EFFECTS OF PROTEIN ON GASTROINTESTINAL FUNCTION AND APPETITE REGULATION

A thesis submitted by

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Abstract

The prevalence of obesity and associated diseases, including type-2 diabetes mellitus, continues to increase at an alarming rate. The available therapies have largely ignored the key role of the gastrointestinal tract in determining appetite and blood glucose regulation in responses to ingested nutrients. A detailed understanding of these gastrointestinal mechanisms is critical in aiding development of new and effective interventions for obesity.

The research presented in this thesis focuses on the complex gastrointestinal mechanisms involved in the regulation of glycaemia, appetite and energy intake in response to protein in lean and obese individuals. In particular, this research explores the gastrointestinal motor and hormonal responses to nutrients involved in energy intake regulation and blood glucose control in both healthy lean and obese individuals. Using the novel, non-invasive technique of 3-dimensional ultrasound, the study described in chapter 5 reports that, in lean individuals, the rate of gastric emptying of drinks containing 30g and 70g of protein was comparable (kcal/min; 30g: 2.6±0.2, 70g: 2.9±0.3), and within the ranges previously observed for fat and carbohydrate (1-4 kcal/min). This was reflected by similar releases of cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), glucose-dependent inhibitory polypeptide (GIP), insulin and glucagon, for ~45 min following the drinks. Beyond 45 min, the 70g load resulted in more sustained hormone release, reflecting greater total calories and thus prolonged delivery of nutrient to the small intestine. Energy intake was comparable between the two loads, suggesting that a threshold amount of protein may exist, beyond which no additional appetite-suppressive benefit occurs.

In the studies described in chapters 6-8, intraduodenal infusions, combined with high-resolution manometry, were used to evaluate the effects of nutrients in the small intestine on

antropyloroduodenal motility and gastrointestinal hormone release. Nutrients were infused directly into the duodenum at standardised rates, reflecting the normal range of gastric emptying; intraduodenal infusion bypasses orosensory and gastric influences, isolating the effects of nutrient to the small intestine.

The first of these studies reported that intraduodenal protein has load-dependent effects on antropyloroduodenal motility, ghrelin, CCK, GLP-1, peptide tyrosine tyrosine (PYY), insulin and glucagon, glycaemia, and energy intake at a subsequent meal in lean individuals. The second study reported that load-dependent effects of protein on antropyloroduodenal motility and CCK, GLP-1, GIP, insulin and glucagon release are also apparent in obese individuals, suggesting that small intestinal sensitivity to protein remains intact in obesity. The final study demonstrated, in lean individuals, that intraduodenal lipid modulates gastrointestinal motor responses and CCK and GLP-1 concentrations more potently than an equicaloric protein load. In contrast, protein had more pronounced effects on insulin and glucagon release. Despite these differences, protein and lipid suppressed energy intake comparably, suggesting that different mechanisms may underlie the suppression of energy intake by these nutrients.

These data provide novel insights into the roles that gastrointestinal motor and hormone responses to dietary protein play in the regulation of blood glucose, appetite and energy intake in lean and obese individuals. These observations provide potential mechanistic explanations for the effects of high-protein diets on glycaemic control, and appetite. Importantly, they provide a basis for future development of nutrition-based interventions for the treatment of obesity.

Declaration of Originality

I, Amy Hutchison, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Amy Hutchison

November 2015

Publications Arising From This Thesis

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

Ryan AT, Feinle-Bisset C, Kallas A, Wishart JM, Clifton PM, Horowitz M, Luscombe Marsh ND. Intraduodenal protein modulates antropyloroduodenal motility, hormone release, glycemia, appetite, and energy intake in lean men. Am J Clin Nutr 2012;96:474-82. DOI:10.3945/ajcn.112.038133

Ryan AT, Luscombe-Marsh ND, Saies AA, Little TJ, Standfield S, Horowitz M, Feinle-Bisset C. Effects of intraduodenal lipid and protein on gut motility and hormone release, glycemia, appetite, and energy intake in lean men. Am J Clin Nutr 2013;98:300-11. DOI:10.3945/ajcn.113.061333

Hutchison AT, Piscitelli D, Horowitz M, Jones KL, Clifton PM, Standfield S, Hausken T, Feinle-Bisset C, Luscombe Marsh ND. Acute load-dependent effects of oral whey protein on gastric emptying, gut hormone release, glycemia, appetite and energy intake in healthy males. Am J Clin Nutr 2015; DOI: 10.3945/ajcn.115.117556

Hutchison AT, Feinle-Bisset C,Fitzgerald PCE, Standfield S, Horowitz M, Clifton PM, Luscombe Marsh ND. Comparative effects of intraduodenal protein on antropyloroduodenal motility, gut hormones, glycemia, appetite and energy intake in lean and obese men. Am J Clin Nutr 2015; DOI:10.3945/ajcn.115.114538

Other Publications

Soenen S, Giezenaar C, **Hutchison AT,** Horowitz M, Chapman I, Luscombe-Marsh ND. Effects of intraduodenal protein on appetite, energy intake, and antropyloroduodenal motility in healthy older compared with young men in a randomized trial. Am J Clin Nutr 2014; 100:1108-15. DOI:10.3945/ajcn.114.087981

Giezenaar C, Trahair LG, Rigda RS, **Hutchison AT,** Feinle-Bisset C, Luscombe-Marsh ND, Hausken T, Jones KL, Horowitz M, Chapman IM, et al. Lesser suppression of energy intake by orally ingested whey protein in healthy older men compared with young controls. Am J Physiol Regul Integr Comp Physiol 2015;309:R845-54.. DOI: 10.1152/ajpregu.00213.2015

Ullrich SS, Otto B, **Hutchison AT,** Luscombe-Marsh ND, Horowitz M, Feinle-Bisset C. Comparative effects of intraduodenal protein and lipid on ghrelin, peptide YY, and leptin release in healthy men. Am J Physiol Regul Integr Comp Physiol 2015; 308:R300-4. DOI:10.1152/ajpregu.00504.2014

Luscombe-Marsh, ND, **Hutchison AT,** Soenen S, Steinert RE, Clifton P, Horowitz M, Feinle-Bisset C. Plasma free amino acid responses to intraduodenal whey protein and relationships with insulin, glucagon-like peptide-1 and energy intake in lean healthy men. Nutrients; submitted 23 October 2015.

Dedication

Ko te manu e kai ana i te miro, nōna te ngahere.
Ko te manu e kai ana i te mātauranga, nōna te ao.

The bird that partakes of the miro berry reigns in the forest.
The bird that partakes of the power of knowledge has access to the world.

This thesis is dedicated to
my husband, Rob,
for always inspiring and challenging me,
and my whānau,
for your unwavering love and support.

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List of abbreviations

APD	Antropyloroduodenal motility
ANOVA	Analysis of Variance
ARC	Arcuate nucleus
AUC	Area under the curve
BMI	Body Mass Index
CCK	Cholecystokinin
CHO	Carbohydrate
CV	Coefficients of Variance
DPP-IV	Dipeptidyl peptidase-IV
EDTA	ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbance assay
FFM	Fat free mass
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
HbA1c	Glycated haemoglobin
HOMA-IR	Homeostatic model assessment of insulin resistance
ID	Intraduodenal
IPPW	Isolated pyloric pressure wave
LCD	Low calorie diet
MI	Motility index
MMC	Migrating motor complex
mV	Millivolt
NIDDM	Non-insulin dependent Diabetes Mellitus
NS	Not significant

PWs	Pressure waves
PYY	Peptide tyrosine tyrosine
RIA	Radioimmunoassay
TAA	Total Free Amino Acids
TFEQ	Three Factor Eating Questionnaire
TMPD	Transmucosal potential difference
T2DM	Type 2 Diabetes Mellitus
VAS	Visual Analog Scale Questionnaire

Chapter 1: Thesis Overview

Both in Australia, and worldwide, the prevalence of overweight and obesity, as well as obesity-related diseases, continues to rise. In the most simplistic sense, weight gain results from an imbalance between energy intake and energy expenditure. To date, considerable effort has been invested in developing surgical and pharmaceutical interventions, however, these are associated with numerous side-effects and, particularly in the case of pharmaceuticals, generally have limited long-term efficacy. As such, lifestyle interventions, including caloric restriction, increased physical activity, or a combination of these, remain the cornerstone of obesity treatment. Moreover, the current obesity epidemic, and the difficulty associated with maintaining weight loss, has generated considerable interest in understanding the regulation of eating behaviour in humans.

Maintaining energy balance requires the brain to detect the status of energy stores and to match energy intake with expenditure. The gut and brain are intimately linked, with signals arising from the gastrointestinal (GI) tract conveyed to the brain, and playing a role in the regulation of appetite and energy intake. The brain initiates responses to eating before any food has even been ingested. For example, visual, olfactory and gustatory stimuli initiate the release of endocrine and exocrine signals in the gut, and modulate GI motility, in anticipation of a meal. Following meal ingestion, a cascade of neural and hormonal responses is activated within, and from, the GI system, to impact responses in the central nervous system. These signals determine how nutrients are utilised, where they are stored, and the amount of food we eat at a meal, and subsequent meals.

After swallowing, the arrival of food in the stomach stimulates mechanoreceptors located in the gastric wall on vagal afferent nerve endings, which initiates a coordinated sequence of physiological changes, including gastric relaxation to accommodate the meal, and propulsion further down the GI tract to ensure digestion and absorption. As food moves from the stomach into the small intestine, peristaltic motor activity propels the nutrients along the small intestine, allowing interaction of nutrients with the mucosa to activate chemoreceptors. The latter sense changes in the GI environment, including nutrient composition, osmolarity and pH, and releases GI hormones in response to these stimuli. These hormones activate receptors located on vagal afferents, and signal to the brain via the vagus nerve. The hormonal signals are then integrated by the brain centres that control food intake, most importantly the medulla and arcuate nucleus (ARC) of the hypothalamus. They are then relayed either directly or via nerve fibres that project to other hypothalamic areas, including the paraventricular nuclei and lateral hypothalamic area. Together, the activation of mechanoreceptors and chemoreceptors induce negative feedback to slow gastric emptying, which in turn prolongs gastric distension, and regulates the rate at which nutrients enter the small intestine. The postprandial glycaemic response is also modulated by changes in gastric emptying and GI hormone release, along with the release of insulin and glucagon. In these ways, GI signals play a fundamental role in appetite regulation as well as blood glucose control. Critically, these signals have the potential to be influenced by the characteristics of a food and/or the diet, with the aim of altering energy intake.

The magnitude and pattern of the signals arising from the GI tract is dependent on the type of nutrient ingested. For example, it is well established that fat infused directly into the small intestine has potent effects on GI motility, the release of anorexigenic hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY),

and the suppression of the release of ghrelin. Intraduodenal carbohydrate (as glucose) has also been shown to modulate GI motor and hormone function, albeit less potently than lipid. In contrast, ingestion of carbohydrate, or protein, stimulates the release of insulin and glucagon more than fat. However, the GI motor and hormone responses to protein have not been comprehensively characterised.

