this may potentially be mediated indirectly by its effect on motility.

2.7 Nutrient tasting in the oral cavity

Taste is an important determinant of the amount of food consumed during a meal. Fat, in particular, increases the palatability of foods and can, therefore, lead to overconsumption. It is well established that the human gustatory system can detect the taste qualities of sweet, sour, bitter, salty and umami or 'glutamate taste', which is the sensation elicited by MSG. More recent evidence supports the existence of a sixth taste modality responsive to fatty acids (Chale-Rush et al. 2007a; Abumrad 2005; Chale-Rush et al. 2007b; Gaillard et al. 2008; Mattes 2009a). Dietary fats, which are predominantly in the form of triacylglycerols, are not an effective taste stimulus (Mattes 2009a), although they contribute to the sensory properties of foods. However, evidence suggests that the detection of fat in the oral cavity appears to be dependent on the presence of free fatty acids, the digestive products of fats. Humans are able to detect a range of fatty acids, including polyunsaturated (linoleic acid [C18:2]), monounsaturated (oleic acid [C18:1]), and saturated (stearic [C18:0], lauric [C12:0], and caproic [C6:0]), even when olfaction is blocked using nose clips, and texture is masked using gum acacia and mineral oil (Chale-Rush et al. 2007a; b; Mattes 2009b), suggesting a true 'taste' component to free fatty acid detection. In humans, sensory detection of free fatty acids occurs within the millimolar range (0.02–0.64 mM) (Stewart et al. 2010). This range of detection is consistent with the concentrations of free fatty acids that are naturally present in food (0.5% free fatty acid) (Mattes 2005), and it has recently been

reported that lipolytic activity in saliva is sufficient to produce micromolar amounts of fatty acids within the detectable range (Stewart et al. 2010).

The following section will discuss our current understanding of the mechanism underlying the detection of oral fatty acids and the physiological responses induced by the detection of fatty acids in the oral cavity.

2.7.1 Oral free fatty acid detection

It is well established that oral detection of sweet, bitter, umami tastants occurs as a result of the interaction of nutrients with specific receptors on the apical surface of taste receptor cells. The understanding of the mechanisms underlying oral fatty acid detection is more limited. However, recently, a number of receptors that interact with fatty acids, including CD36, delayed rectifying potassium channels, and a series of G-protein coupled receptors, including GPR40, GPR41, GPR43 and GPR120, have been reported to be present on taste receptor cells (Mattes 2009a). The detection of free fatty acids by these mechanisms induces a signalling cascade. Fatty acids activate the gustatory nerves that transmit sensory information to the nucleus tractus solitarius in the brainstem (Gaillard et al. 2008) to higher brain centres including the lateral hypothalamus and the nucleus accumbens, both of which play an important role in the regulation of food intake.

CD36, a fatty acid transporter, appears to play a major role in fat detection within the oral cavity, binding long-chain fatty acids with an affinity in the nanomolar range (Baillie et al. 1996; Ibrahimi et al. 1996). Studies have demonstrated that CD36

knockout mice are insensitive to free fatty acids compared with wild type mice, with no difference in their sensitivity to sweet and bitter stimuli (Gaillard et al. 2008; Laugerette et al. 2005). It is still not known whether CD36 serves as a receptor or docking protein. Oral exposure to long-chain fatty acids increases calcium ions in taste receptor cells, and c-fos expression in the solitary tract nucleus, which is abolished in CD36 knockout animals (Gaillard et al. 2008). GPR120 has been reported to be co-localised with phospholipase Cβ2 and α-gustducin in taste receptor cells (Matsumura et al. 2009), both of which are involved in the transduction of other tastes, such as sweetness and bitterness. Compared to wild type controls, GPR40 and GPR120 knockout mice are less sensitive to an array of free fatty acids (Cartoni et al.), but respond equally to sweet, sour, bitter, salty and umami stimuli, demonstrating that GPR40 and GPR120 play an important role in mediating the gustatory responses to fatty acids.

2.7.2 Physiological responses induced by oral fat exposure

In humans, oral stimulation with meals containing fats, using modified sham-feeding techniques, have been reported to induce a number of physiological cephalic-phase responses where the sight, smell and taste of foods stimulate the secretion of digestive juices into the mouth, stomach and intestine, essentially preparing the gastrointestinal tract for nutrient exposure, optimising nutrient digestion and absorption. For example, there is a stimulation of gastric lipase (Wojdemann et al. 1997) and insulin (Smeets et al. 2009) secretion, elevation of serum triglycerides (Mattes 2009c; Chavez-Jauregui et al.), stimulation of pancreatic polypeptide (Crystal and Teff 2006), suppression of ghrelin (Heath et al. 2004) and slowing of gastric emptying (Cecil et al. 1999). For example, gastric emptying of a high fat meal was demonstrated to be much slower when

a meal was ingested orally, when compared with direct intragastric infusion (Cecil et al. 1999), suggesting that orosensory stimulation by fat plays an important role in the regulation of gastrointestinal motor function. In addition, modified sham feeding with high fat meals appears to reduce appetite (Smeets et al. 2009; Heath et al. 2004; Smeets and Westerterp-Plantenga 2006) and energy intake (Crystal and Teff 2006). The studies described above evaluated the effects of meals containing triacylglycerides, rather than free fatty acids; hence, from these data it is not possible to discriminate between the effects of free fatty acids from other sensory factors, such as texture or viscosity. Therefore, it will be important to determine the effects of oral fatty acid exposure on gastrointestinal function, appetite and energy intake.

2.8 Conclusion

It is well established that modulation of gastrointestinal functions, that is, gastrointestinal motility and hormone release, regulate appetite and acute energy intake in humans. However, these relationships do not provide evidence for a causal association between gastrointestinal function and energy intake, and this will be important to clarify.

Chapter 3: Effects of Dietary Excess and Restriction on Gastrointestinal Function and Energy Intake in Obesity

Overweight and obesity occurs, in the broadest sense, as a result of energy intake exceeding energy expenditure. There is evidence that the signals arising from the gastrointestinal tract, which are known to play a fundamental role in the regulation of appetite and energy intake, may be modified by both dietary excess and restriction. The development of obesity may, at least in part, reflect a decreased sensitivity to the gastrointestinal effects of nutrients favouring an increase in appetite and energy intake. Currently, dietary restriction is the most common non-pharmacological approach to weight loss in obesity, and weight loss is usually not sustained in the long term.

This chapter discusses data from experimental induced dietary excess and restriction on gastrointestinal function and energy intake, and gastrointestinal function in obesity.

3.1 Definition of obesity

Obesity is a condition defined as abnormal or excessive fat accumulation that may impair health. Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as an individual's weight in kilograms divided by the square of their height in metres (kg/m^2). A BMI of 19–25 kg/m^2 is considered healthy/normal weight, 25.1–30 kg/m^2 is considered overweight and a BMI of greater than 30.1 kg/m^2 is considered clinically

obese. BMI provides the most useful population-level measure of overweight and obesity, as it is the same for both sexes and for all ages of adults. However, it is considered a rough guide because it is a measurement of body size and does not distinguish between an individual's body fat or lean/muscle mass directly and provides no information relating to the distribution of fat throughout the body. Waist circumference and the waist-to-hip ratio are two other proxy indicators of obesity, and the World Health Organisation (WHO) suggests waist circumference or waist-to-hip ratio to be used in addition to BMI, to provide information about the distribution of excess fat.

3.2 Prevalence of obesity

The worldwide prevalence of obesity continues to increase and has more than doubled since 1980. Current projections from the WHO indicate that in 2008, 1.5 billion adults, 20 years and older, were overweight. Of these, over 200 million males and nearly 300 million females were obese worldwide (WHO 2011). In 2007–2008, results from the Australian Bureau of Statistics' National Health Survey revealed that 61.4% of the Australian population were either overweight or obese. Of these, 42.1% of adult males and 30.9% of adult females were classified as being overweight and 25.6% of males and 24% of females were classified as being obese. Based on current trends, the most recent projections reveal that, in Australia, of adults aged 20 years and older, 83% of males and 67% of females are expected to be overweight and/or obese by 2025 (Victorian Government Department of Human Services 2008).

3.3 Significance of obesity

Obesity has been identified as a risk factor for a number of disorders, including type 2 diabetes (Colditz et al. 1995), cardiovascular diseases (Manson et al. 1990), gallbladder disease (Stampfer et al. 1992), lipid disorders (Despres 1994), hypertension and musculoskeletal diseases (Must et al. 1999). There is also evidence that obesity may be a risk factor for certain types of cancer, including hormone-dependent cancers such as prostrate, breast and uterine cancer, as well as colorectal and kidney cancers (Guh et al. 2009; Pan and DesMeules 2009). Obesity is the fifth-leading risk for global deaths, and at least 2.8 million adults die each year as a result of being overweight or obese. The health, economic and psychosocial consequences of obesity are substantial and associated with considerable, albeit imprecisely defined, costs to the health care system, particularly in Western countries, with the financial cost of obesity in Australia estimated to be $8.28 billion in 2008 (Access Economics 2008).

3.4 Current therapies for obesity

Management strategies for weight reduction in obese individuals include physical interventions, such as exercise and diet, pharmacological treatments and surgery. A number of dietary approaches have been advocated for the treatment of obesity. For example, very low calorie diets (VLCDs) produce greater short-term weight loss, whereas low calorie diets and diets that are low in fat (Avenell et al. 2004; Foster et al. 2003) or high in protein, low in carbohydrate (Johnston et al. 2004; Noakes et al. 2005) only produce modest weight loss. However, weight loss in response to these diets is not sustained in the long term.

Numerous pharmacological treatments for obesity have been developed; however, most have limited efficacy and, often, adverse effects. Pharmacotherapies, taken over a period of 1–2 years, have demonstrated a significant, though modest, decrease in weight, when compared with placebo. However, in the majority of cases, weight loss following pharmacological intervention is not sustained in the longer term once therapy is discontinued, with individuals regaining some or all of the weight that was lost. Over the years, many drugs that have been effective weight loss medications have had to be withdrawn from the market due to serious adverse effects. For example, Fen-Phen, which is a combination of two compounds, fenfluramine and phentermine, was removed from the market in 1997, after it was reported to cause valvular heart disease and pulmonary hypertension (Connolly et al. 1997) and in 2000, phenylpropanolamine, an over-the-counter weight loss drug, was found to be an independent risk factor for hemorrhagic stroke in women (Kernan et al. 2000). The first selective cannabinoid receptor blocker, Rimonabant, was withdrawn from the market because it caused depression and suicidal ideation (Lee et al. 2009). In 2010, Sibutramine, a centrally acting serotonin-norepinephrine reuptake inhibitor structurally related to amphetamines, was withdrawn from the market because of its association with increased cardiovascular events and strokes (James et al. 2010). In Australia, the only drugs approved for the treatment of obesity are phentermine and orlistat. While phentermine is limited to short-term use (up to three months), orlistat can be used for longer-term treatment of obesity, but has a number of gastrointestinal adverse effects, such as diarrhoea, flatulence, bloating, abdominal pain, and dyspepsia, which may not be acceptable to some patients on long-term treatment. Despite these limitations of weight loss drugs, in 2000, anti-obesity drugs still accounted for sales of nearly $0.5 billion in the seven

largest global markets (Hallschmid et al. 2006), with the overall sales of anti-obesity drugs projected to at least triple by 2010 (Chin 2008).

Bariatric surgery is arguably the most successful treatment of obesity for people with severe and complex obesity (BMI $\geq$ 35 kg/m^2). Established procedures are Roux-en-Y gastric bypass, gastric banding and sleeve gastrectomy. Roux-en-Y gastric bypass has been known to be the most effective procedure since the 1980s. The procedure involves creating a small-volume gastric pouch and producing a diversion for food to bypass the duodenum and upper jejunum so that nutrients are diverted directly to the more distal parts of the small intestine. Thus, the increased exposure of the distal small intestine to nutrients is likely to modulate the release of distal gastrointestinal hormones associated with the reduction of energy intake, hence achieving significant long-term weight loss (60–80% of excess body weight loss) (DeMaria et al. 2002). Further, Roux-en-Y gastric bypass leads to almost instant resolution of type 2 diabetes in 85% of patients in the absence of any significant weight loss (Pories et al. 1995).

The available therapies have largely ignored the role of the gastrointestinal tract in the regulation of appetite, as well as the important relationships between gastrointestinal function and energy intake, and that these mechanisms may be compromised in obesity. A greater understanding of the mechanisms that contribute to the pathophysiology of obesity is required, which may result in the identification of novel targets for the treatment of obesity.

3.5 Gastrointestinal function in obesity

As discussed previously, the gastrointestinal tract plays a pivotal role in the regulation of appetite and energy intake in healthy individuals and, therefore, it is important to characterise any disturbances in gastrointestinal function in obesity that may be contributing to the development and maintenance of obesity. However, current studies on gastrointestinal motor and hormonal function in the obese are limited and controversial.

3.5.1 Gastrointestinal motility in obesity

Studies that have evaluated the effects of nutrients on gastric emptying in obese humans have found disturbances; however, data are inconclusive and controversial. For example, gastric emptying has been reported to be similar (French et al. 1993; Zahorska-Markiewicz et al. 1986; Glasbrenner et al. 1993; Hutson and Wald 1993; Verdich et al. 2000), faster (Tosetti et al. 1996; Gryback et al. 1996; Wright et al. 1983; Näslund et al. 1998b) or slower (Maddox et al. 1989; Horowitz et al. 1983) in obese, compared with lean individuals. These inconsistent results may be attributed, at least in part, to differences in methodologies between studies, including meal composition (e.g. solid, semi-solid or liquid meals, with radionuclide labels added to liver, eggs, cereal, porridge, potatoes and pancakes), time of day (after an overnight fast or in the afternoon), methodologies used to assess gastric emptying (e.g. scintigraphy or ultrasonography), differences in selection criteria for obese individuals (e.g. moderately compared with morbidly obese) or other factors that are known to influence gastric emptying, such as habitual diet of individuals. The motor mechanisms underlying changes in gastric emptying have not been studied in the obese. Currently, only one

study has evaluated whether changes in interdigestive motility occur in the obese, when compared with lean individuals (Pieramico et al. 1992). The study reported disturbances in motility patterns in the fasting state, including a diminished phase I, increased phase II, and a less frequent occurrence of a phase III of the MMC in the obese, when compared with lean individuals. However, the clinical significance of these changes is unclear, and antral or duodenal motility were not assessed. No studies have evaluated the potential disturbances in postprandial gastrointestinal motor function in the obese. It is likely that gastrointestinal motility is disturbed in obesity and given that obese individuals are less sensitive to nutrients, the feedback mechanisms regulating gastrointestinal motility may be altered. In addition, in the obese, there is an increased capacity to absorb nutrients from the proximal small intestine (Wisen and Johansson 1992), resulting in reduced exposure of nutrients in the distal small intestinal lumen, thereby reducing feedback signals since effects of nutrients are dependent on the length of small intestinal exposure (Lin et al. 1990; Little et al. 2006b).

3.5.2 Gastrointestinal hormones in obesity

There is evidence that the secretion of gastrointestinal hormones, including CCK, PYY, ghrelin, GLP-1 and GIP, and the secretion of insulin may be altered in obesity. This evidence comes from a number of studies that have investigated both fasting and postprandial gastrointestinal hormones concentrations in the obese. For example, when compared with lean individuals, both fasting and postprandial plasma CCK concentrations were found to be greater in obese (Baranowska et al. 2000). Since CCK suppresses appetite, an increase in plasma CCK concentrations in the obese may reflect a decreased sensitivity to CCK, which may be partly responsible for reduced satiety. In

contrast, it has been reported that both fasting (le Roux et al. 2006; Batterham and Bloom 2003) and postprandial (le Roux et al. 2006) plasma PYY concentrations are reduced in obese individuals (see **Figure 3.1**), suggesting impaired PYY release in the obese, when compared with lean individuals.

Figure 3.1: Plasma PYY concentrations in obese (n = 12) and lean (n = 12) healthy subjects during (t = 0–90 min) and following infusion of saline. * vs lean, $P < 0.001$ (Batterham et al. 2003).

Ghrelin concentrations also appear to be modified in obesity. Plasma ghrelin concentrations have been shown to be inversely related to BMI (Shiiya et al. 2002; Tschop et al. 2001b), with both fasting ghrelin concentrations (Tschop et al. 2001b; Druce et al. 2005; English et al. 2002), and meal-induced suppression of ghrelin (English et al. 2002), lower in obesity.

Following oral carbohydrate, but not fat, studies have reported reduced plasma GLP-1 concentrations in obese, when compared with lean subjects (Ranganath et al. 1996; Verdich et al. 2001a). In response to intraduodenal administration of fat and carbohydrate, fasting GLP-1 concentrations have been reported to be similar, with no differences in plasma GLP-1 concentrations between healthy lean and obese subjects (Feinle et al. 2002). It is likely that the difference in GLP-1 release between lean and obese reflect changes in gastric emptying, rather than an impaired stimulation of GLP-1. The release of plasma GIP concentrations in the obese is inconclusive. For example, some studies have shown plasma GIP concentration to be greater during fasting and in response to meal ingestion in the obese compared with the lean (Elahi et al. 1984; Vilsboll et al. 2003), while other studies found GIP concentrations to be reduced (Carr et al. 2010). In addition, following oral administration of a mixed meal, insulin concentrations are greater in insulin resistant obese, when compared with lean, non-diabetic individuals (Carr et al. 2010), suggesting β cell function is impaired in the obese.

In summary, it appears that the release of gastrointestinal hormones is compromised in obese, compared with lean, individuals. The differences in the secretion/suppression or the sensitivity of gastrointestinal hormones following meal ingestion are likely to contribute to an attenuated suppression of appetite and energy intake and blood glucose homeostasis. These changes in gastrointestinal function may be a result of over eating and this hypothesis is supported by studies evaluating the effects of experimental dietary over- and under-exposure.

3.6 Role of high dietary fat intake in the development of obesity

Although the causes of obesity are heterogeneous, it is generally accepted that one of the main environmental factors contributing to the current epidemic is the increased availability and overconsumption of high fat, energy-dense foods. Human studies have demonstrated a direct relationship between the incidence of overweight and obesity and dietary fat intake (Rolls 1995; Golay and Bobbioni 1997). For example, in countries in which the incidence of obesity is rising rapidly, about 45% of the daily energy intake is obtained by fat (Golay and Bobbioni 1997). There is also evidence that obese individuals display an increased preference for the consumption of fatty foods, when compared with lean individuals, suggesting that fat intake is poorly regulated in the obese (Mela and Sacchetti 1991; Miller et al. 1990). Further, the consumption of a diet high in fat has also consistently been shown to promote an increase in energy intake (Lissner et al. 1987; Tremblay et al. 1989). In animal models, oral fatty acid sensitivity, measured by taste cell electrophysiological activity in response to stimulation with fatty acids, may regulate fat consumption and body weight regulation, that is, greater oral sensitivity in the rats is associated with reduced fat intake, reduced preference for fat, and reduced predisposition for obesity (Gilbertson et al. 1998). Further, animal studies have established that *ad libitum* access to a high fat diet promotes an increase in energy intake and obesity and is associated with leptin and insulin resistance (Woods et al. 2003). Thus, there is evidence to implicate the consumption of a high fat diet in the promotion of increased energy intake.

3.7 Previous patterns of dietary intake in the modulation of gastrointestinal function

It is well established that modifications in the diet can have major influences on gastrointestinal function and energy intake, and these effects have been extensively characterised in animals, particularly rodents (le Roux et al. 2006; Savastano and Covasa 2005; Covasa and Ritter 1998). There is increasing evidence that such changes also occur in humans. Thus, studies have been carried out in healthy, young, older and obese humans, demonstrating that dietary modifications, in excess or restriction, have the ability to modify gastrointestinal function, which may be associated with changes in appetite and energy intake. In the following section, we summarise the current knowledge of the effects of dietary excess, that is, isocaloric high fat diets or hyper-caloric high fat diet, and restriction on gastrointestinal function, including gastrointestinal motor and hormone function, appetite and energy intake.

3.7.1 Effect of dietary excess on gastrointestinal motor function

Studies in animals have indicated that the gastrointestinal motor response to fat is attenuated after acute energy excess. For example, in rats, consumption of a high fat diet for two weeks attenuated the suppressive effects of small intestinal fat on gastric emptying compared with an isocaloric low fat diet (Covasa and Ritter 2000). In addition, infusion of palm oil into the ileum at 0.3 mL/hour for three hours per day for three days per week for four weeks attenuated the lipid-induced slowing of stomach-to-caecum transit in rats (Brown et al. 1994).

In healthy human males, consumption of a high fat, hyper-caloric diet (19.3 MJ/day) for 14 days resulted in marked acceleration of gastric emptying and mouth-to-caecum transit of a high fat test meal (1.4 MJ), when compared with a low fat diet (9.1 MJ/day) (see **Figure 3.2**) (Cunningham et al. 1991a).

Figure 3.2: Gastric emptying (A) and mount-to-caecum transit (B) of a high fat test meal (1.4 MJ) following a 14-day consumption of a low fat (9 MJ/day) or high fat (19.3 MJ/day) diet in healthy male subjects (n = 12). * vs low fat, $P < 0.05$ (Cunningham et al. 1991a).

In another study, gastric emptying was accelerated following consumption of a high fat (55% energy from fat), but not high carbohydrate (62% energy from carbohydrate), test meal, after exposure to a hyper-caloric, high fat diet (55% of energy from fat and an energy intake of 133%), for 14 days, indicating that the changes in gastric emptying following a high fat diet may be nutrient specific (Castiglione et al. 2002). In healthy male subjects, exposure to a high fat, high energy diet (40% of energy from fat, 20.1 MJ/day) for 14 days attenuated the effects of an intraduodenal lipid infusion (6.3

kJ/min) on pyloric pressures, when compared with an isocaloric, low fat diet (11% of energy from fat, 11.2 MJ/day) (Boyd et al. 2003), which is likely to reflect changes in gastric emptying. Further, studies using dietary glucose supplementation have demonstrated changes in gastrointestinal function over short periods. For example, in healthy lean males, supplementation of the diet with 400 g glucose/day for three days accelerated gastric emptying of a glucose drink (68.2 g glucose, 1.07 MJ) (Cunningham et al. 1991b). Therefore, experimentally manipulating the energy content of a diet appears to accelerate small intestinal transit and gastric emptying.

3.7.2 Effect of dietary excess on gastrointestinal hormone secretion

Experimentally-induced overconsumption also appears to affect plasma hormone concentrations. In rats, exposure to a high fat diet (20% energy as fat) for 14 days increased the CCK response to an intraduodenal triacylglycerol infusion by about 1.7-fold (Spannagel et al. 1996). The sensitivity to exogenous CCK also appears to be attenuated in response to a high fat diet (Covasa and Ritter 1998). In this study, the inhibitory effects of an intraperitinal injection of CCK-8 on gastric emptying and energy intake were reduced following exposure to a high fat diet (34 or 54% energy as fat) for two weeks, when compared with an isocaloric low fat diet (5% energy as fat) (Covasa and Ritter 1998). This suggests that both the secretion and sensitivity to the actions of CCK are modulated by a high fat diet.

Only a few studies have investigated the effects of dietary excess on gastrointestinal hormones release in humans. One study demonstrated a modest increase in fasting plasma CCK concentrations in healthy humans, following a three-week period on a high

fat diet (3.1 ± 0.3 pmol/L), when compared with an isocaloric low fat diet (4.3 ± 0.4 pmol/L) (Little et al. 2008). Another study reported increased postprandial CCK concentrations in response to a standard breakfast following exposure to the high fat diet (58% energy from fat) for 14 days, when compared with the pre-diet condition (French et al. 1995). This suggests a decrease in sensitivity to fat occurs even following relatively short periods on high fat diet. However, the CCK response to intraduodenal lipid (2.8 kcal/min), which bypasses the influence of gastric emptying, is not apparently affected by exposure to a high fat diet (Boyd et al. 2003). It is therefore likely that, in humans, the increased postprandial plasma CCK response observed following consumption of a high fat diet (French et al. 1995) is primarily reflective of more rapid gastric emptying (Cunningham et al. 1991a).

The secretion of PYY is also modulated by a high fat diet. For example, in mice who had become obese in response to a high fat diet (60% energy as fat) for 16 weeks, plasma PYY concentrations were less, in both the fasting and postprandial states, when compared with rats that were maintained on an isocaloric low fat diet (2.6% energy as fat) (le Roux et al. 2006). However, no studies have investigated the effects of dietary excess on PYY concentrations in humans.

In rats, a high fat diet for 14 days decreased fasting plasma ghrelin concentrations by ~ 30%, when compared with rats that were fed a control diet (Beck et al. 2002). In humans, plasma ghrelin has been reported to be decreased following exposure to a three-week high fat diet (Robertson et al. 2004).

In summary, observations derived from animal and human studies reviewed in this section indicate that, although some inconsistencies remain, nutrient-induced modulation of gastrointestinal hormones occur following dietary excess, which may contribute to increased energy intake, and thereby, facilitate weight gain in the longer term.

3.7.3 Effect of dietary excess on appetite and energy intake

In humans, there is evidence that exposure to a high fat diet also modifies appetite and energy intake. In healthy females, covert manipulation of the dietary fat content for two weeks resulted in a ~ 15% and 23% increase in total daily energy intake when consuming a high fat diet (45–50% fat), when compared with an isocaloric medium-fat diet (30–35% fat) and an isocaloric low fat diet (15–20% fat) respectively (Lissner et al. 1987) (see **Figure 3.3**). Further, French et al. reported that following exposure to a high fat diet (58% energy as fat) for a period of two weeks, hunger was increased, and fullness decreased, in healthy males who had gained ~ 2 kg of weight (French et al. 1995). They also reported a modest increase in food intake from a pre-selected meal, and an increase in average daily energy intake (0.66 MJ/day), as measured by food diaries, during the two-week period (French et al. 1995).

Figure 3.3: Average daily energy intake over two weeks in response to covert manipulations of the fat content of the diet in healthy female subjects (n = 24). * vs 15–20% and the 30–35% fat diet, P < 0.001 (Lissner et al. 1987).

3.7.4 Effect of dietary excess on oral fat sensitivity

Similar to gastrointestinal and appetite responses, oral sensitivity to fat also appears to be attenuated following exposure to a high fat diet, and this may be an important mechanism mediating changes in energy intake and subsequent weight gain. A very limited number of human studies have investigated the effects of a high fat diet on oral fat sensitivity. One study identified a large diversity in oral sensitivity to fatty acids (oleic acid; C18:1), with detection thresholds ranging from 0.02–12 mM (Stewart et al. 2010; Stewart et al. 2011a). Although all subjects were able to detect the fatty acids across the range, individuals who had significantly lower habitual dietary energy and fat intakes, as assessed in two-day food diaries, were more sensitive to C18:1 (defined in the study as the ability to detect C18:1 at a concentration of 1.4 mM) than those who had greater habitual fat intake (Stewart et al. 2010; Stewart et al. 2011b). A recent

study in humans evaluated the effects of a four-week high fat diet on taste sensitivity to C18:1 in lean and overweight/obese subjects. Oral sensitivity to C18:1 was attenuated following the high fat diet in the lean but not obese, suggesting that the obese/overweight subjects were 'adapted' to high fat exposure, perhaps because of differences in habitual fat consumption (Stewart and Keast 2011c).

Thus, it appears that a high fat diet attenuates both oral and gastrointestinal function, which may contribute to increased energy intake, and thereby, facilitate weight gain in the longer term.

3.7.5 Effects of energy restriction on gastrointestinal function, appetite and energy intake

Since, as described above, overexposure of the small intestine to fat appears to attenuate gastrointestinal sensitivity to fat and increase energy intake and subsequent weight gain, it is conceivable that dietary restriction may reverse these effects and enhance gastrointestinal sensitivity, and thus facilitate appetite suppression. There has been growing interest in understanding the effects of energy restriction on gastrointestinal function and energy intake and how weight loss could be achieved by dietary restriction. The current literature relating to the effects of short- and long-term dietary restrictions on gastrointestinal motor function, gastrointestinal hormone release, appetite, and energy intake will be discussed in the following sections.

3.7.5.1 Effects of acute energy restriction on gastrointestinal function, appetite and energy intake

There is some evidence, although it is limited, that acute dietary restriction in healthy, lean humans has the capacity to increase the sensitivity of gastrointestinal responses to nutrients. For example, a study evaluated the effects of a short-term fast (four days) on gastric emptying in lean and obese subjects (Corvilain et al. 1995). Gastric emptying of a glucose drink was slower after the four-day, when compared with a 12-hour overnight, fast in both lean and obese subjects (Corvilain et al. 1995) (see **Figure 3.4**), suggesting that acute caloric restriction may enhance the sensitivity to the actions of intestinal nutrients.

Figure 3.4: Gastric emptying of 75 g of a glucose load (320 mL), in lean (n = 12) and obese (n = 11) subjects on a four-day fast versus an overnight fast. * vs overnight fast, $P < 0.05$ (Corvilain et al. 1995).

Doucet et al. evaluated the effects of a short-term (four-day) energy-restricted diet (–800 kcal per day of an individual's daily energy intake) on ghrelin concentrations in 15 healthy male subjects, and reported no change in fasting or postprandial ghrelin concentrations following the four-day dietary energy restriction (Doucet et al. 2004). Since it has been shown that only a small increase in circulating ghrelin is observed when a diet is high in carbohydrate (65%) (Weigle et al. 2003), it is possible that the absence of a change in total ghrelin in the study by Doucet et al. (2004) could be explained by the relatively high carbohydrate content of the energy-restricted diet used (55%).

If short-term dietary restriction has the capacity to enhance the sensitivity of the small intestine to the presence of nutrients, for example, the slowing of gastric emptying, it may also have the potential to modify other gastrointestinal functions and this will be evaluated in the study presented in Chapter 8.

3.7.5.2 Effects of long-term energy restriction on gastrointestinal function

It is well documented that a number of metabolic and physiological changes occur in obese subjects in response to weight loss. Weight loss improves glycaemic control and increases insulin sensitivity by stimulating glucose phosphorylation and glucose transport (Williams et al. 2003b). Conversely, there is evidence that during prolonged caloric restriction, adaptive metabolic changes take place, most likely to protect against excessive weight loss (Leibel et al. 1995). For example, resting energy expenditure is reduced with weight loss, even after adjustment for loss of lean and fat mass (Heilbronn et al. 2006) and hunger is increased, driving increased energy intake (Doucet et al.

2003; Anton et al. 2009). For example, Doucet et al. (2003) found desire to eat and hunger perceptions, both measured in a fasting state, to be significantly increased in obese individuals after a 15-week weight loss programme involving energy restriction (Doucet et al. 2003). In addition, another study reported desire to eat significantly increased after a six-month low calorie diet (Anton et al. 2009). Such changes could explain why adherence to weight loss diets are difficult and why body weight often stabilises, or even increases, despite continued adherence of individuals to the prescribed weight loss diet (Sjostrom et al. 1998). Given that such metabolic changes occur in response to longer-term dietary restriction and given the importance of the gastrointestinal tract in appetite regulation, it is likely that, over time, adaptive changes may also take place at oral and gastrointestinal levels that may contribute to increases in energy intake and body weight.

There are only a limited number of studies that have evaluated the effects of prolonged energy restriction on aspects of gastrointestinal function. Studies have reported that both fasting PYY (Roth et al. 2005), and postprandial GLP-1 (Verdich et al. 2001a; Adam and Westerterp-Plantenga 2005), secretion increase following prolonged dietary restriction (3–6 months) in obese subjects. In addition, circulating concentrations of ghrelin over a 24-hour period markedly increased after six months of a diet-induced weight loss (Cummings et al. 2002), suggesting that ghrelin may play a role in the adaptive responses to dietary restriction that limits the amount of weight that may be lost while dieting.

Thus, in summary, following acute energy restriction, there is evidence of increased sensitivity in the gastrointestinal responses to nutrients, whereas longer-term adaptations to dietary restriction seek to prevent further weight loss. How this is related to gastrointestinal functions that play a role in energy intake regulation, including gastrointestinal motility and hormone release, and thus influences energy intake and body weight has not been evaluated comprehensively.

3.8 Conclusion

Investigation in the area of gastrointestinal function and appetite in the obese is critical, as characterisation of changes in the sensitivity of the gastrointestinal tract to the actions of nutrients that may occur during periods of energy restriction. The studies described in the subsequent chapters of this thesis have addressed the following hypotheses:

1) Gastropyloroduodenal motor and gastrointestinal hormonal factors and appetite perceptions are major determinants of acute energy intake (see Chapter 5).
2) Oral ingestion of a nutrient liquid in obese, will be associated with accelerated gastric emptying and oro-caecal transit, diminished GLP-1 and comparable GIP, secretion, attenuated suppression of appetite and energy intake in whom, habitual energy and fat intake will be greater, compared with lean and overweight individuals (see Chapter 6).
3) Gastrointestinal and oral sensitivities to oleic acid are related and sensitivity at both locations is compromised in the obese and directly related to fat consumption (see Chapter 7).
4) Acute energy restriction (four days) will increase the sensitivity of the small intestine to lipid, resulting in increased stimulation of pyloric pressures and

PYY, but greater suppression of ghrelin, as well as reduced appetite and energy intake (see Chapter 8).

5) Acute energy restriction (four days) enhances the effects of duodenal lipid on gastrointestinal function and appetite in lean and obese subjects, while following prolonged 30% energy restriction (12 weeks), associated with weight loss, these effects of energy restriction on gastrointestinal function and appetite would be lost in the obese (see Chapter 9).

Chapter 4: Subjects and Methodologies

4.1 Introduction

This chapter describes the techniques that were used in the studies presented in Chapters 6–9. All techniques were state-of-the-art techniques well established in our laboratory, including high resolution manometry for the measurement of APD motility (Heddle et al. 1988a), scintigraphy for the measurement of gastric emptying (Collins et al. 1991; Collins et al. 1983), radioimmunoassays for the analysis of plasma hormone concentrations (Seimon et al. 2009a; Feltrin et al. 2004; Feltrin et al. 2006), visual analogue scale (VAS) questionnaires for the assessment of appetite perceptions (Parker et al. 2004a), a standardised, cold, buffet-style meal for the assessment of acute energy intake (Feltrin et al. 2004), and diet diaries for the assessment of daily energy intake. Ascending-series three-alternate forced-choice methodology for the assessment of oral fatty acid taste thresholds (Meilgaard et al. 2007) was established in our laboratory in 2009, in collaboration with Dr Russell Keast and Jessica Stewart, School of Exercise & Nutrition Sciences, Deakin University.

4.2 Subjects

4.2.1 Study subjects

For all studies, healthy male subjects, aged 18–60 years, were enrolled. Lean subjects, included in the studies described in Chapters 6, 7 and 9, were of normal body weight for their height with a BMI of 19–25 kg/m^2, overweight subjects, included in the studies

described in Chapters 6 and 7, had a BMI of 25.1–30 kg/m^2, and obese subjects, included in the studies described in Chapters 6–9, had a BMI of 30.1–35 kg/m^2 and a waist circumference of ≥ 102 cm. In studies where lean, overweight and obese subjects were participating subjects were matched for age. The number of subjects required for each study was determined using power calculations based on previous studies as outlined in the individual chapters.

4.2.2 Subject recruitment

Volunteers were recruited from an existing pool of volunteers available in the Discipline, through the use of flyers placed regularly on notice boards within the Royal Adelaide Hospital and local universities (University of Adelaide, University of South Australia and Flinders University) and by advertisements placed in the local *Advertiser* and *Messenger* newspapers and the Career One website, following the guidelines set by the Human Research Ethics Committee.

4.2.3 Exclusion criteria

Prior to the enrolment in a study, each subject underwent a screening process to exclude:

1) significant gastrointestinal symptoms, disease or surgery
2) current use of medications that may alter gastrointestinal motor function or appetite
3) epilepsy
4) cardiovascular or respiratory disease
5) diabetes mellitus

6) any other significant illness as assessed by the investigator
7) allergy to local anaesthetic
8) high performance athletes
9) weight change (increase or decrease) of ≥ 5% of total body weight in the three months prior to enrolment in the study
10) exposure to ionising radiation (from X-ray machines or radioactive substances) as part of a research study in the previous 12 months (see Chapter 6)
11) intake of > 20 g alcohol on a daily basis
12) smoking.

In addition, healthy lean subjects were required to be unrestrained eaters, as determined by a score of ≤ 12 on the eating restraint questionnaire component of the three-factor eating questionnaire (Stunkard and Messick 1985) (see **Appendix I**). While the degree of eating restraint was assessed and recorded in the overweight and obese subjects, it was not used as an exclusion criterion, as overweight and obese subjects were expected to have some degree of eating restraint.

4.3 Ethics committee approval

All subjects provided written, informed consent prior to their inclusion in the study and were advised that they were free to withdraw from the study at any point. All subjects were reimbursed for the time spent in the laboratory by way of an honorarium ($15 per hour for the study assessing gastric emptying using scintigraphy [see Chapter 6] and $18 per hour for all studies involving a nasoduodenal catheter [see Chapters 7–9]).

All studies were approved by the Royal Adelaide Hospital Ethics Committee and the University of Adelaide Human Research Ethics Committee was notified of the approval. One study (described in Chapter 9) was also approved by the CSIRO Research Ethics Committee because dietary assessment for this study was conducted at CSIRO. All clinical studies were registered as clinical trials on the Australian New Zealand Clinical Trials Registry, prior to commencement of the study.