Dietary protein has been shown to have effects on acute suppression of appetite and energy intake, as well as specific metabolic effects, including regulating blood glucose responses to a meal. Moreover, high-protein diets have been shown to induce weight loss, while maintaining fat-free mass, improving glycaemic control and blood lipids under both calorie-restricted and *ad libitum* conditions, for up to 2 years. It has been proposed that both the suppression of appetite, and subsequent reductions in energy intake, are the primary mechanism through which high-protein diets exert their superior weight loss effects. However, despite the known role of the GI tract in modulating energy intake, whether GI mechanisms underlie the appetite-suppressive effects of protein, remains to be established.

The research presented in this thesis addresses two overarching aims: i) to characterise the GI motor and hormonal responses to protein involved in the regulation of energy intake in healthy individuals; ii) to establish whether obese individuals are sensitive to the load of protein, and to explore the comparative effects of protein on GI function, blood glucose control, appetite and energy intake in obese, compared with lean individuals. In addition, the data presented in this thesis also considers the relationship between oral ingestion of protein and GI function, including gastric emptying, and the implications of the amount of protein administered both orally and intraduodenally on these outcomes. As the prevalence of obesity worldwide continues to increase, expanding our knowledge of the physiological

factors involved in appetite regulation will contribute to a better understanding of how these mechanisms might be targeted using nutrition-based strategies to promote satiety, and improve the outcomes of treatment in obesity.

Chapter 2: Human Obesity And Strategies To Achieve And Maintain A Healthy Body Weight

2.1 Introduction

The prevalence of overweight and obesity has increased over the past twenty years at an alarming rate. In Australia, self-reported height and weight, collected during the 2007-08 National Nutrition Survey, and using the World Health Organisation (WHO) classifications for body mass index (BMI) (calculated as weight in kg/height in metres^2) (**Table 2.1**), indicated that, of Australians aged over 18 years, 37 % were overweight and 25 % were obese (Australian Bureau of Statistics 2009).

The current high rates of obesity and their future projections are of considerable relevance. Obesity represents a risk factor for a number of non-communicable diseases, including, but not limited to, metabolic syndrome, non-alcoholic fatty liver disease and type 2 diabetes mellitus (T2DM) (Moayyedi 2008; Colagiuri *et al.* 2010; National Health and Medical Research Council 2013). In addition, obesity is associated with significant financial costs, and a loss of productivity due to poor health (National Health and Medical Research Council 2013). A recent systematic review reported that, when compared with healthy weight individuals, approximately 70 % of obese individuals have at least one established co-morbidity, which results in an average increase in medical costs of ~ 30 % (Withrow and Alter 2011). Perhaps most important to note is that almost all obesity-related conditions are preventable, and most are at least partially reversible through weight loss. Hence, the cost of obesity can be substantially reduced through lifestyle modifications that promote a healthy weight (National Health and Medical Research Council 2013).

While the maintenance of a healthy body weight is achieved by matching energy intake and energy expenditure, obesity, in the most simplistic sense, occurs when energy intake exceeds energy expenditure, over prolonged periods of time. Perturbations in energy balance, and the resultant weight loss (i.e. negative energy balance) or weight gain (i.e. positive energy balance), are influenced directly, and indirectly, by a number of factors. These factors include intrinsic influences such as genetic programming and extrinsic, socio-environmental influences. While genetic factors may increase an individual's susceptibility for becoming overweight, current epidemiological trends suggest that obesity primarily results from a combination of extrinsic lifestyle and environmental factors, such as changes in the food supply (i.e. increases in the abundance of palatable foods (Drewnowski and Specter 2004)), increasing portion sizes influenced by culture and social cues, and the rapid decline in physical activity due to the increasing prevalence of energy-saving technology (National Health and Medical Research Council 2013).

Although the precise contribution of each of these inter-related factors to the development of a positive energy balance and weight gain remains unclear, caloric restriction, in combination with an increase in physical activity via a multi-modal exercise program, remains the cornerstone of obesity management (Furst *et al.* 2005; Valette *et al.* 2012). In more chronically obese individuals, these interventions may support prescribed pharmacological agents or bariatric surgery to achieve sufficient weight loss. Regardless of the nature of the lifestyle therapy, or combination of therapies employed, maintaining weight lost for more than a few years remains a key issue. Even those who undergo bariatric surgery, which generally induces the largest weight loss, can experience weight regain after up to 10 years. Whilst the mechanisms underlying this weight gain probably involve a complex interplay of intrinsic and extrinsic factors, they have not been fully elucidated.

A key factor that has been implicated in driving weight regain following weight loss is the regulation of appetite and energy intake by signals arising from the GI tract (Sumithran *et al.* 2011). A large body of evidence exists to suggest that signals in the body, particularly those arising from the GI tract in response to foods that are consumed, play a critical role in determining energy balance. When food is ingested, neural and hormonal signals from the GI tract determine how the nutrients are utilised, where they are stored, the amount of food we eat at a meal, and also at subsequent meals. The development of obesity may, at least in part, reflect a decreased sensitivity to these GI signals, whereby they are insufficient to maintain energy intake at a level appropriate for weight maintenance. Critically, the magnitude of these signals is dependent on the type of nutrient ingested, so that fat, carbohydrate and protein have differential effects on appetite and energy intake. In particular, protein has been shown to have unique effects on suppressing appetite and energy intake, as well as specific metabolic effects, including regulating blood glucose.

Despite the known role of the GI tract in regulating energy intake (reviewed in Chapter 4), and the reported effects of protein on appetite (reviewed in Chapter 3), the GI mechanisms underlying these effects are poorly characterised. Hence, the main aim of this PhD thesis is to understand how nutrients, particularly dietary protein, modulate the GI mechanisms that are known to be involved in the regulation of energy intake, and how these mechanisms may be implicated in the modulation of body weight. This chapter will outline the definition and prevalence of obesity and its associated economic cost and health consequences. It will also highlight the evidence regarding the efficacy of a number of strategies for obesity treatment, including lifestyle modifications, bariatric surgery, and pharmacological interventions.

2.2 Definition, prevalence and health consequences of obesity

2.2.1 Definition of obesity

The WHO defines obesity as "abnormal or excessive fat accumulation that may impair health" (World Health Organisation 2015). BMI remains the most common method for determining overweight and obesity in individuals within the wider population (World Health Organisation 2015). As indicated in **Table 2.1**, BMI classifications provide an indication of the risk of morbidity and mortality to an individual that result as BMI increases. BMI is also useful as a measure of the risks of overweight and obesity, at a population level, for secondary disease, morbidity and mortality (World Health Organisation 2015).

In addition to BMI, WHO also suggests the use of proxy indicators for health, specifically waist circumference and waist-to-hip ratio, to assess the risk of obesity-related complications within the broader community. These measures account for the relative distribution of excess adipose tissue in the body, and the associated risk for cardiovascular disease, glucose intolerance, hyperinsulinemia, T2DM and other obesity related diseases. They also reduce the likelihood of an individual being misclassified as overweight or obese, for example, athletes with a high proportion of lean muscle mass. It is important to note that these specific BMI classifications apply primarily to Caucasian populations, due to differences in natural body composition between ethnicities. For example, in Asian populations, individuals have been shown to have a tendency for a comparatively higher body fat percentage for a given BMI (Kagawa *et al.* 2006). In Maori, Polynesian and Torres Strait Islander populations, the opposite is true, in that equivalent levels of risk for disease occur at higher BMIs than in Caucasians (Rush *et al.* 1997; Swinburn *et al.* 1999). Conversely, these populations are predisposed to a greater risk of diabetes, despite an equivalent level of body fat, compared with Caucasians (McAuley *et al.* 2002). Thus, assessment of disease risk for non-Caucasian

ethnicities using these BMI classifications should be interpreted with some caution, since it may not reflect the same body fatness in different individuals (He *et al.* 2001). Similarly to BMI, thresholds for waist circumference measures in European populations may differ from those of other ethnicities.

Table 2.1: Proxy indicators for health risk in Adult Caucasian populations

	Classification	**Waist circumference (cm)**		**Waist: Hip ratio**		**Risk of co-morbidities**
BMI (kg/m^2)		*Women*	*Men*	*Women*	*Men*	
<18.5	Underweight					Low (increased risk of other problems)
18.5-24.9	Normal range	<80	<94			Average
25-29.9	Overweight (pre-obese)	≥80	≥94	>0.8	>0.9	Mildly increased
≥30	Obese	≥88	≥102	>0.9	>1.0	Increased
30.0-34.9	Class I					Moderate
35-39.9	Class II					Severe
≥40	Class III					Very severe

2.2.2 Prevalence of obesity

In 2014, over 1.9 billion adults were overweight (~39 % of the worldwide population), and of these over 600 million (~13 %) were obese (World Health Organisation 2015). Of particular concern, projections based on previous population-based data from the National Health Survey (Haby *et al.* 2012) and AusDiab studies (Walls *et al.* 2012) suggest that, if recent patterns of overweight and obesity rates continue, up to 75 % of Australians could be overweight or obese by the year 2025 (Haby *et al.* 2012; Walls *et al.* 2012). Moreover, projections based on international figures suggest that by 2030, up to 58 % of the world's population could be overweight or obese (Appel *et al.* 2005). These projections, as well as

global figures, highlight the urgency to develop robust strategies to both halt and reverse the current rates of weight gain, and thereby reduce the enormous social and economic burdens.

2.2.3 Obesity-related risk factors

Excess adiposity associated with obesity is a risk factor for an array of secondary health complications, including sleep apnoea, joint pain and osteoarthritis, hypertension, hyperlipidaemia, hyperinsulinemia, cardiovascular disease, T2DM, metabolic syndrome (characterised by insulin resistance, dyslipidaemia, hypertension and increased waist circumference), gall bladder disease, non-alcoholic fatty liver disease and some cancers (Thompson *et al.* 1999; Guh *et al.* 2009). Overweight and obesity may also have implications for psychological health, including secondary eating disorders, body dysmorphia, low self-esteem and depression (Black *et al.* 1992; Smith *et al.* 1998; de Zwaan 2001). Generally, the risk for developing these non-communicable diseases increases with increasing BMI (World Health Organisation 2015).

2.3 Strategies for the treatment of obesity

Weight loss is an essential component in the reduction of risk associated with obesity-related co-morbidities. While reducing body weight to a level that places the individual within the normal BMI range (i.e. 18-24.9 kg/m^2 for adults aged 18-65) is the most desirable outcome, reductions in body weight of as little as 10 % can reduce hypertension (Neter *et al.* 2003), and improve cholesterol (Wing *et al.* 2011) glycaemic control (Look Ahead Research Group *et al.* 2007) and psychological outcomes (Yanovski *et al.* 1993; Blaine *et al.* 2007), in overweight and obese individuals. To achieve significant and enduring weight loss, considerable research effort has concentrated on developing a number of interventions, including i) lifestyle changes using varying degrees/ types of caloric restriction, alone or in

combination with increased physical activity; ii) surgical interventions including gastric bypass surgery, gastric banding and sleeve gastrectomy which alter the volume and absorptive capacities of sectors of the GI tract; and iii) pharmacological treatments that enhance metabolism, suppress appetite or inhibit nutrient absorption (or some combination of these), with varying degrees of success (**Figure 2.1**).