4.4 Assessment of gastrointestinal motor function

High resolution manometry and scintigraphy were used to measure gastrointestinal motor function. Manometry was used for the measurement of pressures in the APD region (see Chapters 7–9) and scintigraphy to assess gastric emptying, proximal, distal and total stomach retention and mouth-to-caecum transit (see Chapter 6).

4.4.1 High resolution manometry

High resolution water-perfusion manometry is a technique used to measure pressures in the gastrointestinal tract (Heddle et al. 1988a). Pressures in the APD region were measured using a 16-channel silicone manometric catheter.

4.4.1.1 Catheter design

The manometric catheter (3.5 mm outer diameter, Dentsleeve International Ltd, Mui Scientific, Ontario, Canada) (see **Figure 4.1**) consisted of 16 side-holes (0.1 mm in diameter) separated by 1.5 cm intervals, for the recording of luminal pressures. Six side-holes (channels 1–6) were positioned in the antrum, a 4.5 cm pyloric sleeve sensor (channel 7), with two channels present on the back of the sleeve (channels 8 and 9), was

positioned across the pylorus, and seven side-holes (channels 10–16) were positioned in the duodenum. An additional channel (1 mm in diameter) was positioned 11.75 cm distal to the pylorus, and this was used for the administration of the intraduodenal infusions.

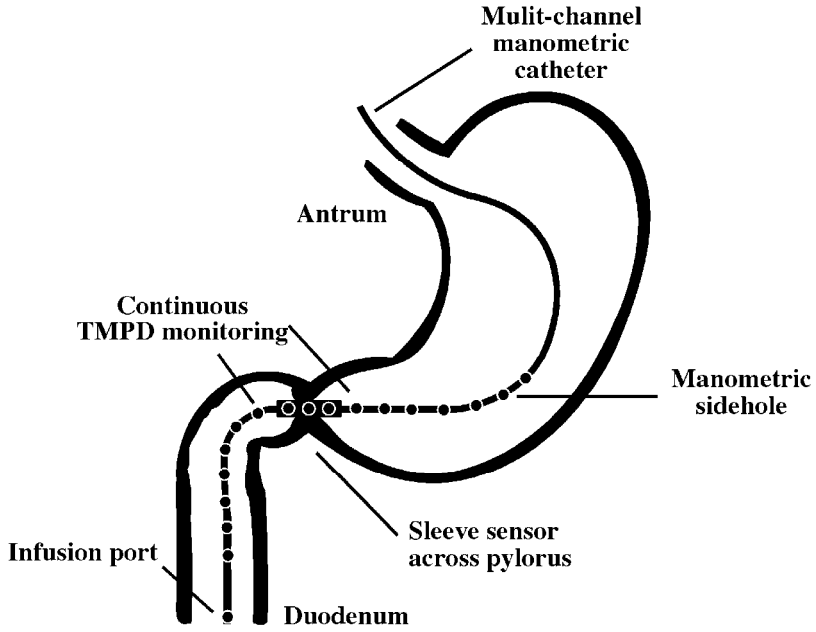


Figure 4.1: Schematic representation of the manometric catheter incorporating six antral and seven duodenal side-holes, a pyloric sleeve sensor and duodenal infusion port.

4.4.1.2 Nasoduodenal intubation and manometry

On the morning of the study, after an overnight fast, the manometry catheter was inserted through a nostril into the stomach and allowed to pass into the duodenum by peristalsis (Heddle et al. 1988a). To minimise discomfort during the intubation, local anaesthetic gel (Lignocaine 2% Gel Sterile, ORION Laboratories Pty Ltd, Balcatta,

Western Australia, Australia) and spray (Co-Phenylcaine Forte Spray, ENT Technologies Pty Ltd, Hawthorn East, Victoria, Australia) were applied to the nostril.

The correct position of the catheter, so that the sleeve sensor straddled the pylorus, was monitored throughout the study by continuous measurement of the transmucosal potential difference (TMPD) (which is ~ –40mV on the most distal antral (channel 6), and ~ 0mV on the most proximal duodenal (channel 10), channel (Heddle et al. 1988a). For this, an intravenous cannula was placed subcutaneously in the left forearm and filled with sterile saline as a reference electrode (Heddle et al. 1988a). All manometric channels were perfused with degassed, distilled water at 0.15 ml/min, except for the two TMPD channels (channel 6 and 10), which were perfused with degassed 0.9% saline (Heddle et al. 1988a). Once the catheter was correctly positioned, fasting motility was monitored until the occurrence of a phase III of the MMC. At the end of phase III, a 15-min baseline was recording during phase I of the MMC, (which is a period of motor quiescence), after which, all study interventions began.

4.4.1.3 Data acquisition and analyses

Manometric pressures were digitised and recorded on a computer-based system, running commercially available software (Flexisoft, Oakfield Instruments, Oxfordshire, United Kingdom, A/Prof GS Hebbard, Melbourne, Australia, written in Labview 3.1.1 [National Instruments]) and stored for subsequent analysis. APD pressures were analysed for i) number and amplitude of pressure waves in the antrum and duodenum, ii) basal pyloric pressure and number and amplitude of isolated pyloric pressure waves (IPPW) and iii) pressure wave sequences (PWS). Pressure waves in the antrum, pylorus

and duodenum were defined by an amplitude ≥ 10 mmHg, with a minimum interval of 15 seconds between peaks for antral and pyloric waves, and three seconds for duodenal waves (Samsom et al. 1998). Basal pyloric pressure ('tone') was calculated for each minute by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral channel from the mean basal pressure recorded at the sleeve (Heddle et al. 1988b), using custom-written software (Professor A Smout, Department of Gastroenterology and Hepatology, University Medical Centre, Amsterdam, Netherlands) modified to our requirements. Pressure waves in the antrum, pylorus and duodenum were considered related and defined as PWS, if they travelled between side-holes at rates of 9–160 mm/s (Samsom et al. 1998). Pressure waves were characterised according to the distance travelled, that is, over two (1.5–< 3 cm), three (3–< 4.5 cm), four (4.5–< 6 cm), …, 15 (21–< 22.5 cm) channels, and expressed as the total number of waves using custom-written software (by Professor A Smout).

4.4.2 Scintigraphy

Scintigraphy is the gold-standard technique for the measurement of gastric emptying, intragastric distribution and mouth-to-caecum transit, and measures the rate at which a radio-labelled meal empties from the stomach to the small intestine, and its transit through the intestine, by acquisition of images on a computer, using a gamma camera (Genie; GE Healthcare Technologies, Milwaukee, WI) (Collins et al. 1983). In scintigraphic studies, radionuclide markers are incorporated into liquid, solid or semi-solid meals. Their course of emptying from the stomach into the small intestine is then tracked using the gamma camera, providing a measure for gastric emptying of the meal. ^{99m}Technetium (Tc) is the most commonly used label for both solid and liquid meals

due to its short half-life of six hours. ^{99m}Technetium is complexed with sulphur colloid to prevent absorption by the gastrointestinal tract, and total body radiation exposure is approximately 0.48 mSv.

In order to establish anatomical reference points, which were used to correct for the movement of the patients when the study was analysed, two markers (lead-lined plastic bottle tops, 1.5 cm in diameter) were taped onto the skin, one situated in the left hypochondrium at the end of the ninth rib, the other over the left anterior superior iliac spine. These remained in position for the entire duration of the study.

4.4.2.1 Data acquisition and analyses

To obtain images, subjects stood with their back against the camera for one min to obtain an anterior image and then turn around facing the camera, with their abdomen against the gamma camera for one min to obtain a posterior image. Images were taken every 15 min for the first 1.5 hours and then every 30 min for the next 3.5 hours. Data were corrected for subject movement and radionuclide decay, and the geometric mean technique of the anterior and posterior data was calculated to correct for attenuation. From the acquired images, regions of interest were drawn around the total stomach, and gastric emptying curves (expressed as % retention over time) derived. To evaluate intragastric meal distribution, the total stomach region of interest was divided into proximal and distal regions, with the proximal region corresponding to the fundus and proximal corpus and the distal region corresponding to the distal corpus and antrum (Collins et al. 1983). The time for 50% of the liquid to empty (T_{50}) from the stomach was determined from the gastric emptying curve (Collins et al. 1983). Mouth-to-

caecum transit was defined as the time from ingestion of the meal to the arrival of the head of the meal at the caecum (Collins et al. 1983).

4.5 Assessment of plasma hormone and blood glucose concentrations

For blood sampling, an intravenous cannula was inserted into an antecubital vein. Blood samples were collected in ice-chilled ethylenediaminetetraacetic acid (EDTA)-treated tubes containing 400 kIU aprotinin (Trasylol; Bayer Australia Ltd, Pymble, Australia) per ml blood. Plasma was obtained by centrifugation of blood samples at 3200 rpm for 15 min at 4^{o} C. The plasma samples were then frozen at –70^{o} C for later analysis. Radioimmunoassays were used to measure plasma concentrations of CCK, PYY, ghrelin, glucagon-like peptide-1 (GLP-1), GIP and insulin. All samples from individual subjects were always measured in the same run. For logistical reasons, plasma CCK, PYY and ghrelin concentrations were determined in different laboratories using two different radioimmunoassays. For the studies described in Chapters 7 and 8, plasma CCK, PYY and ghrelin were determined at the University of Munich, Germany and for the study described in Chapter 9, plasma CCK, PYY and ghrelin were determined at the Department of Medicine, University of Adelaide, Australia.

4.5.1 Plasma cholecystokinin

For the studies described in Chapters 7 and 8, plasma CCK concentrations (pmol/L) were determined by a sensitive and specific radioimmunoassay, as described (Riepl et al. 1996). In short, the antibody (CH40IX), raised in rabbits, was specifically directed to the biologically active site of CCK including the sulphated tyrosyl residue at position 7 from the C-terminal end and showed no cross-reactivity with unsulphated CCK-8,

unsulphated gastrin-17 or unsulphated gastrin-34. The cross-reactivity to sulphated gastrin-17 was less than 1%. The intra-assay coefficient of variation (CV) was 5.6% and the inter-assay CV was 7.2%, with a detection limit of 0.3 pmol/L.

For the study described in Chapter 9, plasma CCK concentrations were measured using a previously adapted method (Santangelo et al. 1998). Samples were extracted in 66% ethanol and extracts were dried down and resuspended in assay buffer (50 mM phosphate, 10 mM EDTA, 2 g/L gelatin, pH 7.4). Standards were prepared using synthetic sulphated CCK-8 (Sigma Chemical, St Louis, MO, USA), antibody (C2581, Lot 105H4852, Sigma Chemical) was added at a working dilution of 1/17,500 and sulphated CCK-8 ^{125}I-labelled with Bolton and Hunter reagent (Perkin Elmer, Boston, MA, USA) was used as tracer. Samples were incubated for seven days at 4˚C. The antibody-bound fraction was separated by the addition of dextran-coated charcoal containing gelatin (0.015 g gelatin, 0.09 g dextran, 0.15 g charcoal in 30 ml assay buffer) and the radioactivity determined in the supernatants following centrifugation. The intra-assay CV was 7.1% and the inter-assay CV was 17.8%, with a detection limit of 1 pmol/L.

4.5.2 Plasma peptide tyrosine tyrosine

For the studies described in Chapters 7 and 8, immunoreactive total human plasma PYY (pmol/L) was measured by a commercially available radioimmunoassay (Linco Research, St Charles, MO) by using ^{125}I-labelled bioactive PYY as the tracer and a PYY antiserum to determine the concentration of active PYY by the double antibody/PEG technique. The PYY antibody was raised in guinea pigs and recognises both the $PYY_{(1-}$

$_{36)}$ and the $PYY_{(3-36)}$ forms of human PYY; that is, the assay does not distinguish between $PYY_{(1-36)}$ and $PYY_{(3-36)}$. The intra-assay CV was 5.3% and the inter-assay CV was 7%, with a detection limit of 10 pg/mL.

For the study described in Chapters 9, plasma PYY was measured by radioimmunoassay using an antiserum raised in rabbits (kindly donated by Dr. B Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) against human $PYY_{(1-36)}$ (Sigma-Aldrich). The antiserum showed < 0.001% cross-reactivity with human pancreatic polypeptide or sulfated CCK-8 and 0.0025% cross-reactivity with human neuropeptide Y. Standards (1.6-50 fmol/tube) or samples (200 µL plasma) were incubated in 200 µl assay buffer (50 mM NaPO4, 10 mM EDTA, 2 g/L gelatin, 0.1 g/L Na-Azide, pH 7.4) and a 1/12000 dilution of antiserum for 24 hours, followed by an incubation with 100 µl of 10000 cpm tracer (NEX3410, Perkin Elmer) for 24 hours. Antibody-bound tracer was separated from free tracer by second antibody precipitation, followed by incubation for 2 hours at room temperature and centrifugation at 4000 rpm for 20 minutes. The supernatant was discarded, and the pellets were counted in a gamma counter (Brennan et al. 2008). The intra-assay CV was 6.5% and the inter-assay CV was 4.2%, with a detectable limit of 1.5 pmol/L.

4.5.3 Plasma ghrelin

For the study described in Chapter 8, total plasma ghrelin concentrations (pmol/L) were measured by a commercially available radioimmunoassay (Phoenix Pharmaceuticals, Mountain View, CA, USA) using ^{125}I-labelled bioactive ghrelin as a tracer and a polyclonal antibody raised in rabbits against the C-terminal end of human ghrelin. No

cross-reactivities with any relevant molecules (i.e. secretin, vasoactive intestinal peptide, prolactin-releasing-peptide-31, galanin, growth hormone releasing factor, neuropeptide Y (NPY), orexin A, orexin B) have been found. Intra-assay CV was 5.3% and inter-assay CV was 13.6%, with a detection limit of 64 pg/mL.

For the study described in Chapter 9, plasma ghrelin concentrations were measured by radioimmunoassay with some modifications to the previously published method (Parker et al. 2005). The radiolabel (NEX388) was purchased from Perkin Elmer (Boston, MA, USA). The standard and samples were incubated with the antibody for 3–4 days prior to incubating with the radiolabel for a further 24 hours at 4^{o} C. The intra-assay CV was 8.5%, the inter-assay CV was 15%, and the detection limit was 40 pg/mL.

4.5.4 Plasma glucagon-like peptide-1 (GLP-1)

Plasma GLP-$1_{(7-36)}$ amide concentrations were determined in Chapter 6 using an antibody supplied by Professor SR Bloom (Hammersmith Hospital, London) which has been shown, using chromatography, to measure intact GLP-$1_{(7-36)}$ amide, and it is likely that this antibody also binds the degraded form of GLP-$1_{(9-36)}$ amide (Wishart et al. 1998). The antibody did not cross-react with glucagon, gastric inhibitory polypeptide, or any other gut or pancreatic peptides. The intra-assay CV was 17% and the inter-assay CV was 18%, with a detection limit of 1.5 pmol/L.

4.5.5 Plasma glucose-dependent insulinotropic polypeptide

Plasma GIP concentrations were determined in Chapter 6 using some modification to the original method (Wishart et al. 1992). The standard curve was prepared in buffer

rather than extracted charcoal-stripped serum and the radioiodinated label was supplied by Perkin Elmer (Boston, Massachusetts, USA). Both the intra-assay and the inter-assay CVs were 15%, and the detection limit was 2 pmol/L.

4.5.6 Plasma insulin

Plasma insulin concentrations (mU/l) were determined in Chapter 6 using ELISA (10-1113, Mercodia, Uppsala, Sweden). The intra-assay CV was 2.6%, the inter-assay CV was 4.9%, and the detection limit was 1.0 mU/L.

4.5.7 Blood glucose concentrations

Venous blood glucose concentrations (mmol/L) were determined in Chapter 6, immediately by the glucose oxidase method using a portable glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA). This technique has a CV between 2.1 and 5.6%. The accuracy of this method has been confirmed in our laboratory using the hexokinase technique (Horowitz et al. 1991).

4.6 Assessment of appetite perceptions

Perceptions of hunger, fullness, desire to eat and prospective consumption were quantified using a validated VAS questionnaire (Parker et al. 2004b) (see **Appendix II**) (see Chapters 6–9). The gastrointestinal symptoms, nausea and bloating, were also assessed using this questionnaire. Other perceptions, such as anxiety, happiness and drowsiness, were assessed, but not evaluated, to distract the subjects from the main purpose of the questionnaire. Each VAS consisted of a 100-mm horizontal line, where 0 represented ‘sensation not felt at all’ and 100 represented ‘sensation felt the greatest’.

Subjects were asked to place a vertical mark along each horizontal line to indicate the strength of the sensation they felt at that particular time point.

4.7 Assessment of dietary intake

Dietary assessment is used to obtain information on individual dietary intakes. The most commonly used method to quantify acute energy intake following a specific treatment, which was a primary outcome in all studies, is *ad libitum* food intake at a buffet meal (see Chapters 6–9) (Feltrin et al. 2004). For assessment of habitual energy intake of individuals, the commonly used methods are food frequency questionnaires (see Chapter 6), dietary recalls and diet histories (see Chapter 7) and weighed food records in terms of diet diaries (see Chapter 9) (Baghurst and Baghurst 1981). Food frequency questionnaires were used to assess habitual energy intake of individuals over a prolonged period (12 months). Dietary recalls and diet histories were used only to estimate recent dietary intake, while weighed food records were used to more precisely evaluate short-term dietary intake.

4.7.1 Buffet meal

Acute energy intake in response to study treatments was assessed in all studies by quantifying the amount consumed by a subject at an *ad libitum* cold, buffet-style meal (Feltrin et al. 2004). The composition of the meal, as well as its energy content (kJ), amount (g) and macronutrient content, is detailed in **Table 4.1**. The quantities of food offered were in excess of what the subject was expected to eat, and subjects were asked to consume the meal freely, for up to 30 min.

The buffet meal was weighed before and after consumption, to quantify the amount eaten (g). Energy intake (kJ) and macronutrient distribution (%energy from fat, carbohydrate and protein) were then calculated using the software programme Foodworks (Xyris Software, Version 3.01, Highgate Hill, Queensland, Australia) (Brennan et al. 2005).

Table 4.1: Composition of the buffet meal

Food items	Amount served (g)	Energy content (kJ)	Fat (g)	Carbohydrate (g)	Protein (g)
Wholemeal bread, 4 slices[a]	125	1,304	3.6	50.0	12.6
White bread, 4 slices[a]	125	1,295	2.9	56.4	11.8
Ham, sliced[b]	100	453	3.6	0	18.8
Chicken, sliced[c]	100	677	7.0	0	24.6
Cheese, 4 slices[d]	85	1,436	28.3	0.9	21.9
Tomato, sliced	100	56	0.1	1.9	1.0
Lettuce	100	27	0	0.4	0.9
Cucumber, sliced	100	44	0.1	1.	0.5
Strawberry yoghurt[e]	200	966	6.2	33.8	9.4
Fruit salad[f]	140	343	0.1	19.3	0.6
Chocolate custard[g]	150	622	5.3	22.7	4.8
Apple	170	359	0.2	21.3	0.5
Banana	190	680	0.2	37.8	3.2
Orange juice, unsweetened[h]	500	800	5.0	42.5	5.0
Iced coffee[i]	600	1,788	10.2	61.8	21.0
Water	600	0	0	0	0
Margarine[j]	20	609	16.4	0.1	0.1
Mayonnaise[k]	20	310	6.5	4.0	0.2
Total	**3,425**	**11,769**	**95.7**	**353.9**	**136.9**
%Energy			16.3%	60.3%	23.3%

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

4.7.2 Dietary questionnaire for epidemiological studies

In the study presented in Chapter 6, a validated dietary questionnaire, developed and validated by the Cancer Council, Victoria, was used to characterise eating habits over the past 12 months, including habitual energy intake and macronutrient distribution (Hodge et al. 2000) (see **Appendix III**). It contains questions on the overall frequency of fruit and vegetable consumption, and questions on consumption of foods that do not fit easily into the frequency format. The questionnaire also contains three photographs of scaled portions for four foods (used to calculate a portion size calibrator) and comprises of a food list of 74 items with 10 frequency response options ranging from 'Never' to '3 or more times per day'. The 74 food items are grouped into four categories: i) cereal foods, sweets and snacks, ii) dairy products, meats and fish, iii) fruit and iv) vegetables. The questionnaires were analysed by the Cancer Council, Victoria. Energy intake (kJ), as well as macronutrient distribution (g and %fat, protein and carbohydrate) was quantified.

4.7.3 Diet diaries

4.7.3.1 Two-day diet recalls

In the study presented in Chapter 7, recent dietary intake was estimated using a two-day dietary recall, during which subjects recalled all foods and beverages consumed the previous day and on one weekend day within the previous week (Gibson 1993) (see **Appendix IV**). To assist in the accurate recall of portion sizes, subjects were provided with validated and quantified pictures of food portions for common foods, such as cereals, meats, take-away foods, spreads, vegetables, rice, pasta and beverages, that were used to calculate the subjects' energy intake using the weights of the foods

provided for each of the portion sizes (University of Otago, Department of Human Nutrition, Dunedin, New Zealand) (see **Appendix V**).

4.7.3.2 Five-day diet diary

In the study presented in Chapter 9, five-day diet diaries were used to assess the habitual diet and determine energy requirements of each subject (see **Appendix VII**). Subjects were instructed to weigh and record all foods and beverages consumed over five consecutive days (three week days and two weekend days). To facilitate this, standardised instructions on how to weigh and record all foods and beverages consumed over the five days were provided along with digital kitchen scales.

4.7.3.3 Data analysis

Data collected from the two-day diet recalls and the five-day diet diaries were analysed using specialised software (Foodworks® Professional Edition, version 5; Xyris Software 1998–2007, Highgate Hill, Queensland, Australia). Daily energy intake (kJ), as well as macronutrient distribution (%fat, protein and carbohydrate) was quantified.

4.8 Dietary restriction

4.8.1 70% VLCD

In Chapter 8, to achieve a period of acute energy restriction, subjects were placed on a four-day VLCD. The VLCD involved a 70% reduction in each individual's energy intake, estimated using the Harris Benedict equation and a physical activity factor between 1.4–1.5 (indicative of light-to-moderate activity) based on an individual's self-reported daily activity (Harris and Benedict 1918). To aid compliance with the VLCD,

subjects were provided with individualised meal plans (**Appendix VI**), detailing the food items and their amount (g) to be consumed at each meal, and the food items required for the diet period, including both liquid meal replacements (KicStart, Pharmacy Health Solutions Pty Ltd., Frenchs Forest, New South Wales, Australia) and standard food items (for example, sliced ham, wholemeal bread, salad items, fruit and pre-packaged frozen meals). These were provided to the subjects and ensured a 'balanced' diet complete in micronutrients and protein. Subjects were permitted to consume an unlimited quantity of non-caloric beverages, which they were required to document (including brand names and quantities), together with all the food consumed, throughout the 4 days, in a food diary. Subjects were contacted by phone during day 2 of the 4-day diet to monitor their progress.

4.8.2 30% energy-restricted diet

The study presented in Chapter 9 involved a dietary restriction protocol, which was designed in collaboration with dieticians from CSIRO, Human Nutrition, Adelaide, Australia. The protocol entailed a 30% reduction of total energy intake, using a macronutrient-balanced diet consisting of approximately 50% carbohydrate, 30% fat and 20% protein (Luscombe-Marsh et al. 2005). Subjects were provided with all foods and snacks using ready-to-eat meals (Lite n' Easy®; Ridleyton, South Australia, Australia). Lite n' Easy® provided for three dietary templates, 1,200, 1,500 and 1,800 calorie meal plans, which, as part of their overall daily energy intake, also included 375 ml skim milk to ensure calcium intake in the diet. In order to match the dietary requirements of individual subjects beyond the energy provided by the Lite n' Easy® meal plan, additional energy was provided (for example, fruit and muesli bars). To

assess dietary compliance, subjects were asked to record any food that was consumed in addition to what was provided in a dietary checklist (see **Appendix VIII**). Lean subjects underwent a four-day period of restriction (to avoid significant weight loss), while obese subjects underwent a 12-week period of dietary restriction that would be associated with weight loss. Obese subjects attended fortnightly, individual counselling sessions with the dietician to review meal plans and dietary checklists and to record body weight.

4.9 Assessment of oral fatty acid detection thresholds

In the studies presented in Chapter 7 and 9, oral sensitivity to oleic acid (C18:1) was determined using a three-alternative forced-choice technique: an established procedure to determine taste thresholds (ASTM 2004). For test sample preparation, C18:1 was mixed at varying concentrations (0.02, 0.06, 1, 1.4, 2, 2.8, 3.8, 5, 6.4, 8, 9.8 and 12 mM) with long-life non-fat milk (Homebrand, Woolworths, Bella Vista, New South Wales, Australia). To minimise textural cues due to the addition of fatty acid, samples were mixed with 5% (w/v) gum acacia (Deltagen, Boronia, Victoria, Australia) and liquid paraffin (Faulding Remedies, Virginia, Queensland, Australia) (Chale-Rush et al. 2007b). To prevent oxidation of C18:1, samples were mixed with 0.01% w/v EDTA (Merck, Darmstadt, Germany). Samples were homogenised for 30 seconds/100 mL solution (Silverson L4RT homogenizer, Longmeadow, Massachusetts, USA) and were prepared fresh on the day of testing and served at room temperature. To prevent confounding from non-oral sensory inputs (e.g. smells), tests were conducted with subjects wearing nose clips. All samples were similar in appearance so subjects could

not visually distinguish between samples. The concentrations of fatty acids used were not expected to cause irritation (Chale-Rush et al. 2007b).

During testing, subjects were presented with three samples per set: two control samples and one 'odd' sample containing C18:1. C18:1 was presented in the samples in ascending order of concentration from the lowest (0.02 mM) to the highest (12 mM). During each presentation, subjects were asked to identify the odd sample; if they did so correctly, they were presented with three more samples, and the C18:1 sample remained at the same concentration; if they did not identify the odd sample, they were presented with three more samples, and the concentration of the C18:1 sample increased. Testing continued, with the concentration of C18:1 increasing each time the subjects picked incorrectly, and ceased once the subject identified the odd sample three consecutive times, at a given concentration, and that concentration was defined as the subject's detection threshold.

4.10 Evaluation of gastrointestinal and appetite responses to oral and intraduodenal nutrients

Orally administered nutrients were used to assess gastric emptying, small intestinal transit, caecal arrival, gastrointestinal hormones, appetite and energy intake. Intraduodenal infusion allowed assessment of gastrointestinal function, appetite and energy intake without orosensory and gastric influences such as gastric distension and gastric emptying.

4.10.1 Oral test meal

For the study presented in Chapter 6, subjects ingested a liquid meal comprising 500 ml (~ 532 kcal) of Ensure® (Abbott Australasia Pty Ltd, Botany, NSW, Australia). The meal was macronutrient balanced, consisting of 15% protein, 60% carbohydrate and 25% fat with an energy density load of 1 kcal/mL. The meal was labelled with 20 MBq ^{99m}Technetium sulphur colloid to allow scintigraphic evaluation of gastric emptying.

4.10.2 Intraduodenal infusions

4.10.2.1 Triglyceride emulsion

Intralipid® (10%, 300 mOsmol/kg, 1.1 kcal/mL, Fresenius Medical Care Australia Pty Ltd, Smithfield, NSW, Australia), a commercially available lipid emulsion consisting predominantly of long-chain triglycerides extracted from soy bean oil (50 g/500 mL), egg phospholipids (1.2 g/500 mL) and glycerol anhydrous (2.25 g/500 mL) was used as the nutrient infusion in Chapters 8 and 9. Intralipid was administered at a rate of 2.86 kcal/min (2.6 mL/min) for 120 min. The infusion rate was selected to reflect the average rate of gastric emptying in humans (Brener et al. 1983), and has been used previously in other studies; hence, there is a body of data on its effects on gastrointestinal and appetite in lean individuals (MacIntosh et al. 2001a; Seimon et al. 2009b).

4.10.2.2 Sodium oleate solution

The sodium oleate (C18:1) solution, presented in Chapter 7, was prepared by dissolving 12.9 g of C18:1 with 3 ml 1 M sodium hydroxide (Sigma-Aldrich, St Louis, Missouri, USA) in distilled water to a volume of 300 mL (resulting pH: 7.9). The C18:1 was kept

in solution by continuous stirring throughout the study. The pH of the saline control was adjusted to 7.9 by addition of 50 µl 1 M sodium hydroxide solution. The solutions were administered at a rate of 2 ml/min (total volume: 180 ml over 90 min) using a volumetric infusion pump (Imed Gemini PC-1, C&A Company, Royse City, TX, USA), and C18:1 was delivered at 0.78 kcal/min (2 mL/min) for 90 min (Matzinger et al. 2000). The infusion rate was selected on the basis of a previous study in humans, which showed significant suppression of energy intake without any adverse effects (Matzinger et al. 2000).

4.11 Statistical analysis

The statistical analysis in each study is described in detail in individual chapters. Data were analysed using commercially available statistical software, SPSS version 17 (SPSS Inc, Chicago, Illinois, USA). Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

Chapter 5: Pooled-Data Analysis Identifies Pyloric Pressures and Plasma CCK Concentrations as Major Determinants of Acute Energy Intake in Healthy Lean Males

Published: American Journal of Clinical Nutrition, 2010, 92(1), 61–68.

Radhika V Seimon, Kylie Lange, Tanya J Little, Ixchel M Brennan, Amelia N Pilichiewicz, Kate L Feltrin, Astrid J Smeets, Michael Horowitz, and Christine Feinle-Bisset (2010) Pooled-data analysis identifies pyloric pressures and plasma cholecystokinin concentrations as major determinants of acute energy intake in healthy, lean men.
American Journal of Clinical Nutrition, v. 92 (1), pp. 61-68, July 2010

Chapter 6: Gastric Emptying, Oro-caecal Transit, Blood Glucose, Gut Hormones, Appetite and Energy Intake Responses to a Nutrient Liquid Drink in Lean, Overweight and Obese Males

6.1 Summary

Signals arising from the gastrointestinal tract contribute to the suppression of appetite and energy intake, which are disturbed in obesity. Although many studies have addressed aspects of gastrointestinal function in the obese, many of these have substantial limitations, and there is a lack of studies that have evaluated gastric emptying and gastrointestinal hormone release in lean, overweight and obese individuals as well as previous patterns of nutrient intake concurrently. Gastric emptying is known to be a major determinant of postprandial blood glucose in healthy and type 2 diabetes. We evaluated the hypothesis that in healthy obese males, oral ingestion of a nutrient liquid will be associated with accelerated gastric emptying and oro-caecal transit, diminished GLP-1 and comparable GIP, secretion, attenuated suppression of appetite and energy intake in whom, habitual energy and fat intake will be greater, compared with lean and overweight individuals. We also hypothesised that the glycaemic response to the drink would be related to gastric emptying in the obese. Twenty lean, 20 overweight and 20 obese subjects were studied once during which time gastric emptying, intragastric distribution, oro-caecal transit and gastrointestinal hormone release were measured for 5 hours after ingestion of an oral mixed-nutrient

drink, Ensure® (500 ml [532 kcal]); energy intake at a buffet lunch was determined between t = 300 and 330 min and habitual energy intake was also quantified. There were no differences in gastric emptying, intragastric distribution or oro-caecal transit between the lean, overweight and obese groups. After the drink, blood glucose and plasma insulin were greater in the obese ($P < 0.05$ for both), when compared with both the lean and overweight groups; however, there were no differences in plasma GLP-1 or GIP concentrations, appetite and energy intake at the buffet meal or habitual energy intake between the groups. In the obese, the magnitude of the rise in blood glucose was inversely related to the gastric emptying T_{50} ($r = -0.55$, $P < 0.55$). This study concludes that: i) obesity per se, in the absence of differences in habitual energy intake, has no effect on gastric emptying or incretin hormone release; ii) gastric emptying influences postprandial blood glucose in the obese.

6.2 Introduction

Dietary patterns, particularly fat content, have been shown to modify gastrointestinal function and thus compromise appetite regulation. For example, in rats, exposure to a high fat diet is associated with attenuation of the suppressive effects of small intestinal fat on gastric emptying (Covasa et al. 2000a) and energy intake (Covasa and Ritter 1999). Further, although high fat feeding has been reported to increase the CCK response to small intestinal oleate (Spannagel et al. 1996), the effects of intraperitoneal administration of CCK-8 on gastric emptying (Covasa et al. 2000a) and energy intake (Covasa and Ritter 1998; Covasa et al. 2001) in rats are attenuated by high fat feeding, indicating that sensitivity to CCK is reduced by an excess dietary fat intake. A small number of short-term studies have investigated the effects of a high fat diet on

gastrointestinal function in humans, with inconsistent observations (Cunningham et al. 1991a; Castiglione et al. 2002; Boyd et al. 2003). Nonetheless, these studies suggest that sensitivity to the effects of fat on gastrointestinal function and energy intake is reduced by a high fat diet. For example, the slowing of gastric emptying by fat (Cunningham et al. 1991a), and the stimulatory effects of intraduodenal lipid infusion on pyloric pressures (Boyd et al. 2003), have been reported to be attenuated following a high fat diet.

There is evidence that human obesity is associated with an increased preference for the consumption of fatty foods, when compared with lean individuals, suggesting that fat intake is poorly regulated in this group (Mela and Sacchetti 1991; Miller et al. 1990). Thus, it is conceivable that gastrointestinal responses to nutrients are diminished in the obese; however, evidence is inconclusive and controversial. For example, gastric emptying in obesity has been reported to be similar (Tosetti et al. 1996), faster (French et al. 1993) and slower (Maddox et al. 1989) compared with lean humans. Studies have also reported reduced plasma PYY concentrations in response to a meal (le Roux et al. 2006) in obese compared with lean individuals. There is evidence that obesity is also associated with compromised nutrient sensing, particularly that of fatty acids, in both the oral cavity and small intestinal lumen (Stewart et al. 2011a). Obesity is well recognised as a risk factor for a number of disorders, perhaps most importantly type 2 diabetes (Ford et al. 1997; Resnick et al. 2000). Gastric emptying is known to be a major determinant of postprandial blood glucose in health and type 2 diabetics (Korosi et al. 2001; Ma et al. 2011; Schirra et al. 1996; Horowitz et al. 1993; Chaikomin et al.

2005; Jones et al. 1995; Vollmer et al. 2008) and relatively more rapid emptying may potentially predispose to the development of diabetes (Phillips et al. 1993).

The 'incretin' hormones, GLP-1 and GIP, account for the substantially greater incretin response to enteral, as opposed to an isoglycaemic, intravenous load (Creutzfeldt 2005) and accordingly, play a major role in blood glucose homeostasis. The 'incretin-effect' is diminished in type 2 diabetes (Bagger et al. 2011) at least in part because the insulinotropic capacity of GIP is markedly diminished (Nauck et al. 1993). There is limited, and inconsistent, information about GLP-1 (Feinle et al. 2002; Ranganath et al. 1996; Verdich et al. 2001a) and GIP (Elahi et al. 1984; Vilsboll et al. 2003) secretion in obesity.

While many studies have addressed specific aspects of gastrointestinal function in the obese, many of these have substantial limitations and there is a lack of studies that have evaluated changes in gastrointestinal function in lean, overweight and obese individuals as well as previous patterns of nutrient intake concurrently. Accordingly, the aim of this study was to evaluate the hypothesis that in healthy obese males, oral ingestion of a nutrient liquid will be associated with accelerated gastric emptying and oro-caecal transit, diminished GLP-1 and comparable GIP, secretion, and attenuated suppression of appetite and energy intake. We also hypothesised that the glycaemic response to a carbohydrate-containing drink would be dependent on the rate of gastric emptying in the obese.

6.3 Materials and methods

6.3.1 Subjects

A total of 60 adult males, including 20 lean (median age (range) 35 (19–60) years, median BMI (range) 23.4 (19.3–25) kg/m^2), 20 overweight (median age (range) 36 (21–60) years, median BMI (range) 27.5 (25.8–29.7) kg/m^2) and 20 obese (median age (range) 37 (22–59) years, median BMI (range) 34.5 (30.7–37.6) kg/m^2), were recruited according to guidelines described in Chapter 4 (section 4.2). Only males were included, as they may be more sensitive to dietary manipulation than are females (Rolls et al. 1994) and to avoid any influence of the menstrual cycle (Brennan et al. 2009).

6.3.2 Study protocol

Each subject was provided with a standardised meal (Beef lasagne, McCain Foods, Wendouree, Victoria, Australia), to be consumed on the evening prior to each study at 2000 h, and were instructed to fast overnight from solids and liquids thereafter before attending the laboratory at 0830 h. Once subjects arrived at the Department of Nuclear Medicine and Bone Densitometry, an intravenous cannula was inserted into a forearm vein for regular blood sampling. At t = –15 min, a baseline blood sample was taken and a VAS questionnaire, for the measurement of appetite perceptions, as described in Chapter 4 (section 4.6), was completed (Parker et al. 2004a). In order to establish anatomical reference points, two markers were taped onto the skin as described in Chapter 4 (section 4.4.2). After a 10 min baseline period (i.e. at t = –5 min), subjects ingested the test drink comprising 500 ml (532 kcal) of Ensure® (Abbott Australasia Pty Ltd, Botany, NSW, Australia) and labelled with 20 MBq ^{99m}Technetium sulphur colloid, as described in Chapter 4 (section 4.10.1). At t = 0 min, measurement of gastric

emptying commenced, as described in Chapter 4 (section 4.4.2.1). Radioisotopic images, 10 ml blood samples and VAS were obtained at t = 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 270 and 300 min. At t = 300 min, subjects were offered a cold buffet-style meal to consume freely for up to 30 min (t = 300–330 min) until comfortably full, as described in Chapter 4 (section 4.7.1). After completion of the meal, at t = 330 min, another blood sample was obtained and VAS questionnaire completed; subjects were then allowed to leave the laboratory.

6.3.3 Data analysis

6.3.3.1 Gastric emptying, intragastric distribution and mouth-to-caecum transit

From the acquired images, gastric emptying, intragastric distribution and oro-caecal transit were determined, as described in Chapter 4 (section 4.4.2.1).

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

Blood glucose, plasma GLP-1, GIP and insulin were measured on the blood samples, as described in Chapter 4 (sections 4.5.4, 4.5.5, 4.5.6 and 4.5.7).

6.3.3.3 Insulin resistance

The homeostasis model assessment (HOMA) was used to quantify insulin resistance (Matthews et al. 1985), as calculated from fasting glucose and insulin concentrations using the formula: Insulin resistance (HOMA) = (fasting insulin [mU/L] x fasting glucose [mmol/L]/22.5).

6.3.3.4 Appetite and energy intake

Perceptions of hunger and fullness were rated using a validated VAS questionnaire (Parker et al. 2004a), as described in Chapter 4 (section 4.6). Energy intake at the buffet meal was assessed, as described in Chapter 4 (section 4.7.1)

6.3.3.5 Habitual energy intake

Habitual energy intake and macronutrient distribution were quantified using validated dietary questionnaires, as described in Chapter 4 (section 4.7.2). Data from one overweight and two obese subjects could not be obtained, as there were errors detected in the completed dietary questionnaires from these subjects.

6.3.3.6 Statistical analysis

All data were analysed using SPSS version 17 (SPSS Inc, Chicago, Illinois, USA). Repeated-measures analysis of variance (ANOVA) was used to evaluate total, proximal and distal stomach, blood glucose, plasma hormones and insulin, and VAS scores with time as within-subject factor and group (lean, overweight and obese) as between-subject factor. AUCs (using the trapezoidal rule) were calculated for blood glucose, plasma hormone and insulin concentrations. One-way ANOVA was used to analyse gastric emptying (T_{50}), mouth-to-caecum transit, insulin resistance, AUC for blood glucose, plasma hormones and insulin, energy intake (kJ), amount eaten (g) and macronutrient distribution (%) from the buffet meal, and habitual energy intake (kJ) and macronutrient distribution (g and %) with group as a factor. *Post-hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects. Linear regression analysis was used to evaluate

relationships between gastric emptying, oro-caecal transit and blood glucose, plasma GLP-1, GIP and insulin. Statistical significance was accepted at $P < 0.05$.

6.4 Results

All subjects tolerated the study well, except one lean volunteer who experienced mild nausea soon after consumption of the test drink. The nausea was transient (the subject felt better within 30 min and continued with the study) and this subject was, accordingly, included.

6.4.1 Gastric emptying, intragastric distribution and oro-caecal transit

Gastric emptying was non-linear and approximated an overall monoexponential pattern. There was no difference in total stomach (see **Figure 6.1A**) and gastric emptying (T_{50}: lean: 85 ± 4; overweight: 77 ± 4; obese: 81 ± 7 min). Similarly, there was no difference in the amount of meal remaining in the proximal (see **Figure 6.1B**) or distal (see **Figure 6.1C**) stomach between the groups. There was also no difference in oro-caecal transit time (lean: 111 ± 11; overweight: 120 ± 15; obese: 86 ± 9 min) between the groups.

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

Blood glucose: There was no difference in baseline glucose concentrations (lean: 5.5 ± 0.1; overweight: 5.6 ± 0.2; obese: 5.9 ± 0.2 mmol/l) between groups (see **Figure 6.2A**). In all three groups there was a rise in blood glucose ($P < 0.001$) after the drink and blood glucose had returned to baseline by ~ t = 150 min. There was a significant group * time interaction for blood glucose concentrations ($P < 0.001$), so that blood glucose

was greater in the obese, compared with the lean between t = 45 and 90 min ($P < 0.05$) and, compared with the overweight between t = 30 and 45 min and at t = 75 min ($P < 0.05$) and tended to be greater at t = 60, 90 and 120 min (all $P \leq 0.08$), with no difference between the overweight and lean group. Peak blood glucose was 9.3 ± 0.5 mmol/L in the obese, 7.8 ± 0.3 mmol/L in the overweight and 8 ± 0.3 mmol/L in the lean ($P < 0.05$)

Plasma GLP-1: There was no difference in baseline GLP-1 concentrations (lean: 21.3 ± 1.7; overweight: 21.9 ± 1.7; obese: 24.7 ± 2.1 mmol/L) between groups (see **Figure 6.2B**). There was a significant time effect for plasma GLP-1 concentrations ($P < 0.001$). Plasma GLP-1 concentrations increased in all groups promptly following the drink. GLP-1 concentrations increased between t = 15 and 30 min and at t = 60 and 90 min in lean, between 15 and 90 min in overweight and between t = 15 and 30 min in the obese ($P < 0.05$ for all), with no difference between groups. In all three groups, plasma GLP-1 had reduced baseline by ~ t = 210 min.

Plasma GIP: There was no difference in baseline GIP concentrations (lean: 17.5 ± 1.7; overweight: 18.2 ± 1.3; obese: 20.9 ± 2.3 mmol/l) between groups. There was a significant time effect for plasma GIP concentrations ($P < 0.001$) (see **Figure 6.2C**). Plasma GIP concentrations increased in all groups following the test meal. GIP concentrations increased between t = 15 and 180 min in lean and obese and between 15 and 120 min in overweight ($P < 0.05$ for all) with no difference between groups. At t =

300 min there was no difference in plasma GIP concentrations from baseline in all groups.

Plasma insulin: There was a difference in baseline insulin concentrations (lean: 3.7 ± 0.4; overweight: 4.4 ± 0.5; obese: 10.7 ± 1.5 mmol/l) between groups ($P < 0.001$), so that baseline plasma insulin concentrations were greater in obese, when compared with both lean and overweight ($P < 0.001$ for both). There was a significant group * time interaction for insulin ($P < 0.001$) (see **Figure 6.2D**). Plasma insulin concentrations were greater in the obese, when compared with the lean between t = 0 and 300 min ($P < 0.01$) and compared with the overweight between t = 0 and 75 min and between t = 180 and 300 min ($P < 0.05$), with no difference between the overweight and lean group. In all thress groups plasma insulin had returned to baseline by ~ 180 min.

6.4.3 Insulin resistance

There were differences in the HOMA (lean: 0.9 ± 0.1; overweight: 1.1 ± 0.1; obese: 2.9 ± 0.5 min), between groups so that HOMA was greater in the obese compared with both the lean and overweight (both $P < 0.001$) with no difference between lean and overweight.

6.4.4 Appetite and energy intake

There was no difference in baseline hunger (lean: 37 ± 7; overweight: 46 ± 8; obese: 38 ± 5 mm) or fullness (lean: 12 ± 3; overweight: 15 ± 5; obese: 11 ± 5 mm), scores between groups. Hunger decreased ($P < 0.001$) and fullness increased ($P < 0.001$) after

the drink, with no differences between the groups (data not shown). There was an effect of time, but not group, on hunger and fullness ($P < 0.001$ for both) so that hunger increased and fullness decreased progressively in all groups (time effect: $P < 0.001$).

While there were no differences in energy intake (kJ) or the amount eaten (g) at the buffet meal (see **Table 6.1**), there was a difference on %energy consumed from fat and carbohydrate at the buffet meal ($P < 0.05$ for both) so that %energy from fat was slightly greater ($P < 0.05$) and %energy from carbohydrate was slightly less ($P < 0.05$), in the obese, compared with the lean and overweight group, with no difference between the overweight and obese or lean groups (see **Table 6.1**). There was no difference in %energy from protein consumed at the buffet meal between groups.

6.4.5 Habitual energy intake and macronutrients distribution

There were no differences in habitual energy intake or macronutrient distribution (g) between obese, overweight and lean subjects (see **Table 6.2**), although there was a trend for a difference in %energy from fat ($P = 0.058$) and carbohydrate ($P = 0.06$) between groups (see **Table 6.2**). Obese subjects tended to consume greater %energy from fat ($P = 0.052$) and carbohydrate ($P = 0.086$) than overweight subjects, with no difference between lean and obese or overweight. There was no difference in %energy from protein or between obese, overweight or lean subjects.

6.4.6 Relation between other variables with gastric emptying

There was no significant relationship between habitual energy intake with the gastric emptying T_{50} in any of the groups. There was a significant direct relationship between

the time to peak blood glucose concentrations and T_{50} with all three groups combined ($r = 0.73$, $P < 0.001$). There were inverse relationships between peak blood glucose concentrations and T_{50} in the lean ($r = 0.48$, $P < 0.05$) and obese ($r = –0.53$, $P < 0.05$), but not in the overweight group. There was a direct relationship between time for blood glucose concentrations to peak and T_{50} ($r = 0.73$, $P < 0.001$) and inverse relationships between magnitude of the rise in blood glucose at $t = 30$ ($r = –0.55$, $P < 0.05$) and $t = 45$ min ($r = –0.48$, $P < 0.05$) with T_{50}, in the obese group, but not in the lean or overweight group.

There was a significant relationship between magnitude of the rise in GLP-1 concentrations at $t = 30$ min and the T_{50} ($r = 0.55$, $P < 0.05$) in the obese, but not in the lean or overweight, group.

There was no relationship between magnitude of the rise in GIP concentrations with the T_{50} in any of the groups.

There were a direct relationship between rise in plasma insulin with rise in blood glucose at $t = 30$, 45, 60 and 75 min in the overweight and obese ($P < 0.05$ for all), but not in the lean group.

Table 6.1: Energy intake and macronutrient distribution at the buffet meal (t = 300 – 330 min) following ingestion of 500 ml (532 kcal) of Ensure® test drink in lean, overweight and obese subjects

	Lean	**Overweight**	**Obese**
Energy intake (kJ)	4717 ± 259	4722 ± 347	5387 ± 328
Amount eaten (g)	1053 ± 53	1116 ± 75	1105 ± 101
Fat (g)	41 ± 3	43 ± 4	51 ± 3
Carbohydrate (g)	127 ± 7	119 ± 8	131 ± 9
Protein (g)	58 ± 4	61 ± 5	70 ± 4
Fat (%)	32 ± 1	33 ± 1	36 ± 1*
Carbohydrate (%)	47 ± 2	44 ± 1	42 ± 3*
Protein (%)	21 ± 1	22 ± 1	23 ± 2

Data are means ± SEM (n = 20 lean, 20 overweight and 20 obese). * vs lean, $P < 0.05$.

Table 6.2: Habitual energy and macronutrient distribution in lean, overweight and obese subjects, quantified using validated dietary questionnaires

	Lean	**Overweight**	**Obese**
Energy intake (kJ)	9627 ± 712	10684 ± 1636	10564 ± 720
Fat (g)	99 ± 8	109 ± 20	116 ± 8
Carbohydrate (g)	246 ± 22	260 ± 37	242 ± 19
Protein (g)	109 ± 7	135 ± 24	133 ± 9
Fat (%)	37 ± 1	36 ± 2	40 ± 1
Carbohydrate (%)	42 ± 1	43 ± 2	38 ± 1
Protein (%)	20 ± 1	21 ± 1	21 ± 1

Data are means ± SEM (n = 20 lean, 19 overweight and 18 obese).

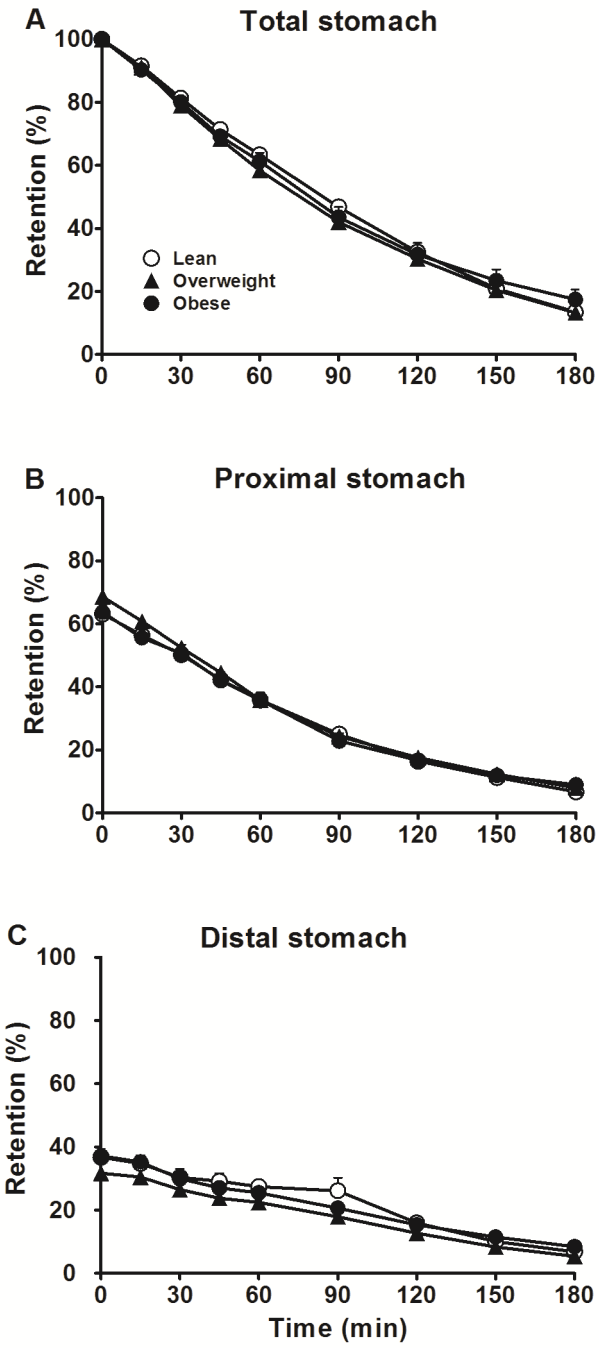


Figure 6.1: Total (A), distal (B) and proximal (C) stomach retention following oral ingestion of 500 ml (532 kcal) of Ensure® in lean, overweight and obese subjects. Repeated-measures ANOVA with time as factors was used to determine statistical difference. If ANOVAs revealed significant effects, pairwise comparisons were performed. Data are means ± SEM (n = 20 lean, 20 overweight and 20 obese).

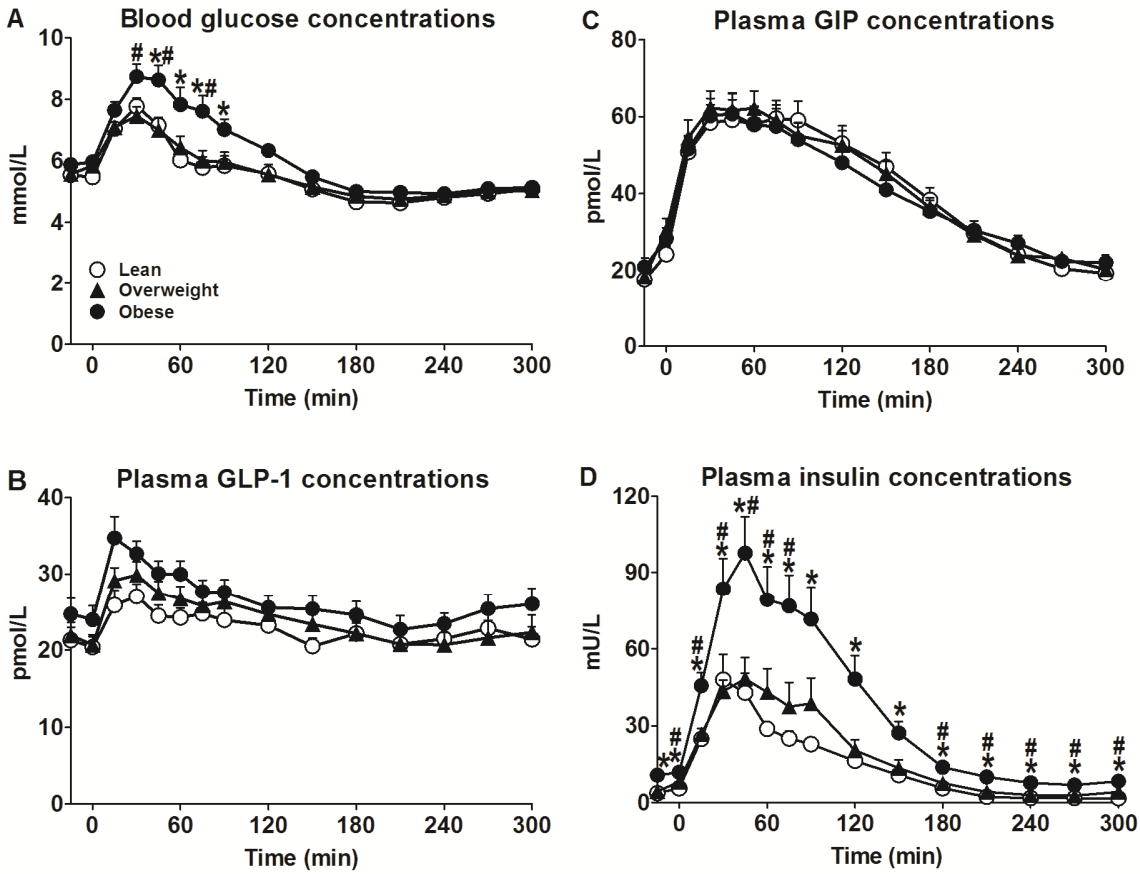


Figure 6.2: Blood glucose (A), plasma GLP-1 (B) GIP (C) and insulin (D) concentrations, following oral ingestion of 500 ml (532 kcal) of Ensure® in lean, overweight and obese subjects. Repeated-measures ANOVA with time as factors was used to determine statistical difference. If ANOVAs revealed significant effects, pairwise comparisons were performed. Data are means ± SEM (n = 20 lean, 20 overweight and 20 obese). * vs lean, $P < 0.05$; # vs overweight $P < 0.05$.

6.5 Discussion

This study has evauated a number of aspects of gastrointestinal function relevant to appetite regulation and glycaemic control in the obese—including gastric emptying, oro-caecal transit, gastrointestinal hormone release, appetite and energy intake—following ingestion of a high nutrient liquid in healthy lean, overweight and obese male humans. We found no differences in gastric emptying, oro-caecal transit, plasma GLP-1 or GIP, appetite or energy intake between the groups. Blood glucose and plasma insulin concentrations were presumably greater in the obese compared with the lean and overweight group, and the postprandial glycaemic response was shown to be related to gastric emptying in the obese.

Previous studies on the relationship between gastric emptying and body weight have yielded inconsistent observations, with gastric emptying being reported to be comparable (French et al. 1993; Zahorska-Markiewicz et al. 1986; Glasbrenner et al. 1993; Hutson and Wald 1993; Verdich et al. 2000), faster (Tosetti et al. 1996; Gryback et al. 1996; Wright et al. 1983; Näslund et al. 1998b) or slower (Maddox et al. 1989; Horowitz et al. 1983) in obese, when compared with lean humans. In most cases, the magnitude of reported differences was relatively modest. These inconsistent results may be attributable to, at least in part, differences in meal composition, time of day, methodologies used to assess gastric emptying, different criteria used in the selection of obese individuals or, perhaps most importantly, other factors that may affect gastric emptying, particularly changes in weight or the habitual diet of individual subjects (Cunningham et al. 1991a; Cunningham et al. 1991b), which were not quantified in the majority of studies. For example, there is evidence that the effects of fat on

gastrointestinal function are attenuated following consumption of a high fat diet so that, in healthy males, consumption of a high fat, hyper-caloric diet for 14 days was associated with marked acceleration of gastric emptying and mouth-to-caecum transit of a high fat solid test meal, when compared with a low fat diet (Cunningham et al. 1991a). In our study, there were no differences in habitual fat or energy intake between the lean, overweight and obese group; this could well account for the absence of any difference in gastric emptying between the groups.

Wright et al. (Wright et al. 1983) reported that gastric emptying of solids was more rapid in the obese group than in the non-obese controls, whereas no difference was seen in gastric emptying of a low nutrient liquid between the two groups. The physiological mechanisms regulating gastric emptying of liquid meals differ from that of solid meals. Gastric emptying of low nutrient liquids is dependent on fundic tone, whereas digestable solid gastric emptying is characterised by an initial lag phase, which reflects the transit of food from the fundus to the antrum and the time in which solid food is reduced to small particles by antral peristalsis. However, high nutrient liquids and semi-solids or solid meals empty from the stomach at comparable rates, after the lag phase, which is more prominent for solids (Horowitz and Dent 1991; Collins et al. 1991), as their emptying is dependent primarily on neural/humoral feedback arising from the interaction of nutrients with the small intestine (Lin et al. 1990; Little et al. 2006b; Meyer et al. 1998). Hence, our observations are likely to also apply to solid meals.

We assessed small intestinal transit by caecal arrival of the radioisotopically labelled meal, which is known to have limitations. Nevertheless, there was no suggestion of any difference between the groups. Previous studies have reported that post-prandial GLP-1 concentrations are reduced in the obese (Näslund et al. 1998b; Ranganath et al. 1996; Verdich et al. 2001a; Carr et al. 2010; Lugari et al. 2004), with no differences in GIP concentrations between lean and obese (Carr et al. 2010), whereas we found that GLP-1 and GIP concentrations in lean, overweight and obese subjects were comparable. GLP-1 and GIP secretion is highly dependent on the rate of small intestinal carbohydrate delivery in healthy (Pilichiewicz et al. 2007a), type 2 diabetic (Ma et al. 2011) and older subjects (Vanis et al. 2011). Moreover, in type 2 diabetic and older subjects, at a particular glucose load, GLP-1 and GIP concentrations are comparable to that of healthy young lean individuals. A major limitation of the studies referred to above (Pilichiewicz et al. 2007a; Ma et al. 2011; Vanis et al. 2011) is that gastric emptying was not quantified and our study shows that in obese and overweight subjects, GLP-1 and GIP secretion is similar to that of lean individuals. Hence, there is no evidence that a diminished incretin hormone response is associated with obesity and the predisposition to type 2 diabetes.

Consistent with other studies, blood glucose and plasma insulin concentrations were substantially higher in the obese compared with the lean and overweight group following oral ingestion and the obese subjects were insulin-resistant as assessed by HOMA. Postprandial blood glucose concentrations are known to be a major determinant of gastric emptying, which is affected by elevations of blood glucose within the physiological range, such that gastric emptying of both solids and liquids are slower

at blood glucose concentrations of 8 mmol/L compared with 4 mmol/L (Schvarcz et al. 1997). Hence, it remains possible that gastric emptying would have been relatively faster in the obese if they had been studied during euglycaemia. Our study confirms that in the obese, gastric emptying is a determinant of, as well as being determined by, the blood glucose concentrations, as is known to be the case in health (Pilichiewicz et al. 2007a; Horowitz et al. 1993; O'Donovan et al. 2004) and type 2 diabetes (Jones et al. 1995; O'Donovan et al. 2004; Pilichiewicz et al. 2003). We would, accordingly, speculate that more obese subjects who have relatively more rapid gastric emptying (within the normal range) are at greater risk for post-prandial hypoglycaemia, which should be normalised by 'short-acting' GLP-1 analogues such as exenatide (Edwards et al. 1999).

Surprisingly, we did not observe differences in appetite perceptions and energy intake between lean, overweight and obese groups in response to the drink. It should be recognised that in retrospect, the timing of the buffet meal at 5 hours was less than optimal given that gastric emptying was complete and glucose, GLP-1 and GIP concentrations had returned to baseline; however, there was also no difference in habitual energy or macronutrient intake between the groups, which was surprising. Accordingly, it would be of interest to study an obese group who did exhibit an increased energy and fat intake.

In conclusion, in the absence of differences in habitual energy intake: i) obesity has no effect on gastric emptying or incretin hormone release; and ii) gastric emptying is a determinant of postprandial blood glucose.

Chapter 7: Marked Differences in Gustatory and Gastrointestinal Sensitivity to Oleic Acid between Lean and Obese Men

Published: American Journal of Clinical Nutrition, 2011, 93(4), 703-711.

Jessica E Stewart, Radhika V Seimon, Bärbel Otto, Russell SJ Keast, Peter M Clifton and Christine Feinle-Bisset (2011) Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men.
American Journal of Clinical Nutrition, v. 93 (4), pp. 703-711, April 2011

Chapter 8: Effects of Acute Dietary Restriction on Gut Motor, Hormone and Energy Intake Responses to Duodenal Fat in Obese Men

Published: *International Journal of Obesity*, 2010, 1–9.

8.1 Summary

Previous patterns of energy intake influence gastrointestinal function and appetite, probably reflecting changes in small intestinal nutrient-mediated feedback. Obese individuals consume more fat and may be less sensitive to its gastrointestinal and appetite-suppressant effects than lean individuals. To evaluate the hypothesis that in obese individuals, the effects of duodenal fat on gastrointestinal motor and hormone function, and appetite would be enhanced by a short period on a VLCD. Eight obese males (BMI 34 ± 0.6 kg/m^2) were studied on two occasions, before (V1), and immediately after (V2), a four-day VLCD. On both occasions, APD motility, plasma CCK, PYY and ghrelin and appetite perceptions were measured during a 120-min intraduodenal fat infusion (2.86 kcal/min). Immediately afterwards, energy intake was quantified. During V2, basal pyloric pressure and the number and amplitude of IPPW were greater, while the number of antral and duodenal pressure waves were less, compared with V1 (all $P < 0.05$). Moreover, during V2, baseline ghrelin was higher, and the stimulation of PYY, and suppression of ghrelin, by lipid were greater, with no difference in CCK, and hunger and energy intake (kJ; V1: 4378 ± 691, V2: 3634 ± 700)

were less (all $P < 0.05$), compared with V1. In obese males, the effects of small intestinal lipid on gastrointestinal motility and some hormone responses and appetite are enhanced after a four-day VLCD.

8.2 Introduction

The prevalence of obesity has assumed enormous proportions; current projections from the WHO indicate that, in 2005, more than 400 million adults were obese worldwide, with numbers forecast to rise to 700 million by 2015 (Bray 2003). Current therapeutic interventions for the treatment of obesity are of limited efficacy (Kaplan 2005) and, with the exception of bariatric surgery, have largely ignored the pivotal role of the gastrointestinal tract in the regulation of appetite (Brennan et al. 2005; Sturm et al. 2004).

In health, the interaction of nutrients, including fat, with the small intestine has potent effects on gastrointestinal function. When infused at an energy load of 2–3 kcal/min, duodenal lipid slows gastric emptying, associated with suppression of antral and duodenal pressures waves and the stimulation of phasic and tonic pyloric motility (Pilichiewicz et al. 2007b; Cunningham et al. 1991a; Little et al. 2007a), and suppresses appetite and subsequent energy intake (Feinle et al. 2003; Feltrin et al. 2007). The effects are mediated, at least in part, by the release of a number of gastrointestinal hormones, including CCK, glucagon-like peptide-1 (GLP-1) and PYY, and the suppression of ghrelin (Beglinger et al. 2001; Batterham et al. 2002; Schirra et al. 2006; Wren et al. 2001b). Recent evidence suggests that changes in upper gut motility, particularly stimulation of the pylorus, may affect energy intake (Seimon et al. 2010).

Studies evaluating gastrointestinal function and appetite in the obese have yielded inconsistent information. A recent study in a large cohort found gastric emptying of both solids and liquids to be accelerated with increasing body weight (Vazquez Roque et al. 2006), while others have reported gastric emptying in the obese to be either similar (French et al. 1993) or slower (Maddox et al. 1989), when compared with lean subjects. The outcome of gut hormone measurements is also inconsistent, for example, some studies have reported lower fasting ghrelin (Vazquez Roque et al. 2006), higher fasting (Baranowska et al. 2000) and postprandial (French et al. 1993) plasma CCK, and lower postprandial PYY (le Roux et al. 2006), GLP-1 (Verdich et al. 2001a) and ghrelin (Cummings et al. 2001), concentrations, while others found no differences in PYY or GLP-1 (Vazquez Roque et al. 2006).

There is evidence that previous patterns of energy intake, both in excess and restriction and even when sustained for short periods, have the capacity to modify gastrointestinal function (Cunningham et al. 1991a; Corvilain et al. 1995; Nguyen et al. 2007), and this may be of particular relevance to the inconsistent observations from studies relating to gastrointestinal function in the obese given that previous nutrient intake has not been quantified. For example, in healthy subjects a two-week period on a high fat diet accelerates gastric emptying of a high fat meal (Cunningham et al. 1991a). In contrast, fasting appears to have the opposite effect, so that following a four-day fast, gastric emptying of glucose is slower in both lean and obese subjects (Corvilain et al. 1995). The nutrient deprivation of critical illness is associated with delayed gastric emptying and increased plasma CCK and PYY (Nguyen et al. 2007), with evidence that increased

small intestinal feedback contributes to the slowing of gastric emptying (Chapman et al. 2005).

We have now evaluated, in obese subjects, the effects of short-term energy restriction on antropyloroduodenal motility, plasma CCK, PYY and ghrelin concentrations, and appetite and energy intake, in response to administration of intraduodenal lipid. We hypothesised that acute energy restriction would increase the sensitivity of the small intestine to lipid, resulting in increased stimulation of pyloric pressures, increased stimulation of PYY, but greater suppression of ghrelin, as well as reduced appetite and energy intake.

8.3 Methods

8.3.1 Subjects

Ten obese males (aged 50 ± 1 (range 45–55) years; BMI 34 ± 0.6 (range 32–36) kg/m^2) were recruited according to guidelines described in Chapter 4 (section 4.2). One subject failed to adhere to the VLCD prescribed and was excluded from the study, and one withdrew for personal reasons; thus, eight subjects completed the study. Based on data derived from a pilot study in four obese subjects (within-subject standard deviation in energy intake: 700 kJ), we calculated that a mean difference in energy intake between visits of 800 kJ would be detectable with a sample size of eight subjects at 80% power and a Bonferroni adjusted significance level of 5%.

8.3.2 Study design

Each subject attended the laboratory on two occasions, once after an overnight fast (visit 1, day 1) and again following four days of a VLCD (visit 2, day 6. On both visits, the effects of a 120-min intraduodenal infusion of a lipid emulsion (10% Intralipid, Baxter Healthcare, Old Toongabbie, NSW, Australia), infused at 2.86 kcal/min, on APD motility, plasma CCK, PYY and ghrelin concentrations, appetite, and energy intake were quantified.

8.3.3 Very low calorie diet

The four-day VLCD involved a 70% reduction of each individual's energy intake as described in Chapter 4 (section 4.8.1).

8.3.4 Study protocol for visits 1 and 2

On each study day, subjects attended the laboratory at 0830 hours after fasting from solid and liquid food from 2200 hours the previous night. On the morning of each study day, subjects were intubated via an anaesthetised nostril, with a 16-channel manometric catheter as described in Chapter 4 (section 4.4.1). An intravenous cannula was inserted into a forearm vein for blood sampling.

Once the catheter was positioned correctly, at t = –15 min, a baseline blood sample was taken and a validated VAS questionnaire, assessing perceptions of appetite, as described in Chapter 4 (section 4.6), administered. At *t* = 0 min, intraduodenal infusion of the lipid emulsion commenced and was continued for 120 min. During the infusion, blood samples were obtained and VAS completed every 15 min between t = 0–90 min, and

again at t = 120 min. At *t* = 120 min, subjects were extubated and immediately offered a standardised, cold, buffet-style, meal to consume until comfortable full between (t = 120–150 min) as described in Chapter 4 (section 4.7.1). A final blood sample was collected and VAS completed following the meal (t = 150 min), after which the intravenous cannula was removed, and the subject allowed to leave the laboratory.

8.3.5 Measurements

8.3.5.1 Antropyloroduodenal pressures

Manometric pressures were digitised and recorded on a computer-based system and stored for subsequent analysis. APD pressures were analysed for i) the number and amplitude of pressure waves (PWs) in the antrum and duodenum; ii) basal pyloric pressure (pyloric 'tone'); iii) the number and amplitude of IPPW; and iv) PWS, as described in Chapter 4 (section 4.4.1.4).

8.3.5.2 Plasma hormone concentrations

Blood samples were collected for the measurement of plasma CCK, PYY and ghrelin, as described in Chapter 4 (sections 4.5.1, 4.5.2 and 4.5.3).

8.3.5.3 Appetite and energy intake

Hunger and fullness were assessed using validated VAS, as described in Chapter 4 (section 4.6). Energy intake was assessed as described in Chapter 4 (section 4.7.1).

8.3.6 Statistical analysis

Baseline values ('0') were calculated as the mean of values obtained at t = –15 and 0 min for plasma hormone concentrations and VAS, and between t = –15 to 0 min for the total number and mean amplitude of antral and duodenal PW, IPPW, basal pyloric pressures and total number of PWS. The number and amplitude of antral and duodenal PW were expressed as total, and mean, values, respectively, during the infusion period. IPPW and basal pyloric pressure were expressed as mean values of 15 min segments between 0 and 120 min (i.e. 0–15, 15–30, … , 105–120 min), and PWS as mean numbers of waves travelling over defined distances (i.e. over two (1.5 to < 3 cm), three (3 to < 4.5 cm), four (4.5 to < 6 cm), … , 15 (21 to < 22.5 cm) channels). For IPPW and basal pyloric pressures, peak values were also determined by identifying in each individual the peak number and amplitude of IPPW as well as peak basal pyloric pressure and then calculating mean values. To evaluate temporal differences in the responses during the infusion period, data were divided into two periods, that is, from t = 0–60 min and t = 60–120 min. IPPW, basal pyloric pressures, PWS, plasma hormones and VAS were analysed by repeated-measures ANOVA, with time (for PWS, distance of propagation) and visit as factors. Number and amplitude of antral and duodenal PW, and energy intake were analysed by one-way ANOVA. *Post-hoc* paired comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed when ANOVAs revealed significant effects. Statistical significance was accepted at $P < 0.05$, and data are presented as means ± SEM.

8.4 Results

All subjects completed the study and tolerated the experimental conditions well. The mean score for eating restraint was 5 ± 0.4 (range 3–7), that is, all were unrestrained eaters. The average 30% daily energy requirement was 3938 ± 63 kJ. Based on the dietary records maintained by the subjects, 100% compliance with the VLCD was achieved. There was no difference in body weight between visit 1 (102.9 ± 2.4 kg) and visit 2 (100.8 ± 2.2 kg).

8.4.1 Antropyloroduodenal pressures

Antral pressures: There was an effect of treatment on the number ($P < 0.05$), but not the amplitude, of antral PW (see **Table 8.1**). The number was much less on visit 2 (by ~ 69%), compared with visit 1.

Basal pyloric pressures: During visit 1, basal pyloric pressure increased in response to intraduodenal lipid until t = 30 min (time effect: $P < 0.05$), before decreasing to baseline levels by t = 120 min (see **Figure 8.1A**). During visit 2, basal pyloric pressure increased markedly until t = 45 min (time effect: $P < 0.01$), after which levels declined. Peak basal pyloric pressure was greater on visit 2 (13 ± 2 mmHg), when compared with visit 1 (8 ± 2 mmHg) ($P < 0.05$). There was an effect of treatment on basal pyloric pressure ($P < 0.05$). Between t = 0–60 min, basal pyloric pressure was greater during visit 2, compared with visit 1 ($P < 0.01$), while there was no difference between t = 60–120 min, although mean values were higher during visit 2.

Isolated pyloric pressures: The number of IPPW increased in response to lipid until t = 45 min during visit 1, and until t = 30 min during visit 2 (time effect: $P < 0.01$ for both), and subsequently declined gradually (see **Figure 8.1B**). Peak number of IPPW was greater on visit 2 (37 ± 3 / 15 min), when compared with visit 1 (28 ± 2 / 15 min) ($P < 0.05$). There was an effect of treatment on the number of IPPW ($P < 0.05$). Between t = 0–60 min, the number of IPPW was greater during visit 2, when compared with visit 1 ($P < 0.05$), while there was no difference between t = 60–120 min, although mean values were higher during visit 2.

In response to lipid the amplitude of IPPW rose until t = 45 min during visit 1, and until t = 30 min during visit 2 (time effect: $P < 0.01$ for both); the responses then declined gradually (see **Figure 8.1C**). The peak amplitude of IPPW was greater on visit 2 (63 ± 7 mmHg), when compared with visit 1 (51 ± 6 mmHg) ($P < 0.05$). There was an effect of treatment on the amplitude of IPPW ($P < 0.01$). Between t = 0–60 min, the amplitude of IPPW was higher during visit 2, when compared with visit 1 ($P < 0.05$), while there was no difference between t = 60 and 120 min, although mean values were higher during visit 2.

Duodenal pressures: There was an effect of treatment on the number ($P < 0.05$), but not the amplitude, of duodenal PW (see **Table 8.1**). The number was less on visit 2 (by ~ 36%), when compared with visit 1.