As mentioned earlier, lifestyle changes are the first avenue in treating obesity. Current Australian obesity guidelines suggest that intensive interventions (i.e. Very low calorie diets [VLCD], pharmacological interventions, bariatric surgery) may be used as an adjunct to lifestyle interventions in individuals with a BMI $\geq$ 30 kg/m^2, or with a BMI $\geq$ 27 kg/m^2 with a co-existing health condition, whom have previously been unsuccessful in reducing weight or mitigating weight gain using lifestyle interventions (National Health and Medical Research Council 2013).

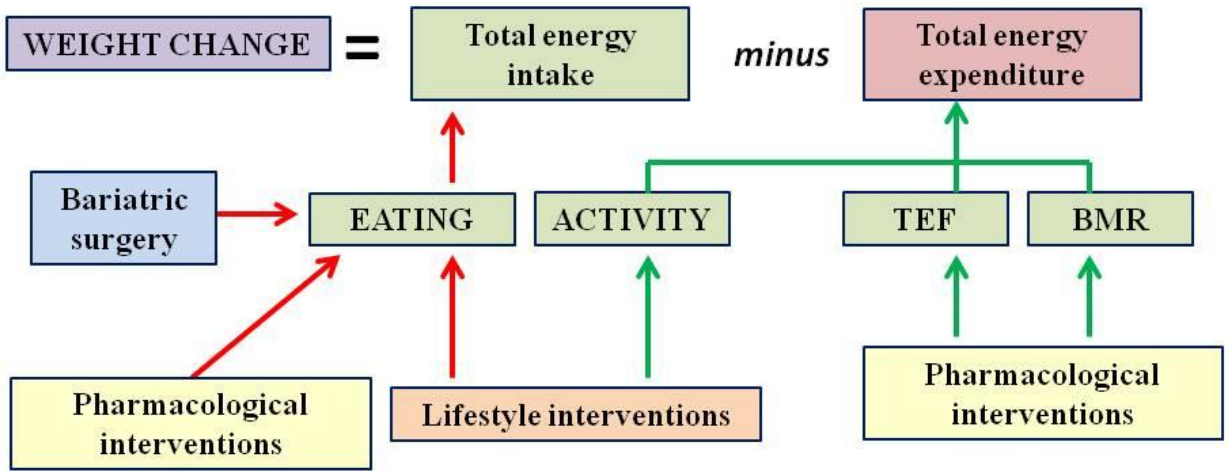

Figure 2.1: Model of energy balance in the treatment of obesity, and effects of therapeutic interventions. Changes in weight are resultant of inducing a negative energy balance (i.e. total energy intake > total energy expenditure). Therapeutic interventions target reducing energy intake (red arrows) and increasing activity (green arrows) through increasing physical activity, the thermic effect of food (TEF) and basal metabolic rate (BMR). Reproduced from Bray (2008).

2.3.1 Lifestyle modifications

Weight loss through lifestyle modification remains the safest and most accessible method in reducing both the co-morbidities of obesity, and excess body weight. While exercise alone may induce a small energy deficit via an increase in fat oxidation, this deficit is often insufficient to maintain weight loss. Instead, it is the sustained negative energy balance through energy restriction that is the most critical factor in inducing weight loss (Jakicic *et al.* 2001; Layman *et al.* 2005). Importantly, since physical activity offers additional advantages of maintaining metabolically active lean tissue and improving cardiovascular and metabolic health outcomes, it is widely recommended that individuals use a combination of both caloric restriction and increased physical activity (as both aerobic and resistance exercise) to gain maximum improvements in health from the lifestyle modifications they undertake (Furst *et al.* 2005; National Health and Medical Research Council 2013).

Weight loss diets

A number of dietary approaches have been advocated for the treatment of obesity, including very low calorie (VLCD), low calorie (LCD), low-fat/ high-carbohydrate, low-carbohydrate and high-protein, diets, with varying degrees of efficacy. For example, VLCDs (i.e. 400-800 kcal/day) produce greater short-term weight loss (i.e. up to 15 kg over 12 weeks) (Mathus-Vliegen and Balance Study 2005), whereas LCDs (800-1200kcal/day) (Johnston *et al.* 2004; Noakes *et al.* 2005) produce a comparatively modest weight loss (i.e. up to ~ 7kg over 12 weeks), but are comparatively easier to sustain, for at least 6-12 months (Johnston *et al.* 2004; Noakes *et al.* 2005). Moderate energy restriction (i.e. a reduction of 500-1000 kcal per day) has also been shown to induce clinically significant weight loss. Often, weight loss up to, and beyond 12 months is similar between VLCD and more moderate calorie-controlled diets, while almost all weight-loss diets are associated with a weight loss plateau (Curioni and

Lourenco 2005). Regardless of the degree of caloric restriction, weight loss of between 5 and 10 % of initial body weight has been shown to improve fasting plasma glucose and insulin, fasting lipids and blood pressure, independent of physical activity (McLaughlin *et al.* 2001; Reaven *et al.* 2001; Luscombe-Marsh *et al.* 2005; Noakes *et al.* 2005).

The macronutrient composition of the diet has been manipulated extensively in a number of studies to establish the most effective weight loss diet, which also produces significant changes in health outcomes. Considerable evidence has indicated that higher fat intakes contribute to increased body weight, as well as an increased risk of cardiovascular disease, while diets low in fat and high in complex-carbohydrates were thought to be protective against weight gain (Astrup and Raben 1995). Diets prescribing low-fat and high-carbohydrate intakes have been popular since the 1970's when fat was first implicated as a factor in increasing body weight and cardiovascular disease. In contrast, the Atkins diet, formulated in 1972, prescribes large amounts of protein and fat in the diet, while restricting carbohydrates to < 20 g per day, based on the notion that hyperinsulinemia and insulin resistance are the primary causes of obesity.

More recently, high-protein diets, with more moderate amounts of fat and carbohydrate have come to the fore in light of the increasing rates of obesity. High-protein diets have been shown, in both normal and overweight populations, to have superior effects in maintaining satiety, suppressing appetite and improving a number of metabolic outcomes. Importantly, there is considerable evidence to suggest that energy-restricted, high-protein diets are an effective nutrient-based therapy for managing appetite, reducing weight and improving glycaemic control in the obese, at least over 6-12 months (Wycherley *et al.* 2012b). High-protein diets also favour retention of fat-free mass, which contributes considerably to energy

expenditure and metabolism. Finally, high-protein diets have been shown to reduce energy intake, and induce weight loss under *ad libitum* conditions, suggesting that high-protein diets have superior appetite suppressive effects that allow a negative energy balance to be induced and maintained (Weigle *et al.* 2005).

Given the effects of dietary protein on appetite, glycaemia and metabolism, diets with an emphasis on high-protein foods may be advantageous in the treatment of obesity. While the effects of dietary protein and high-protein diets on energy expenditure and metabolism have been widely examined (Barrows and Snook 1987; Westerterp *et al.* 1999; Mikkelsen *et al.* 2000; Luscombe *et al.* 2003b), these mechanisms do not appear to fully explain the effects of high-protein diets on satiation, weight loss and reduction in metabolic risk. In light of this, additional factors, such as GI mechanisms, are likely to underpin the metabolic effects of protein. High protein diets, their effects on metabolic outcomes and use in weight management are reviewed in **Chapter 3.**

2.3.2 Surgical interventions

Bariatric surgery is arguably the most successful treatment for individuals with severe and complex obesity; however, it is associated with higher risks of both acute and longer-term complications than both pharmacological treatments and lifestyle interventions. Whilst surgical interventions have been shown to have a relatively high long-term success rate (i.e. up to 20 % weight loss for 10 years), in Australia bariatric surgery is currently only indicated in the very obese (BMI $\geq$ 40 kg/m^{2,} or $\geq$ 35 kg/m^2 with co-morbidities), or in individuals for whom other interventions are contraindicated, or have been unsuccessful (Sjostrom *et al.* 2004; National Health and Medical Research Council 2013). Established surgical procedures

currently utilised for treatment of obesity are Roux-en-Y gastric bypass (RYGB), gastric banding, and sleeve gastrectomy.

Roux-en-Y gastric bypass

The RYGB procedure involves creating a small-volume gastric pouch and producing a ~80-100 cm diversion, termed the alimentary limb, for food to bypass the duodenum and upper jejunum (**Figure 2.2B**), thus diverting nutrients directly to the more distal parts of the small intestine. The biliopancreatic limb (~60-80 cm in length), consists of the resected portion of the stomach, duodenum and jejunum, and joins the alimentary limb to transport bile and pancreatic secretions to the distal small intestine (Simons *et al.* 1999). RYGB induces weight loss primarily through the diminished size of the gastric pouch, which restricts intake, and the bypassing of nutrients to beyond the proximal small intestine (Elder and Wolfe 2007). In addition, the increased exposure of the distal small intestine to nutrients, resulting from the surgical redirection modulates the release of GI hormones, including CCK, PYY, and GLP-1, as well as leptin from adipose tissue, which ultimately result in reduced energy intake (Rubino *et al.* 2004; Beckman *et al.* 2010; Dirksen *et al.* 2013).

RYGB surgery has been shown to achieve significant long-term weight loss (up to 76 % of excess body weight loss at one year) (Cottam *et al.* 2006; Rosenthal *et al.* 2006) and has been shown to resolve type 2 diabetes in up to 85 % of patients almost immediately after surgery, even in the absence of any significant weight loss (Saliba *et al.* 2009; Jacobsen *et al.* 2012). This rapid reversal has been attributed to enhanced GLP-1, glucose-dependent insulinotropic peptide (GIP), PYY and insulin responses (Peterli *et al.* 2009). Risks of RYGB include micronutrient deficiencies, including ferritin, vitamin B_{12}, vitamin D and red blood cell folate deficiencies (Toh *et al.* 2009), gastric pouch enlargement, marginal ulceration, and dilation of

the gastrojejunostomy (Griffith *et al.* 2012). Of critical importance, RYGB may be complicated in some individuals by dumping syndrome, whereby food enters the small intestine too quickly, and which may cause nausea, cramping and diarrhoea, and presents a risk of inappropriate glycaemic responses in patients with T2DM (Clegg *et al.* 2002).

Adjustable gastric banding

Adjustable gastric banding (AGB) has been performed since ~1983, and involves the placement of an adjustable silicone cuff around the stomach, approximately 2 cm below the gastro-oesophageal junction at a 55° angle to the midline (Mehanna *et al.* 2006) (**Figure 2.2C**). A thin tube connects with an injection port located at the skin, allowing injection of saline for the adjustment of the tightness of the band. AGB modulates a complex interplay of mechanisms to reduce weight, including activation of peripheral satiety signals and is mediated, at least in part, by vagal mechanisms (Dixon *et al.* 2005).