PWSs: Only PWS that spanned 2–6 channels (1.5–9 cm) were analysed statistically, as PWS spanning 7–15 channels were infrequent (no / 120 min: visit 1, 6 ± 1; visit 2, 2 ±

1). There was an effect of treatment on the number of PWS travelling over two (i.e. 1.5 < 3 cm), three (i.e. 3 < 4.5 cm), four (i.e. 4.5 < 6 cm) and five (i.e. 6 < 7.5 cm) ($P < 0.05$ for all), channels, which were substantially less on visit 2 when compared with visit 1 (see **Figure 8.2**).

8.4.2 Gastrointestinal hormone concentrations

Plasma CCK: There was no difference in CCK concentrations at baseline, or in response to lipid, between visit 1 and visit 2 (see **Figure 8.3A**). During both visits, plasma CCK concentrations increased in response to lipid until t = 30 min (time effect: $P < 0.001$ for both), after which levels decreased and then plateaued. Immediately after the buffet meal (i.e. t = 150 min), plasma CCK concentrations were lower on both visit 1 and visit 2 ($P < 0.05$ for both), when compared with pre-meal concentrations (i.e. t = 120 min), with no difference between visits.

Plasma PYY: There was no difference in baseline PYY concentrations between visit 1 and visit 2 (see **Figure 8.3B**). There was a treatment-by-time interaction for plasma PYY concentrations ($P < 0.05$). During both visits, plasma PYY concentrations increased in response to lipid across the entire infusion period (time effect: $P < 0.01$ for both). The magnitude of the rise was greater during visit 2, so that plasma PYY was higher between t = 90 and 120 min, when compared with visit 1 ($P < 0.05$). Immediately after the buffet meal, there was no difference in plasma PYY, when compared with pre-meal concentrations, during either visit 1 or visit 2.

Plasma ghrelin: There was an effect of treatment on baseline ghrelin concentrations ($P < 0.001$), which were higher on visit 2 when compared with visit 1 (see **Figure 8.3C**). There was a treatment-by-time interaction for plasma ghrelin concentrations ($P < 0.001$). During visit 1, plasma ghrelin decreased, albeit very slightly, between t = 30 and 120 min, while during visit 2, plasma ghrelin decreased steadily across the entire infusion period (time effect: $P < 0.05$). The magnitude of the decrease was greater during visit 2, so that plasma ghrelin was higher between t = 0–60 min ($P < 0.05$), when compared with visit 1, but there was no difference between visits immediately prior to the buffet meal. Immediately after the buffet meal, there was no difference in plasma ghrelin, when compared with pre-meal concentrations, during either visit 1 or visit 2.

8.4.3 Gastrointestinal perceptions

There was an effect of treatment on baseline scores for hunger ($P < 0.001$), which were higher on visit 2 when compared with visit 1 (see **Figure 8.4A**). There was a treatment-by-time interaction for hunger ($P < 0.001$). During visit 1, hunger increased slightly, while during visit 2, hunger declined gradually during the entire infusion period (time effect: $P < 0.05$). Hunger was greater during visit 2 between t = 0–60 min ($P < 0.05$), when compared with visit 1, but there was no difference between visits between t = 75–120 min.

There were no differences in scores for fullness, bloating (data not shown) or nausea (see **Figure 8.4B**) between visits.

8.4.4 Energy intake

There was an effect of treatment on both the amount eaten (g) and energy intake (kJ) at the buffet meal ($P < 0.05$ for both) (see **Table 8.2**). Both were less on visit 2 when compared with visit 1, by 8% and 17%, respectively.

Table 8.1: Total number and mean amplitude of antral and duodenal pressure waves during 120-min intraduodenal infusion of 10% Intralipid® (2.86 kcal/min) before (visit 1) and after (visit 2) a four-day VLCD [2].

	Visit 1	**Visit 2**	**Δ(Visit 2-Visit 1)**
Antral pressure waves			
Number	87 ± 29	27 ± 11*	–60 ± 23
Amplitude (mmHg)	26 ± 2	20 ± 4	–6 ± 4
Duodenal pressure waves			
Number	374 ± 91	238 ± 66*	–135 ± 52
Amplitude (mmHg)	25 ± 2	25 ± 2	–1 ± 2

[1]Data are mean ± SEM (n = 8).

[2]One-way ANOVA was used to determine statistical difference. * $P < 0.05$ vs visit 1.

Table 8.2: Energy intake at a buffet meal immediately following 120-min intraduodenal infusion of 10% Intralipid® (2.86 kcal/min) before (visit 1) and after (visit 2) a four-day VLCD [2].

	Visit 1	**Visit 2**	**Δ(Visit 2-Visit 1)**
Amount eaten (g)	1022 ± 114	943 ± 115*	–69 ± 64
Energy intake (kJ)	4378 ± 691	3634 ± 700*	–744 ± 302
Energy (%)			
Fat	35 ± 2	33 ± 4	–3 ± 3
Carbohydrate	44 ± 2	44 ± 5	–1 ± 4
Protein	22 ± 2	24 ± 2	2 ± 1

[1]Data are mean ± SEM (n = 8).

[2]One-way ANOVA was used to determine statistical difference. * $P < 0.05$ vs visit 1.

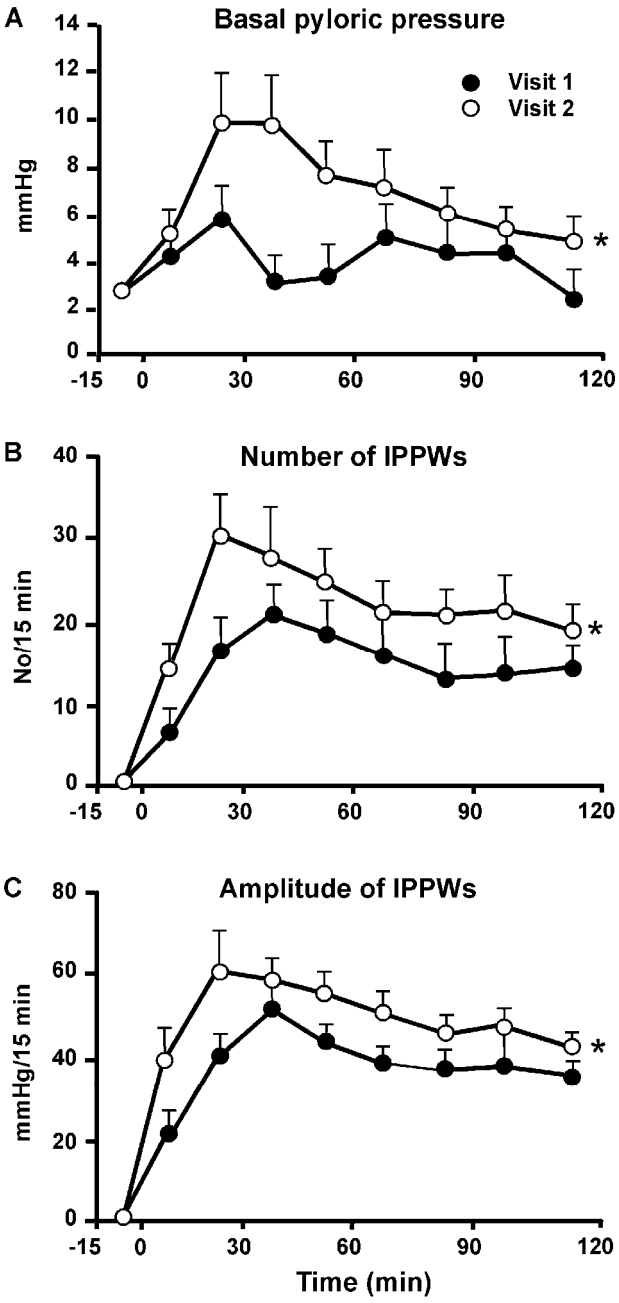


Figure 8.1: Basal pyloric pressure (**A**) and number (**B**), and amplitude (**C**), of IPPW during 120-min intraduodenal infusion of 10% Intralipid® (2.86 kcal/min) before (visit 1) and after (visit 2) a four-day VLCD. * $P < 0.05$ vs visit 1. Data are mean ± SEM (n = 8). *Please, note that the data shown in this figure refer to means at defined time points, while the peak data reported in the text are based on actual peak values in individuals, which did not necessarily occur at the same time points across individuals, thus the maximum values in the figure do not reflect actual peak values.

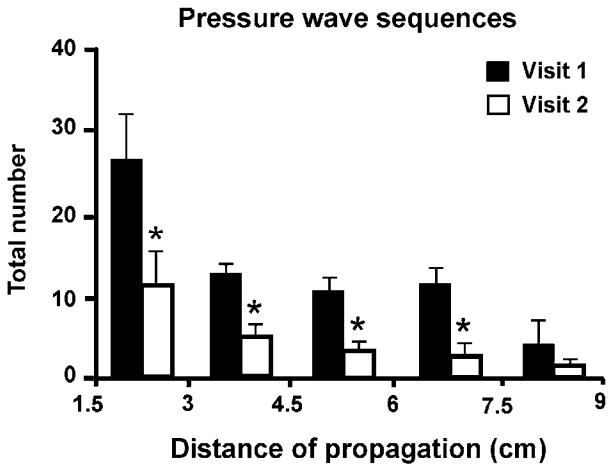


Figure 8.2: Number of PWS during 120-min intraduodenal infusion of 10% Intralipid® (2.86 kcal/min) before (visit 1) and after (visit 2) a four-day VLCD. * $P < 0.05$ vs visit 1. Data are mean ± SEM (n = 8).

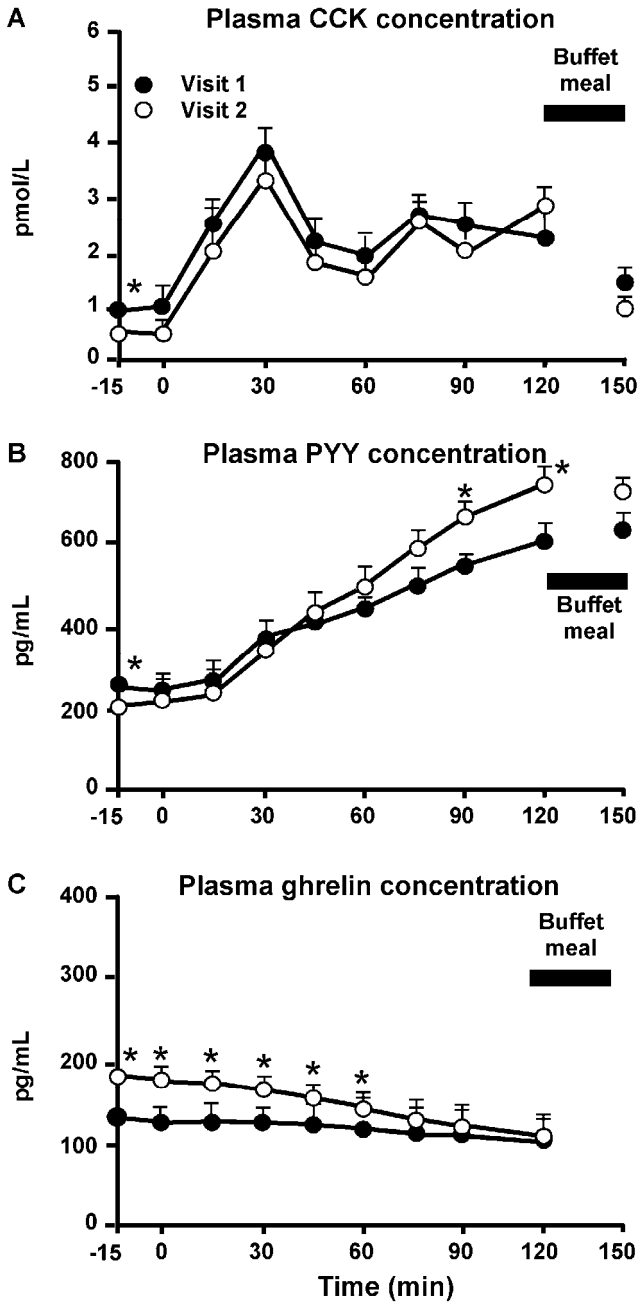


Figure 8.3: Plasma CCK (**A**), PYY (**B**) and ghrelin (**C**) during 120-min intraduodenal infusion of 10% Intralipid® (2.86 kcal/min) before (visit 1) and after (visit 2) a four-day VLCD. * $P < 0.05$ vs visit 1. Data are mean ± SEM (n = 8).

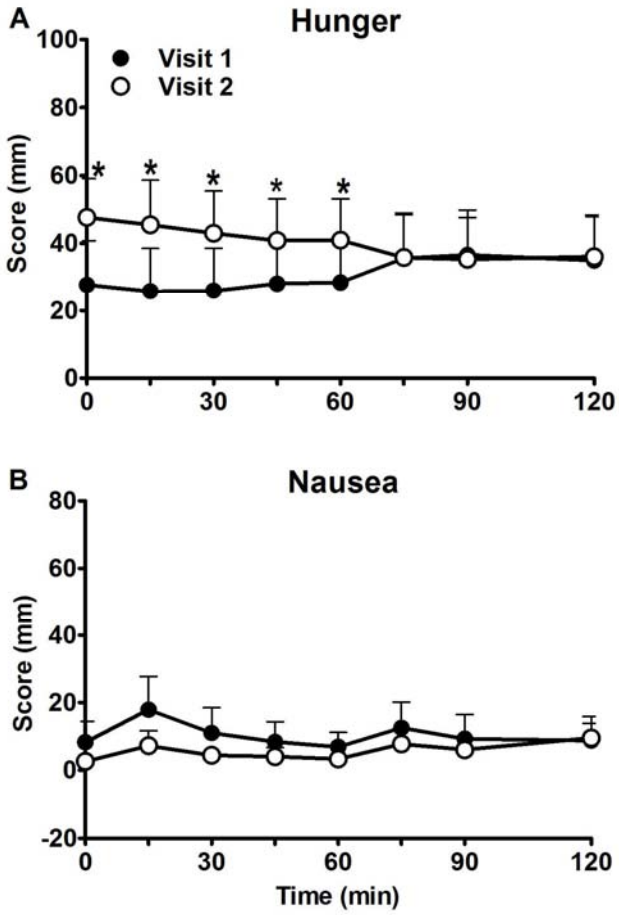


Figure 8.4: Scores for hunger (**A**) and nausea (**B**) during 120-min intraduodenal infusion of 10% Intralipid® (2.86 kcal/min) before (visit 1) and after (visit 2) a four-day VLCD. * $P < 0.05$ vs visit 1. Data are mean ± SEM (n = 8).

8.5 Discussion

This study evaluated the effects of a four-day VLCD on APD motor, gastrointestinal hormone, appetite and energy intake responses to an intraduodenal lipid infusion in obese males. Arguably the most important, and novel, finding of our study is that, within the short period of four days, a VLCD increases the 'sensitivity' to small intestinal fat in obese individuals, associated with enhanced modulation of gastrointestinal motor and hormone function and potentiation of the suppression of hunger and energy intake.

A number of modifications in gastrointestinal function have been described in the obese (French et al. 1993; Baranowska et al. 2000; le Roux et al. 2006; English et al. 2002; Vazquez Roque et al. 2006), which may potentially be attributable to gastrointestinal adaptation to high nutrient exposure. Whether such changes are reversible in response to energy restriction, has not been investigated in detail, but is conceivable. That a four-day fast slowed gastric emptying of glucose in both lean and obese subjects (Corvilain et al. 1995), suggests that acute dietary restriction is associated with adaptive changes in the mechanisms responsible for the feedback slowing of gastric emptying. Our data relating to gastrointestinal motility support this hypothesis. Following acute energy restriction, the stimulation of tonic and phasic pyloric pressures and the suppression of antral and duodenal pressures by small intestinal lipid were markedly greater. These motor events underlie the slowing of gastric emptying induced by small intestinal lipid (Heddle et al. 1989), consistent with the reported slowing of gastric emptying following a four-day fast (Corvilain et al. 1995). Moreover, our recent study (Seimon et al. 2010)

identified the magnitude of peak pyloric stimulation in response to duodenal nutrients as an independent predictor of subsequent energy intake.

PYY and ghrelin, but not CCK, responses were modified following acute dietary restriction. While baseline PYY concentrations were unchanged, its release in response to the lipid infusion was increased following the four-day fast. PYY is released from the distal small intestine, indirectly via CCK (Verdich et al. 2000), or directly by the interaction of nutrients with PYY-releasing L-cells in the distal small intestine (Aponte et al. 1985). Since CCK concentrations were unchanged, it would suggest that either CCK was not involved in enhancing PYY release, or the sensitivity to the actions of CCK was enhanced, following the four-day diet. The difference in PYY concentrations between the two study days was evident between 90–120 min, that is, at a time when some lipid would almost certainly have reached the distal small intestine. In this context, it is interesting to note that, following the four-day diet, the number of PWS in response to lipid was less, suggesting that small intestinal transit, which we did not measure, was likely to be slowed, potentially as an effect of elevated PYY (Spiller et al. 1988). Interestingly, despite this, PYY release was enhanced, supporting the hypothesis that the sensitivity of the small intestine to nutrients was enhanced by the dietary intervention. Plasma ghrelin concentrations are high in the fasting state and suppressed following meal ingestion (Cummings et al. 2001) or direct small intestinal lipid infusion (Feinle-Bisset et al. 2005), with the latter suggesting that ghrelin suppression arises from the small intestine. In obesity, fasting ghrelin concentrations have been reported to be reduced, and postprandial suppression may (English et al. 2002) or may not (Cummings et al. 2002) be absent. In our study, lipid-induced ghrelin suppression prior

to the four-day diet was minimal. In contrast, both fasting ghrelin concentrations and the magnitude of suppression during the lipid infusion were greater after the four-diet diet, suggesting that even a short period of dietary restriction can modulate ghrelin release towards normality. Not unexpectedly, the higher fasting ghrelin concentrations were associated with increased hunger scores, and both fell in parallel during the lipid infusion to levels not different from those before the VLCD. Taken together, it appears that of absolute plasma hormone concentrations, only PYY can be implicated in the observed reduction in energy intake.

The mechanisms that mediate the gastrointestinal and appetite responses to a VLCD are unknown. Studies in rats have demonstrated that both myenteric neuronal and vagal afferent activation, as measured by Fos-like immunoreactivity in the dorsal hindbrain and the myenteric plexus, in response to small intestinal oleate, are diminished after a period on a high fat diet, compared with an isocaloric low fat diet (Covasa et al. 2000a). Further, the high fat diet reduced the expression of Fos-like immunoreactivity in the area postrema and the nucleus of the solitary tract in response to exogenous CCK (Covasa et al. 2000b), suggesting that, in response to a high fat diet, adaptations occur in the transmission processes involved in conveying luminal signals to the brain, associated with a reduced sensitivity to small intestinal nutrients. Thus, it is likely that changes in the opposite direction occur in response to dietary restriction.

The focus of our study was to determine whether acute dietary restriction can modify gastrointestinal functions associated with energy intake reduction. If these changes were to be sustained over a prolonged period, substantial weight loss would be

expected. However, it is also possible that, during a longer period of energy restriction, adaptive mechanisms develop, which aim to preserve energy stores. For example, resting energy expenditure is reduced with weight loss, even after adjustment for loss of lean and fat mass (Heilbronn et al. 2006). Whether adaptive changes in gastrointestinal function occur during prolonged weight loss periods accordingly warrants evaluation.

In interpreting our data, it is important to consider the experimental design of the study. We administered the lipid emulsion directly into the duodenum, as our primary focus was to identify potential changes in small intestinal sensitivity to lipid. As a result, we bypassed potential gustatory and gastric mechanisms and cannot comment on any effect(s) on gastric emptying. These issues should be addressed in subsequent studies. Only one dose of intraduodenal lipid, one type of caloric restriction and one period of dietary restriction were employed. Hence, the effects of higher, or lower, lipid loads, other macronutrients, and other degrees of energy restriction and duration, are uncertain. The order of the study days was not randomised; however, we have demonstrated, that our test meal used to evaluate energy intake, does so reliably when administered repeatedly under identical study conditions (Nair et al. 2009), and the observed differences in the current study were substantial. Only male volunteers were included to avoid any confounding effects of the menstrual cycle (Brennan et al. 2009) and, accordingly, our observations may not be applicable to females, albeit unlikely. Finally, our study did not include a control arm to exclude the possibility that the observed effects may reflect habituation to the test procedures or a time effect. However, this is highly unlikely given that the parameters measured have been shown to be highly reproducible in the short term in healthy subjects (Nair et al. 2009), the

differences in the responses between study days were substantial and comparable in magnitude to the responses observed in our previous studies comparing the effects of intraduodenal nutrients with control infusions (Brennan et al. 2007; Riepl et al. 1996; Seimon et al. 2009b), and a previous study showed that short-term (four-day) starvation in obese subjects had no effect on gastric emptying of saline, while gastric emptying of glucose was slowed substantially (Corvilain et al. 1995).

Chapter 9: Effects of Acute and Longer-Term Dietary Restriction On Antropyloroduodenal Motility, Gut Hormones, Appetite and Energy Intake Responses To Duodenal Lipid, and on Oral Fat Perception, in Lean and Obese Males

9.1 Summary

Short-term dietary restriction enhances gastrointestinal sensitivity to nutrients, and thereby, suppresses energy intake. Whether these acute changes are sustained in the longer term is currently unknown. We aimed to evaluate the hypotheses that: i) acute (four days) energy restriction enhances the effects of intraduodenal fat, on gastrointestinal function and appetite, in lean and obese subjects, and ii) following prolonged (12 weeks) energy restriction, associated with weight loss, these effects of acute energy restriction on gastrointestinal function and appetite would be lost in the obese. Twelve obese and 12 lean males participated in the study. The obese group were studied on four occasions, before (visit 1) and after four days (visit 2), four weeks (visit 3) and 12 weeks (visit 4) on a 30% energy-restricted diet, while the lean group were studied on two occasions, before and after four days on a 30% energy-restricted diet. On all occasions, APD pressures, plasma gastrointestinal hormones and appetite were measured during 120-min intraduodenal fat infusion (rate: 2.86 kcal/min). Energy intake was determined immediately afterwards at a buffet lunch. After acute energy

restriction (visit 2) there were no changes in APD motility in either group. Fasting and postprandial plasma CCK concentrations were reduced (both $P < 0.05$), and fasting and postprandial ghrelin increased (both $P < 0.05$) in lean, but not obese, desire to eat and hunger was greater in both groups (both $P < 0.05$), with no difference in energy intake. After prolonged energy restriction, there were no changes in APD motility or hormone release, desire to eat and hunger were greater at visit 3, and amount eaten reduced at visit 3 and 4, compared to visit 1 (all $P < 0.05$), with no difference in energy intake. A 30% energy-restricted diet diminishes gastrointestinal hormone responses in lean, but not obese, which may suggest that obese are less sensitive to this caloric restriction and that a greater energy restriction may be necessary in obese to observe changes in gastrointestinal function.

9.2 Introduction

Obesity occurs, in the broadest sense, as a result of energy intake exceeding energy expenditure, with the most common cause being the increased availability and overconsumption of high fat, energy-dense foods (Mela and Sacchetti 1991; Miller et al. 1990). Dietary management remains the most common approach to obesity. However, despite continued adherence to weight loss diets, body weight often stabilises over time, or even increases. This may, at least in part, be due to an adaptive response to reduced energy availability. For example, during dietary restriction, there is a fall in basal metabolic rate (Leibel et al. 1995; Elliot et al. 1989), resulting in reduced energy requirements and an increase in hunger (Doucet et al. 2003; Anton et al. 2009).

Gastrointestinal factors play an important role in the regulation of appetite and acute energy intake (Kissileff et al. 1981; Batterham et al. 2002; Seimon et al. 2010). The presence of nutrients in the small intestine has potent effects on gastrointestinal motor (Heddle et al. 1988b) and hormonal function (Pilichiewicz et al. 2007b; Little et al. 2007a), and these, particularly the stimulation of pyloric motility and CCK release, are major determinants of the suppression of further energy intake (Seimon et al. 2010). There is evidence, albeit inconsistent, that gastrointestinal motor and hormonal functions are disturbed in the obese. For example, studies have reported accelerated (Vazquez Roque et al. 2006), normal (Verdich et al. 2000), and delayed (Jackson et al. 2004) gastric emptying and lower fasting and postprandial PYY and GLP-1 concentrations (le Roux et al. 2006) in obesity, suggesting reduced sensitivity in obese, when compared with lean individuals. Obese individuals have also been reported to have higher dietary energy and fat intakes (Mela and Sacchetti 1991; Miller et al. 1990); therefore, it is likely that obese individuals could be 'desensitised' to the gastrointestinal effects of nutrients, resulting in greater capacity for energy intake.

Previous patterns of energy intake, in excess or restriction, have the capacity to modify gastrointestinal function (Cunningham et al. 1991a; Corvilain et al. 1995; Nguyen et al. 2007; Chapman et al. 2005). There is evidence that short-term (four days) dietary restriction enhances sensitivity to nutrients, which is associated with increased gastrointestinal responses and reduced energy intake. For example, short-term (four days) fasting slows gastric emptying of glucose in both lean and obese individuals (Corvilain et al. 1995). In another study, following 70% energy restriction (four-day very low calorie diet) an intraduodenal lipid infusion had significantly greater effects on

the stimulation of pyloric pressure and PYY, as well as more potent suppression of ghrelin, and this was associated with an enhanced suppressive effect of lipid on appetite perceptions and energy intake (Brennan et al. 2010). Taken together, these studies suggest that acute caloric restriction enhances sensitivity to the actions of intestinal nutrients. It is currently unknown whether a more moderate and sustainable dietary regimen (~ 30% energy restriction) has similar effects on gastrointestinal function and energy intake, nor if these effects are sustained over a longer-term period (Sumithran et al. 2011).

Given that body weight stabilises following long-term dietary restriction, it is likely that, over time, adaptive changes occur in gastrointestinal factors that influence energy intake. For example, while both fasting PYY (Roth et al. 2005), and postprandial GLP-1 (Verdich et al. 2001a), secretion have been reported to increase following prolonged dietary restriction (three to six months) in obese subjects, the levels were less, when compared with lean individuals. Further, both fasting and postprandial ghrelin levels are increased by weight loss (Cummings et al. 2002). These changes could potentially reduce dietary compliance as they may lead to increased hunger and contribute to the reduction in the effect of dietary restriction on weight loss occurring during long-term (~ three months) energy restriction. However, no studies have evaluated the effects on gastrointestinal function over a longer period comprehensively and related these to energy intake and weight loss, in obese subjects.

Therefore, the aims of this study were to evaluate the hypotheses that acute energy restriction (four days) enhances the effects of duodenal nutrients, on gastrointestinal

function and appetite, in lean and obese subjects, while following prolonged energy restriction (12 weeks), the effects of energy restriction on gastrointestinal function and appetite would be lost in obesity, associated with weight loss.

9.3 Methods

9.3.1 Subjects

Twelve healthy lean (median age [range] 44 [35–60] years, median BMI [range] 23 [20.9–25.7] kg/m^2) and 12 obese (median age [range] 49 [24–59] years, median BMI [range] 32.6 [30.9–37.6] kg/m^2), but otherwise healthy, males were recruited according to guidelines described in Chapter 4 (section 4.2). Only males were included, as they have been reported to be more sensitive to dietary manipulation than females (Rolls et al. 1994).

9.3.2 Study outline

The study evaluated the effects of a four-day 'acute' (in lean and obese subjects) and a 12-week 'prolonged' (in obese subjects only), 30% energy restriction on gastrointestinal motility, hormone release, appetite and energy intake responses to a 120-min intraduodenal infusion (2.86 kcal/min) of a long-chain triglyceride emulsion (10% Intralipid®, 300 mOsmol/kg, 1.1 kcal/mL, Fresenius Medical Care Australia Pty Ltd, Smithfield, NSW, Australia) and on body weight.

9.3.3 Determination of energy requirements and diet plans

To assess habitual diet and determine energy requirements, subjects completed a weighed food diary over five consecutive days (three weekdays and two weekend days) prior to study commencement as described in Chapter 4 (section 4.7.3.2).

9.3.4 Dietary intervention

Lean subjects underwent a four-day period of dietary restriction (to avoid significant weight loss), while obese subjects underwent a 12-week period of dietary restriction that would be associated with weight loss. Dietary restriction entailed a 30% reduction of total energy intake, using a macronutrient-balanced diet as described in Chapter 4 (section 4.8.2).

9.3.5 Study protocol

During the dietary intervention period (see **Figure 9.1**), obese subjects attended the laboratory on four occasions, that is, day 0, before starting the diet (visit 1), day 5 (visit 2), day 29 (visit 3) and day 85 (visit 4), to evaluate the effects of acute (visit 2) and prolonged (visits 3 and 4) dietary restriction, while lean subjects attended the laboratory on two occasions, that is, on day 0 (visit 1) and day 5 (visit 2), to evaluate the effects of acute dietary restriction on gastrointestinal function and energy intake in response to duodenal nutrients. In addition, obese subjects underwent fortnightly counselling sessions on days 13, 27, 41, 55 and 69. Following the 12-week period of dietary restriction, obese subjects received standardised, and structured, advice on how to continue with a balanced diet, as outlined by Australian Healthy eating guidelines (**Appendix IX**).

9.3.6 Study day protocol for visits 1–4

Subjects were provided with a standardised meal (Beef lasagne, McCain Foods, Wendouree, Victoria, Australia), for dinner on the evening prior to each study. On each of the study visits 1–4, subjects attended the laboratory, at 08.30 hours after an overnight fast (14 hours from solids and liquids). A silicone rubber manometry catheter incorporating 16 channels (Dentsleeve International Ltd, Ontario, Canada) was inserted through an anaesthetised nostril as described in Chapter 4 (section 4.4.1). An intravenous cannula was then inserted into a forearm vein for regular blood sampling.

Once the catheter was positioned correctly, at t = –15 min, a baseline blood sample was taken and the subject completed a VAS questionnaire, for the assessment of appetite perceptions (Parker et al. 2004b), as described in Chapter 4 (section 4.6). At t = 0 min, duodenal infusion of lipid (rate: 2.86 kcal/min, reflecting the average rate of gastric emptying in humans) commenced for 120 min as described in Chapter 4 (section 4.10.2.1). During the infusion, 10 ml blood samples were collected, and VAS completed, every 15 min for the first 60 min (i.e. 15, 30, 45 and 60 min) and then at t = 90 and 120 min. At t = 120 min, the infusion ceased and the subject was extubated and offered a cold, buffet-style meal to consume until comfortably full (between t = 120 and 150 min) as described in Chapter 4 (section 4.7.1). After ingestion of the meal, at t = 150 min, another blood sample was taken and VAS completed. Thereafter, the subject was allowed to leave the laboratory.

9.3.7 Data analysis

9.3.7.1 Antropyloroduodenal pressures

APD pressures were digitised and recorded on a computer-based system, and analysed for i) number and amplitude of antral pressure waves (PWs); ii) number and amplitude of IPPW; iii) basal pyloric pressure; and iv) number and amplitude of duodenal PW, as described in Chapter 4 (section 4.4.1.4).

9.3.7.2 Gastrointestinal hormone concentrations

Blood samples were collected for the measurement of plasma CCK, PYY and ghrelin, as described in Chapter 4 (section 4.5.1, 4.5.2 and 4.5.3). The results of the PYY analysis are not yet available.

9.3.7.3 Appetite, energy intake and habitual dietary intake

Appetite perceptions, including hunger, fullness and desire to eat, were assessed using a validated VAS questionnaire (Parker et al. 2004b), as described in Chapter 4 (section 4.6). Energy intake in response to intraduodenal lipid was quantified from the amount eaten at the buffet meal, as described in Chapter 4 (section 4.7.1). Habitual dietary intake was assessed using five-day diet diaries, as described in Chapter 4 (section 4.7.3.2).

9.3.8 Statistical analysis

All data were analysed using SPSS version 17 (SPSS Inc, Chicago, Illinois, USA). For missing data (for two subjects, data from t = 60 and 75 min after commencement of the

lipid infusion) the last measured data value was carried forward. Baseline values for VAS and hormone concentrations were calculated as the means of values at t = –15 min and t = 0 min. Baseline values for basal pyloric pressures and IPPW were obtained from the means of values between t = –15 and 0 min. During the 120-min infusion period, antral and duodenal PW were expressed as total numbers and mean amplitudes, which were used to calculate the MI (Camilleri and Malagelada 1984), while IPPW were expressed as total number and amplitude and basal pyloric pressures were expressed as AUCs over the 120-min infusion period. AUCs were calculated (using the trapezoidal rule) amplitude of IPPW and basal pyloric pressures.

Repeated-measures ANOVA were used to evaluate total number and amplitude of IPPW, basal pyloric pressures, plasma hormones and VAS scores with time and visit as factors. One-way ANOVA was used to analyse, MI for antral and duodenal pressures, energy intake (kJ), amount eaten (g) and macronutrient distribution (%) from the buffet meal and body weight and waist circumference. *Post-hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects. Differences between lean and obese groups in number of IPPW, basal pyloric pressures, plasma hormones, VAS scores during the acute dietary restriction period were analysed using repeated-measured ANOVA with group (lean and obese) as between-subject, factors. MI for antral and duodenal pressures, energy intake and macronutrient distribution from the buffet meal between lean and obese subjects, were compared using independent samples *t*-tests. Statistical significance was accepted at $P < 0.05$.

9.4 Results

9.4.1 Habitual energy intake and macronutrient distribution

There were no differences in reported habitual energy intake or amount (g) of fat or carbohydrate consumed between the lean and obese groups. There was a significant difference in the amount (g) of protein consumed (see **Table 9.1**). The obese group consumed greater amounts of protein when compared with the lean group ($P < 0.05$). There were no differences in the amount (%) of fat, carbohydrate or protein consumed between the groups.

9.4.2 Part 1: Effects of acute dietary restriction in the lean and obese

The lean and obese subjetcs complied with the acute 30% dietary restriction period. All subjects tolerated the acute experimental conditions well.

9.4.2.1 Body weight and waist circumference

There was a significant effect of visit on body weight in the lean (visit 1: 76 ± 2 kg; visit 2: 75 ± 2 kg; $P < 0.01$) and obese (visit 1: 106 ± 4 kg; visit 2: 104 ± 3 kg), group, and weight circumference in the obese (visit 1: 114 ± 2 cm; visit 2: 111 ± 2 cm), but not the lean (visit 1: 86 ± 2 cm; visit 2: 86 ± 1 cm), group.

9.4.2.2 Antropyloroduodenal pressures

Antral and duodenal pressures: There was no effect of visit, or group, on the MI of antral or duodenal pressure waves (see **Table 9.2**).

IPPW: There were no differences in baseline values between visits in the lean (visit 1: 0.3 ± 0.1; visit 2: 0.5 ± 0.5) or the obese (visit 1: 0 ± 0; visit 2: 0 ± 0), group. There was no effect of visit on total number, or amplitude, of IPPW in either the lean or obese group (see **Table 9.2**). There was no difference in the total number, or amplitude, of IPPW between the lean and obese group on visit 1 or visit 2.

Basal pyloric pressures: There were no differences in baseline values between visits in the lean (visit 1: –0.6 ± 1; visit 2: –1 ± 1.6) or obese (visit 1: 1 ± 3; visit 2: 2 ± 3), group. There was a trend for an effect of visit on the AUC of basal pyloric pressures, in the lean ($P = 0.09$), but not the obese (see **Table 9.2**), group, such that, the AUC of basal pyloric pressures tended to be greater on visit 2 compared with visit 1 in the lean group ($P = 0.09$). There was no difference in the AUC of basal pyloric pressures between the lean and obese group at visit 1. However, on visit 2, AUC of basal pyloric pressures was greater in the lean, when compared with the obese group ($P < 0.05$).

9.4.2.3 Gastrointestinal hormones

Plasma CCK concentrations: There was a small, but significant, difference in baseline CCK concentrations between visits in the lean (visit 1: 2.3 ± 0.3 pmol/L; visit 2: 1.9 ± 0.2 pmol/L; $P < 0.05$), but not the obese (visit 1: 2.9 ± 0.7 pmol/L; visit 2: 2.6 ± 0.8 pmol/L), group. There was a visit * time interaction for plasma CCK concentrations in the lean group ($P < 0.01$) (see **Figure 9.2A**). Plasma CCK concentrations were lower between t = 0 and 120 min on visit 2 compared with visit 1 ($P < 0.05$). In the obese group, there was an effect of time ($P < 0.01$), but not visit, for plasma CCK concentrations (see **Figure 9.2A**). Plasma CCK concentrations increased in response to

lipid during all visits, peaking at ~ 30 min, after which time concentrations plateaued. There was no difference in CCK concentrations between the lean and obese group on visit 1 or visit 2.