The placement of the band distal to the gastro-oesophageal junction impacts the types of foods able to be consumed, and elicits behavioural adaptations, such as more restrained eating patterns and consumption of softer, less solid foods. In addition, the location of the band impacts intraluminal pressures of the oesophagus. These intraluminal pressures delay transit of food, particularly semi-solid food, into the portion of the stomach below the band (Burton and Brown 2011). Moreover, since only small portions can cross the AGB, the remaining portion of the bolus is refluxed into the oesophagus, which triggers additional peristalsis. This delayed transit results in a slowed eating pace, as patients must wait for the bolus to clear the AGB before consuming more food (Burton *et al.* 2010). Despite these changes, AGB has little impact on overall gastric emptying rate, at least in the regions of the stomach below the band (de Jong *et al.* 2009). Moreover, fasting concentrations of

anorexigenic GI hormones, including PYY and GLP-1, do not appear to be altered by AGB (Korner *et al.* 2006).

In a randomised, blinded, cross over trial, patients were allocated to have their bands inflated to an optimal level, or to have the fluid partially, or completely removed. When the bands were optimally adjusted, subjects rated satiety higher, and hunger lower, before and after a standardised meal, when compared with sub-optimal band adjustment (Dixon *et al.* 2005). A recent publication of a 15-year-total follow-up of ~3200 patients who underwent adjustable gastric banding procedures reported that 714 patients completed at least 10 years follow-up, and after 10-years, excess weight lost by those patients averaged 47 %.

Complications associated with gastric banding include perforation of the stomach wall, slippage, pouch dilatation, oesophageal dysmotility and reflux, and erosion of the stomach wall by the band (Mehanna *et al.* 2006). In some patients, insufficient weight loss, or weight regain after band deflation, symptomatic proximal gastric pouch dilatation, intragastric band migration and psychological band intolerance necessitate conversion of the AGB to RYGB (Mognol *et al.* 2004).

Sleeve gastrectomy

Sleeve gastrectomy involves resection of the greater curvature and fundus of the stomach, leaving the remaining stomach fashioned into an elongated tube with limited accommodative space (~60-80 mL) (**Figure 2.2D**). Sleeve gastrectomy offers almost immediate weight loss, maintains GI continuity (i.e. does not bypass regions of the GI tract) thus preventing dumping syndrome, and does not involve implantation of foreign material (i.e. the silicone band as in AGB).

Sleeve gastrectomy employs a combination of both restrictive and neurohormonal mechanisms to induce weight loss. The reduced volume of the post-operative "stomach", particularly the removal of the accommodative region, the fundus, restricts the amount of food able to be consumed as a single meal (Melissas *et al.* 2007). Removing the fundus also decreases release of the anorexigenic hormone ghrelin, and thus reduces baseline levels of hunger (Langer *et al.* 2005; Padwal *et al.* 2011). Sleeve gastrectomy has also been shown to result in accelerated gastric emptying of both liquid and solid portions of a meal (Melissas *et al.* 2008), probably as a result of the absence of accommodative relaxation, and altered contractile activity in the proximal stomach (Melissas *et al.* 2008). This increase in gastric emptying has been hypothesised to result in greater amounts of undigested food reaching the small intestine, stimulating increased release of GLP-1, GIP and PYY postprandially (Ramon *et al.* 2012).

Long-term weight loss following sleeve gastrectomy is generally between 60 – 85 % of excess weight lost. The sleeve gastrectomy procedure is advantageous in that, similarly to RYGB, glucose homeostasis and type 2 diabetes are promptly corrected in up to 67 % of patients (Gill *et al.* 2010). It is likely that enhanced GI hormone release, particularly GLP-1 and GIP, underpins these effects (Romero *et al.* 2012). Importantly, the potent stimulation of GI hormone release and the recovery of normal glucose homeostasis following both sleeve gastrectomy and RYGB highlight the critical importance of GI mechanisms in both blood glucose control and energy balance.

Risks associated with sleeve gastrectomy surgery include possibilities of staple-line leaks or bleeds, abscesses and, in the longer term, stricture (narrowing of the gastric lumen due to scar tissue), gastro-oesophageal reflux and nutritional deficiencies (Sarkhosh *et al.* 2013). The

procedure is also irreversible since a majority of the stomach is resected, and has a higher operative risk compared to other bariatric procedures (Noah *et al.* 2013).

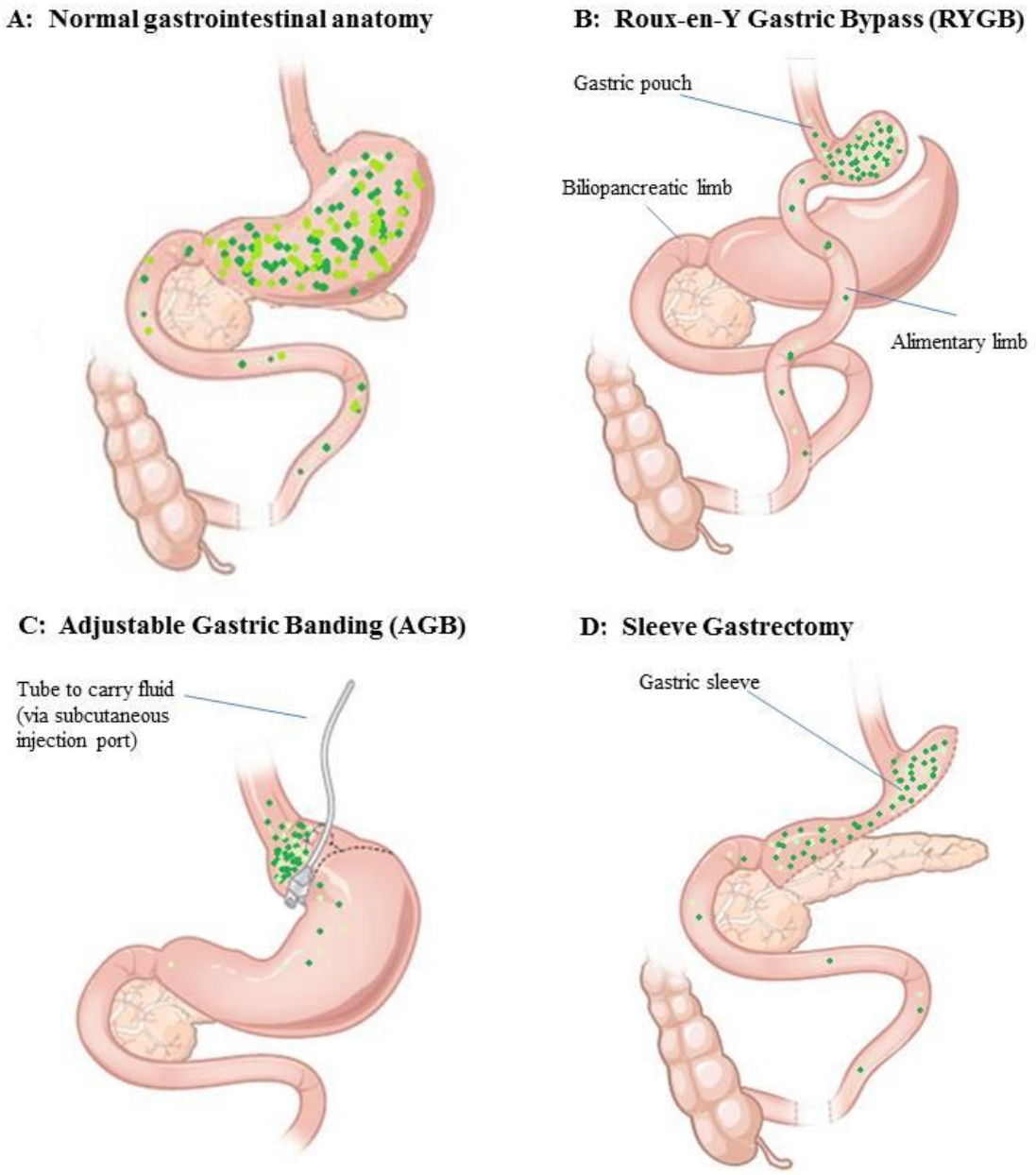

Figure 2.2: Graphical depiction of commonly used bariatric surgery techniques. (**A**): Normal GI anatomy; (**B**): Roux-en-Y Gastric Bypass (RYGB); (**C**): Adjustable Gastric Banding (AGB); (**D**): Sleeve Gastrectomy. (adapted from Cummings (2012).

2.3.3 Pharmacological interventions

Pharmacotherapies represent a third avenue of intervention for overweight and obesity, and may be used in conjunction with lifestyle therapies, and surgery, to enhance weight loss outcomes. To correct the imbalance of energy intake with energy expenditure associated with weight gain, pharmacotherapies have been developed that increase energy expenditure, reduce energy intake, or target both aspects of the energy balance equation. Typically, energy expenditure in an individual remains relatively stable from day to day, while energy intake can vary greatly due to social, behavioural and hedonic factors. Previously utilised drugs targeting increased energy expenditure include thyroid hormone, amphetamines, and dinitrophenol to stimulate basal metabolic rate and reduce body weight. These therapies are no longer approved for use in treating obesity, due to a considerable number of side effects, including addiction (Adan 2013). Accordingly, targeting mechanisms regulating appetite and energy intake remains the primary role of current pharmacotherapies.

In general, pharmacotherapies, taken over a period of 1–2 years, have demonstrated a significant, albeit modest (<5 kg), decrease in weight, when compared with placebo, with attrition rates of up to 43 % in some clinical trials (Padwal *et al.* 2004). In the majority of cases, weight loss following pharmacological intervention is not sustained once the drug is withdrawn, with many individuals regaining some, if not all plus more, of the weight that was lost. Weight loss drugs are classified by their effects, including fat absorption inhibitors and appetite suppressants with, and without, stimulant effects on the central nervous system. Currently, the only drugs approved for the treatment of obesity in Australia are phentermine (Duromine™, Metermine™), a central nervous system stimulant, and Orlistat (Xenical®), a fat absorption inhibitor. Off-label usage of drugs that have known secondary effects on

weight loss is also becoming increasingly popular as a mechanism for inducing weight loss where diet and lifestyle interventions alone have failed.

Fat absorption inhibitors

Orlistat acts to reduce fat digestion and absorption within the small intestine, by inactivating gastric and pancreatic lipases, thereby inhibiting hydrolysis and absorption of dietary triacylglycerols by around 30 % (Hauptman *et al.* 1992; Jandacek and Woods 2004). Orlistat is approved for longer-term treatment of obesity, and has been shown, in a meta-analysis of 11 placebo-controlled 12-month trials, to reduce weight by around 3 % in ~6000 patients (Padwal *et al.* 2004). Whilst this weight loss is relatively modest, Orlistat has also been shown to have beneficial effects on blood pressure, cholesterol and blood glucose in type 2 diabetics (Padwal and Majumdar 2007). Compliance of patients with the regimen can be limited due to adverse GI effects (i.e. diarrhoea, flatulence, bloating, oily stools, abdominal pain, and dyspepsia), which are greater following high-fat foods, due to the amount of unabsorbed fat passing through the GI tract, and being excreted from the body (Jandacek and Woods 2004). Prolonged use can also result in deficiency in the fat soluble vitamins A, D, E and K.

Appetite suppressants

Over the last 40 years, a vast number of anorexiants have been developed for obesity treatment, which target various neurotransmitters and receptors, including dopamine, serotonin and noradrenalin (Haddock *et al.* 2002). Such medications include phentermine, sibutramine (Reductil®, Meridia®), a monoamine reuptake inhibitor primarily affecting noradrenaline, dopamine and serotonin, rimonabant (Acomplia®), an endocannabinoid receptor inhibitor, and lorcaserin (Belviq®), a selective serotonin receptor inhibitor.