Plasma ghrelin concentrations: There was a significant difference in baseline ghrelin concentrations between visits in the lean (visit 1: 2063 ± 221 pg/mL; visit 2: 2317 ± 241 pg/mL; $P < 0.05$), but not the obese (visit 1: 1321 ± 242 pg/mL; visit 2: 1254 ± 224 pg/mL), group. There was a visit * time interaction for plasma ghrelin concentrations in the lean group ($P < 0.05$) (see **Figure 9.2B**). Plasma ghrelin concentrations were greater between t = 0 and 30 min and at t = 60 min on visit 2 compared with visit 1 ($P < 0.05$). In the obese group, there was an effect of time ($P < 0.01$), but not visit, for plasma ghrelin concentrations (see **Figure 9.2B**). Ghrelin concentrations decreased slightly in response to lipid during all visits. There was a significant difference in baseline plasma ghrelin concentrations between visits 1 ($P < 0.05$) and visits 2 ($P < 0.01$) in the lean and obese group. There was a visit * group interaction for ghrelin concentrations ($P < 0.05$). Plasma ghrelin was greater during visit 1 and visit 2 in the lean group, compared with visit 1 and visit 2 in the obese group (both $P < 0.05$). The magnitude of ghrelin suppression (i.e. ghrelin at t = 0 min – ghrelin at t = 120 min) was greater at visit 2 ($P < 0.001$) and tended to be greater at visit 1 ($P = 0.07$), in the lean, when compared to the obese group.

9.4.2.4 Appetite perceptions

Desire to eat: There was a difference in baseline scores for desire to eat between visits in the lean (visit 1: 45 ± 8; visit 2: 64 ± 8), but not the obese (visit 1: 38 ± 6; visit 2: 51

± 8), group. There was a significant effect of visit on desire to eat in the lean and obese group ($P < 0.05$ for both) (see **Figure 9.3A**). Desire to eat was greater in both the lean and obese group on visit 2 compared with visit 1 ($P < 0.05$). There was no difference in desire to eat scores between the lean and obese group on visit 1 or visit 2.

Hunger: There were differences in baseline scores for hunger between visits in the lean (visit 1: 34 ± 8; visit 2: 59 ± 7; $P < 0.01$), but not the obese (visit 1:32 ± 6; visit 2: 44 ± 8) group. There was a visit * time interaction for hunger scores in the lean ($P < 0.05$), but not the obese group (see **Figure 9.3B**). Hunger was greater on visit 2 between t = 30 and 60 min ($P < 0.05$), and tended to be greater at t = 15 min ($P = 0.08$), when compared with visit 1, in the lean group. There was a trend for an effect of visit on hunger in the obese group ($P = 0.052$), such that hunger tended to be greater on visit 2, when compared with visit 1 ($P = 0.08$). There was no difference in hunger scores between the lean and obese group on visit 1 or visit 2 or in magnitude suppression between the lean and obese group on visit 1 or visit 2.

There were no effects of visit, or group, on fullness, nausea or bloating (data not shown).

9.4.2.5 Energy and macronutrient intake

There was no effect of visit on energy intake, amount of food consumed (g), protein or fat (g) intake from the buffet meal in lean or obese (see **Table 9.3**). There was an effect of visit on carbohydrate intake (g) in the obese ($P < 0.05$), but not the lean group. Carbohydrate intake was reduced following the energy restriction, when compared with

visit 1, in the obese group. There was an effect of visit on %energy from carbohydrate intake in the lean group ($P < 0.05$) and a trend in the obese group ($P = 0.08$), with no difference in %fat or %protein intake in either the lean or obese group. There was no effect of visit on energy intake, amount eaten or protein and carbohydrate intake (g or %) during the buffet meal between the lean and obese group. There was a trend for an effect of visit for fat (g and %). Fat intake tended to be greater in the obese group during visit 1 ($P = 0.07$) and visit 2 ($P = 0.06$), when compared with the lean group.

9.4.3 Part 2: Effects of prolonged dietary restriction in the obese

The obese subjects complied with the prolonged dietary restriction period (28.8%) except for three subjects who had a dietary restriction of 16%, 18% and 20%. All subjects tolerated the prolonged experimental conditions well, except for two obese subjects, one who vomited at 75 min after commencement of the lipid infusion during visit 4, and one who experienced diarrhoea 60 min after commencement of the lipid infusion during visit 3, at which time the infusion was discontinued.

9.4.3.1 Body weight and waist circumference

There was a significant effect of visit on body weight and waist circumference ($P < 0.001$ for both). Body weight and waist circumference were significantly less on visits 3 (100 ± 3 kg; 109 ± 2 cm) and 4 (96 ± 3 kg; 103 ± 2 cm), when compared with visit 1 (106 ± 4 kg; 114 ± 2 cm) ($P < 0.01$ for all), and less on visit 4, when compared with visit 3 ($P < 0.01$).

9.4.3.2 Antropyloroduodenal pressures

Antral and duodenal pressures: There was no effect of visit on the MI of antral pressure or duodenal waves (see **Table 9.2**).

IPPW: There were no differences in baseline values between visits (visit 1: 0 ± 0; visit 3: 0 ± 0; visit 4: 0 ± 0). There was no effect of visit on the total number, or amplitude, of IPPW (see **Table 9.2**).

Basal pyloric pressures: There were no differences in baseline values between visits (visit 1: 1 ± 3; visit 3: –0.3 ± 2; visit 4: –2 ± 0.3). There was a trend for an effect of visit on AUC of basal pyloric pressures ($P = 0.08$) (see **Table 9.2**).

9.4.3.3 Gastrointestinal hormones

Plasma CCK concentrations: There were no differences in baseline concentrations between visits (visit 1: 2.9 ± 0.7 pmol/L; visit 3: 2.6 ± 0.6 pmol/L; visit 4: 2.6 ± 0.6 pmol/L). There was an effect of time ($P < 0.01$), but not visit, for plasma CCK concentration (see **Figure 9.2C**). Plasma CCK concentrations increased in response to lipid during all visits, peaking at ~ 30 min, after which time concentrations plateaued.

Plasma ghrelin concentrations: There were differences in baseline concentrations between visits (visit 1: 1321 ± 242 pg/mL; visit 3: 1513 ± 265 pg/mL; visit 4: 1502 ± 246 pg/mL) ($P < 0.05$), although pairwise comparisons revealed no significant difference between visits. There was an effect of time ($P < 0.01$), but not visit, for

plasma ghrelin concentration (see **Figure 9.2D**). Plasma ghrelin concentrations decreased in response to lipid during all visits.

9.4.3.4 Appetite perceptions

Desire to eat: There were differences in baseline scores between visits ($P < 0.001$). Baseline desire-to-eat scores were greater at visit 3 (60 ± 8, $P < 0.01$) and 4 (62 ± 9, $P < 0.05$) when compared with visit 1 (38 ± 6), with no differences between visit 3 and visit 4. There was a significant effect of visit on scores for desire to eat ($P < 0.05$) (see **Figure 9.3C**). There was a trend for desire to eat to be greater at visit 3, when compared with visit 1 ($P = 0.054$) with no differences between visit 1 and visit 4 or between visit 3 and visit 4.

Hunger: There were differences in baseline scores between visits ($P < 0.01$). Baseline hunger scores were greater at visit 3 (56 ± 9, $P < 0.01$), when compared with visit 1 (32 ± 6), with no difference between visit 1 and visit 4 (58 ± 11) and visit 3 and visit 4. There was a significant effect of visit for hunger scores ($P < 0.05$), although pairwise comparisons revealed no significant differences (see **Figure 9.3D**).

There were no effects of visit on fullness, nausea or bloating (data not shown).

9.4.3.5 Energy and macronutrient intake

There was a trend for a significant effect of visit on energy intake ($P = 0.06$) and protein intake (g) ($P = 0.06$), a significant effect of visit on the amount of food consumed (g) ($P < 0.01$) and % and amount (g) of carbohydrate intake ($P < 0.05$), with no difference in

fat intake (g) between visits during the buffet meal (see **Table 9.3**). The amount of food consumed was significantly reduced during visit 3 and 4 (for both $P < 0.05$), when compared with visit 1, with no differences between visit 3 and 4. Carbohydrate intake was reduced during visit 4 ($P < 0.05$) compared with visit 1, with no difference between any of the other visits. There were no differences in %fat or protein between visits.

Table 9.1: Habitual energy intake and macronutrient distribution in lean and obese[1].

	LEAN	OBESE
Energy intake (kJ)	10456 ± 636	10705 ± 460
Amount eaten (g)	2581 ± 228	2955 ± 185
Fat (g)	100 ± 7	98 ± 6
Protein (g)	105 ± 6	123 ± 4[2]
CHO (g)	267 ± 25	257 ± 16
Fat (% TEI)	21 ± 1	21 ± 1
Protein (% TEI)	23 ± 2	26 ± 1
CHO (% TEI)	56 ± 2	53 ± 2

[1]Data are means ± SEM, n = 12 lean and 12 obese subjects. CHO, carbohydrates; TEI, total energy intake.

[2]Significantly different from lean, group effect: $P<0.05$ (repeated-measures ANOVA). There were no significant interactions or main effects on energy intake or the amount of fat or carbohydrate (g), or the %energy from fat, carbohydrate or protein (repeated-measures ANOVA).

Table 9.2: Antral and duodenal motility indices during 120-min intraduodenal infusion of 10% Intralipid (2.86 kcal/min) on day 0 (visit 1), day 5 (visit 2) (lean and obese), day 29 (visit 3) and day 85 (visit 4) (obese only), on a four-day, for lean, and 12-week, for obese, 30% energy-restricted diet[1].

	VISIT 1	VISIT 2	VISIT 3	VISIT 4
Lean				
Antral MI (mmHg)	6.6 ± 0.5	6.9 ± 0.4		
Duodenal MI (mmHg)	8.0 ± 0.4	8.4 ± 0.3		
Total number IPPW	125 ± 29	179 ± 44		
AUC Amp IPPW (mmHg)	3848 ± 971	5272 ± 1200		
AUC BPP (mmHg)	247 ± 115	453 ± 121		
Obese				
Antral MI (mmHg)	5.7 ± 0.8	7 ± 0.3	6.6 ± 0.4	6.3 ± 0.3
Duodenal MI (mmHg)	8.3 ± 0.4	8.6 ± 0.3	8.1 ± 0.2	8.1 ± 0.2
Total number IPPW	167 ± 32	149 ± 25	191 ± 26	168 ± 22
AUC Amp IPPW (mmHg)	3841 ± 730	5024 ± 804	5553 ± 979	4771 ± 572
AUC BPP (mmHg)	204 ± 93	129 ± 118[3]	230 ± 132	352 ± 91

[1]Data are means ± SEM, n = 12 lean and 12 obese subjects. MI, motility index; AUC, area under the curve; BPP, basal pyloric pressures; Amp, amplitude.

There was no significant effect of visit for antral or duodenal MI, number or amplitude or IPPW and basal pyloric pressures (repeated-measures ANOVA).

[3]Significantly different from visit 2 lean, group effect: $P<0.05$ (independent sample t - test).

Table 9.3: Energy and macronutrient intake from the buffet meal following a 120-min intraduodenal infusion of 10% Intralipid (2.86 kcal/min) on day 0 (visit 1), day 5 (visit 2) (lean and obese), day 29 (visit 3) and day 85 (visit 4) (obese only), on a four-day, for lean, and 12-week, for obese, 30% energy-restricted diet[1].

	VISIT 1	VISIT 2	VISIT 3	VISIT 4
Lean				
Energy intake (kJ)	4370 ± 376	4199 ± 471		
Amount eaten (g)	1073 ± 88	974 ± 124		
Fat (g)	35 ± 4	37 ± 4		
Protein (g)	53 ± 4	55 ± 7		
CHO (g)	122 ± 11	108 ± 13		
Fat (% TEI)	30 ± 1	33 ± 1		
Protein (% TEI)	22 ± 2	22 ± 1		
CHO (% TEI)	48 ± 2	45 ± 2[2]		
Obese				
Energy intake (kJ)	4579 ± 436	4306 ± 459	3854 ± 502	4146 ± 505
Amount eaten (g)	1037 ± 109	966 ± 113	798 ± 107[2]	839 ± 110[2]
Fat (g)	41 ± 5	42 ± 5	36 ± 5	41 ± 5
Protein (g)	58 ± 6	55 ± 6	49 ± 6	56 ± 6
CHO (g)	119 ± 11	103 ± 13	105 ± 13	95 ± 14[2]
Fat (% TEI)	33 ± 1	37 ± 2	35 ± 1	36 ± 2
Protein (% TEI)	22 ± 1	22 ± 1	22 ± 2	24 ± 2
CHO (% TEI)	45 ± 2	41 ± 3	43 ± 2	39 ± 3

[1]Data are means ± SEM, n = 12 lean and 12 obese subjects. CHO, carbohydrates; TEI, total energy intake.

[2]Significantly different from visit 1, visit effect: $P<0.05$ (repeated-measures ANOVA).

There were no significant interactions or main effects on energy intake or the amount of fat or protein (g), or the %energy from fat, carbohydrate or protein consumed at the buffet meal (repeated-measures ANOVA).

Figure 9.1: Schematic representation of the study protocol for lean and obese subjects. Obese subjects attended the laboratory on four occasions: day 0, before starting the diet (visit 1), day 5 (visit 2), day 29 (visit 3) and day 85 (visit 4). They also attended the laboratory each fortnight (days 13, 27, 41, 55 and 69) during the study for a meeting with a dietician to review their diet and record their body weight. Lean subjects attended the laboratory on two occasions: day 0 (visit 1), and day 5 (visit 2). During each study visit the effects of acute dietary restriction on GI function and energy intake in response to 120-min intraduodenal infusions of 10% Intralipid (2.86 kcal/min) were evaluated.

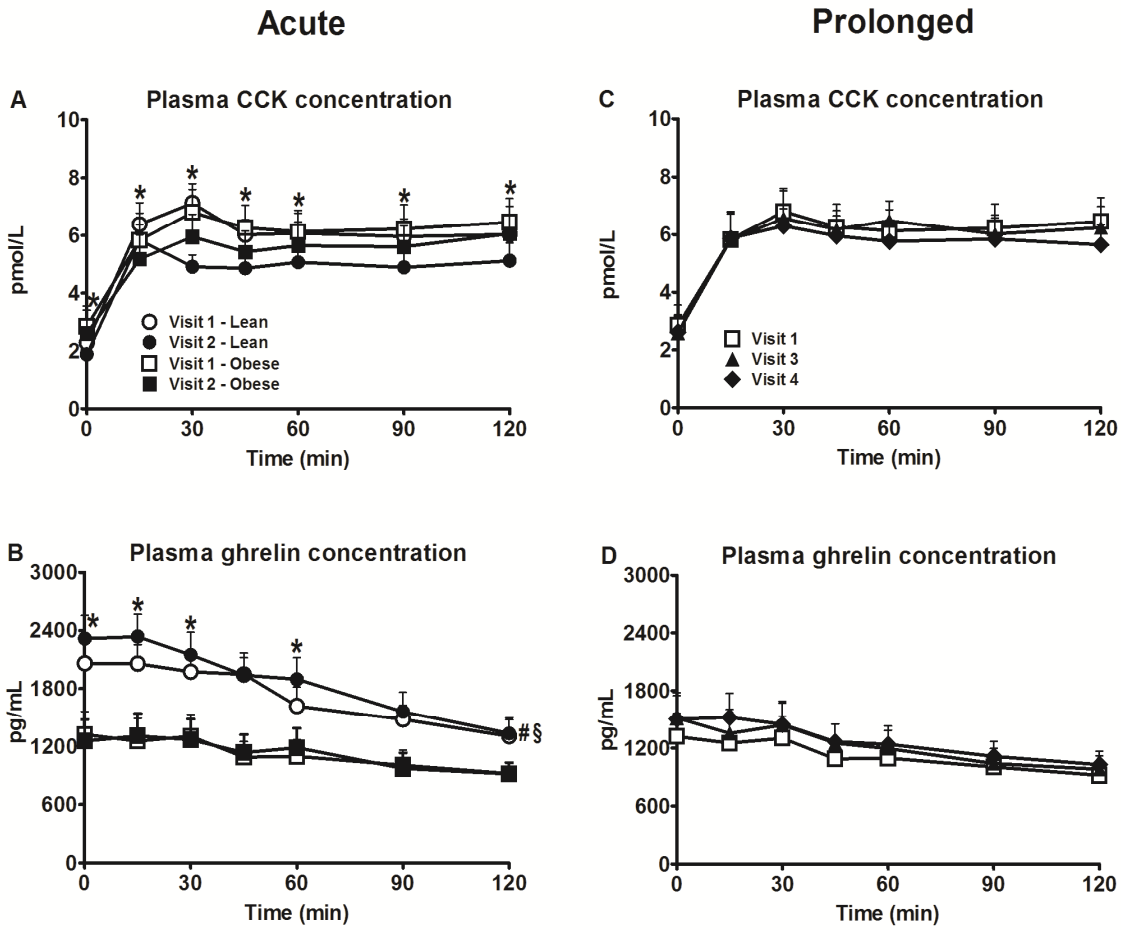


Figure 9.2: Plasma CCK and ghrelin concentrations during 120-min intraduodenal infusions of 10% Intralipid (2.86 kcal/min) on day 0 (visit 1) and day 5 (visit 2) (A and B), on a four-day (acute), in lean and obese, and on day 1 (visit 1), day 29 (visit 3) and day 85 (visit 4) (C and D), on a 12-week (prolonged), in obese, 30% energy-restricted diet. * $P < 0.05$ vs visit 1 in lean; # vs visit 1 in obese; § vs visit 2 in obese. Data are mean ± SEM. (n = 12 lean and 12 obese).

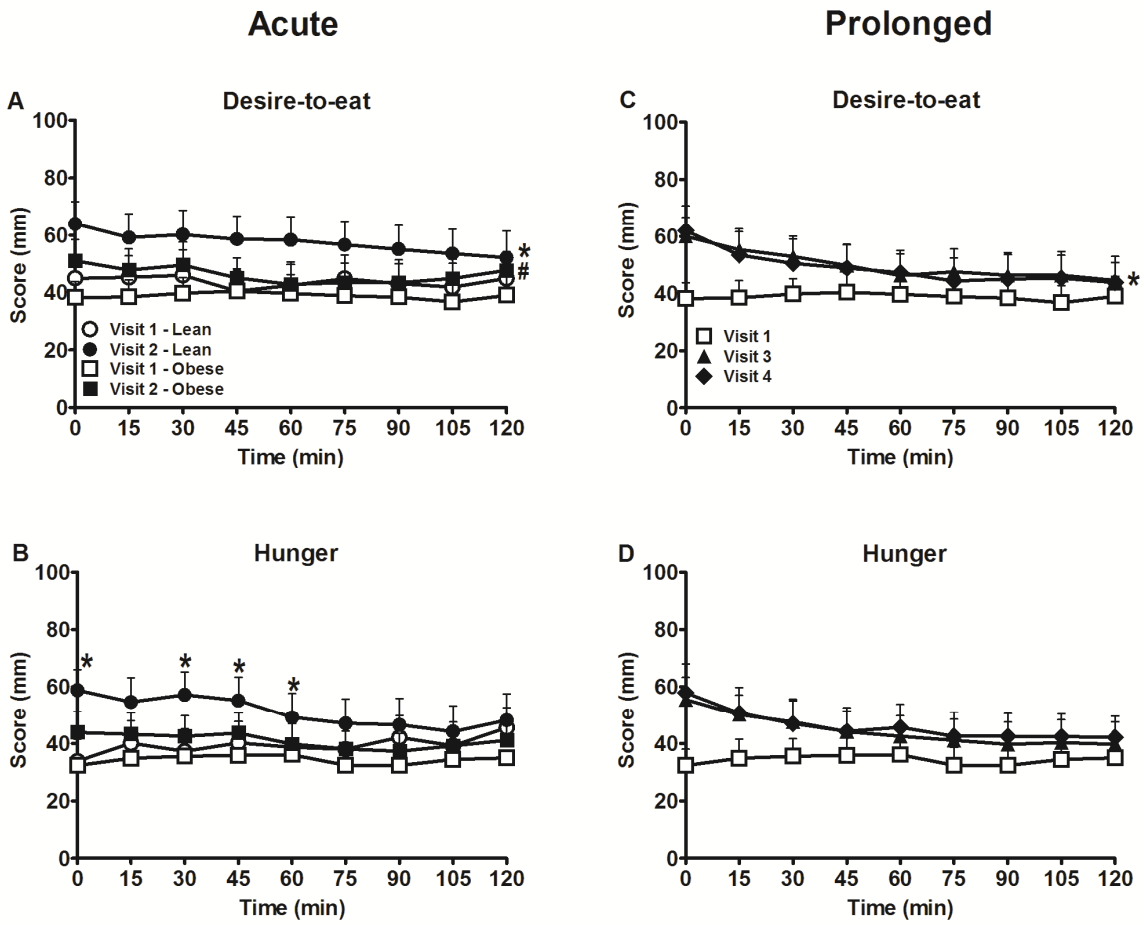


Figure 9.3: Desire to eat and hunger scores during 120-min intraduodenal infusions of 10% Intralipid (2.86 kcal/min) on day 0 (visit 1) and day 5 (visit 2) (A and B), on a four-day (acute), in lean and obese, and on day 0 (visit 1), day 29 (visit 3) and day 85 (visit 4) (C and D), on a 12-week (prolonged), in obese, 30% energy-restricted diet. * $P < 0.05$ vs visit 1 in lean, # vs visit 1 in obese. Data are mean ± SEM. (n = 12 lean and 12 obese).

9.5 Discussion

This study evaluated the effects of acute (in lean and obese) and prolonged (in obese) 30% energy restriction on APD motility, gastrointestinal hormone release, appetite and energy intake in response to an intraduodenal lipid infusion. In lean individuals, acute energy restriction had no effect on energy intake, although there was a reduced plasma CCK response and attenuated suppression of ghrelin during the intraduodenal lipid infusion. In contrast, in the obese group, acute and prolonged energy restriction had no effect on gastrointestinal motor or hormonal responses to intraduodenal lipid, although the amount of food eaten at the buffet meal was reduced following prolonged energy restriction.

We hypothesised that acute 30% energy restriction (four days) would enhance the effects of intraduodenal lipid on gastrointestinal function and appetite in lean and obese subjects, but that following prolonged 30% energy restriction (12 weeks), associated with weight loss, these effects of energy restriction on gastrointestinal function and appetite would be lost in the obese. Prolonged caloric restriction results in marked increases in ghrelin (Cummings et al. 2002), rapid reductions in circulating levels of leptin (Havel et al. 1996) and energy expenditure (Leibel et al. 1995) and an increase in appetite (Keim et al. 1998), which could explain why body weight often stabilises, or even increases, despite continued adherence to prescribed weight loss diet (Sjostrom et al. 1998). We had previously demonstrated that following a four-day 70% VLCD, the effects of small intestinal lipid on gastrointestinal motility and hormone responses were enhanced (Brennan et al. 2010), suggesting that acute caloric restriction increases sensitivity to the actions of intestinal nutrients. However, this was a very low calorie

diet, and it was unclear if similar changes in gastrointestinal nutrient sensitivity would occur with more moderate and sustainable dietary regimens. Therefore, we used a moderate dietary regimen (30% energy restriction), more realistic of real life, which is commonly recommended on the grounds that greater degrees of energy restriction do not achieve better long-term weight loss (National Institutes of Health 2002) and may result in a greater loss of fat-free mass (Forbes 2000).

In lean, but not obese, subjects, acute energy restriction diminished hormonal responses to intraduodenal lipid, suggesting that even a short period of dietary restriction can modulate hormone release in this group. However, this is in contrast to the acute 70% energy-restricted diet in obese where hormonal responses were enhanced (Brennan et al. 2010). We found fasting and postprandial plasma CCK concentrations to be reduced after the dietary restriction period. This observation is consistent with a study in rats in which food deprivation for three days was associated with a rapid decrease in plasma CCK concentrations. The reason for this is unclear but duodenal levels of CCK mRNA were decreased, suggesting a reduction in the rate of CCK gene transcription (Kanayama and Liddle 1991). In addition, lipid-induced ghrelin suppression was reduced after the four day dietary restrcition in the lean group. There is evidence that intravenous administration of CCK has the capacity to suppress plasma ghrelin concentrations (Brennan et al. 2007). Since plasma CCK stimulation was reduced following acute energy restriction, this may have contributed to the failure of lipid to further suppress ghrelin. Not unexpectedly, the higher fasting ghrelin concentrations following the energy restriction period were associated with increased hunger scores. However, although fasting hunger was greater in the lean subjects following the dietary

restriction, lipid failed to suppress hunger to the same extent following energy restriction. The reduced stimulation of plasma CCK following energy restriction in the lean may, at least in part, underlie the increased perception of hunger and desire to eat. It is known that gastrointestinal motility and hormone release, particularly the stimulation of pyloric pressures and plasma CCK release, are major determinants of subsequent energy intake (Seimon et al. 2010). However, the changes in plasma CCK release and the lack of gastrointestinal motor responses may be a reason why changes in energy intake at the buffet meal were not observed.

There were no significant changes in gastrointestinal motor or hormonal function in the obese group following acute 30% dietary restriction. This is in contrast to the four-day 70% VLCD in obese that increased pyloric pressure, plasma PYY and the suppression of ghrelin, and reduced appetite perceptions and energy intake in response to intraduodenal lipid (Brennan et al. 2010). Similarly, in another study, gastric emptying of a 75 g glucose drink was slower following a four-day fast in both lean and obese subjects, when compared with an overnight, 12 hour fast (Corvilain et al. 1995). This may suggest that the moderate 30% energy restriction may not have been sufficient to induce changes in gastrointestinal function and subsequent energy intake in this group and may suggest that for short term weight loss diets, more severe dietary restrictions would be more beneficial in terms of changes in gastrointestinal function and subsequent energy intake.

In addition, at baseline, there were no differences in APD motility between the lean and obese group, which was inconsistent with a previous study in our laboratory in which

the stimulation of pyloric pressures, in response to intraduodenal oleic acid, was reduced in overweight or obese, compared with lean, subjects (Stewart et al. 2011a). The study by Stewart et al (2011) reported that overweight and obese subjects had significantly greater habitual energy intake than lean subjects, whereas in this present study, there were no differences in habitual energy intake between the lean and obese subjects, which may explain why there were no differences in gastrointestinal motility between the groups. Although, it is important to acknowledge that self-reported questionnaires may not necessarily reflect dietary intake, habits or behaviour, as they may be prone to bias and under-reporting (Gibson 2005). However, baseline CCK concentrations dropped in response to acute dietary restriction in the lean, but not obese, which also suggests that the obese may be less sensitive to the dietary restriction compared with the lean at least in terms of gastrointestinal hormone responses.

Following prolonged energy restriction, fasting and postprandial ghrelin concentrations have been reported to be increased (Cummings et al. 2002; Sumithran et al. 2011) and fasting and postprandial plasma CCK concentrations reduced in obese (Sumithran et al. 2011). In our study, lipid-induced ghrelin suppression and CCK stimulation before and after, the dietary restriction was unchanged in the obese. This may have been due to the moderate caloric restriction that we used in this study, whereas the above-mentioned studies used very low caloric diets. Surprisingly, despite no changes in any of the gastrointestinal motor or hormone responses, the amount eaten at the buffet meal was significantly reduced in the obese during the prolonged energy-restricted diet. It may be possible that other hormones including PYY and GLP-1 may have had an effect on this suppression.

There are some limitations of the study that need to be recognised. We administered the lipid emulsion directly into the duodenum, as our primary focus was to identify potential changes in small intestinal sensitivity to lipid. As a result, we bypassed potential gustatory and gastric mechanisms and cannot comment on any effects on gastric emptying. Due to the small sample size in this study, it is possible that some findings may have reached statistical significance if a larger sample size was used. Only one degree (30%) and one period (12 weeks) of energy restriction were employed; therefore, the effects of higher degrees of energy restrictions and more prolonged periods remain uncertain. We did monitor dietary compliance during the study; although this is difficult with intervention studies, the significant changes in weight that occurred as a result of the dietary restriction suggest that subjects were compliant to their prescribed diets. Self-reported dietary records may be prone to bias and under-reporting; nevertheless, they are an acceptable method of dietary assessment.

Therefore, in conclusion, a 30% acute energy-restricted diet diminished gastrointestinal hormone responses in the lean, while in the obese subjects, acute and prolonged 30% energy restriction had no effect on gastrointestinal function. This may suggest that obese are less sensitive to caloric restriction and it may be that greater degrees of energy restriction for short term periods may be more beneficial in obese to observe changes in gastrointestinal function. This information may be important when considering weight loss interventions.

Chapter 10: Conclusion

The studies reported in this thesis have evaluated aspects of the complex and interrelated oral and gastrointestinal mechanisms involved in the regulation of appetite and energy intake in lean and obese individuals. The three broad areas of research that have been investigated in the thesis include: i) the gastrointestinal motor and hormonal functions involved in the regulation of energy intake in healthy individuals; ii) the effects of oral and intraduodenal nutrients on gastrointestinal motility and hormone release, appetite and energy intake in obese compared with lean individuals; and iii) the effects of acute and prolonged energy restriction on gastrointestinal function, appetite and energy intake.

The study in **Chapter 5** was carried out to determine if any gastrointestinal motor or hormone functions and appetite were independent determinants of acute energy intake in healthy, lean men. Although there were correlations between energy intake and APD pressures, plasma hormone concentrations, and gastrointestinal perceptions, only the peak number of IPPW, peak plasma CCK concentration, and area under the curve of nausea were identified as independent predictors of energy intake. The evaluation of these variables as determinants of energy intake and their potential as screening tools for the appetite-suppressant potency of novel, gut-focused, therapeutic agents in prospective studies would be of interest.

In **Chapter 6**, we demonstrated that following oral ingestion of the nutrient liquid drink, there were no differences in gastric emptying, intragastric distribution or oro-caecal transit between the lean, overweight and obese groups. After the drink, blood glucose and plasma insulin were greater in the obese, when compared with both the lean and overweight groups, however, there were no differences in plasma GLP-1 or GIP concentrations, appetite and energy intake at the buffet meal or habitual energy intake between the groups. In the obese, the magnitude of the rise in blood glucose was inversely related to the gastric emptying. This study suggests that obesity per se, in the absence of differences in habitual energy intake, has no effect on gastric emptying or incretin hormone release and that gastric emptying influences postprandial blood glucose in the obese.

The study presented in **Chapter 7** evaluated the hypothesis that overweight or obese subjects would be less sensitive to both oral and intraduodenal oleic acid exposure than lean subjects. The study demonstrated that during a 90-min intraduodenal fatty acid (oleic acid [C18:1]) infusion, the number of IPPW was greater than during saline infusion, in lean subjects, with no differences between the C18:1 and saline infusions in the overweight or obese subjects. In both groups, C18:1 stimulated plasma CCK and PYY and suppressed energy intake compared with saline, with trends for reduced CCK and energy intake responses in the overweight or obese subjects. Oral fatty acid detection thresholds for C18:1 was greater in overweight or obese than in lean subjects. Overweight or obese subjects had greater energy and fat intakes than did lean subjects. There was a direct relation of BMI with C18:1 detection thresholds and inverse relations of pyloric pressures with BMI and C18:1 detection thresholds. The ability to detect

C18:1 both orally and within the gastrointestinal tract is compromised in obese men, and oral and gastrointestinal responses to C18:1 is related.

The study described in **Chapter 8** demonstrated that following a 70% four-day VLCD there was a significant increase in basal pyloric pressures and the number and amplitude of IPPW, and a decrease in the number of antral and duodenal pressure waves and PWS, and the stimulation of PYY and suppression of ghrelin was greater, during a 120-min intraduodenal lipid infusion. In addition, following the four-day diet, hunger and prospective consumption scores were lower, and energy intake was reduced, indicating that gastrointestinal function, appetite and energy intake in the obese can be modified over a short period.

We evaluated the effects of an acute (in lean and obese) and prolonged (in obese only) 30% energy restriction on gastrointestinal function and appetite in response to an intraduodenal lipid infusion in **Chapter 9**. In contrast to the previous 70% very low calorie diet presented in Chapter 8, there were no differences in gastrointestinal motor or hormonal function in the obese following the acute or prolonged 30% dietary restriction period, although there was a trend for energy intake to be reduced. However, in lean, there was a decrease in plasma CCK and an increase in ghrelin concentrations following the acute period of dietary restriction with no differences in gastrointestinal motility or energy intake. This suggests that obese individuals are less sensitive to the presence of small intestinal nutrients compared with lean and that this moderate 30% energy restriction was insufficient to observe changes in gastrointestinal function in the obese.

The studies reported in this thesis provide novel insights relating to the regulation of appetite and energy intake by gastrointestinal motor and hormones release and/or suppression in healthy lean, overweight and obese subjects. These observations will contribute to advances in knowledge regarding basic appetite physiology. Further, the data presented in this thesis have clinical implications for management of obesity and support dietary interventions as potential treatments for obesity.

Appendix I: Three-Factor Eating Questionnaire

Name: Date:

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

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

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

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

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

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

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

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

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

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

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

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

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

13. When I am with someone who is overeating, I usually overeat too.
(T) (F)

14. I have a pretty good idea of the number of calories/grams of fat in common foods.
(T) (F)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Each question in this section is followed by a number of options. After reading each question carefully, choose <u>one</u> 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 never	seldom	usually	almost always

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

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

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

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

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

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

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

1	2	3	4
never	rarely	sometimes	at least once a week

50. To what extent does this statement describe your eating behaviour?
'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

1	2	3	4
not like me	little like me	pretty good description of me	describes me perfectly

51. On a scale of 1 to 6, where 1 means no restraint in eating (eat whatever you want, whenever you want it) and 6 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?

1 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 Scale Questionnaire

Name (Initials): Visit: Time:

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

I feel nauseated

Not at all — Very much

I feel drowsy

Not at all — Very much

I feel bloated

Not at all — Very much

I feel anxious

Not at all — Very much

I feel hungry

Not at all — Very much

I feel full

Not at all — Very much

I feel happy

Not at all — Very much

I feel energetic

Not at all — Very much

How strong is your desire to eat?

Non existent — Very strong

I feel comfortable

Not at all — Very much

How much food do you think you could eat?

None — A large amount

Appendix III: Dietary Questionnaire for Epidemiological Studies

Dietary Questionnaire

QUESTIONS ABOUT WHAT YOU USUALLY EAT AND DRINK

Please fill in the date you completed this questionnaire:

DAY	MTH	YEAR
	○ JAN	○ 2004
	○ FEB	○ 2005
0 0	○ MAR	○ 2006
1 1	○ APR	○ 2007
2 2	○ MAY	○ 2008
3 3	○ JUN	○ 2009
4	○ JUL	○ 2010
5	○ AUG	○ 2011
6	○ SEP	○ 2012
7	○ OCT	○ 2013
8	○ NOV	○ 2014
9	○ DEC	○ 2015

INSTRUCTIONS:

This questionnaire is about your **usual** eating habits **over the past 12 months**. Where possible give only **one answer per question** for the type of food you eat **most often.**
(If you can't decide which type you have most often, answer for the types you usually eat.)

- Use a soft pencil only, preferably 2B.
- Do not use **any** biro or felt tip pen.
- Erase mistakes fully.
- Make no stray marks.

Please MARK LIKE THIS: ○ ● ○ ○

1. How many pieces of fresh fruit do you usually eat per day? *(Count 1/2 cup of diced fruit, berries or grapes as one piece.)*

- ○ I didn't eat fruit
- ○ less than 1 piece of fruit per day
- ○ 1 piece of fruit per day
- ○ 2 pieces of fruit per day
- ○ 3 pieces of fruit per day
- ○ 4 or more pieces of fruit per day

2. How many different vegetables do you usually eat per day? *(Count all types, fresh, frozen or tinned.)*

- ○ less than 1 vegetable per day
- ○ 1 vegetable per day
- ○ 2 vegetables per day
- ○ 3 vegetables per day
- ○ 4 vegetables per day
- ○ 5 vegetables per day
- ○ 6 or more vegetables per day

3. What type of milk do you usually use?

- ○ none
- ○ full cream milk
- ○ reduced fat milk
- ○ skim milk
- ○ soya milk

4. How much milk do you usually use per day? *(Include flavoured milk and milk added to tea, coffee, cereal, etc.)*

- ○ none
- ○ less than 250 ml *(1 large cup or mug)*
- ○ between 250 and 500 ml *(1-2 cups)*
- ○ between 500 and 750 ml *(2-3 cups)*
- ○ 750 ml *(3 cups)* or more

5. What type of bread do you usually eat?

- ○ I don't eat bread
- ○ high fibre white bread
- ○ white bread
- ○ wholemeal bread
- ○ rye bread
- ○ multi-grain bread

6. How many slices of bread do you usually eat per day? *(Include all types, fresh or toasted and count one bread roll as 2 slices.)*

- ○ less than 1 slice per day
- ○ 1 slice per day
- ○ 2 slices per day
- ○ 3 slices per day
- ○ 4 slices per day
- ○ 5-7 slices per day
- ○ 8 or more slices per day

7. Which spread do you usually put on bread?

- ○ I don't usually use any fat spread
- ○ margarine of any kind
- ○ polyunsaturated margarine
- ○ monounsaturated margarine
- ○ butter and margarine blends
- ○ butter

8. On average, how many teaspoons of sugar do you usually use per day? *(Include sugar taken with tea and coffee and on breakfast cereal, etc.)*

- ○ none
- ○ 1 to 4 teaspoons per day
- ○ 5 to 8 teaspoons per day
- ○ 9 to 12 teaspoons per day
- ○ more than 12 teaspoons per day

9. On average, how many eggs do you usually eat per week?

- ○ I don't eat eggs
- ○ less than 1 egg per week
- ○ 1 to 2 eggs per week
- ○ 3 to 5 eggs per week
- ○ 6 or more eggs per week

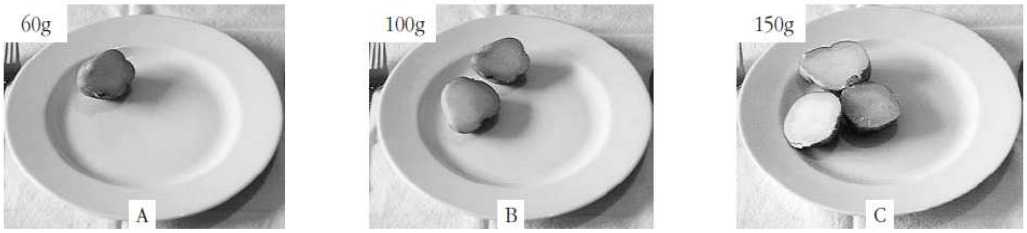
10. What types of cheese do you usually eat?

- ○ I don't eat cheese
- ○ hard cheeses, e.g. parmesan, romano
- ○ firm cheeses, e.g. cheddar, edam
- ○ soft cheeses, e.g. camembert, brie
- ○ ricotta or cottage cheese
- ○ cream cheese
- ○ low fat cheese

DO NOT WRITE IN THIS AREA.

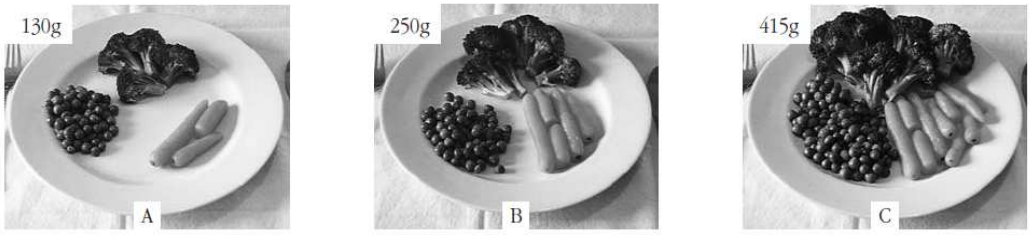
For each food shown on this page, indicate ***how much on average you would usually have eaten at main meals during the past 12 months.*** *When answering each question, think of the* ***amount*** *of that food you usually ate, even though you may rarely have eaten the food on its own.*
If you usually ate more than one helping, fill in the oval for the serving size closest to the ***total amount*** *you ate.*

11. When you ate potato, did you usually eat: ○ I never ate potato



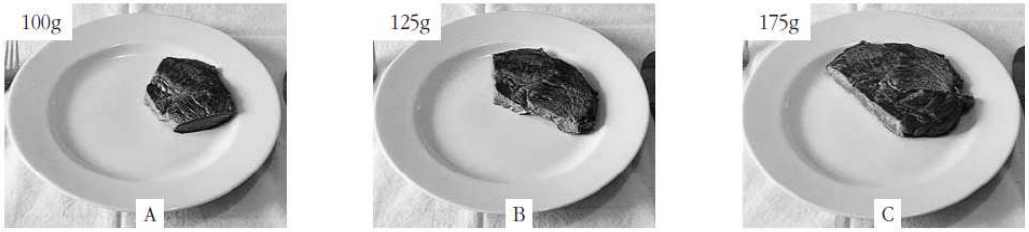
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

12. When you ate vegetables, did you usually eat: ○ I never ate vegetables



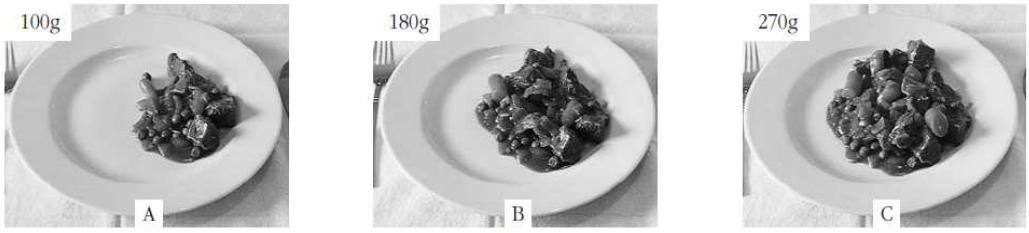
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

13. When you ate steak, did you usually eat: ○ I never ate steak



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

14. When you ate meat *or* vegetable casserole, did you usually eat: ○ I never ate casserole



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

15. **Over the last 12 months, on average, *how often* did you eat the following foods?** *Please completely fill one oval in every line.* Please MARK LIKE THIS: ● NOT LIKE THIS: ✓ ✗ ⊖

Times You Have Eaten		NEVER	less than once per month	1 to 3 times per month	1 time per week	2 times per week	3 to 4 times per week	5 to 6 times per week	1 time per day	2 times per day	3 or more times per day
CEREAL FOODS, SWEETS & SNACKS											
All Bran™	A1	○	○	○	○	○	○	○	○	○	○
Sultana Bran™, FibrePlus™, Branflakes™	A2	○	○	○	○	○	○	○	○	○	○
Weet Bix™, Vita Brits™, Weeties™	A3	○	○	○	○	○	○	○	○	○	○
Cornflakes, Nutrigrain™, Special K™	A4	○	○	○	○	○	○	○	○	○	○
Porridge	A5	○	○	○	○	○	○	○	○	○	○
Muesli	A6	○	○	○	○	○	○	○	○	○	○
Rice	A7	○	○	○	○	○	○	○	○	○	○
Pasta or noodles (include lasagne)	A8	○	○	○	○	○	○	○	○	○	○
Crackers, crispbreads, dry biscuits	A9	○	○	○	○	○	○	○	○	○	○
Sweet biscuits	A10	○	○	○	○	○	○	○	○	○	○
Cakes, sweet pies, tarts and other sweet pastries	A11	○	○	○	○	○	○	○	○	○	○
Meat pies, pasties, quiche and other savoury pastries	A12	○	○	○	○	○	○	○	○	○	○
Pizza	A13	○	○	○	○	○	○	○	○	○	○
Hamburger with a bun	A14	○	○	○	○	○	○	○	○	○	○
Chocolate	A15	○	○	○	○	○	○	○	○	○	○
Flavoured milk drink (cocoa, Milo™, etc.)	A16	○	○	○	○	○	○	○	○	○	○
Nuts	A17	○	○	○	○	○	○	○	○	○	○
Peanut butter or peanut paste	A18	○	○	○	○	○	○	○	○	○	○
Corn chips, potato crisps, Twisties™, etc.	A19	○	○	○	○	○	○	○	○	○	○
Jam, marmalade, honey or syrups	A20	○	○	○	○	○	○	○	○	○	○
Vegemite™, Marmite™ or Promite™	A21	○	○	○	○	○	○	○	○	○	○
DAIRY PRODUCTS, MEAT & FISH											
Cheese	B1	○	○	○	○	○	○	○	○	○	○
Ice-cream	B2	○	○	○	○	○	○	○	○	○	○
Yoghurt	B3	○	○	○	○	○	○	○	○	○	○
Beef	B4	○	○	○	○	○	○	○	○	○	○
Veal	B5	○	○	○	○	○	○	○	○	○	○
Chicken	B6	○	○	○	○	○	○	○	○	○	○
Lamb	B7	○	○	○	○	○	○	○	○	○	○
Pork	B8	○	○	○	○	○	○	○	○	○	○
Bacon	B9	○	○	○	○	○	○	○	○	○	○
Ham	B10	○	○	○	○	○	○	○	○	○	○
Corned beef, luncheon meats or salami	B11	○	○	○	○	○	○	○	○	○	○
Sausages or frankfurters	B12	○	○	○	○	○	○	○	○	○	○
Fish, steamed, grilled or baked	B13	○	○	○	○	○	○	○	○	○	○
Fish, fried (include take-away)	B14	○	○	○	○	○	○	○	○	○	○
Fish, tinned (salmon, tuna, sardines, etc.)	B15	○	○	○	○	○	○	○	○	○	○
FRUIT											
Tinned or frozen fruit (any kind)	C1	○	○	○	○	○	○	○	○	○	○
Fruit juice	C2	○	○	○	○	○	○	○	○	○	○
Oranges or other citrus fruit	C3	○	○	○	○	○	○	○	○	○	○
Apples	C4	○	○	○	○	○	○	○	○	○	○
Pears	C5	○	○	○	○	○	○	○	○	○	○
Bananas	C6	○	○	○	○	○	○	○	○	○	○
Watermelon, rockmelon (cantaloupe), honeydew, etc.	C7	○	○	○	○	○	○	○	○	○	○
Pineapple	C8	○	○	○	○	○	○	○	○	○	○
Strawberries	C9	○	○	○	○	○	○	○	○	○	○
Apricots	C10	○	○	○	○	○	○	○	○	○	○
Peaches or nectarines	C11	○	○	○	○	○	○	○	○	○	○
Mango or paw paw	C12	○	○	○	○	○	○	○	○	○	○
Avocado	C13	○	○	○	○	○	○	○	○	○	○

Times You Have Eaten CONTINUED		NEVER	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
			per month		per week				per day		
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)											
Potatoes, roasted or fried (include hot chips)	D1	○	○	○	○	○	○	○	○	○	○
Potatoes cooked without fat	D2	○	○	○	○	○	○	○	○	○	○
Tomato sauce, tomato paste or dried tomatoes	D3	○	○	○	○	○	○	○	○	○	○
Fresh or tinned tomatoes	D4	○	○	○	○	○	○	○	○	○	○
Peppers (capsicum)	D5	○	○	○	○	○	○	○	○	○	○
Lettuce, endive, or other salad greens	D6	○	○	○	○	○	○	○	○	○	○
Cucumber	D7	○	○	○	○	○	○	○	○	○	○
Celery	D8	○	○	○	○	○	○	○	○	○	○
Beetroot	D9	○	○	○	○	○	○	○	○	○	○
Carrots	D10	○	○	○	○	○	○	○	○	○	○
Cabbage or Brussels sprouts	D11	○	○	○	○	○	○	○	○	○	○
Cauliflower	D12	○	○	○	○	○	○	○	○	○	○
Broccoli	D13	○	○	○	○	○	○	○	○	○	○
Silverbeet or spinach	D14	○	○	○	○	○	○	○	○	○	○
Peas	D15	○	○	○	○	○	○	○	○	○	○
Green beans	D16	○	○	○	○	○	○	○	○	○	○
Bean sprouts or alfalfa sprouts	D17	○	○	○	○	○	○	○	○	○	○
Baked beans	D18	○	○	○	○	○	○	○	○	○	○
Soy beans, soy bean curd or tofu	D19	○	○	○	○	○	○	○	○	○	○
Other beans (include chick peas, lentils, etc.)	D20	○	○	○	○	○	○	○	○	○	○
Pumpkin	D21	○	○	○	○	○	○	○	○	○	○
Onion or leeks	D22	○	○	○	○	○	○	○	○	○	○
Garlic (not garlic tablets)	D23	○	○	○	○	○	○	○	○	○	○
Mushrooms	D24	○	○	○	○	○	○	○	○	○	○
Zucchini	D25	○	○	○	○	○	○	○	○	○	○

16. Over the last 12 months, how often did you drink beer, wine and/or spirits?

Times That You Drank		NEVER	less than once a month	1-3 days per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	every day
Beer (low alcohol)	1	○	○	○	○	○	○	○	○	○	○
Beer (full strength)	2	○	○	○	○	○	○	○	○	○	○
Red wine	3	○	○	○	○	○	○	○	○	○	○
White wine (include sparkling wines)	4	○	○	○	○	○	○	○	○	○	○
Fortified wines, port, sherry, etc.	5	○	○	○	○	○	○	○	○	○	○
Spirits, liqueurs, etc.	6	○	○	○	○	○	○	○	○	○	○

When answering the next two questions, please convert the amounts you drank into glasses using the examples given below. For spirits, liqueurs, and mixed drinks containing spirits, please count each nip (30 ml) as one glass.

1 can or stubby of beer = 2 glasses
1 large bottle beer (750 ml) = 4 glasses
1 bottle wine (750 ml) = 6 glasses
1 bottle of port or sherry (750 ml) = 12 glasses

17. Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you *usually* drink?

TOTAL NUMBER OF GLASSES PER DAY	1	2	3	4	5	6	7	8	9	10 or more
	○	○	○	○	○	○	○	○	○	○

18. Over the last 12 months, what was the *maximum* number of glasses of beer, wine and/or spirits that you drank in 24 hours?

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more
	○	○	○	○	○	○	○	○	○	○

Thank You for completing this questionnaire

DO NOT WRITE IN THIS AREA.

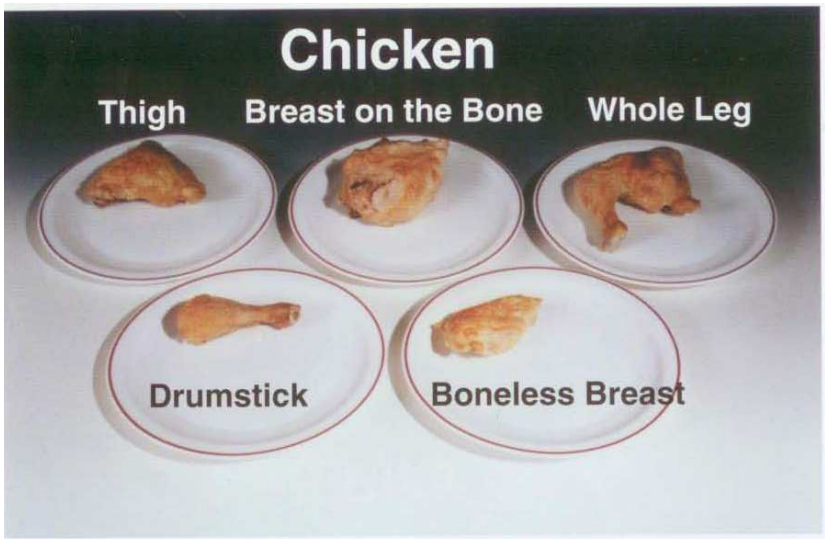
□□□□□□□□□□□□□□□□□□□□□□□□

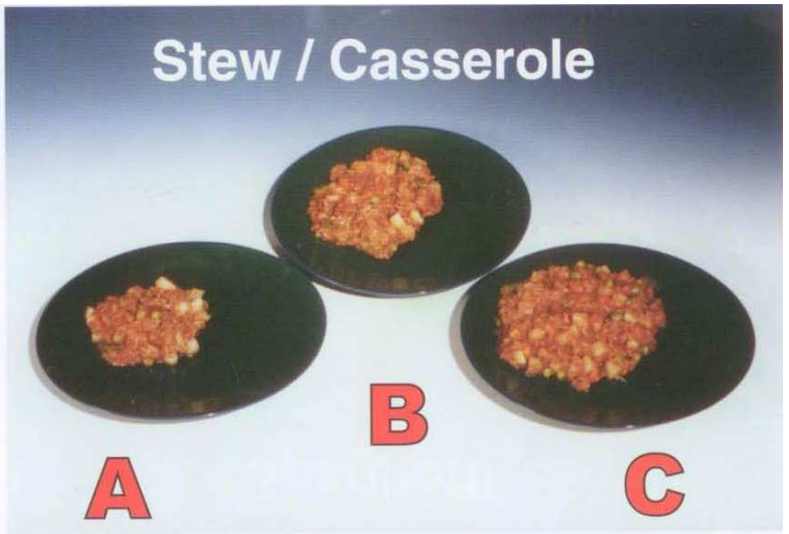
Appendix IV: Diet Diary for Food Recall

Sample Diet record

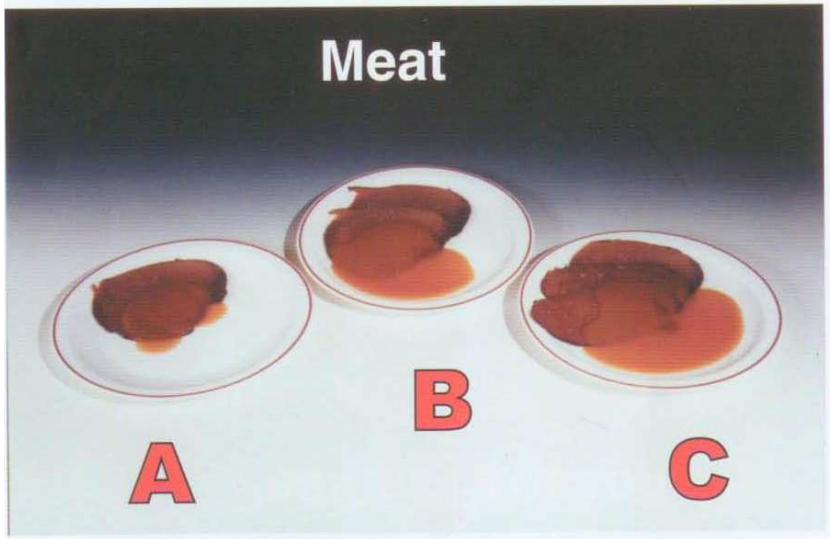
MEAL	TIME	FOOD OR DRINK	BRAND AND DETAILS	PREPARATION COOKING	AMOUNT
Breakfast	7am	Bread	Wholemeal, sandwich, Tip Top	Toasted	4 slices
		Butter	Devondale, reduced salt		Spread lightly on all 4 pieces of bread
		Coffee	Cappuccino with full cream milk and 2 tablespoons of sugar. (Gloria Jeans)		500 ml
Lunch	12pm	Ham and cheese roll	1 x bakers delight white bread roll, 3 slices of Don ham (smoked, lean), 2 slices of cheese (Coon), 1 small plastic tub of mayonnaise (Praise). No butter	-	1 roll
		Coca-cola	Coca-cola	-	600 ml
		Mars bar	Mars	-	1 king size bar

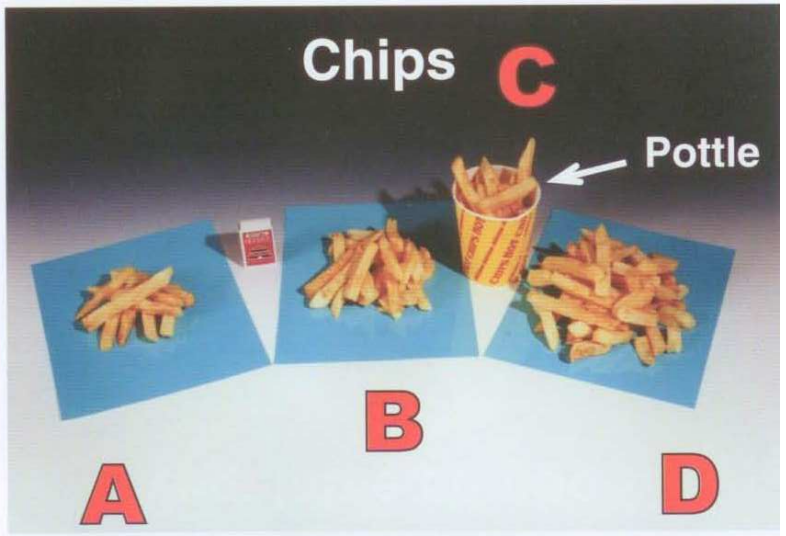
Appendix V: Quantified Food Portion Pictures





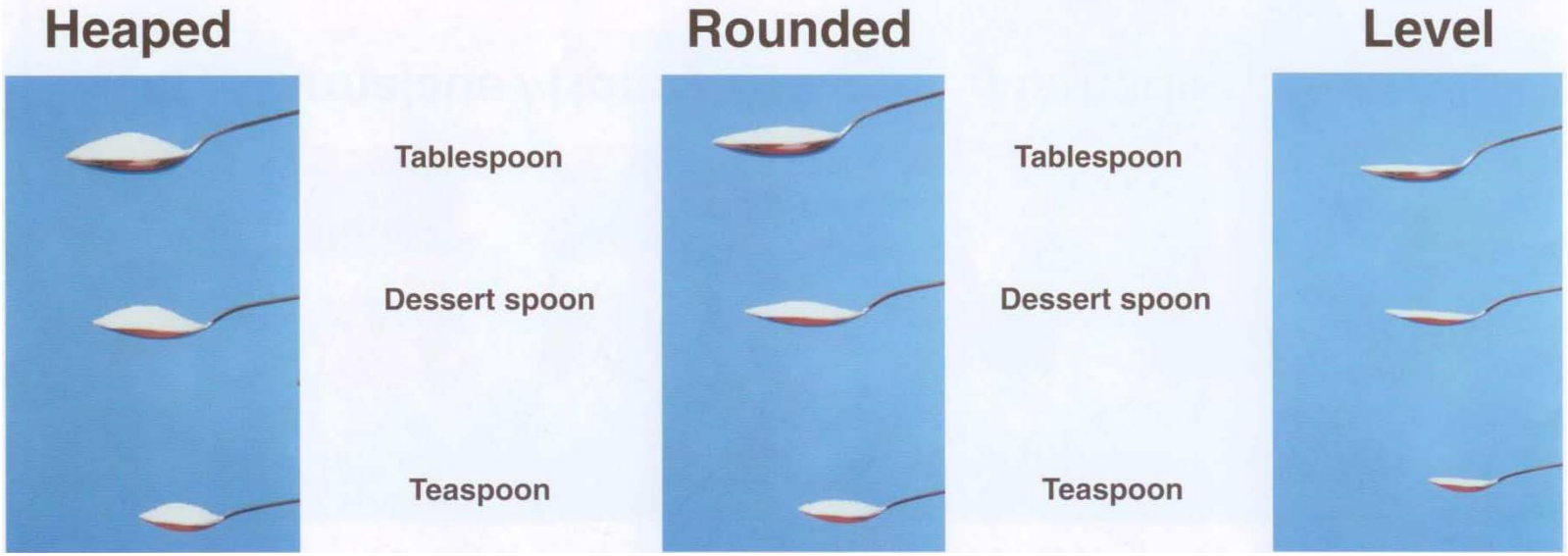
All plates are dinner size, diameter 27cm






215mL
355mL
390 / 610 / 715mL
710mL
A
B
C
D
Takeaway Containers


Muffins
A
B
C
50g
40g
150g
200g
250g
350g
Chocolate


Heaped
Rounded
Level
Tablespoon
Dessert spoon
Teaspoon
Tablespoon
Dessert spoon
Teaspoon

Peanut Butter
A
B
C
Jam / Marmalade / Honey
Vegemite / Marmite
A
B
C
Margarine / Butter
Cornflakes
A
B
C
Muesli
A
B
C

PORTION QUANTITIES

Peas & broccoli
A: 37 g
B: 63 g
C: 87 g

Vegetable combo
A: 72 g
B: 127 g
C: 208 g

Rice
A: 108 g
B: 144 g
C: 216 g

Spaghetti
A: 100 g
B: 145 g
C: 224 g

Chicken
A: Drumstick, cooked weight with skin 58 g, without skin 49 g
B: Boneless breast, cooked weight with skin 192 g, without skin 176 g
C: Thigh, cooked weight with skin, 119g, without skin 95 g
D: Breast on bone, cooked weight with skin 230 g, without skin, 200 g
E: Maryland, cooked weight with skin 177 g, without skin, 144 g

Stew
A 162 g
B: 256 g
C: 351 g

Meat
A: 36 g + 31 g gravy
B: 72 g + 63 g gravy
C: 108 g + 84 g gravy

Hot chips
A: 75 g
B: 150 g
C: 170 g
D: 300 g

Containers
Coffee 215 ml
Drink can 355 ml
Coke cups 390 ml, 610 ml, 715 ml
Milkshake 710 ml
Black 230 ml
White base 315 ml, lid 315 ml, total 630 ml
Cream, base 850 ml, lid 100 ml, total 950 ml
Clear base 1000 ml, lid 550 ml, total 1550 ml

Muffins
A: 62 g
B: 160 g
C: 185 g

Chocolate
Family block total, 350 g, row 33.6 g, piece, 4.2 g
King block, total 250 g, row 25.2 g, piece 4.2 g
Nestlé block, total 200 g, row 32.5 g, piece 4.7 g
Hershey block, total 40 g, row 9.6 g, piece 3.2 g
Chunky bar 50 g

Spoon volumes
Teaspoon, flat 4 ml, rounded 6 ml. heaped 14 ml
Dessert spoon, flat 7 ml, rounded 15 ml, heaped 20 ml
Table spoon, flat 14 ml, rounded 30 ml, heaped 60 ml

Peanut butter
A: 3 g
B: 6 g
C: 10 g

Jam/marmalade/honey
A: 4 g
B: 13 g
C: 23 g

Vegemite/marmite
A: 2 g
B: 3.5 g
C: 6 g

Margarine/butter
A: 3.5 g
B: 6 g
C: 9.9 g

Cornflakes
A: 30 g
B: 45 g
C: 60 g

Muesli
A: 50 g
B: 75 g
C: 100g

Volume of sphere = 4/3 πr^3

Area of circle = πr^2

Appendix VI: Very Low Calorie Diet Meal Plan

FOUR-DAY MEAL PLAN

Name: _____

Address: _____

_____

Phone: _____

Study code: _____

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

GUIDELINES

Food intake

1. This is meal plan for you to follow for the next **4** days.
2. You are required to follow **ALL** dietary intake instructions as they are detailed in the meal plan.
4. Please fill in the diary **IMMEDIATELY** after eating (i.e. tick off the food items consumed at each meal).
5. Please **record ALL drinks** such as tea/coffee (with or without milk), and any non-caloric beverages consumed (water or diet soft drinks).

Appetite

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

I feel satisfied **Not at all** ⊢——————————————⊣ **Very much**

Energy expenditure

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

SEDENTARY / EASY / MODERATE / HARD / VERY HARD

Morning ketone reading

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

Daily weight record

Please weigh yourself **EVERY DAY** immediately when you awake (before ingestion of any food or drinks)

DAY 1

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

WEIGHT: _____

BREAKFAST	TIME	FOOD ITEM	AMOUNT	Check ⊗
		Kicstart drink	1 sachet + 200 ml skim milk	

I feel satisfied Not at all ⊢——————————⊣ Very much

LUNCH	TIME	FOOD ITEM	AMOUNT	Check ⊗
		Mixed grain bread	2 slice	
		Ham	2 slice	
		Tomato	25 g	
		Cucumber	20 g	
		Lettuce	60 g	
		Carrot	20 g	
		Apple	1 medium	

I feel satisfied Not at all  Very much

DINNER	TIME	FOOD ITEM	AMOUNT	Check ⊗
		Lean Cuisine Beef Lasagne	300 g	
		Lettuce	140 g	
		Tomato	75 g	
		Cucumber	40 g	
		Carrot	40 g	
		Salad Dressing	30 g	

I feel satisfied Not at all ——— Very much

Please record below any drinks consumed throughout the day

DRINKS	TIME	TYPE	AMOUNT	Check ⊗
		Milk (daily allocation)	100 g	

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

SEDENTARY / EASY / MODERATE / HARD / VERY HARD

NOTE:
Appendices VII - IX are included in the print copy of the thesis held in the University of Adelaide Library.

CSIRO Food and Nutritional Sciences ©
PO Box 10041 Adelaide BC, South Australia 5000

References

Abbott, C.R., Small, C.J., Kennedy, A.R., Neary, N.M., Sajedi, A., Ghatei, M.A. and Bloom, S.R. (2005) *Blockade of the neuropeptide Y Y2 receptor with the specific antagonist BIIE0246 attenuates the effect of endogenous and exogenous peptide YY(3-36) on food intake*. Brain Res, 1043, 139–144.

Abumrad, N.A. (2005) *CD36 may determine our desire for dietary fats*. J Clin Invest, 115, 2965–2967.

Access Economics (2008) *The growing cost of obesity in 2008: three years on*. Diabetes Australia.

Adam, T.C. and Westerterp-Plantenga, M.S. (2005) *Glucagon-like peptide-1 release and satiety after a nutrient challenge in normal-weight and obese subjects*. Br J Nutr, 93, 845–851.

Adrian, T.E., Bloom, S.R., Hermansen, K. and Iversen, J. (1978) *Pancreatic polypeptide, glucagon and insulin secretion from the isolated perfused canine pancreas*. Diabetologia, 14, 413–417.

Adrian, T.E., Ferri, G.L., Bacarese-Hamilton, A.J., Fuessl, H.S., Polak, J.M. and Bloom, S.R. (1985a) *Human distribution and release of a putative new gut hormone, peptide YY*. Gastroenterology, 89, 1070–1077.

Adrian, T.E., Savage, A.P., Sagor, G.R., Allen, J.M., Bacarese-Hamilton, A.J., Tatemoto, K., Polak, J.M. and Bloom, S.R. (1985b) *Effect of peptide YY on gastric, pancreatic, and biliary function in humans*. Gastroenterology, 89, 494–499.

Anderson, E. and Long, J.A. (1947) *The effect of hyperglycemia on insulin secretion as determined with the isolated rat pancreas in a perfusion apparatus*. Endocrinology, 40, 92–97.

Andrews, J.M., Doran, S., Hebbard, G.S., Rassias, G., Sun, W.M. and Horowitz, M. (1998) *Effect of glucose supplementation on appetite and the pyloric motor response to intraduodenal glucose and lipid.* Am J Physiol Gastrointest Liver Physiol, 274, G645–G652.

Anton, S.D., Han, H., York, E., Martin, C.K., Ravussin, E. and Williamson, D.A. (2009) *Effect of calorie restriction on subjective ratings of appetite*. J Hum Nutr Diet, 22, 141–147.

Anvari, M., Horowitz, M., Fraser, R., Maddox, A., Myers, J., Dent, J. and Jamieson, G.G. (1995) *Effects of posture on gastric emptying of nonnutrient liquids and antropyloroduodenal motility.* Am J Physiol, 268, G868–G871.

Aponte, G.W., Fink, A.S., Meyer, J.H., Tatemoto, K. and Taylor, I.L. (1985) *Regional distribution and release of peptide YY with fatty acids of different chain length*. Am J Physiol Gastrointest Liver Physiol, 249, G745–750.

ASTM (2004) *Standard Practice for Determination of Odor and Taste Thresholds By a Forced-Choice Ascending Concentration Series Method of Limits*. American Society for Testing and Materials, E679.

Avenell, A., Brown, T.J., McGee, M.A., Campbell, M.K., Grant, A.M., Broom, J., Jung, R.T. and Smith, W.C. (2004) *What are the long-term benefits of weight reducing diets in adults? A systematic review of randomized controlled trials*. J Hum Nutr Diet, 17, 317–335.

Azpiroz, F. and Malagelada, J.R. (1987) *Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis*. Gastroenterology, 92, 934–943.

Bagger, J.I., Knop, F.K., Lund, A., Vestergaard, H., Holst, J.J. and Vilsboll, T. (2011) *Impaired regulation of the incretin effect in patients with type 2 diabetes.* J Clin Endocrinol Metab, 96, 737–745.

Baghurst, K.L. and Baghurst, P.A. (1981) *The measurement of usual dietary intake in individuals and groups.* Transactions of the Menzies Foundation, 3, 139–160.

Baillie, A.G., Coburn, C.T. and Abumrad, N.A. (1996) *Reversible binding of long-chain fatty acids to purified FAT, the adipose CD36 homolog*. J Membr Biol, 153, 75–81.

Baranowska, B., Radzikowska, M., Wasilewska-Dziubinska, E., Roguski, K. and Borowiec, M. (2000) *Disturbed release of gastrointestinal peptides in anorexia nervosa and in obesity*. Diabetes Obes Metab, 2, 99–103.

Batterham, R.L., Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A., Dakin, C.L., Wren, A.M., Brynes, A.E., Low, M.J., Ghatei, M.A., Cone, R.D. and Bloom, S.R. (2002) *Gut hormone PYY(3-36) physiologically inhibits food intake*. Nature, 418, 650–654.

Batterham, R.L. and Bloom, S.R. (2003) *The gut hormone peptide YY regulates appetite*. Ann N Y Acad Sci, 994, 162–168.

Batterham, R.L., Cohen, M.A., Ellis, S.M., Le Roux, C.W., Withers, D.J., Frost, G.S., Ghatei, M.A. and Bloom, S.R. (2003) *Inhibition of food intake in obese subjects by peptide YY3-36*. N Engl J Med, 349, 941–948.

Beck, B., Musse, N. and Stricker-Krongrad, A. (2002) *Ghrelin, macronutrient intake and dietary preferences in long-evans rats*. Biochem Biophys Res Commun, 292, 1031–1035.

Beglinger, C., Degen, L., Matzinger, D., D'Amato, M. and Drewe, J. (2001) *Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans*. Am J Physiol Regul Integr Comp Physiol, 280, R1149–1154.

Bertino, M., Beauchamp, G.K. and Engelman, K. (1982) *Long-term reduction in dietary sodium alters the taste of salt*. Am J Clin Nutr, 36, 1134–1144.

Bertino, M., Beauchamp, G.K. and Engelman, K. (1986) *Increasing dietary salt alters salt taste preference*. Physiol Behav, 38, 203–213.

Bland, J.M. and Altman, D.G. (1995) *Calculating correlation coefficients with repeated observations: Part 1--Correlation within subjects*. BMJ, 310, 446.

Borgstrom, B., Dahlqvist, A., Lundh, G. and Sjovall, J. (1957) *Studies of intestinal digestion and absorption in the human*. J Clin Invest, 36, 1521–1536.

Bowen, J., Noakes, M. and Clifton, P.M. (2006) *Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake*. J Clin Endocrinol Metab, 91, 2913–2919.

Boyd, K.A., O'Donovan, D.G., Doran, S., Wishart, J., Chapman, I.M., Horowitz, M. and Feinle, C. (2003) *High-fat diet effects on gut motility, hormone, and appetite responses to duodenal lipid in healthy men*. Am J Physiol Gastrointest Liver Physiol, 284, G188–196.

Bray, G.A. (2003) *Risks of obesity*. Endocrinol Metab Clin North Am, 32, 787–804, viii.

Brener, W., Hendrix, T.R. and McHugh, P.R. (1983) *Regulation of the gastric emptying of glucose.* Gastroenterology, 85, 76–82.

Brennan, I.M., Feltrin, K.L., Horowitz, M., Smout, A.J., Meyer, J.H., Wishart, J. and Feinle-Bisset, C. (2005) *Evaluation of interactions between CCK and GLP-1 in their effects on appetite, energy intake and antropyloroduodenal motility in healthy men*. Am J Physiol Regul Integr Comp Physiol, 288, R1477–1485.

Brennan, I.M., Otto, B., Feltrin, K.L., Meyer, J.H., Horowitz, M. and Feinle-Bisset, C. (2007) *Intravenous CCK-8, but not GLP-1, suppresses ghrelin and stimulates PYY release in healthy men*. Peptides, 28, 607–611.

Brennan, I.M., Little, T.J., Feltrin, K.L., Smout, A.J., Wishart, J.M., Horowitz, M. and Feinle-Bisset, C. (2008) *Dose-dependent effects of cholecystokinin-8 on antropyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men*. Am J Physiol Endocrinol Metab, 295, E1487–1494.

Brennan, I.M., Feltrin, K.L., Nair, N.S., Hausken, T., Little, T.J., Gentilcore, D., Wishart, J.M., Jones, K.L., Horowitz, M. and Feinle-Bisset, C. (2009) *Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women*. Am J Physiol Gastrointest Liver Physiol, 297, G602–610.

Brennan, I.M., Seimon, R.V., Luscombe-Marsh, N.D., Otto, B., Horowitz, M. and Feinle-Bisset, C. (2010) *Effects of acute energy restriction on gastrointestinal motor and hormone, and appetite responses to intraduodenal lipid in obese men* Int J Obes, 1–9.

Brown, J.C., Mutt, V. and Pederson, R.A. (1970) *Further purification of a polypeptide demonstrating enterogastrone activity*. J Physiol, 209, 57–64.

Brown, N.J., Rumsey, R.D. and Read, N.W. (1994) *Gastrointestinal adaptation to enhanced small intestinal lipid exposure*. Gut, 35, 1409–1412.

Camilleri, M. and Malagelada, J.R. (1984) *Abnormal intestinal motility in diabetics with the gastroparesis syndrome*. Eur J Clin Invest, 14, 420–427.

Carr, R.D., Larsen, M.O., Jelic, K., Lindgren, O., Vikman, J., Holst, J.J., Deacon, C.F. and Ahren, B. (2010) *Secretion and dipeptidyl peptidase-4-mediated metabolism of incretin hormones after a mixed meal or glucose ingestion in obese compared to lean, nondiabetic men*. J Clin Endocrinol Metab, 95, 872–878.

Cartoni, C., Yasumatsu, K., Ohkuri, T., Shigemura, N., Yoshida, R., Godinot, N., le Coutre, J., Ninomiya, Y. and Damak, S. *Taste preference for fatty acids is mediated by GPR40 and GPR120*. J Neurosci, 30, 8376–8382.

Castiglione, K.E., Read, N.W. and French, S.J. (2002) *Adaptation to high-fat diet accelerates emptying of fat but not carbohydrate test meals in humans*. Am J Physiol Regul Integr Comp Physiol, 282, R366–371.

Cecil, J.E., Francis, J. and Read, N.W. (1999) *Comparison of the effects of a high-fat and high-carbohydrate soup delivered orally and intragastrically on gastric emptying, appetite, and eating behaviour*. Physiol Behav, 67, 299–306.