Phentermine is currently the only available prescription appetite suppressant in Australia. Phentermine is a sympathomimetic amine that stimulates increased hypothalamic release of norepinephrine, with no detectable effect on serotonin (Rothman and Baumann 2009). Phentermine has been shown to have favourable effects on lipid profiles (i.e. lowering of total cholesterol and low-density lipoprotein [LDL-] cholesterol) and glycaemic control, thereby reducing the risk of diabetes (Kim *et al.* 2006). Phentermine is also associated with a number of side effects including palpitations, tachycardia, increased blood pressure, tremor, excessive sweating, anxiety, irritability, restlessness, dizziness, insomnia, euphoria, dysphoria, headache, dryness of the mouth and GI complaints (i.e. diarrhoea and constipation) (Snow *et al.* 2005; Kang *et al.* 2010). Phentermine is also being studied in combination with other drugs to treat obesity, and was approved by the Food and Drug Authority (FDA) as a combination drug (Qsymia®) with topiramate (an anti-epileptic) in the United States in 2012.

A number of previously approved weight loss drugs have been withdrawn from use in many countries, due to their limited efficacy (i.e. a placebo-corrected weight loss of <-4.0 kg) and considerable associated adverse effects. For example, 'Fen-Phen', a combination of the drugs fenfluramine and phentermine was shown to induce an average placebo-corrected weight loss of ~10 kg over 32 weeks (~300 g/week weight loss) when used in conjunction with lifestyle modifications (Weintraub *et al.* 1992). The drug combination was withdrawn from the market in 1997, after it was reported to cause valvular heart disease and pulmonary hypertension (Connolly *et al.* 1997). Phenylpropanolamine (Acutrim®), a potent selective adrenaline, noradrenaline and dopamine mimetic, was found to be associated with an increased risk of haemorrhagic stroke in women (Kernan *et al.* 2000), and is no longer available as a weight loss treatment; however it is present in small doses in some cough medicines. Rimonabant, which suppresses appetite by inhibiting cannabinoid receptors, and

also increases thermogenesis, was withdrawn from the market after it was associated with depression and suicidal ideation (Padwal and Majumdar 2007). More recently, sibutramine, a centrally acting serotonin-norepinephrine and dopamine reuptake inhibitor, which induced weight loss of ~ 5 % per year, was withdrawn from the market due to an increased risk of cardiovascular events and strokes (James *et al.* 2010).

Pharmacological treatments with secondary effects on weight loss

There is considerable evidence to suggest that the mechanisms underlying food intake are tightly linked to those that also regulate mood (McElroy *et al.* 2004). For example, as serotonin is highly involved in the regulation of energy intake, and since many anti-psychotics target the serotonin system, it is conceivable that these drugs may have secondary effects on appetite and body weight (Jandacek and Woods 2004). In addition, drugs normally prescribed to treat neurological or mental disorders (i.e. epilepsy or depression), or T2DM have been noted for their effects on appetite or energy intake, and are increasingly being prescribed "off-label" to specifically achieve weight loss. Such drugs, including topiramate (an anti-epileptic), tesofensin and fluoxetine (anti-depressant medications) are used either as monotherapies, or in combination with, for example, phentermine, to suppress appetite (Sjodin *et al.* 2010; Allison *et al.* 2012). In the case of drugs targeting the treatment of T2DM, GLP-1 agonists, such as liraglutide and exenatide, dipeptidyl peptidase IV (DPP-IV, an enzyme that deactivates GLP-1) inhibitors, and metformin appear to have secondary effects on weight loss. Liraglutide (3.0 mg) has recently been approved for use in the USA, specifically for weight loss. Long-term efficacy of these drugs as a monotherapy for obesity, rather than diabetes, requires further substantiation.

2.4 Concluding remarks

The available evidence suggests that current therapies used for obesity vary in their efficacy, side effects and associated risks. Bariatric surgery represents an effective intervention to induce significant weight loss that is mostly sustained in the long term. Moreover, the changes in GI hormone release, and subsequent improvements in glycaemic control following RYGB and sleeve gastrectomy highlight the importance of GI neural and hormonal signals in determining energy intake. Unfortunately, bariatric surgery is only indicated in individuals with a high BMI, where previous lifestyle interventions have failed, and is associated with a number of risks. Pharmacological interventions are also able to induce modest weight loss; however the options for these are limited, and are also associated with a number of undesirable side effects. Conversely, lifestyle modifications have no qualification criteria, and in most individuals (excluding the very obese) present minimal risk. Hence, they remain the first avenue of treatment for obesity. In particular, dietary modification has beneficial effects for weight loss, glycaemic control and plasma lipids, however the magnitude of these improvements is dependent on the macronutrient profile of the diet. Some evidence suggests protein in the diet may offer metabolic advantages, as well as suppressing energy intake. High-protein diets, the importance of protein in the diet, physiological advantages of protein and the mechanisms underlying these, and the optimal amounts (i.e. grams) and percentage of energy intake of protein are reviewed in **Chapter 3**.

Chapter 3: Diets High In Protein As A Strategy For The Management of Obesity

3.1 Introduction

In light of the increasing prevalence of obesity, and the costs and risks associated with bariatric surgery and pharmaceutical treatments, much attention has fallen on dietary interventions to improve both body composition and metabolic health. While the most important factor in inducing weight loss is a negative energy balance, current literature underscores the controversy regarding the optimal macronutrient composition of weight loss diets, and whether an emphasis on protein, fat or carbohydrate in the diet is most effective in both reducing body weight and improving health (Gardner *et al.* 2007; Sacks *et al.* 2009). For example, for many years, low-fat diets with a moderate protein, and high carbohydrate (i.e. < 20 % fat, 20 % protein, > 60 % carbohydrate) content were advocated to promote weight loss, through lowered energy density, and increased fibre in the diet. Later, diets with low (i.e. < 100 g/day), or very low (i.e. < 20g/day), carbohydrate, and moderate to high (i.e. up to 40 %) fat and protein contents became of interest.

The WHO recommends a protein intake of 10-15 % of total daily energy, or a minimum of 0.83 g/kg body weight for healthy adults in energy balance (Joint WHO FAO UNU Expert Consultation 2007). Considerable evidence, however, suggests that the optimal intake for health indicators, including body composition, and maintaining satiety, may be in the range of 0.8 -1.6 g/kg body weight (Layman *et al.* 2003; Layman *et al.* 2009), while a protein intake of up to 2 g/kg body weight per day may be consumed without adverse effects (Poortmans and Dellalieux 2000; Bilsborough and Mann 2006). A large body of evidence suggests that increasing the protein, and reducing the carbohydrate, content of a meal, can reduce hunger

(Weigle *et al.* 2005; Veldhorst *et al.* 2008) and energy intake (Weigle *et al.* 2005), and improve plasma lipid profiles (Brinkworth *et al.* 2004a), postprandial glycaemic control (Farnsworth *et al.* 2003; Layman 2003), insulin resistance (Brinkworth *et al.* 2004a) and promote thermogenesis and energy expenditure (Lasker *et al.* 2008). In addition, increased protein in the diet may reduce the catabolism of fat-free mass and blunt the decrease in resting energy expenditure that usually occur during weight loss (Krieger *et al.* 2006; Leidy *et al.* 2007). It has been proposed that both the acute, and long-term, suppression of appetite, and subsequent reductions in energy intake, are the primary mechanism through which high-protein diets exert their superior weight loss effects (Weigle *et al.* 2005; Paddon-Jones *et al.* 2008). This chapter will discuss the effects of protein, consumed either as a single meal, or as part of a diet over a period of days to weeks, on appetite, energy intake and weight loss, as well as the metabolic advantages of an increased amount of protein in the diet. Potential risks and adverse effects of higher-protein diets will also be discussed.

3.2 Satiety and appetite responses to protein

Human eating behaviour is complex, and influenced by a number of factors. Typically, humans eat in episodes, i.e., distinct meals and snacks (de Graaf *et al.* 2004). The drive to eat determines individual eating episodes, and is influenced by, for example, environmental cues (i.e. time of day), sensory hedonic, emotional (including boredom) and social cues (de Graaf *et al.* 2004). The ingestion of a meal initiates the "satiety cascade", which describes the physiological processes involved in modulating food intake (**Figure 3.1**). The satiety cascade is characterised by two distinct phases, satiation, and satiety, which are mediated by sensory, cognitive, post-ingestive and post-absorptive factors (Blundell *et al.* 1996). With respect to a meal, humans tend to eat until they are comfortably full (termed satiation), upon which the meal is usually terminated (de Graaf *et al.* 2004). The degree of satiation is determined by

the measured meal size (as volume, weight or caloric value of the meal). Immediately after a meal, the drive to eat is at its lowest, and satiation at its highest. Satiety refers to the inhibition of hunger and appetite between meals, and determines both the length of time between meals (inter-meal interval) and meal frequency. Both satiation and satiety, and the reciprocal suppression of appetite are modulated and maintained by sensory, cognitive, pre-ingestive (i.e. hormonal), and post-absorptive (i.e. the presence of absorbed nutrient in the blood) processes, as described in the "satiety cascade" (**Figure 3.1**) (Blundell *et al.* 1993).

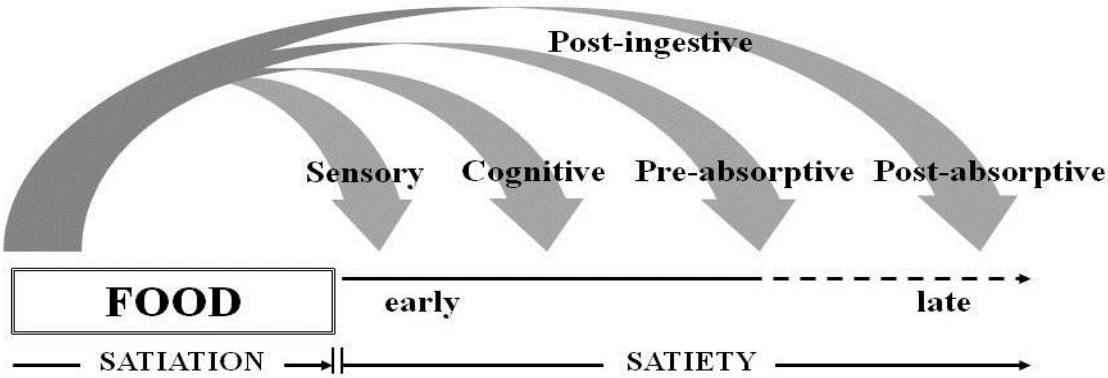

Figure 3.1: Satiety cascade (recreated from Blundell, 1996). The satiety cascade identifies the series of processes related to food intake. Satiation occurs during an eating episode and brings that meal or eating occasion to an end; satiety refers to the drive to eat in between meals. Satiation is influenced by meal characteristics. Mediating processes include sensory, cognitive, post-ingestive and post-absorptive factors, and these play a role at different stages during the food intake cycle. Sensory and cognitive factors play a role during satiation and contribute to early satiety. Post-ingestive signals may play a role during satiation, but are mainly involved during the mid- to late stages of satiety. Post-absorptive effects are primarily implicated in later stages of satiety (Blundell *et al.* 1996).

3.2.1 Pre–absorptive satiety signals in response to protein

The ingestion of a meal generates absorptive signals from the GI tract. Pre-absorptive signals are generated as the nutrient is ingested and digested. With respect to protein, pre-absorptive signals include the release of GI hormones (CCK, GLP-1, GIP, PYY, ghrelin, neuropeptide Y), as well as regulatory peptides implicated in energy balance (leptin, orexin, insulin), and these signals reach the brain via vagal afferent signalling. Collectively, these vagal signals

converge on the brainstem, where afferent vagal fibres terminate. In addition, ghrelin and PYY also directly stimulate the ARC of the hypothalamus and the area postrema of the brainstem, where the blood-brain barrier is incomplete (Journel *et al.* 2012). The hypothalamus plays a role in regulating appetite and food intake; the area postrema, tractus solitarius and ARC are involved in the regulation of satiety, while the mesolimbic reward system, including the nucleus accumbens, which is also stimulated by the presence of protein-digestive products in the bloodstream, appears to decrease the motivation to eat (Tome *et al.* 2009).

3.2.2 Post–absorptive satiety signals in response to protein

During the absorptive phase of digestion, individual amino acids are sensed in the small intestine and hepatoportal regions, and have been shown to have both excitatory and inhibitory effects on hepatic vagal afferent fibres (Chaudhari *et al.* 2000; Uneyama *et al.* 2006). Non-vagal, post-absorptive signals may also play a role in regulating the responses to dietary protein, since an intact vagal nerve is not required for protein to elicit effects on food intake (L'Heureux-Bouron *et al.* 2003). In rodents, elevations of plasma amino acid concentrations, as a result of protein ingestion, are directly detected by the ARC and the area postrema, and PYY-detecting neurons may play a critical role in initiating these effects (Tome *et al.* 2009).

In humans, as early as 1956, Melinkoff *et al* observed that a rise in serum amino acid concentrations correlated with a reduction in appetite (Mellinkoff *et al.* 1956). Melinkoff termed this the “aminostatic hypothesis” and postulated that a centre in the brain may sense changes in amino acid concentrations in the blood, and that a threshold concentration of amino acids signalled the termination of eating (Mellinkoff *et al.* 1956). Following this,

ingestion of protein has been shown to alter plasma amino acid concentrations (Fernstrom *et al.* 1979), and when mixed amino acids were consumed as a preload 30 min prior to a meal, food intake was reduced by ~ 22 % (Butler *et al.* 1981). Whether these effects are due solely to direct stimulation of specific areas of the brain by various amino acids is unclear, and it is likely that both direct stimulation of regions of the brain by plasma amino acids, and indirect vagal afferent signals generated from GI hormone release are implicated (Tome 2004). Additional post-absorptive mechanisms, including increased thermogenesis (Tome 2004), glucose signalling and homeostasis, via amino-acid induced gluconeogenesis, may also influence the satiating effects of protein (Tome 2004; Potier *et al.* 2009). The maintenance of satiety through these mechanisms is thought to enhance the effects of both energy-restricted diets for weight loss, and the maintenance of ideal body composition following weight loss. While it is known that both pre-, peri- and post-absorptive satiety signals play a role in determining the appetite responses to nutrient, the contributions of these signals to the superior satiating effects of protein remain unclear.

3.2.3 Protein-specific appetite – the protein leverage hypothesis

There is a large body of evidence to show that a number of animal species, including rodents, non-human primates, pigs, birds, fish and insects, will self-select diets to maintain protein intake within an optimal physiological range (Anderson *et al.* 1990). The protein leverage hypothesis, postulated by Simpson and Raubenheimer (2005), has suggested an integral role of "protein-specific appetite" in determining energy intake, whereby a species will aim to consume a target amount of protein within the diet. When protein sources are limited, they may increase total energy intake beyond that required, to ensure this protein threshold is reached. This protein-specific appetite is thought to exist to ensure adequate protein is consumed within the diet to meet physiological demand (Gosby *et al.* 2014).

In humans, only a handful of studies to date have directly tested the protein leverage hypothesis (Gosby *et al.* 2011; Griffioen-Roose *et al.* 2012; Martens *et al.* 2013a; Martens *et al.* 2013b; Martens *et al.* 2014). Gosby *et al*, using a 4-day dietary manipulation, showed that subjects consuming a low-protein (10 % total daily energy [TDE]) as compared to a normal-protein (15 % TDE) diet, increased energy intake by around 12 % (Gosby *et al.* 2011). However, this increase in energy intake was insufficient to maintain protein intake at a constant. Griffioen-Roose *et* al reported that subjects consuming a low-protein diet (0.5g/kg body weight/day) for 14 days, followed by 2.5 days of *ad libitum* feeding, increased their protein intake by ~13 % during the *ad libitum* feeding period, however, total energy intake remained unchanged (Griffioen-Roose *et al.* 2012).

In contrast, in two separate studies, Martens *et al* reported that individuals under-ate relative to energy balance, following a diet containing 30 % protein, compared with diets containing normal (15 %) and low (5%) amounts of protein respectively, however, in response to the low-protein diet they did not find evidence for a protein leveraging effect on energy intake (Martens *et al.* 2013a; Martens *et al.* 2013b). Collectively, while current examinations of the protein leverage hypothesis reinforce the effects of dietary protein on regulating appetite, no single study has provided evidence supporting protein leverage in both directions, and hence it remains unclear whether protein intake is regulated in humans for the purposes of protein balance.

3.3 Effects of high-protein meals on appetite and subsequent food intake

A macronutrient satiating hierarchy is well established. Laboratory studies utilising pure nutrient infusions have demonstrated that, kJ for kJ, regardless of the route of administration (i.e. oral vs. intravenous), protein is more satiating than fat, and fat more satiating than carbohydrate, at least in rodents (Walls and Koopmans 1992). A small number of human

studies that have simultaneously compared the effects of the three macronutrients on appetite and energy intake have also confirmed this hierarchy. For example, when compared with carbohydrate or fat preloads, a protein preload elicited greater subjective "satiety" ratings and resulted in fewer kJ being consumed *ad libitum* over the rest of the day (Rolls *et al.* 1988; Poppitt *et al.* 1998). This section will review the evidence for effects of high-protein meals on appetite and energy intake, as well as the importance of protein source, and quantity in a meal, for effects on satiety.

3.3.1 Effects of high-protein meals on appetite and energy intake

Protein exhibits a dose-dependent effect on satiety, across a range of concentrations offered acutely to weight-stable subjects in a single meal (Hill 1986; Barkeling *et al.* 1990; Westerterp-Plantenga *et al.* 2006; Smeets *et al.* 2008; Veldhorst *et al.* 2008) as well as an increased satiety over 24 hours (Westerterp-Plantenga *et al.* 1999; Westerterp-Plantenga *et al.* 2004; Lejeune *et al.* 2006). An overview of the results from a number of studies assessing the acute effects of high-protein meals on appetite energy intake is presented in **Table 3.1**.

In acute studies, mixed-nutrient meals containing up to 68 % protein have been shown to induce greater postprandial satiety than meals containing moderate amounts of protein (19 %) as quantified using visual analogue scales (VAS) (Stubbs *et al.* 1996; Crovetti *et al.* 1998), and to reduce the amount of energy consumed at a subsequent *ad libitum* meal (Porrini *et al.* 1995; Poppitt *et al.* 1998). For example, Latner and Schwartz (1999) reported that, following isocaloric (450 kcal), sensory-matched (taste, texture) liquid meals that were either high-protein (71.5 %), high-carbohydrate (99 %), or a balanced-protein/carbohydrate meal (50 % / 50 %), subjects consumed less energy at an *ad libitum* dinner following the high-protein and mixed-nutrient meals. In both lean and obese subjects, Batterham *et al* reported that a high-

protein mixed-nutrient meal (~65 % protein, 17 % carbohydrate, 17 % fat) suppressed hunger more than a high-fat (65 % fat, 17 % protein, 17 % carbohydrate) or high-carbohydrate (65 % carbohydrate, 17 % protein, 17 % fat) meal (Batterham *et al.* 2006). While all of these studies report potent effects on appetite in response to protein, the meals used contained very high amounts of protein, which are not sustainable to consume longer term, and over multiple meals.

Using protein loads that were more representative of the average protein load for a meal, a number of researchers also report potent effects on appetite and energy intake (Porrini *et al.* 1997; Veldhorst *et al.* 2009c; Veldhorst *et al.* 2009b; Brennan *et al.* 2012; Douglas *et al.* 2013; Ortinau *et al.* 2014). For example, Brennan *et al* administered a pasta, mince and tomato sauce meal with a vanilla yoghurt dessert, covertly manipulated to alter the macronutrient content, to lean and obese males. The meals were high- (45 %) or adequate-protein (30 %), high-carbohydrate/low-protein (10 % protein, 60 % carbohydrate), or high-fat (55 % fat, 15 % protein) (Brennan *et al.* 2012). The authors reported that, in lean subjects, the high-protein and high-fat meals reduced subsequent energy intake compared with the adequate protein meal by ~ 14 % and 9 % respectively. In obese participants, the high-protein meal reduced energy intake compared with the high-carbohydrate/low-protein (-23 %) and high-fat meals (-18 %), while the adequate protein meal also reduced energy intake compared with the high-carbohydrate meal (-10 %) (**Figure 3.2**) (Brennan *et al.* 2012).

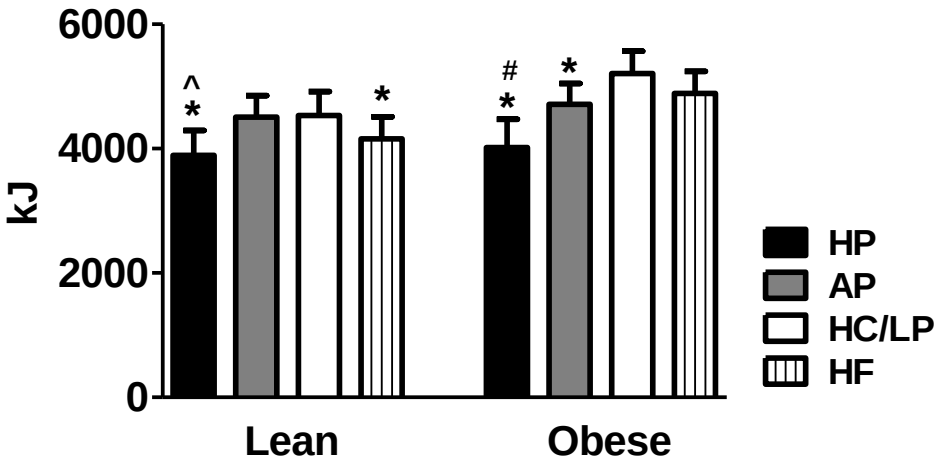

Figure 3.2: Energy intake (kJ) in lean and obese individuals following high-protein (HP, % energy from protein/fat/carbohydrate/ 45/25/30), adequate-protein (AP, 30/30/40) high-carbohydrate/low-protein (HC/LP 10/30/60) or high-fat (HF 15/55/30) test meals. Lean: * P<0.05 vs. HC/LP, ^ P<0.05 vs. AP; Obese: * P<0.05 vs. HC/LP, # P<0.05 vs HF (Brennan et al. 2012).