Chaikomin, R., Doran, S., Jones, K.L., Feinle-Bisset, C., O'Donovan, D., Rayner, C.K. and Horowitz, M. (2005) *Initially more rapid small intestinal glucose delivery increases plasma insulin, GIP and GLP-1 but does not improve overall glycemia in healthy subjects*. Am J Physiol Endocrinol Metab 289: E504–507.

Chale-Rush, A., Burgess, J.R. and Mattes, R.D. (2007a) *Evidence for human orosensory (taste?) sensitivity to free fatty acids*. Chem Senses, 32, 423–431.

Chale-Rush, A., Burgess, J.R. and Mattes, R.D. (2007b) *Multiple routes of chemosensitivity to free fatty acids in humans*. Am J Physiol Gastrointest Liver Physiol, 292, G1206–1212.

Chandrashekar, J., Hoon, M.A., Ryba, N.J. and Zuker, C.S. (2006) *The receptors and cells for mammalian taste*. Nature, 444, 288–294.

Chapman, I.M., Goble, E.A., Wittert, G.A. and Horowitz, M. (1999) *Effects of small-intestinal fat and carbohydrate infusions on appetite and food intake in obese and nonobese men*. Am J Clin Nutr, 69, 6–12.

Chapman, M., Fraser, R., Vozzo, R., Bryant, L., Tam, W., Nguyen, N.Q., Zacharakis, B., Butler, R., Davidson, G.P. and Horowitz, M. (2005) *Antro-pyloro-duodenal motor responses to gastric and duodenal nutrient in critically ill patients*. Gut.

Chavez-Jauregui, R.N., Mattes, R.D. and Parks, E.J. *Dynamics of fat absorption and effect of sham feeding on postprandial lipema*. Gastroenterology, 139, 1538–1548.

Chen, M., Yang, Y., Braunstein, E., Georgeson, K.E. and Harmon, C.M. (2001) *Gut expression and regulation of FAT/CD36: possible role in fatty acid transport in rat enterocytes*. Am J Physiol Endocrinol Metab, 281, E916–923.

Chin, K.V. (2008) *Small molecule intervention for obesity*.

Colditz, G.A., Willett, W.C., Rotnitzky, A. and Manson, J.E. (1995) *Weight gain as a risk factor for clinical diabetes mellitus in women*. Ann Intern Med, 122, 481–486.

Collins, P.J., Horowitz, M., Cook, D.J., Harding, P.E. and Shearman, D.J. (1983) *Gastric emptying in normal subjects—a reproducible technique using a single scintillation camera and computer system*. Gut, 24, 1117–1125.

Collins, P.J., Houghton, L.A., Read, N.W., Horowitz, M., Chatterton, B.E., Heddle, R. and Dent, J. (1991) *Role of the proximal and distal stomach in mixed solid and liquid meal emptying*. Gut, 32, 615–619.

Connolly, H.M., Crary, J.L., McGoon, M.D., Hensrud, D.D., Edwards, B.S., Edwards, W.D. and Schaff, H.V. (1997) *Valvular heart disease associated with fenfluramine-phentermine*. N Engl J Med, 337, 581–588.

Cook, C.G., Andrews, J.M., Jones, K.L., Wittert, G.A., Chapman, I.M., Morley, J.E. and Horowitz, M. (1997) *Effects of small intestinal nutrient infusion on appetite and pyloric motility are modified by age*. Am J Physiol, 273, R755–761.

Corvilain, B., Abramowicz, M., Fery, F., Schoutens, A., Verlinden, M., Balasse, E. and Horowitz, M. (1995) *Effect of short-term starvation on gastric emptying in humans: relationship to oral glucose tolerance*. Am J Physiol, 269, G512–517.

Covasa, M. and Ritter, R.C. (1998) *Rats maintained on high-fat diets exhibit reduced satiety in response to CCK and bombesin*. Peptides, 19, 1407–1415.

Covasa M and Ritter RC. (1999) *Reduced sensitivity to the satiation effect of intestinal oleate in rats adapted to high-fat diet*. Am J Physiol, 277, R279–R285.

Covasa, M. and Ritter, R.C. (2000) *Adaptation to high-fat diet reduces inhibition of gastric emptying by CCK and intestinal oleate*. Am J Physiol, 278, R166–R170.

Covasa, M., Grahn, J. and Ritter, R.C. (2000a) *Reduced hindbrain and enteric neuronal response to intestinal oleate in rats maintained on high-fat diet*. Auton Neurosci, 84, 8–18.

Covasa, M., Grahn, J. and Ritter, R.C. (2000b) *High fat maintenance diet attenuates hindbrain neuronal response to CCK.* Regul Pept, 86, 83–88.

Covasa, M., Marcuson, J.K. and Ritter, R.C. (2001) *Diminished satiation in rats exposed to elevated levels of endogenous or exogenous cholecystokinin*. Am J Physiol Regul Integr Comp Physiol, 280, R331–337.

Covington, D.K., Briscoe, C.A., Brown, A.J. and Jayawickreme, C.K. (2006) *The G-protein-coupled receptor 40 family (GPR40-GPR43) and its role in nutrient sensing*. Biochem Soc Trans, 34, 770–773.

Creutzfeldt, W. (2005) *The [pre-] history of the incretin concept*. Regul Pept, 128, 87–91.

Crystal, S.R. and Teff, K.L. (2006) *Tasting fat: cephalic phase hormonal responses and food intake in restrained and unrestrained eaters*. Physiol Behav, 89, 213–220.

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E. and Weigle, D.S. (2001) *A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans*. Diabetes, 50, 1714–1719.

Cummings, D.E., Weigle, D.S., Frayo, R.S., Breen, P.A., Ma, M.K., Dellinger, E.P. and Purnell, J.Q. (2002) *Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery*. N Engl J Med, 346, 1623–1630.

Cunningham, K.M., Horowitz, M. and Read, N.W. (1991a) *The effect of short-term dietary supplementation with glucose on gastric emptying in humans*. Br J Nutr, 65, 15–19.

Cunningham, K.M., Daly, J., Horowitz, M. and Read, N.W. (1991b) *Gastrointestinal adaptation to diets of differing fat composition in human volunteers*. Gut, 32, 483–486.

D'Alessio, D.A., Kahn, S.E., Leusner, C.R. and Ensinck, J.W. (1994) *Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal*. J Clin Invest, 93, 2263–2266.

Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M.S., Suganuma, T., Matsukura, S., Kangawa, K. and Nakazato, M. (2000) *Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans*. Endocrinology, 141, 4255–4261.

Degen, L., Oesch, S., Casanova, M., Graf, S., Ketterer, S., Drewe, J. and Beglinger, C. (2005) *Effect of peptide YY3-36 on food intake in humans*. Gastroenterology, 129, 1430–1436.

Degen, L., Drewe, J., Piccoli, F., Grani, K., Oesch, S., Bunea, R., D'Amato, M. and Beglinger, C. (2007) *Effect of CCK-1 receptor blockade on ghrelin and PYY secretion in men*. Am J Physiol Regul Integr Comp Physiol, 292, R1391–1399.

Delgado-Aros, S., Kim, D.Y., Burton, D.D., Thomforde, G.M., Stephens, D., Brinkmann, B.H., Vella, A. and Camilleri, M. (2002) *Effect of GLP-1 on gastric volume, emptying, maximum volume ingested, and postprandial symptoms in humans*. Am J Physiol Gastrointest Liver Physiol, 282, G424–431.

DeMaria, E.J., Sugerman, H.J., Kellum, J.M., Meador, J.G. and Wolfe, L.G. (2002) *Results of 281 consecutive total laparoscopic Roux-en-Y gastric bypasses to treat morbid obesity*. Ann Surg, 235, 640–645; discussion 645–647.

Despres, J.P. (1994) *Dyslipidaemia and obesity*. Baillieres Clin Endocrinol Metab, 8, 629–660.

Dinehart, M.E., Hayes, J.E., Bartoshuk, L.M., Lanier, S.L. and Duffy, V.B. (2006) *Bitter taste markers explain variability in vegetable sweetness, bitterness, and intake*. Physiol Behav, 87, 304–313.

Dornonville de la Cour, C., Lindstrom, E., Norlen, P. and Hakanson, R. (2004) *Ghrelin stimulates gastric emptying but is without effect on acid secretion and gastric endocrine cells*. Regul Pept, 120, 23–32.

Doucet, E., St-Pierre, S., Almeras, N. and Tremblay, A. (2003) *Relation between appetite ratings before and after a standard meal and estimates of daily energy intake in obese and reduced obese individuals*. Appetite, 40, 137–143.

Doucet, E., Pomerleau, M. and Harper, M.E. (2004) *Fasting and postprandial total ghrelin remain unchanged after short-term energy restriction*. J Clin Endocrinol Metab, 89, 1727–1732.

Drewnowski, A., Henderson, S.A., Levine, A. and Hann, C. (1999) *Taste and food preferences as predictors of dietary practices in young women*. Public Health Nutr, 2, 513–519.

Druce, M.R., Wren, A.M., Park, A.J., Milton, J.E., Patterson, M., Frost, G., Ghatei, M.A., Small, C. and Bloom, S.R. (2005) *Ghrelin increases food intake in obese as well as lean subjects*. Int J Obes (Lond), 29, 1130–1136.

Dupre, J., Ross, S.A., Watson, D. and Brown, J.C. (1973) *Stimulation of insulin secretion by gastric inhibitory polypeptide in man*. J Clin Endocrinol Metab, 37, 826–828.

Edelbroek, M., Horowitz, M., Maddox, A. and Bellen, J. (1992) *Gastric emptying and intragastric distribution of oil in the presence of a liquid or a solid meal*. J Nucl Med, 33, 1283–1290.

Edwards, C.M., Todd, J.F., Mahmoudi, M., Wang, Z., Wang, R.M., Ghatei, M.A. and Bloom, S.R. (1999) *Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9-39*. Diabetes, 48, 86–93.

Eissele, R., Goke, R., Willemer, S., Harthus, H.P., Vermeer, H., Arnold, R. and Goke, B. (1992) *Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man*. Eur J Clin Invest, 22, 283–291.

Ekblad, E. and Sundler, F. (2002) *Distribution of pancreatic polypeptide and peptide YY*. Peptides, 23, 251–261.

Elahi, D., Andersen, D.K., Muller, D.C., Tobin, J.D., Brown, J.C. and Andres, R. (1984) *The enteric enhancement of glucose-stimulated insulin release. The role of GIP in aging, obesity, and non-insulin-dependent diabetes mellitus*. Diabetes, 33, 950–957.

Elliot, D.L., Goldberg, L., Kuehl, K.S. and Bennett, W.M. (1989) *Sustained depression of the resting metabolic rate after massive weight loss*. Am J Clin Nutr, 49, 93–96.

English, P.J., Ghatei, M.A., Malik, I.A., Bloom, S.R. and Wilding, J.P. (2002) *Food fails to suppress ghrelin levels in obese humans*. J Clin Endocrinol Metab, 87, 2984.

Erdmann, J., Lippl, F. and Schusdziarra, V. (2003) *Differential effect of protein and fat on plasma ghrelin levels in man*. Regul Pept, 116, 101–107.

Eysselein, V.E., Eberlein, G.A., Hesse, W.H., Schaeffer, M., Grandt, D., Williams, R., Goebell, H. and Reeve, J.R., Jr. (1990) *Molecular variants of cholecystokinin after endogenous stimulation in humans: a time study*. Am J Physiol, 258, G951–957.

Falko, J.M., Crockett, S.E., Cataland, S. and Mazzaferri, E.L. (1975) *Gastric inhibitory polypeptide (GIP) stimulated by fat ingestion in man*. J Clin Endocrinol Metab, 41, 260–265.

Fehmann, H.C., Goke, R. and Goke, B. (1995) *Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide*. Endocr Rev, 16, 390–410.

Feinle, C., D'Amato, M. and Read, N.W. (1996) *Cholecystokinin-A receptors modulate gastric sensory and motor responses to gastric distension and duodenal lipid*. Gastroenterology, 110, 1379–1385.

Feinle, C., Grundy, D. and Read, N.W. (1997) *Effects of duodenal nutrients on sensory and motor responses of the human stomach to distension*. Am J Physiol Gastrointest Liver Physiol, 273, G721–G726.

Feinle, C., Meier, O., Otto, B., D'Amato, M. and Fried, M. (2001) *Role of duodenal lipid and cholecystokinin A receptors in the pathophysiology of functional dyspepsia.* Gut, 48, 347–355.

Feinle, C., Chapman, I.M., Wishart, J. and Horowitz, M. (2002) *Plasma glucagon-like peptide-1 (GLP-1) responses to duodenal fat and glucose infusions in healthy and obese men*. Peptides, 23, 1491–1495.

Feinle, C., O'Donovan, D., Doran, S., Andrews, J.M., Wishart, J., Chapman, I. and Horowitz, M. (2003) *Effects of fat digestion on appetite, APD motility, and gut hormones in response to duodenal fat infusion in humans*. Am J Physiol Gastrointest Liver Physiol, 284, G798–807.

Feinle-Bisset, C., Patterson, M., Ghatei, M.A., Bloom, S.R. and Horowitz, M. (2005) *Fat digestion is required for the suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid*. Am J Physiol Endocrinol Metab, 289, E948–E953.

Feltrin, K.L., Little, T.J., Meyer, J.H., Horowitz, M., Smout, A.J., Wishart, J., Pilichiewicz, A.N., Rades, T., Chapman, I.M. and Feinle-Bisset, C. (2004) *Effects of intraduodenal fatty acids on appetite, antropyloroduodenal motility, and plasma CCK and GLP-1 in humans vary with their chain length*. Am J Physiol Regul Integr Comp Physiol, 287, R524–533.

Feltrin, K.L., Patterson, M., Ghatei, M.A., Bloom, S.R., Meyer, J.H., Horowitz, M. and Feinle-Bisset, C. (2006) *Effect of fatty acid chain length on suppression of ghrelin and stimulation of PYY, GLP-2 and PP secretion in healthy men*. Peptides, 27, 1638–1643.

Feltrin, K.L., Little, T.J., Meyer, J.H., Horowitz, M., Rades, T., Wishart, J. and Feinle-Bisset, C. (2007) *Effects of lauric acid on upper gut motility, plasma*

cholecystokinin and peptide YY, and energy intake are load, but not concentration, dependent in humans. J Physiol, 581, 767–777.

Feltrin, K.L., Little, T.J., Meyer, J.H., Horowitz, M., Rades, T., Wishart, J. and Feinle-Bisset, C. (2008) *Comparative effects of intraduodenal infusions of lauric and oleic acids on antropyloroduodenal motility, plasma cholecystokinin and peptide YY, appetite, and energy intake in healthy men*. Am J Clin Nutr, 87, 1181–1187.

Flint, A., Raben, A., Astrup, A. and Holst, J.J. (1998) *Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans*. J Clin Invest, 101, 515–520.

Forbes, G.B. (2000) *Body fat content influences the body composition response to nutrition and exercise*. Ann N Y Acad Sci, 904, 359–365.

Ford, E.S., Williamson, D.F. and Liu, S. (1997) *Weight change and diabetes incidence: findings from a national cohort of US adults*. Am J Epidemiol, 146, 214–222.

Foster, G.D., Wyatt, H.R., Hill, J.O., McGuckin, B.G., Brill, C., Mohammed, B.S., Szapary, P.O., Rader, D.J., Edman, J.S. and Klein, S. (2003) *A randomized trial of a low-carbohydrate diet for obesity*. N Engl J Med, 348, 2082–2090.

Fraser, R., Fone, D., Horowitz, M. and Dent, J. (1993) *Cholecystokinin octapeptide stimulates phasic and tonic pyloric motility in healthy humans.* Gut, 34, 33–37.

French, S.J., Murray, B., Rumsey, R.D., Sepple, C.P. and Read, N.W. (1993) *Preliminary studies on the gastrointestinal responses to fatty meals in obese people*. Int J Obes Relat Metab Disord, 17, 295–300.

French, S.J., Murray, B., Rumsey, R.D., Fadzlin, R. and Read, N.W. (1995) *Adaptation to high-fat diets: effects on eating behaviour and plasma cholecystokinin*. Br J Nutr, 73, 179–189.

Fried, M., Schwizer, W., Beglinger, C., Keller, U., Jansen, J.B. and Lamers, C.B. (1991a) *Physiological role of cholecystokinin on postprandial insulin secretion and gastric meal emptying in man. Studies with the cholecystokinin receptor antagonist loxiglumide*. Diabetologia, 34, 721–726.

Fried, M., Erlacher, U., Schwizer, W., Lochner, C., Koerfer, J., Beglinger, C., Jansen, J.B., Lamers, C.B., Harder, F., Bischof-Delaloye, A., Stalder, G.A. and Rovati, L. (1991b) *Role of cholecystokinin in the regulation of gastric emptying and pancreatic enzyme secretion in humans. Studies with the cholecystokinin-receptor antagonist loxiglumide.* Gastroenterology, 101, 503–511.

Fujino, K., Inui, A., Asakawa, A., Kihara, N., Fujimura, M. and Fujimiya, M. (2003) *Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats*. J Physiol, 550, 227–240.

Gaillard, D., Laugerette, F., Darcel, N., El-Yassimi, A., Passilly-Degrace, P., Hichami, A., Khan, N.A., Montmayeur, J.P. and Besnard, P. (2008) *The gustatory pathway is involved in CD36-mediated orosensory perception of long-chain fatty acids in the mouse*. FASEB J, 22, 1458–1468.

Gaudreau, P., Quirion, R., St-Pierre, S. and Pert, C.B. (1983) *Characterization and visualization of cholecystokinin receptors in rat brain using [3H]pentagastrin*. Peptides, 4, 755–762.

Gibbs, J., Young, R.C. and Smith, G.P. (1973) *Cholecystokinin elicits satiety in rats with open gastric fistulas*. Nature, 245, 323–325.

Gibson, R.S. (1993) *Nutritional assessment : a laboratory manual*. In 2 (ed.). Oxford University Press, New York, p. 196.

Gibson, R.S. (2005) *Principles of nutritional assessment*. Oxford University Press.

Gilbertson, T.A., Fontenot, D.T., Liu, L., Zhang, H. and Monroe, W.T. (1997) *Fatty acid modulation of K+ channels in taste receptor cells: gustatory cues for dietary fat*. Am J Physiol, 272, C1203–1210.

Gilbertson, T.A., Liu, L., York, D.A. and Bray, G.A. (1998) *Dietary fat preferences are inversely correlated with peripheral gustatory fatty acid sensitivity*. Ann N Y Acad Sci, 855, 165–168.

Glasbrenner, B., Pieramico, O., Brecht-Krauss, D., Baur, M. and Malfertheiner, P. (1993) *Gastric emptying of solids and liquids in obesity*. Clinical Investigation, 71, 542–546.

Golay, A. and Bobbioni, E. (1997) *The role of dietary fat in obesity*. Int J Obes Relat Metab Disord, 21 Suppl 3, S2–11.

Grandt, D., Schimiczek, M., Beglinger, C., Layer, P., Goebell, H., Eysselein, V.E. and Reeve, J.R., Jr. (1994) *Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36*. Regul Pept, 51, 151–159.

Greenman, Y., Golani, N., Gilad, S., Yaron, M., Limor, R. and Stern, N. (2004) *Ghrelin secretion is modulated in a nutrient- and gender-specific manner*. Clin Endocrinol (Oxf), 60, 382–388.

Grudell, A.B. and Camilleri, M. (2007) *The role of peptide YY in integrative gut physiology and potential role in obesity*. Curr Opin Endocrinol Diabetes Obes, 14, 52–57.

Gryback, P., Naslund, E., Hellstrom, P.M., Jacobsson, H. and Backman, L. (1996) *Gastric emptying of solids in humans: improved evaluation by Kaplan-Meier plots, with special reference to obesity and gender*. Eur J Nucl Med, 23, 1562–1567.

Guh, D.P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C.L. and Anis, A.H. (2009) *The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis*. BMC Public Health, 9, 88.

Gutniak, M., Orskov, C., Holst, J.J., Ahren, B. and Efendic, S. (1992) *Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus*. N Engl J Med, 326, 1316–1322.

Gutzwiller, J.P., Goke, B., Drewe, J., Hildebrand, P., Ketterer, S., Handschin, D., Winterhalder, R., Conen, D. and Beglinger, C. (1999) *Glucagon-like peptide-1: a potent regulator of food intake in humans*. Gut, 44, 81–86.

Hallschmid, M., Smolnik, R., McGregor, G., Born, J. and Fehm, H.L. (2006) *Overweight humans are resistant to the weight-reducing effects of melanocortin4-10*. J Clin Endocrinol Metab, 91, 522–525.

Harper, A.A. and Raper, H.S. (1943) *Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine*. J Physiol, 102, 115–125.

Harris, J.A and Benedict, F.G. (1918) *A Biometric Study of Human Basal Metabolism*. Proc Natl Acad Sci USA, 4, 370–373.

Havel, P.J., Kasim-Karakas, S., Mueller, W., Johnson, P.R., Gingerich, R.L. and Stern, J.S. (1996) *Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss*. J Clin Endocrinol Metab, 81, 4406–4413.

Heath, R.B., Jones, R., Frayn, K.N. and Robertson, M.D. (2004) *Vagal stimulation exaggerates the inhibitory ghrelin response to oral fat in humans*. J Endocrinol, 180, 273–281.

Heddle, R., Dent, J., Read, N.W., Houghton, L.A., Toouli, J., Horowitz, M., Maddern, G.J. and Downton, J. (1988a) *Antropyloroduodenal motor responses to intraduodenal lipid infusion in healthy volunteers*. Am J Physiol, 254, G671–679.

Heddle, R., Dent, J., Toouli, J. and Read, N.W. (1988b) *Topography and measurement of pyloric pressure waves and tone in humans*. Am J Physiol, 255, G490–497.

Heddle, R., Collins, P.J., Dent, J., Horowitz, M., Read, N.W., Chatterton, B. and Houghton, L.A. (1989) *Motor mechanisms associated with slowing of the gastric emptying of a solid meal by an intraduodenal lipid infusion*. J Gastroenterol Hepatol, 4, 437–447.

Heilbronn, L.K., de Jonge, L., Frisard, M.I., DeLany, J.P., Larson-Meyer, D.E., Rood, J., Nguyen, T., Martin, C.K., Volaufova, J., Most, M.M., Greenway, F.L., Smith, S.R., Deutsch, W.A., Williamson, D.A. and Ravussin, E. (2006) *Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial*. JAMA, 295, 1539–1548.

Hellstrom, P.M. and Naslund, E. (2001) *Interactions between gastric emptying and satiety, with special reference to glucagon-like peptide-1*. Physiol Behav, 74, 735–741.

Hill, D.R., Campbell, N.J., Shaw, T.M. and Woodruff, G.N. (1987) *Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists*. J Neurosci, 7, 2967–2976.

Hirasawa, A., Tsumaya, K., Awaji, T., Katsuma, S., Adachi, T., Yamada, M., Sugimoto, Y., Miyazaki, S. and Tsujimoto, G. (2005) *Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120*. Nat Med, 11, 90–94.

Hodge, A., Patterson, A.J., Brown, W.J., Ireland, P. and Giles, G. (2000) *The Anti-Cancer Council of Victoria FFQ: relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation*. Aust N Z J Public Health, 24, 576–583.

Holt, S., Reid, J., Taylor, T.V., Tothill, P. and Heading, R.C. (1982) *Gastric emptying of solids in man*. Gut, 23, 292–296.

Horowitz, M., Collins, P.J., Cook, D.J., Harding, P.E. and Shearman, D.J. (1983) *Abnormalities of gastric emptying in obese patients*. Int J Obes, 7, 415–421.

Horowitz, M., Maddox, A.F., Wishart, J.M., Harding, P.E., Chatterton, B.E. and Shearman, D.J.C. (1991) *Relationships between oesophageal transit and solid and liquid gastric emptying in diabetes mellitus*. Eur J Nucl Med, 18, 229–234.

Horowitz, M. and Dent, J. (1991) *Disordered gastric emptying: mechanical basis, assessment and treatment*. Baillieres Clin Gastroenterol, 5, 371–407.

Horowitz, M., Edelbroek, M.A., Wishart, J.M. and Straathof, J.W. (1993) *Relationship between oral glucose tolerance and gastric emptying in normal healthy subjects*. Diabetologia, 36, 857–862.

Horowitz, M., Dent, J., Fraser, R., Sun, W. and Hebbard, G. (1994) *Role and integration of mechanisms controlling gastric emptying*. Dig Dis Sci, 39, 7S–13S.

Houghton, L.A., Read, N.W., Heddle, R., Horowitz, M., Collins, P.J., Chatterton, B. and Dent, J. (1988) *Relationship of the motor activity of the antrum, pylorus, and duodenum to gastric emptying of a solid-liquid mixed meal.* Gastroenterology, 94, 1285–1291.

Hunt, J.N., Smith, J.L. and Jiang, C.L. (1985) *Effect of meal volume and energy density on the gastric emptying of carbohydrates*. Gastroenterology, 89, 1326–1330.

Hutson, W.R. and Wald, A. (1993) *Obesity and weight reduction do not influence gastric emptying and antral motility*. Am J Gastroenterol, 88, 1405–1409.

Ibrahimi, A., Sfeir, Z., Magharaie, H., Amri, E.Z., Grimaldi, P. and Abumrad, N.A. (1996) *Expression of the CD36 homolog (FAT) in fibroblast cells: effects on fatty acid transport*. Proc Natl Acad Sci U S A, 93, 2646–2651.

Jackson, S.J., Leahy, F.E., McGowan, A.A., Bluck, L.J., Coward, W.A. and Jebb, S.A. (2004) *Delayed gastric emptying in the obese: an assessment using the non-invasive (13)C-octanoic acid breath test*. Diabetes Obes Metab, 6, 264–270.

James, W.P., Caterson, I.D., Coutinho, W., Finer, N., Van Gaal, L.F., Maggioni, A.P., Torp-Pedersen, C., Sharma, A.M., Shepherd, G.M., Rode, R.A. and Renz, C.L. (2010) *Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects*. N Engl J Med, 363, 905–917.

Jesudason, D.R., Monteiro, M.P., McGowan, B.M., Neary, N.M., Park, A.J., Philippou, E., Small, C.J., Frost, G.S., Ghatei, M.A. and Bloom, S.R. (2007) *Low-dose pancreatic polypeptide inhibits food intake in man*. Br J Nutr, 97, 426–429.

Johnston, C.S., Tjonn, S.L. and Swan, P.D. (2004) *High-protein, low-fat diets are effective for weight loss and favorably alter biomarkers in healthy adults*. J Nutr, 134, 586–591.

Jones, K.L., Horowitz, M., Wishart, M.J., Maddox, A.F., Harding, P.E. and Chatterton, B.E. (1995) *Relationships between gastric emptying, intragastric meal distribution and blood glucose concentrations in diabetes mellitus*. J Nucl Med, 36, 2220–2228.

Jones, K.L., Doran, S.M., Hveem, K., Bartholomeusz, F.D., Morley, J.E., Sun, W.M., E., C.B. and Horowitz, M. (1997) *Relation between postprandial satiation and antral area in normal subjects.* Am J Clin Nutr, 66, 127–132.

Kanayama, S. and Liddle, R.A. (1991) *Influence of food deprivation on intestinal cholecystokinin and somatostatin*. Gastroenterology, 100, 909–915.

Kaplan, L.M. (2005) *Pharmacological therapies for obesity*. Gastroenterol Clin North Am, 34, 91–104.

Kare, M.R. and Mattes, R.D. (1990) *A selective overview of the chemical senses*. Nutr Rev, 48, 39–48; discussion 114–131.

Katschinski, M., Schirra, J., Begliner, C., Langbein, S., Wank, U., D'Amato, M. and Arnold, R. (1996) *Intestinal phase of human antro-pyloro-duodenal motility: cholinergic and CCK-mediated regulation*. Eur J Clin Invest, 26, 574–583.

Keim, N.L., Stern, J.S. and Havel, P.J. (1998) *Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women*. Am J Clin Nutr, 68, 794–801.

Kernan, W.N., Viscoli, C.M., Brass, L.M., Broderick, J.P., Brott, T., Feldmann, E., Morgenstern, L.B., Wilterdink, J.L. and Horwitz, R.I. (2000) *Phenylpropanolamine and the risk of hemorrhagic stroke*. N Engl J Med, 343, 1826–1832.

Kissileff, H.R., Pi-Sunyer, F.X., Thornton, J. and Smith, G.P. (1981) *C-terminal octapeptide of cholecystokinin decreases food intake in man.* Am J Clin Nutr, 34, 154–160.

Kissileff, H.R., Carretta, J.C., Geliebter, A. and Pi-Sunyer, F.X. (2003) *Cholecystokinin and stomach distension combine to reduce food intake in humans*. Am J Physiol Regul Integr Comp Physiol, 285, R992–998.

Kobayashi, C., Kennedy, L.M. and Halpern, B.P. (2006) *Experience-induced changes in taste identification of monosodium glutamate (MSG) are reversible*. Chem Senses, 31, 301–306.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K. (1999) *Ghrelin is a growth-hormone-releasing acylated peptide from stomach*. Nature, 402, 656–660.

Korosi, J., McIntosh, C.H., Pederson, R.A., Demuth, H.U., Habener, J.F., Gingerich, R., Egan, J.M., Elahi, D. and Meneilly, G.S. (2001). *Effect of aging and diabetes on the enteroinsular axis*. J Gerontol A Biol Sci Med Sci, 56, M575–579.

Kreymann, B., Williams, G., Ghatei, M.A. and Bloom, S.R. (1987) *Glucagon-like peptide-1 7-36: a physiological incretin in man*. Lancet, 2, 1300–1304.

Kumar, D., Ritman, E.L. and Malagelada, J.R. (1987) *Three-dimensional imaging of the stomach: role of pylorus in the emptying of liquids*. Am J Physiol, 253, G79–85.

Kuvshinoff, B.W., Rudnicki, M., McFadden, D.W., Nussbaum, M.S. and Fischer, J.E. (1990) *Release of intraluminal and circulatory peptide YY after intravenous CCK-8 in conscious dogs*. Curr Surg, 47, 338–340.

Lal, S., McLaughlin, J., Barlow, J., D'Amato, M., Giacovelli, G., Varro, A., Dockray, G.J. and Thompson, D.G. (2004) *Cholecystokinin pathways modulate*

sensations induced by gastric distension in man. Am J Physiol Gastrointest Liver Physiol.

Larsson, L.I. and Rehfeld, J.F. (1978) *Distribution of gastrin and CCK cells in the rat gastrointestinal tract. Evidence for the occurrence of three distinct cell types storing COOH-terminal gastrin immunoreactivity*. Histochemistry, 58, 23–31.

Laugerette, F., Passilly-Degrace, P., Patris, B., Niot, I., Febbraio, M., Montmayeur, J.P. and Besnard, P. (2005) *CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions*. J Clin Invest, 115, 3177–3184.

Lavin, J.H., Wittert, G., Sun, W.M., Horowitz, M., Morley, J.E. and Read, N.W. (1996) *Appetite regulation by carbohydrate: role of blood glucose and gastrointestinal hormones*. Am J Physiol, 271, E209–214.

Lavin, J.H., Wittert, G.A., Andrews, J.M., Yeap, B., Wishart, J.M., Morris, H.A., Morley, J.E., Horowitz, M. and Read, N.W. (1998) *Interaction of insulin, glucagon-like peptide 1, gastric inhibitory polypeptide, and appetite in response to intraduodenal carbohydrate.* Am J Clin Nutr, 68, 591–598.

le Roux, C.W., Batterham, R.L., Aylwin, S.J., Patterson, M., Borg, C.M., Wynne, K.J., Kent, A., Vincent, R.P., Gardiner, J., Ghatei, M.A. and Bloom, S.R. (2006) *Attenuated peptide YY release in obese subjects is associated with reduced satiety*. Endocrinology, 147, 3–8.

Ledikwe, J.H., Ello-Martin, J., Pelkman, C.L., Birch, L.L., Mannino, M.L. and Rolls, B.J. (2007) *A reliable, valid questionnaire indicates that preference for dietary fat declines when following a reduced-fat diet*. Appetite, 49, 74–83.

Lee, H.K., Choi, E.B. and Pak, C.S. (2009) *The current status and future perspectives of studies of cannabinoid receptor 1 antagonists as anti-obesity agents*. Curr Top Med Chem, 9, 482–503.

Leibel, R.L., Rosenbaum, M. and Hirsch, J. (1995) *Changes in energy expenditure resulting from altered body weight*. N Engl J Med, 332, 621–628.

Levin, F., Edholm, T., Schmidt, P.T., Gryback, P., Jacobsson, H., Degerblad, M., Hoybye, C., Holst, J.J., Rehfeld, J.F., Hellstrom, P.M. and Naslund, E. (2006) *Ghrelin stimulates gastric emptying and hunger in normal-weight humans*. J Clin Endocrinol Metab, 91, 3296–3302.

Li, Z., Maglione, M., Tu, W., Mojica, W., Arterburn, D., Shugarman, L.R., Hilton, L., Suttorp, M., Solomon, V., Shekelle, P.G. and Morton, S.C. (2005) *Meta-analysis: pharmacologic treatment of obesity*. Ann Intern Med, 142, 532–546.

Liddle, R.A., Goldfine, I.D., Rosen, M.S., Taplitz, R.A. and Williams, J.A. (1985) *Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction*. J Clin Invest, 75, 1144–1152.

Liddle, R.A., Morita, E.T., Conrad, C.K. and Williams, J.A. (1986) *Regulation of gastric emptying in humans by cholecystokinin*. J Clin Invest, 77, 992–996.

Lieverse, R.J., Jansen, J.B., Masclee, A.A., Rovati, L.C. and Lamers, C.B. (1994a) *Effect of a low dose of intraduodenal fat on satiety in humans: studies using the type A cholecystokinin receptor antagonist loxiglumide*. Gut, 35, 501–505.

Lieverse, R.J., Jansen, J.B., Masclee, A.M. and Lamers, C.B. (1994b) *Satiety effects of cholecystokinin in humans*. Gastroenterology, 106, 1451–1454.

Lin, H.C., Doty, J.E., Reedy, T.J. and Meyer, J.H. (1990) *Inhibition of gastric emptying by sodium oleate depends on length of intestine exposed to nutrient.* Am J Physiol Gastrointest Liver Physiol, 259, G1031–1036.

Lin, H.C., Chey, W.Y. and Zhao, X. (2000) *Release of distal gut peptide YY (PYY) by fat in proximal gut depends on CCK*. Peptides, 21, 1561–1563.

Lindemann, B. (1996) *Taste reception*. Physiological Reviews, 76, 718–766.

Lissner, L., Levitsky, D.A., Strupp, B.J., Kalkwarf, H.J. and Roe, D.A. (1987) *Dietary fat and the regulation of energy intake in human subjects*. Am J Clin Nutr, 46, 886–892.

Little, T.J., Feltrin, K.L., Horowitz, M., Smout, A.J., Rades, T., Meyer, J.H., Pilichiewicz, A.N., Wishart, J. and Feinle-Bisset, C. (2005) *Dose-related effects of lauric acid on antropyloroduodenal motility, gastrointestinal hormone release, appetite, and energy intake in healthy men.* Am J Physiol Regul Integr Comp Physiol, 289, R1090–R1098.

Little, T.J., Pilichiewicz, A.N., Russo, A., Phillips, L., Jones, K.L., Nauck, M.A., Wishart, J., Horowitz, M. and Feinle-Bisset, C. (2006a) *Effects of intravenous*

glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. J Clin Endocrinol Metab, 91, 1916–1923.

Little, T.J., Doran, S., Meyer, J.H., Smout, A.J., O'Donovan, D.G., Wu, K.L., Jones, K.L., Wishart, J., Rayner, C.K., Horowitz, M. and Feinle-Bisset, C. (2006b) *The release of GLP-1 and ghrelin, but not GIP and CCK, by glucose is dependent upon the length of small intestine exposed*. Am J Physiol Endocrinol Metab, 291, E647–655.

Little, T.J., Russo, A., Meyer, J.H., Horowitz, M., Smyth, D.R., Bellon, M., Wishart, J.M., Jones, K.L. and Feinle-Bisset, C. (2007a) *Free fatty acids have more potent effects on gastric emptying, gut hormones, and appetite than triacylglycerides*. Gastroenterology, 133, 1124–1131.

Little, T.J., Horowitz, M. and Feinle-Bisset, C. (2007b) *Modulation by high-fat diets of gastrointestinal function and hormones associated with the regulation of energy intake: implications for the pathophysiology of obesity*. Am J Clin Nutr, 86, 531–541.

Little, T.J., Feltrin, K.L., Horowitz, M., Meyer, J.H., Wishart, J., Chapman, I.M. and Feinle-Bisset, C. (2008) *A high-fat diet raises fasting plasma CCK but does not affect upper gut motility, PYY, and ghrelin, or energy intake during CCK-8 infusion in lean men*. Am J Physiol Regul Integr Comp Physiol, 294, R45–51.

Long, S.J., Sutton, J.A., Amaee, W.B., Giouvanoudi, A., Spyrou, N.M., Rogers, P.J. and Morgan, L.M. (1999) *No effect of glucagon-like peptide-1 on short-term satiety and energy intake in man.* Br J Nutr, 81, 273–279.

Lugari, R., Dei Cas, A., Ugolotti, D., Barilli, A.L., Camellini, C., Ganzerla, G.C., Luciani, A., Salerni, B., Mittenperger, F., Nodari, S., Gnudi, A. and Zandomeneghi, R. (2004) *Glucagon-like peptide 1 (GLP-1) secretion and plasma dipeptidyl peptidase IV (DPP-IV) activity in morbidly obese patients undergoing biliopancreatic diversion*. Horm Metab Res, 36, 111–115.

Luscombe-Marsh, N.D., Noakes, M., Wittert, G.A., Keogh, J.B., Foster, P. and Clifton, P.M. (2005) *Carbohydrate-restricted diets high in either monounsaturated fat or protein are equally effective at promoting fat loss and improving blood lipids*. Am J Clin Nutr, 81, 762–772.

Ma, J., Pilichiewicz, A.N., Feinle-Bisset, C., Wishart, J.M., Jones, K.L., Horowitz, M. and Rayner, C.K. (2011) *Effects of variations in duodenal glucose load on glycaemic, insulin, and incretin responses in type 2 diabetes*. Diabet Med.

MacIntosh, C.G., Horowitz, M., Verhagen, M.A., Smout, A.J., Wishart, J., Morris, H., Goble, E., Morley, J.E. and Chapman, I.M. (2001a) *Effect of small intestinal nutrient infusion on appetite, gastrointestinal hormone release, and gastric myoelectrical activity in young and older men*. Am J Gastroenterol, 96, 997–1007.

MacIntosh, C.G., Morley, J.E., Wishart, J., Morris, H., Jansen, J.B., Horowitz, M. and Chapman, I.M. (2001b) *Effect of exogenous cholecystokinin (CCK)-8 on food intake and plasma CCK, leptin, and insulin concentrations in older and young adults: evidence for increased CCK activity as a cause of the anorexia of aging.* J Clin Endocrinol Metab, 86, 5830–5837.

Maddox, A., Horowitz, M., Wishart, J. and Collins, P. (1989) *Gastric and oesophageal emptying in obesity.* Scand J Gastroenterol, 24, 593–598.

Manson, J.E., Colditz, G.A., Stampfer, M.J., Willett, W.C., Rosner, B., Monson, R.R., Speizer, F.E. and Hennekens, C.H. (1990) *A prospective study of obesity and risk of coronary heart disease in women*. N Engl J Med, 322, 882–889.

Masuda, Y., Tanaka, T., Inomata, N., Ohnuma, N., Tanaka, S., Itoh, Z., Hosoda, H., Kojima, M. and Kangawa, K. (2000) *Ghrelin stimulates gastric acid secretion and motility in rats*. Biochem Biophys Res Commun, 276, 905–908.

Matsumura, S., Mizushige, T., Yoneda, T., Iwanaga, T., Tsuzuki, S., Inoue, K. and Fushiki, T. (2007) *GPR expression in the rat taste bud relating to fatty acid sensing*. Biomed Res, 28, 49–55.

Matsumura, S., Eguchi, A., Mizushige, T., Kitabayashi, N., Tsuzuki, S., Inoue, K. and Fushiki, T. (2009) *Colocalization of GPR120 with phospholipase-Cbeta2 and alpha-gustducin in the taste bud cells in mice*. Neurosci Lett, 450, 186–190.

Mattes, R.D. (1993) *Fat preference and adherence to a reduced-fat diet*. Am J Clin Nutr, 57, 373–381.

Mattes, R.D. (2005) *Fat taste and lipid metabolism in humans*. Physiol Behav, 86, 691–697.

Mattes, R.D. (2009a) *Is there a fatty acid taste?* Annu Rev Nutr, 29, 305–327.

Mattes, R.D. (2009b) *Oral detection of short-, medium-, and long-chain free fatty acids in humans*. Chem Senses, 34, 145–150.

Mattes, R.D. (2009c) *Brief oral stimulation, but especially oral fat exposure, elevates serum triglycerides in humans*. Am J Physiol Gastrointest Liver Physiol, 296, G365–371.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. and Turner, R.C. (1985) *Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man*. Diabetologia, 28, 412–419.

Matzinger, D., Gutzwiller, J.P., Drewe, J., Orban, A., Engel, R., D'Amato, M., Rovati, L. and Beglinger, C. (1999) *Inhibition of food intake in response to intestinal lipid is mediated by cholecystokinin in humans.* Am J Physiol Regul Integr Comp Physiol, 277, R1718–R1724.

Matzinger, D., Degen, L., Drewe, J., Meuli, J., Duebendorfer, R., Ruckstuhl, N., D'Amato, M., Rovati, L. and Beglinger, C. (2000) *The role of long chain fatty acids in regulating food intake and cholecystokinin release in humans*. Gut, 46, 688–693.

McFadden, D.W., Rudnicki, M., Kuvshinoff, B. and Fischer, J.E. (1992) *Postprandial peptide YY release is mediated by cholecystokinin*. Surg Gynecol Obstet, 175, 145–150.

McHugh, P.R. and Moran, T.H. (1986) *The stomach, cholecystokinin, and satiety*. Fed Proc, 45, 1384–1390.

Meier, J.J., Gallwitz, B., Salmen, S., Goetze, O., Holst, J.J., Schmidt, W.E. and Nauck, M.A. (2003) *Normalization of glucose concentrations and deceleration of*

gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. J Clin Endocrinol Metab, 88, 2719–2725.

Meier, J.J. and Nauck, M.A. (2004) *GIP as a potential therapeutic agent?* Horm Metab Res, 36, 859–866.

Meier, J.J., Goetze, O., Anstipp, J., Hagemann, D., Holst, J.J., Schmidt, W.E., Gallwitz, B. and Nauck, M.A. (2004) *Gastric inhibitory polypeptide does not inhibit gastric emptying in humans*. Am J Physiol Endocrinol Metab, 286, E621–625.

Meier, J.J., Kemmeries, G., Holst, J.J. and Nauck, M.A. (2005) *Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects*. Diabetes, 54, 2212–2218.

Meilgaard, M., Civille, C.G., Carr, B.T. (2007) *Sensory evaluation techniques*. Boca Raton (FL): CRC Press.

Mela, D.J. and Sacchetti, D.A. (1991) *Sensory preferences for fats: relationships with diet and body composition*. Am J Clin Nutr, 53, 908–915.

Mentlein, R., Dahms, P., Grandt, D. and Kruger, R. (1993) *Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV*. Regul Pept, 49, 133–144.

Meyer, J.H., Thomson, J.B., Cohen, M.B., Shadchehr, A. and Mandiola, S.A. (1979) *Sieving of solid food by the canine stomach and sieving after gastric surgery*. Gastroenterology, 76, 804–813.

Meyer, B.M., Werth, B.A., Beglinger, C., Hildebrand, P., Jansen, J.B., Zach, D., Rovati, L.C. and Stalder, G.A. (1989) *Role of cholecystokinin in regulation of gastrointestinal motor functions*. Lancet, 2, 12–15.

Meyer, J.H., Hlinka, M., Tabrizi, Y., DiMaso, N. and Raybould, H.E. (1998) *Chemical specificities and intestinal distributions of nutrient-driven satiety*. Am J Physiol Gastrointest Liver Physiol, 275, R1293–1307.

Miki, T., Minami, K., Shinozaki, H., Matsumura, K., Saraya, A., Ikeda, H., Yamada, Y., Holst, J.J. and Seino, S. (2005) *Distinct effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 on insulin secretion and gut motility*. Diabetes, 54, 1056–1063.

Miller, W.C., Lindeman, A.K., Wallace, J. and Niederpruem, M. (1990) *Diet composition, energy intake, and exercise in relation to body fat in men and women*. Am J Clin Nutr, 52, 426–430.

Monteleone, P., Bencivenga, R., Longobardi, N., Serritella, C. and Maj, M. (2003) *Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women*. J Clin Endocrinol Metab, 88, 5510–5514.

Moran, T.H., Robinson, P.H., Goldrich, M.S. and McHugh, P.R. (1986) *Two brain cholecystokinin receptors: implications for behavioral actions*. Brain Res, 362, 175–179.

Moran, T.H. and McHugh, P.R. (1988) *Gastric and nongastric mechanisms for satiety action of cholecystokinin*. Am J Physiol, 254, R628–632.

Moran, T.H. and Kinzig, K.P. (2004) *Gastrointestinal satiety signals II. Cholecystokinin*. Am J Physiol Gastrointest Liver Physiol, 286, G183–188.

Moran, T.H., Smedh, U., Kinzig, K.P., Scott, K.A., Knipp, S. and Ladenheim, E.E. (2005) *Peptide YY(3-36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys*. Am J Physiol Regul Integr Comp Physiol, 288, R384–388.

Morgan, L.M. (1996) *The metabolic role of GIP: physiology and pathology*. Biochem Soc Trans, 24, 585–591.

Must, A., Spadano, J., Coakley, E.H., Field, A.E., Colditz, G. and Dietz, W.H. (1999) *The disease burden associated with overweight and obesity*. JAMA, 282, 1523–1529.

Nagell, C.F., Pedersen, J.F. and Holst, J.J. (2007) *The antagonistic metabolite of GLP-1, GLP-1 (9-36)amide, does not influence gastric emptying and hunger sensations in man*. Scand J Gastroenterol, 42, 28–33.

Nair, N.S., Brennan, I.M., Little, T.J., Gentilcore, D., Hausken, T., Jones, K.L., Wishart, J.M., Horowitz, M. and Feinle-Bisset, C. (2009) *Reproducibility of energy intake, gastric emptying, blood glucose, plasma insulin and cholecystokinin responses in healthy young males*. Br J Nutr, 101, 1094–1102.

Näslund, E., Gutniak, M., Skogar, S., Rossner, S. and Hellström, P.M. (1998a) *Glucagon-like peptide-1 increases the period of postprandial satiety and slows gastric emptying in obese men.* Am J Clin Nutr, 68, 525–530.

Näslund, E., Grybäck, P., Backman, L., Jacobsson, H., Holst, J.J., Theodorsson, E. and Hellström, P.M. (1998b) *Distal small bowel hormones: correlation with fasting antroduodenal motility and gastric emptying.* Dig Dis Sci, 43, 945–952.

National Institutes of Health NH, Lung and Blood Institute. (2002) *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults*. The Evidence Report 2002.

Nauck, M.A., Heimesaat, M.M., Orskov, C., Holst, J.J., Ebert, R. and Creutzfeldt, W. (1993) *Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus*. J Clin Invest, 91, 301–307.

Nauck, M.A., Niedereichholz, U., Ettler, R., Holst, J.J., Orskov, C., Ritzel, R. and Schmiegel, W.H. (1997) *Glucagon-like peptide-1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans*. Am J Physiol, 273, E981–988.

Nauck, M.A., Weber, I., Bach, I., Richter, S., Orskov, C., Holst, J.J. and Schmiegel, W. (1998) *Normalization of fasting glycaemia by intravenous GLP-1 ([7-36 amide] or [7-37]) in type 2 diabetic patients.* Diabet Med, 15, 937–945.

Nauck, M.A. (1998) *Glucagon-like peptide-1 (GLP-1): a potent gut hormone with a possible therapeutic perspective.* Acta Diabetol, 35, 117–129.

Neary, N.M., Small, C.J., Druce, M.R., Park, A.J., Ellis, S.M., Semjonous, N.M., Dakin, C.L., Filipsson, K., Wang, F., Kent, A.S., Frost, G.S., Ghatei, M.A. and Bloom, S.R. (2005) *Peptide YY3-36 and glucagon-like peptide-17-36 inhibit food intake additively*. Endocrinology, 146, 5120–5127.

Nguyen, N.Q., Fraser, R.J., Chapman, M.J., Bryant, L.K., Holloway, R.H., Vozzo, R., Wishart, J., Feinle-Bisset, C. and Horowitz, M. (2007) *Feed intolerance in critical illness is associated with increased basal and nutrient-stimulated plasma cholecystokinin concentrations*. Critical care medicine, 35, 82–88.

Noakes, M., Keogh, J.B., Foster, P.R. and Clifton, P.M. (2005) *Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women*. Am J Clin Nutr, 81, 1298–1306.

O'Donovan, D.G., Doran, S., Feinle-Bisset, C., Jones, K.L., Meyer, J.H., Wishart, J.M., Morris, H.A. and Horowitz, M. (2004) *Effect of variations in small intestinal glucose delivery on plasma glucose, insulin, and incretin hormones in healthy subjects and type 2 diabetes*. J Clin Endocrinol Metab, 89, 3431–3435.

Overduin, J., Frayo, R.S., Grill, H.J., Kaplan, J.M. and Cummings, D.E. (2005) *Role of the duodenum and macronutrient type in ghrelin regulation*. Endocrinology, 146, 845–850.

Pan, S.Y. and DesMeules, M. (2009) *Energy intake, physical activity, energy balance, and cancer: epidemiologic evidence*. Methods Mol Biol, 472, 191–215.

Pappas, T.N., Debas, H.T., Chang, A.M. and Taylor, I.L. (1986) *Peptide YY release by fatty acids is sufficient to inhibit gastric emptying in dogs*. Gastroenterology, 91, 1386–1389.

Parker, B.A., Sturm, K., MacIntosh, C.G., Feinle, C., Horowitz, M. and Chapman, I.M. (2004) *Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects.* Eur J Clin Nutr, 58, 212–218.

Parker, B.A., Doran, S., Wishart, J., Horowitz, M. and Chapman, I.M. (2005) *Effects of small intestinal and gastric glucose administration on the suppression of plasma ghrelin concentrations in healthy older men and women*. Clin Endocrinol (Oxf), 62, 539–546.

Pepino, M.Y., Finkbeiner, S., Beauchamp, G.K. and Mennella, J.A. *Obese women have lower monosodium glutamate taste sensitivity and prefer higher concentrations than do normal-weight women*. Obesity (Silver Spring), 18, 959–965.

Phillips, W.T., Schwartz, J.G. and McMahan, C.A.. (1993) *Reduced postprandial blood glucose levels in recently diagnosed non-insulin-dependent diabetics secondary to pharmacologically induced delayed gastric emptying*. Dig Dis Sci, 38, 51–58.

Pieramico, O., Malfertheiner, P., Nelson, D.K., Glasbrenner, B. and Ditschuneit, H. (1992) *Interdigestive gastroduodenal motility and cycling of putative regulatory hormones in severe obesity*. Scand J Gastroenterol, 27, 538–544.

Pilichiewicz, A., O'Donovan, D., Feinle, C., Lei, Y., Wishart, J.M., Bryant, L., Meyer, J.H,. Horowitz, M. and Jones, K.L. (2003) *Effect of lipase inhibition on gastric emptying of, and the glycemic and incretin responses to, an oil/aqueous drink in type 2 diabetes mellitus*. J Clin Endocrinol Metab, 88, 3829–3834.

Pilichiewicz, A.N., Little, T.J., Brennan, I.M., Meyer, J.H., Wishart, J.M., Otto, B., Horowitz, M. and Feinle-Bisset, C. (2005) *Effects of load, and duration, of duodenal*

lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men. Am J Physiol Regul Integr Comp Physiol, 290, R668–677.

Pilichiewicz, A.N., Chaikomin, R., Brennan, I.M., Wishart, J.M., Rayner, C.K., Jones, K.L., Smout, A.J., Horowitz, M. and Feinle-Bisset, C. (2007a) *Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men*. Am J Physiol Endocrinol Metab, 293, E743–753.

Pilichiewicz, A.N., Papadopoulos, P., Brennan, I.M., Little, T.J., Meyer, J.H., Wishart, J.M., Horowitz, M. and Feinle-Bisset, C. (2007b) *Load-dependent effects of duodenal lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men*. Am J Physiol Regul Integr Comp Physiol, 293, R2170–2178.

Pories, W.J., Swanson, M.S., MacDonald, K.G., Long, S.B., Morris, P.G., Brown, B.M., Barakat, H.A., deRamon, R.A., Israel, G., Dolezal, J.M. and et al. (1995) *Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus*. Ann Surg, 222, 339–350; discussion 350–332.

Ranganath, L.R., Beety, J.M., Morgan, L.M., Wright, J.W., Howland, R. and Marks, V. (1996) *Attenuated GLP-1 secretion in obesity: cause or consequence?* Gut, 38, 916–919.

Raudenbush, S.W. and Bryk, A.S. (2002) Hierarchical linear models: applications and data analysis methods. SAGE Publications Inc, 2002, Thousand Oaks, California.

Rayner, C.K., Park, H.S., Doran, S.M., Chapman, I.M. and Horowitz, M. (2000) *Effects of cholecystokinin on appetite and pyloric motility during physiological hyperglycemia.* Am J Physiol, 278, G98–G104.

Rayner, C.K. and Horowitz, M. (2005) *New management approaches for gastroparesis*. Nat Clin Pract Gastroenterol Hepatol, 2, 454–462; quiz 493.

Read, N.W., McFarlane, A., Kinsman, R.I., Bates, T.E., Blackhall, N.W., Farrar, G.B., Hall, J.C., Moss, G., Morris, A.P., O'Neill, B., Welch, I., Lee, Y. and Bloom, S.R. (1984) *Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon*. Gastroenterology, 86, 274–280.

Rehfeld, J.F. (1978) *Immunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog*. J Biol Chem, 253, 4022–4030.

Resnick, H.E., Valsania, P., Halter, J.B. and Lin, X. (2000) *Relation of weight gain and weight loss on subsequent diabetes risk in overweight adults*. J Epidemiol Community Health, 54, 596–602.

Riepl, R.L., Fiedler, F., Ernstberger, M., Teufel, J. and Lehnert, P. (1996) *Effect of intraduodenal taurodeoxycholate and L-phenylalanine on pancreatic secretion and on gastroenteropancreatic peptide release in man*. Eur J Med Res, 1, 499–505.

Riley, R.D., Lambert, P.C. and Abo-Zaid, G. (2010) *Meta-analysis of individual participant data: rationale, conduct, and reporting*. BMJ, 340, c221.

Robertson, M.D., Henderson, R.A., Vist, G.E. and Rumsey, R.D. (2004) *Plasma ghrelin response following a period of acute overfeeding in normal weight men*. Int J Obes Relat Metab Disord, 28, 727–733.

Rolls, B.J., Kim-Harris, S., Fischman, M.W., Foltin, R.W., Moran, T.H. and Stoner, S.A. (1994) *Satiety after preloads with different amounts of fat and carbohydrate: implications for obesity.* Am J Clin Nutr, 60, 476–487.

Rolls, B.J. (1995) *Carbohydrates, fats, and satiety.* Am J Clin Nutr, 61, Suppl, 960S–967S.

Roth, C.L., Enriori, P.J., Harz, K., Woelfle, J., Cowley, M.A. and Reinehr, T. (2005) *Peptide YY is a regulator of energy homeostasis in obese children before and after weight loss*. J Clin Endocrinol Metab, 90, 6386–6391.

Samsom, M., Roelofs, J.M., Akkermans, L.M., van Berge Henegouwen, G.P. and Smout, A.J. (1998) *Proximal gastric motor activity in response to a liquid meal in type I diabetes mellitus with autonomic neuropathy*. Dig Dis Sci, 43, 491–496.

Santangelo, A., Peracchi, M., Conte, D., Fraquelli, M. and Porrini, M. (1998) *Physical state of meal affects gastric emptying, cholecystokinin release and satiety*. Br J Nutr, 80, 521–527.

Savage, A.P., Adrian, T.E., Carolan, G., Chatterjee, V.K. and Bloom, S.R. (1987) *Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers*. Gut, 28, 166–170.

Savastano, D.M. and Covasa, M. (2005) *Adaptation to a high-fat diet leads to hyperphagia and diminished sensitivity to cholecystokinin in rats*. J Nutr, 135, 1953–1959.

Scarpignato, C., Kisfalvi, I., D'Amato, M. and Varga, G. (1996) *Effect of dexloxiglumide and spiroglumide, two new CCK-receptor antagonists, on gastric emptying and secretion in the rat: evaluation of their receptor selectivity in vivo*. Aliment Pharmacol Ther, 10, 411–419.

Schirra, J., Katschinski, M., Weidmann, C., Schafer, T., Wank, U., Arnold, R. and Goke, B. (1996) *Gastric emptying and release of incretin hormones after glucose ingestion in humans*. J Clin Invest, 97, 92–103.

Schirra, J., Sturm, K., Leicht, P., Arnold, R., Goke, B. and Katschinski, M. (1998) *Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans.* J Clin Invest, 101, 1421–1430.

Schirra, J., Houck, P., Wank, U., Arnold, R., Goke, B. and Katschinski, M. (2000) *Effects of glucagon-like peptide-1(7-36)amide on antro-pyloro-duodenal motility in the interdigestive state and with duodenal lipid perfusion in humans.* Gut, 46, 622–631.

Schirra, J., Wank, U., Arnold, R., Goke, B. and Katschinski, M. (2002) *Effects of glucagon-like peptide-1(7-36)amide on motility and sensation of the proximal stomach in humans*. Gut, 50, 341–348.

Schirra, J. and Goke, B. (2005) *The physiological role of GLP-1 in human: incretin, ileal brake or more?* Regul Pept, 128, 109–115.

Schirra, J., Nicolaus, M., Roggel, R., Katschinski, M., Storr, M., Woerle, H.J. and Goke, B. (2006) *Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans*. Gut, 55, 243–251.

Schwartz, G.J., Whitney, A., Skoglund, C., Castonguay, T.W. and Moran, T.H. (1999) *Decreased responsiveness to dietary fat in Otsuka Long-Evans Tokushima fatty rats lacking CCK-A receptors*. Am J Physiol, 277, R1144–1151.

Schwizer, W., Borovicka, J., Kunz, P., Fraser, R., Kreiss, C., D'Amato, M., Crelier, G., Boesiger, P. and Fried, M. (1997) *Role of cholecystokinin in the regulation of liquid gastric emptying and gastric motility in humans: studies with the CCK antagonist loxiglumide*. Gut, 41, 500–504.

Schvarcz, E., Palmer, M., Aman, J., Horowitz, M., Stridsberg, M. and Berne, C. (1997) *Physiological hyperglycemia slows gastric emptying in normal subjects and patients with insulin-dependent diabetes mellitus*. Gastroenterology, 113, 60–66.

Seimon, R.V., Feltrin, K.L., Meyer, J.H., Brennan, I.M., Wishart, J.M., Horowitz, M. and Feinle-Bisset, C. (2009a) *Effects of varying combinations of intraduodenal lipid and carbohydrate on antropyloroduodenal motility, hormone release, and appetite in healthy males*. Am J Physiol Regul Integr Comp Physiol, 296, R912–920.

Seimon, R.V., Wooster, T., Otto, B., Golding, M., Day, L., Little, T.J., Horowitz, M., Clifton, P.M. and Feinle-Bisset, C. (2009b) *The droplet size of intraduodenal fat emulsions influences antropyloroduodenal motility, hormone release, and appetite in healthy males*. Am J Clin Nutr, 89, 1729–1736.

Seimon, R.V., Lange, K., Little, T.J., Brennan, I.M., Pilichiewicz, A.N., Feltrin, K.L., Smeets, A.J., Horowitz, M. and Feinle-Bisset, C. (2010) *Pooled-data analysis identifies pyloric pressures and plasma cholecystokinin concentrations as major determinants of acute energy intake in healthy, lean men*. Am J Clin Nutr, 92, 61–68.

Shiiya, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M.S., Tanaka, M., Nozoe, S., Hosoda, H., Kangawa, K. and Matsukura, S. (2002) *Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion*. J Clin Endocrinol Metab, 87, 240–244.

Simmonds, M.C., Higgins, J.P., Stewart, L.A., Tierney, J.F., Clarke, M.J. and Thompson, S.G. (2005) *Meta-analysis of individual patient data from randomized trials: a review of methods used in practice*. Clin Trials, 2, 209–217.

Sjostrom, L., Rissanen, A., Andersen, T., Boldrin, M., Golay, A., Koppeschaar, H.P. and Krempf, M. (1998) *Randomised placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. European Multicentre Orlistat Study Group*. Lancet, 352, 167–172.

Smeets, A.J. and Westerterp-Plantenga, M.S. (2006) *Satiety and substrate mobilization after oral fat stimulation*. Br J Nutr, 95, 795–801.

Smeets, A.J., Lejeune, M.P. and Westerterp-Plantenga, M.S. (2009) *Effects of oral fat perception by modified sham feeding on energy expenditure, hormones and appetite profile in the postprandial state*. Br J Nutr, 101, 1360–1368.

Smith, G.T., Moran, T.H., Coyle, J.T., Kuhar, M.J., O'Donahue, T.L. and McHugh, P.R. (1984) *Anatomic localization of cholecystokinin receptors to the pyloric sphincter.* Am J Physiol, 264, R127–R130.

Smith, D.V. and Margolskee, R.F. (2001) *Making sense of taste*. Sci Am, 284, 32–39.

Spannagel, A.W., Nakano, I., Tawil, T., Chey, W.Y., Liddle, R.A. and Green, G.M. (1996) *Adaptation to fat markedly increases pancreatic secretory response to intraduodenal fat in rats.* Am J Physiol, 270, G128–G135.

Spiller, R.C., Trotman, I.F., Higgins, B.E., Ghatei, M.A., Grimble, G.K., Lee, Y.C., Bloom, S.R., Misiewicz, J.J. and Silk, D.B. (1984) *The ileal brake – inhibition of jejunal motility after ileal fat perfusion in man*. Gut, 25, 365–374.

Spiller, R.C., Trotman, I.F., Adrian, T.E., Bloom, S.R., Misiewicz, J.J. and Silk, D.B. (1988) *Further characterisation of the 'ileal brake' reflex in man—effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY*. Gut, 29, 1042–1051.

Stampfer, M.J., Maclure, K.M., Colditz, G.A., Manson, J.E. and Willett, W.C. (1992) *Risk of symptomatic gallstones in women with severe obesity*. Am J Clin Nutr, 55, 652–658.

Sternini, C. (2007) *Taste receptors in the gastrointestinal tract. IV. Functional implications of bitter taste receptors in gastrointestinal chemosensing*. Am J Physiol Gastrointest Liver Physiol, 292, G457–461.

Sternini, C., Anselmi, L. and Rozengurt, E. (2008) *Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing*. Curr Opin Endocrinol Diabetes Obes, 15, 73–78.

Stewart, J.E., Feinle-Bisset, C., Golding, M., Delahunty, C., Clifton, P.M. and Keast, R.S. (2010) *Oral sensitivity to fatty acids, food consumption and BMI in human subjects*. Br J Nutr, 104, 145–152.

Stewart, J.E., Seimon, R.V., Otto, B., Keast, R.S., Clifton, P.M. and Feinle-Bisset, C. (2011a) *Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men*. Am J Clin Nutr, 93, 703–711.

Stewart, J.E., Newman, L.P. and Keast, R.S. (2011b) *Oral sensitivity to oleic acid is associated with fat intake and body mass index*. Clin Nutr, 30, 838–844.

Stewart, J.E. and Keast, R.S. (2011c) *Recent fat intake modulates fat taste sensitivity in lean and overweight subjects*. Int J Obes (Lond).

Stoeckel, L.E., Weller, R.E., Giddings, M. and Cox, J.E. (2008) *Peptide YY levels are associated with appetite suppression in response to long-chain fatty acids*. Physiol Behav, 93, 289–295.

Stunkard, A.J. and Messick, S. (1985) *The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger*. J Psychosom Res, 29, 71–83.

Sturm, K., Parker, B., Feinle-Bisset, C., Jones, K.L., Chapman, I. and Horowitz, M. (2004) *Energy intake and appetite are related to antral area in healthy young and older subjects*. Am J Clin Nutr, 80, 656–667.

Sumithran, P., Prendergast, L.A., Delbridge, E., Purcell ,K., Shulkes, A., Kriketos, A. and Proietto, J. (2011) *Long-term persistence of hormonal adaptations to weight loss*. N Engl J Med, 365, 1597–1604.

Swartz, T.D., Hajnal, A. and Covasa, M. (2010) *Altered orosensory sensitivity to oils in CCK-1 receptor deficient rats*. Physiol Behav, 99, 109–117.

Tack, J., Depoortere, I., Bisschops, R., Delporte, C., Coulie, B., Meulemans, A., Janssens, J. and Peeters, T. (2006) *Influence of ghrelin on interdigestive gastrointestinal motility in humans*. Gut, 55, 327–333.

Taminato, T., Seino, Y., Goto, Y., Inoue, Y. and Kadowaki, S. (1977) *Synthetic gastric inhibitory polypeptide. Stimulatory effect on insulin and glucagon secretion in the rat*. Diabetes, 26, 480–484.

Tanaka, R., Inui, A., Asakawa, A., Atsuchi, K., Ataka, K. and Fujimiya, M. (2009) *New method of manometric measurement of gastroduodenal motility in conscious mice: effects of ghrelin and Y2 depletion*. Am J Physiol Gastrointest Liver Physiol, 297, G1028–1034.

Tolessa, T., Gutniak, M., Holst, J.J., Efendic, S. and Hellstrom, P.M. (1998) *Glucagon-like peptide-1 retards gastric emptying and small bowel transit in the rat: effect mediated through central or enteric nervous mechanisms.* Dig Dis Sci, 43, 2284–2290.

Tosetti, C., Corinaldesi, R., Stanghellini, V., Pasquali, R., Corbelli, C., Zoccoli, G., Di Febo, G., Monetti, N. and Barbara, L. (1996) *Gastric emptying of solids in morbid obesity.* Int J Obes Relat Metab Disord, 20, 200–205.

Travers, J.B., Travers, S.P. and Norgren, R. (1987) *Gustatory neural processing in the hindbrain*. Annu Rev Neurosci, 10, 595–632.

Tremblay, A., Plourde, G., Despres, J.P. and Bouchard, C. (1989) *Impact of dietary fat content and fat oxidation on energy intake in humans*. Am J Clin Nutr, 49, 799–805.

Tritos, N.A. and Kokkotou, E.G. (2006) *The physiology and potential clinical applications of ghrelin, a novel peptide hormone*. Mayo Clin Proc, 81, 653–660.

Tschop, M., Wawarta, R., Riepl, R.L., Friedrich, S., Bidlingmaier, M., Landgraf, R. and Folwaczny, C. (2001a) *Post-prandial decrease of circulating human ghrelin levels*. J Endocrinol Invest, 24, RC19–21.

Tschop, M., Weyer, C., Tataranni, P.A., Devanarayan, V., Ravussin, E. and Heiman, M.L. (2001b) *Circulating ghrelin levels are decreased in human obesity*. Diabetes, 50, 707–709.

Tschop, M., Castaneda, T.R., Joost, H.G., Thone-Reineke, C., Ortmann, S., Klaus, S., Hagan, M.M., Chandler, P.C., Oswald, K.D., Benoit, S.C., Seeley, R.J., Kinzig, K.P., Moran, T.H., Beck-sickinger, A.G., Koglin, N., Rodgers, R.J., Blundell, J.E., Ishii, Y., Beattie, A.H., Holch, P., Allison, D.B., Raun, K., Madsen, K., Wulff, B.S., Stidsen, C.E., Birringer, M., Kreuzer, O.J., Schindler, M., Arndt, K., Rudolf, K., Mark, M., Deng, X.Y., Whitcomb, D.C., Halem, H., Taylor, J., Dong, J., Datta, R., Culler, M., Craney, S., Flora, D., Smiley, D. and Heiman, M.L. (2004) *Physiology: does gut hormone PYY3-36 decrease food intake in rodents?* Nature, 430, 1 p following 165; discussion 162 p following 165.

Turton, M.D., O'Shea, D., Gunn, I., Beak, S.A., Edwards, C.M., Meeran, K., Choi, S.J., Taylor, G.M., Heath, M.M., Lambert, P.D., Wilding, J.P., Smith, D.M., Ghatei, M.A., Herbert, J. and Bloom, S.R. (1996) *A role for glucagon-like peptide-1 in the central regulation of feeding.* Nature, 379, 69–72.

Ueno, H., Yamaguchi, H., Mizuta, M. and Nakazato, M. (2008) *The role of PYY in feeding regulation*. Regul Pept, 145, 12–16.

Vanis, L., Gentilcore, D., Rayner, C.K., Wishart, J.M., Horowitz, M., Feinle-Bisset, C. and Jones, K.L. (2011) *Effects of small intestinal glucose load on blood pressure, splanchnic blood flow, glycemia, and GLP-1 release in healthy older subj*ects. Am J Physiol Regul Integr Comp Physiol, 300, R1524–1531.

Vazquez Roque, M.I., Camilleri, M., Stephens, D.A., Jensen, M.D., Burton, D.D., Baxter, K.L. and Zinsmeister, A.R. (2006) *Gastric sensorimotor functions and hormone profile in normal weight, overweight, and obese people*. Gastroenterology, 131, 1717–1724.

Verdich, C., Madsen, J.L., Toubro, S., Buemann, B., Holst, J.J. and Astrup, A. (2000) *Effect of obesity and major weight reduction on gastric emptying*. Int J Obes Relat Metab Disord, 24, 899–905.

Verdich, C., Toubro, S., Buemann, B., Lysgard Madsen, J., Juul Holst, J. and Astrup, A. (2001a) *The role of postprandial releases of insulin and incretin hormones in meal-induced satiety—effect of obesity and weight reduction.* Int J Obes Relat Metab Disord, 25, 1206–1214.

Verdich, C., Flint, A., Gutzwiller, J.P., Naslund, E., Beglinger, C., Hellstrom, P.M., Long, S.J., Morgan, L.M., Holst, J.J. and Astrup, A. (2001b) *A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans*. J Clin Endocrinol Metab, 86, 4382–4389.

Victorian Government Department of Human Services (2008) *Future prevalence of overweight and obesity in Australian children and adolescents, 2005–2025*.

Vilsboll, T., Krarup, T., Sonne, J., Madsbad, S., Volund, A., Juul, A.G. and Holst, J.J. (2003) *Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus*. J Clin Endocrinol Metab, 88, 2706–2713.

Wang, Y. and Beydoun, M.A. (2007) *The obesity epidemic in the United States--gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis*. Epidemiol Rev, 29, 6–28.

Wank, S.A., Pisegna, J.R. and de Weerth, A. (1994) *Cholecystokinin receptor family. Molecular cloning, structure, and functional expression in rat, guinea pig, and human*. Ann N Y Acad Sci, 713, 49–66.

Weigle, D.S., Cummings, D.E., Newby, P.D., Breen, P.A., Frayo, R.S., Matthys, C.C., Callahan, H.S. and Purnell, J.Q. (2003) *Roles of leptin and ghrelin in the loss of body weight caused by a low fat, high carbohydrate diet*. J Clin Endocrinol Metab, 88, 1577–1586.

Welch, I., Saunders, K. and Read, N.W. (1985) *Effect of ileal and intravenous infusions of fat emulsions on feeding and satiety in human volunteers*. Gastroenterology, 89, 1293–1297.

WHO (2011) *WHO Fact Sheet No.311: Obesity and overweight, http://www.who.int/mediacentre/factsheets/fs311/en/index.html*.

Williams, D.L., Cummings, D.E., Grill, H.J. and Kaplan, J.M. (2003a) *Meal-related ghrelin suppression requires postgastric feedback*. Endocrinology, 144, 2765–2767.

Williams, K.V., Bertoldo, A., Kinahan, P., Cobelli, C. and Kelley, D.E. (2003b) *Weight loss-induced plasticity of glucose transport and phosphorylation in the insulin resistance of obesity and type 2 diabetes*. Diabetes, 52, 1619–1626.

Wisen, O. and Johansson, C. (1992) *Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal.* Metabolism, 41, 390–395.

Wishart, J., Morris, H.A. and Horowitz, M. (1992) *Radioimmunoassay of gastric inhibitory polypeptide in plasma*. Clin Chem, 38, 2156–2157.

Wishart, J.M., Horowitz, M., Morris, H.A., Jones, K.L. and Nauck, M.A. (1998) *Relation between gastric emptying of glucose and plasma concentrations of glucagon-like peptide-1*. Peptides, 19, 1049–1053.

Wojdemann, M., Olsen, O., Norregaard, P., Sternby, B. and Rehfeld, J.F. (1997) *Gastric lipase secretion after sham feeding and cholinergic blockade*. Dig Dis Sci, 42, 1070–1075.

Woods, S.C., Seeley, R.J., Rushing, P.A., D'Alessio, D. and Tso, P. (2003) *A controlled high-fat diet induces an obese syndrome in rats*. J Nutr, 133, 1081–1087.

Wren, A.M., Small, C.J., Abbott, C.R., Dhillo, W.S., Seal, L.J., Cohen, M.A., Batterham, R.L., Taheri, S., Stanley, S.A., Ghatei, M.A. and Bloom, S.R. (2001a) *Ghrelin causes hyperphagia and obesity in rats*. Diabetes, 50, 2540–2547.

Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillo, W.S., Ghatei, M.A. and Bloom, S.R. (2001b) *Ghrelin enhances appetite and increases food intake in humans*. J Clin Endocrinol Metab, 86, 5992–5995.

Wright, R.A., Krinsky, S., Fleeman, C., Trujillo, J. and Teague, E. (1983) *Gastric emptying and obesity.* Gastroenterology, 84, 747–751.

Xu, X., Zhu, H. and Chen, J.D. (2005) *Pyloric electrical stimulation reduces food intake by inhibiting gastric motility in dogs*. Gastroenterology, 128, 43–50.

Zahorska-Markiewicz, B., Jonderko, K., Lelek, A. and Skrzypek, D. (1986) *Gastric emptying in obesity.* Hum Nutr Clin Nutr, 40, 309–313.