In contrast, a number of researchers have found no significant differences in the effects of high-protein versus high-carbohydrate or high-fat meals on satiety and energy intake (Rolls *et al.* 1988; Johnson and Vickers 1993; Raben *et al.* 2003). For example, Raben *et al* gave 20 lean, healthy subjects isocaloric meals (595 kcal for females, 714 kcal for males) that were high in either protein (crisp bread with cheese, yogurt with muesli, boiled egg, and skim milk; 31.8 % protein, 37.2 % carbohydrate, 31.1 % fat), carbohydrate (corn flakes with skim milk, white bread with butter, cheese, jam, and honey; 12.2 % protein, 65.4 % carbohydrate, 23.7 % fat), fat (yogurt mixed with double cream and grated apple, honeydew melon, rye bread with butter, cream cheese, and whole milk; 11.6 % protein, 23.9 % carbohydrate, 64.6 % fat), or alcohol (rye and whole-grain bread with butter and cheese, yogurt with muesli, honeydew melon, and orange juice with vodka; 12.1 % protein, 42.9 % carbohydrate, 24.3 % fat, 23 % alcohol), and reported no significant differences in hunger, satiety or fullness or energy intake between the macronutrient enriched meals (Raben *et al.* 2003). Of considerable importance, it should be noted that the foods used in the above described studies were whole foods, which

varied greatly in taste, texture and appearance, and thus it is not clear to what extent the type of food, and associated hedonic influences, or the macronutrient content of the food, affected satiety and subsequent energy intake.

Overall, the collective evidence presented here suggests that meals containing ≥ 25 % protein may modulate appetite sensations and decrease subsequent energy intake, in both lean and obese individuals. More recent evidence suggests that the absolute amount of protein (i.e. grams), may be more important than the proportion (i.e. %) of a meal, which may explain why some groups, but not others, reported effects of protein-containing meals on appetite and subsequent energy intake. In addition, differences in methodology, for example the use of whole foods versus liquid meals, the palatability of the test meals offered, the sources of protein administered, the timing of subsequent meals, the study population (i.e. lean vs. obese, mixed or single gender) and often small sample sizes make it difficult to determine the optimum percentage contribution, or dosage of protein, to induce a significant effect on satiety and energy intake at a later meal. The potential importance of the amount of protein is discussed in the following section.

3.3.2 The importance of protein quantity and timing in appetite regulation

Recent data have highlighted the importance of ensuring an absolute quantity of protein is consumed at each individual meal during the day, not only as a critical factor for protection of lean tissues during weight loss, but to induce satiety and suppress appetite (Schoeller and Buchholz 2005; Weigle *et al.* 2005). To stimulate muscle protein synthesis, adults probably require a minimum of 15 g of essential amino acids, and a total of 30 g of protein in a single meal (Rasmussen *et al.* 2000; Paddon-Jones and Rasmussen 2009). Based on 7-day food diary data of ~870 individuals, De Castro *et al* reported that current dietary patterns generally result in protein intakes of ~10 g protein at the breakfast meal, ~20 g at the lunch meal, and

~60 g at the evening meal. With respect to appetite control, in this pattern, the ingestion of the majority of daily protein intake at the evening meal, i.e. before sleep, limits the satiating benefits of the meal (de Castro 2004). In contrast, spreading the total daily intake of protein evenly across the day, may promote more stable appetite control. Thus, consuming ~30 g of protein at each meal may improve the satiety response to that meal, and, in particular, consuming this amount at the breakfast meal, when the body is in a catabolic state after an overnight fast, may have the greatest impact on total daily energy intake (Rolls *et al.* 1988; Layman 2009). This information has specific implications for the short-term (between-meal) regulation of appetite, as well as energy intake at subsequent meals.

There is also some evidence, albeit limited, that there is a ceiling effect for protein, whereby additional protein consumption beyond a threshold does not further suppress energy intake. For example, Belza *et al* have observed that *ad libitum* energy intake was not significantly different in response to equicaloric normal (24 g), medium-high (44 g), and high- (80 g) protein mixed-meals, despite protein-load-dependent suppression of hunger and prospective consumption, and stimulation of satiety and fullness (Belza *et al.* 2013). In addition, Akhavan *et al* reported that 20 g, but not 10 g, of whey protein, when consumed 30 min before a buffet meal, resulted in suppression of energy intake compared with a non-nutritive control. However, they also found no additional suppression of energy intake following 30 g, as compared with 20 g (Akhavan *et al.* 2010). While the data are limited, the above studies suggest that a protein ceiling may exist, and while increased amounts of protein may suppress appetite, approximately 30 g of protein appears to represent the maximum threshold amount of protein to significantly suppress energy intake, beyond which additional protein intake has no further effects. This requires further investigation.

By comparison, little is known regarding the minimum amount of protein required to elicit effects on appetite and energy intake. For example, Douglas *et al* report that 160 kcal of Greek yoghurt containing 24 g of protein increased fullness, and reduced hunger and delayed the request of dinner compared with a low (5 g) or moderate (14 g) protein yoghurt snack, or no snack at all (Douglas *et al.* 2013). Interestingly however, they report that the energy content of the snacks was not fully compensated for at an *ad libitum* dinner. In overweight women, Poppitt *et al* showed that a 500 mL liquid drink containing 1, 2 or 4 % w/w whey protein decreased hunger, and suppressed it for longer compared with a water control (Poppitt *et al.* 2011). Moreover, fullness and satisfaction were increased in response to the 2 and 4 % drinks. All of the protein drinks reduced energy intake at a subsequent *ad libitum* meal in a dose-dependent manner, however the energy content of all of the drinks was not entirely compensated for. This suggests that, while modest amounts of protein may alter short term satiety, the effects are insufficient to induce a significant reduction in energy intake at a subsequent meal. Evidence for the minimum protein dose however is scant, and further research is required.

3.3.3 Effects of protein source on appetite

Within the diet, there is a wide variety of foods that provide protein, and different sources of protein have different effects on satiety. For example, almost all animal-derived proteins have been shown to maintain post-absorptive satiety for longer than plant-derived proteins (Mikkelsen *et al.* 2000; Anderson *et al.* 2004). In particular, whey protein (a derivative of milk) has been shown to suppress short-term energy intake, increase subjective satiety and stimulate satiety signalling mechanisms more than other protein sources, including casein (Hall *et al.* 2003), egg albumin (Anderson *et al.* 2004) soy protein (Anderson *et al.* 2004), tuna (Pal and Ellis 2010) or turkey (Pal and Ellis 2010). Furthermore, whey proteins have

been shown to have a faster rate of digestion and absorption than other sources of protein, eliciting a rapid peak in plasma amino acid concentrations, which may contribute to their satiating effect (Boirie *et al.* 1997; Hall *et al.* 2003). Current evidence also suggests that increases in plasma amino acid concentrations, although not reflective of the amino acid composition of the food consumed, are associated with decreases in appetite (Mikkelsen *et al.* 2000; Anderson *et al.* 2004; Veldhorst *et al.* 2008). Moreover, the differences in magnitude of satiety are associated with the amino acid profile of the food source (Luhovyy *et al.* 2007; Veldhorst *et al.* 2008). Overall, studies investigating the effects of protein source on appetite and energy intake have generally reported whey to be more potent than other proteins. In light of this evidence, the effects of intraduodenal and oral protein in lean and obese individuals was examined using whey protein as the protein source for all studies described in this thesis.

Table 3.1: Studies examining acute effects of high-protein meals on appetite and energy intake

Author	Subjects	Meal type, energy content	Test meal		Comparative test meals		Time to next meal	Satiety outcomes	Subsequent energy intake
			composition	protein content	composition	protein content			
Batterham, 2006	19 males (10 normal weight, 9 obese	Pasta (normal vs high-protein), tomato sauce, dessert 1088 ± 2 kcal	65 % P 17 % C 17 % F	187 g	**HC:** 65 % C, 17 % P, 17 % F **HF:** 65 % F, 17 % P, 17 % C	**HC:** 49 g **HF:** 51 g	*Not measured*	**Lean:** HP significantly reduced hunger **Obese:** HP significantly reduced hunger	*Not measured*
Belza, 2013	25 males (13 normal weight, 12 overweight and obese [BMI 25.2 -37 kg/m^2])	Pork rice and mushroom pate; covertly manipulated 720 kcal + 300 mL water	50 % P 30 % F 20 % C	88.4 g	**MHP:**25 % P, 30 % F, 45 % C **NP:**14 % P, 30 % F, 66 % C	**MHP:** 44.5 g **NP:** 24 g	4 h	Significant dose dependent increases in satiety and fullness	NP: 997 ± 77 kcal MHP: 986 ± 72 kcal; HP: 830 ± 79 kcal **(NS)**
Brennan, 2012	32 males (16 normal weight, 16 obese [BMI 30-35.6 kg/m^2])	Pasta, mince and tomato sauce, vanilla yoghurt; covertly manipulated; ~10 % total daily energy requirements (TDE) lean: 299 ± 5 kcal obese: 314 ± 6 kcal	45 % P 25 % F 30 % C	1.35 g/kg bw	**AP:**30 % P, 30 % F, 40 % C; **HC/LP:**10 % P, 30 % F, 60 % C **HF:**15 % P, 55 % F, 30 % C	**AP:** 0.8 g/kg bw **HC/LP:** 0.2 g/kg bw **HF:** 0.4 g/kg bw	3 hours	**Lean:** HP significantly reduced hunger and increased fullness **Obese:** HP significantly reduced hunger	**Lean:** HP:926 kcal HF: 989 kcal AP: 1079 kcal HC: 1073 kcal **P<0.05 vs HF,AP, HC meals** **Obese:** HP: 957 kcal HF:1163 kcal AP: 1121 kcal HC: 1239 kcal **P<0.05 vs. HF,AP, HC meals**
Crovetti, 1998	10 normal weight women	Isocaloric (557 ± 9 kcal) **HP:** cured beef, crackers **HC:** pasta, tomato sauce, olive oil **HF:** mascarpone, crackers	68 % P 19 % F 13 % C	97 g	**HC:**10 % P, 21% F, 69 % C **HF:**9 % P, 70 % F, 21 % C	**HC:** 14 g **HF:** 12 g	7 hours	HP significantly increased satiety	HP: 1192 kcal HC: 1130 kcal HF: 1043 kcal **(NS)**

Table 3.1 continued: studies examining acute effects of high-protein meals on appetite and energy intake

Author	Subjects	Meal type, energy content	Test meal		Comparative test meals		Time to next meal	Satiety outcomes	Subsequent energy intake
			composition	protein content	composition	protein content			
Johnson, 1993	14 (8 females, 6 males) BMI 18.5-27.1 kg/m^2	150 and 300 kcal preloads **HP:** chicken **HC:** pasta, tomato sauce, onion, herbs **HF:** whipped cream, blueberries	72 % P 18 % F 0 % C	300 kcal preload: 57 g 150 kcal preload: 28.5 g	**HC:**12 % P, 3 % F, 86 % C **HF:**2 % P, 89 % F, 10 % C	**HC:** 300 kcal preload: 10.2 g 150 kcal preload: 5.1 g **HF:** 300 kcal preload: 2 g 150 kcal preload: 1 g	90 min	HP significantly increased satiety	*150kcal:* HP 753 kcal HF 897 kcal HC 877 kcal **P<0.05 vs HF, HC** *300kcal:* HP 641 kcal HF 814 kcal HC 674 kcal **P<0.05 vs HF, HC**
Latner, 1999	12 females; BMI 19-29 kg/m^2	Isocaloric (450 kcal) sensory-matched liquid preloads	71 % P 19 % F 10 % C	80.4 g	**HC:** 99% C; **Mixed:** 35.7 % P, 9.6 % F, 55.1 % C	**HC:** 0 g **Mixed:** 40.2 g	4.5 hours	HP significantly reduced hunger	HP: 943 kcal HC: 1239 kcal Mixed:1034 kcal **P<0.05 vs HC**
Ortinau, 2014	12 normal weight females	Isocaloric (160kcal) **HP:** yoghurt **HF(1):** crackers **HF(2):** chocolate	36 % P 0 % F 6 % C	14 g	**HF(1):** 0 % P, 32 % F, 68 % C; **HF(2):** 6.6 % P, 30.1 % F, 63.3 % C	**HF(1):** 0 g **HF(2):** 2g	Meal requested	HP reduced hunger vs HF(2); HP delayed dinner by 30 min vs HF(2) (P<0.01)	HP: HF(1): HF(2): **P<0.05 vs HF(2)** **P=0.08 VS HF(1)**
Poppitt, 1998	12 normal weight females	Isocaloric (476 kcal); Fish and potato pie, gin and tonic flavoured drink, covertly manipulated, sensory-matched	60 % P 20 % F 20 % C	71 g	**HC:** 12 % P, 20 % F, 68 % C **HF:** 12 % P, 68 % F, 20 % C **HAl:** 12 % P, 20 % F, 21 % C, 47 % Al.	**HC:** 16 g **HF:** 14 g **HA:** 15 g	1.5 hours	HP significantly increased satiety	HP 523 kcal HC 596 kcal HF 607 kcal HA 660 kcal **P<0.05 vs. HC, HF, HA**

Table 3.1 continued: studies examining acute effects of high-protein meals on appetite and energy intake

Author	Subjects	Meal type, energy content	Test meal		Comparative test meals		Time to next meal	Satiety outcomes	Subsequent energy intake
			composition	protein content	composition	protein content			
Porrini, 1997	14 normal weight males	Omelette, covertly manipulated, **HP:** 273.5 kcal **HF:** 284.3 kcal	54 % P 45 % F 1% C	37 g	15 % P, 79 % F, 6 % C	10.5 g	2 hours	*Not measured*	HP 1385 kcal HF 1596 kcal **(NS)**
Porrini, 1995	12 normal weight males	**HC:** Baked macaroni (960 kcal) **HP:** Meatballs in sauce (880 kcal)	56 % P 25 % F 19 % C	122 g	17 % P, 27 % F, 56 % C	41 g	2 hours	HP significantly increased satiety	HP 437 kcal HC 808 kcal **P<0.01 vs HC**
Potier, 2010	56 normal weight (12 men, 44 women)	Isocaloric hot chocolate drink (245 kcal), covertly manipulated	50 g protein	50 g	**HC:** 50 g maltodextrin **HF:** 22.3 g oil	**HC:** 0 g **HF:** 0 g	1 hour	No differences in satiety	HP 590 kcal HC 558 kcal HF 609 kcal **P<0.05 HC vs. HF**
Raben, 2003	19 normal weight (9 women, 10 men)	Unmatched food components (described in text); isocaloric (595 kcal for females; 714 kcal for males)	32 % P 31 % F 37 % C	*Not reported*	**HC:** 12 % P, 24 % F, 64 % C; **HF:** 12 % P, 64 % F, 24 % C; **HA:** 12 % P, 24 % F, 43 % C, 23 % Al.	*Not reported*	5 hours	No differences in satiety (VAS)	No differences in energy intake (actual figures not reported)

Table 3.1 continued: studies examining acute effects of high-protein meals on appetite and energy intake

Author	Subjects	Meal type, energy content	Test meal		Comparative test meals		Time to next meal	Satiety outcomes	Subsequent energy intake
			composition	protein content	composition	protein content			
Rolls, 1988	10 normal weight females	300 kcal **HP:** chicken **HC (starch):** pasta **HF:** cream cheese **HC (sucrose):** Turkish delight **HC/HF:** chocolate	74 % P 26 % F 0 % C	**55.6 g**	**HC(st):** 9 % P, 8 % F, 70 % C **HF:** 3 % P, 97 % F, 0 % C **HC(su):** 2 % P, 0 % F, 91 % C **HC/HF:** 5 % P, 38 % F, 60 % C	**HC (st):** 6.6 g **HF:** 2.0 g **HC (su):** 1.8 g **HC/HF:** 3.6 g	2 hours	HP significantly increased fullness and decreased hunger	**P<0.05 HP and HC(st) vs. all** (actual intakes not reported)
Smeets, 2008	30 (19 women, 11 men) BMI 20-30 kg/m^2	Pasta, sausages, tomato sauce, covertly manipulated; ~15 % TDE	25 % P 30 % F 45 % C	*Not reported*	10 % P, 30 % F, 60 % C	*Not reported*	4.5 hours	HP significantly increased satiety	*Not measured*
Veldhorst, 2009a	25 normal weight subjects	Custard, 20 % TDE (602 ± 17 kcal)	25 % P 55 % C 20 % F	15 g (whey)	10 % P, 35 % F, 55 % C	38 g	4 h	LP increased satiety	**NS** (actual intakes not reported)
Veldhorst, 2009b	25 normal weight subjects	Custard, 20 % TDE (602 ± 17 kcal)	25 % P 55 % C 20 % F	15 g (casein)	10 % P, 35 % F, 55 % C	38 g	4 h	HP increased fullness and satiety	**NS** **HP:** 734 ± 55 kcal **LP:** 749 ± 54 kcal

Al, alcohol; BW, body weight; C carbohydrate; F, fat; HA, high alcohol; HC, high carbohydrate; HC(st), high carbohydrate (starch); HC(su), high carbohydrate (sucrose); HF, high fat; HP, high protein; MHP, moderate-high protein; NP, normal protein; P, protein.

3.4 Longer-term effects of high-protein diets on appetite, food intake and body weight

In addition to the acute, meal-induced effects of protein on appetite and energy intake, chronic consumption of a diet higher in protein, under both energy-restricted and *ad* libitum conditions has been shown to have advantageous effects on the suppression of appetite and subsequent energy intake, as well as weight loss, retention of fat free mass, and improvements in body composition. The following sections will review the evidence for the effects of both *ad libitum*, and energy-restricted, high-protein diets.

3.4.1 Effects of high-protein diets on appetite and food intake

In the context of energy balance, a diet with a protein intake of 10-15 % of total daily energy intake is considered a standard- or adequate-protein diet, while > 15 % is considered a high-protein diet. High-protein diets with ≥ 30 % total daily energy intake from protein, when compared with a normal-protein diet, and administered for durations ranging between 24 hours and 5 days, have been shown to result in consistently higher satiety ratings throughout the day (Westerterp-Plantenga *et al.* 1999; Lejeune *et al.* 2006; Westerterp-Plantenga *et al.* 2009a). For example, in the controlled environment of a respiration chamber, healthy, lean women in energy balance were allocated to either a high-protein diet (30 % protein, 40 % carbohydrate, 30 % fat) or adequate-protein diet (10 % protein, 60 % carbohydrate, 30 % fat) over a period of 4 days. Throughout the day, satiety and fullness were rated higher, hunger and appetite rated lower, and energy intake was reduced with the high-protein diet (Westerterp-Plantenga *et al.* 1999). Similar effects (using a diet with macronutrient compositions identical to those used in women) have also been reported in lean men (Westerterp-Plantenga *et al.* 2009a). In a similar respiration chamber study, lean women fed either a high-protein (30 %), or adequate protein (10 %)/high carbohydrate, fat matched (30

%), diet over 4 days, reported an increased 24-hour satiety, and decreased hunger and energy intake, over the duration of the study period (Lejeune *et al.* 2006). In what is considered to be a landmark trial, Weigle *et al* explored the effects of both an isocaloric, and *ad libitum*, high-protein diet on weight loss, appetite and energy intake over a 4 month period. Subjects consumed, sequentially, a weight-maintaining, adequate-protein diet (15 % protein, 35 % fat and 50 % carbohydrate) for 2 weeks, followed by 2 weeks of an isoenergetic high-protein diet (30 % protein, 20 % fat, 50 % carbohydrate). They then consumed an *ad libitum* diet with 30 % protein, 20 % fat, 50 % carbohydrate for 12 weeks. Satiety was markedly increased with the isocaloric high-protein diet, while energy intake was spontaneously reduced by ~ 441 kcal on the *ad libitum* phase of the diet (Weigle *et al.* 2005). Together, these results suggest that increasing the protein content of the diet may have potent anorectic effects, and can result in spontaneous reductions in energy intake.

3.4.2 Effects of high-protein *ad-libitum*, and energy restricted, diets on weight loss and changes in body composition

In energy-restricted diets, where the overall macronutrient balance is manipulated, it is not uncommon for the absolute protein content (i.e. as grams of protein) to remain the same, but for the percentage contribution of protein content to reach up to 40 % of total daily energy intake, as fat and carbohydrate are generally the macronutrients that are reduced to induce a negative energy balance.

3.4.2.1 Effects of high-protein ad-libitum diets

Longer-term, high-protein, *ad libitum* trials are limited, however, in the trial by Weigle *et al*, subjects lost an average of 4.9 kg at the end of the 16 weeks, as a result of reduced hunger and sustained feelings of fullness, and decreased energy intake (**Figure 3.4**) (Weigle *et al.*

2005). Skov *et al* randomised 60 free living overweight men and women to either a high-protein (25 % total intake) or moderate-protein (12 % total intake) *ad libitum* diet for 6 months. Subjects in the high-protein group lost considerably more weight (8.8 kg vs. 5.1 kg) and fat (7.6 kg vs 4.3 kg) compared with the low protein diet, and consumed, on average, ~450 kcal less per day (Skov *et al.* 1999b). In an extension of this study, the authors followed up the participants for an additional 6 months, with less stringent intervention. While there were no differences between the high-protein and moderate-protein groups for weight loss (6.2 kg vs. 4.3 kg) the high-protein group had a 10% greater reduction in intra-abdominal adipose tissue (Due *et al.* 2004).

In the large scale, multi-centre DIOGENES trial, the role of protein intake in weight maintenance after weight loss has also been examined (Aller *et al.* 2014). After an 8 week LCD weight loss phase, subjects were randomised to one of five weight maintenance diets, i) low-protein, low-GI (LP/LGI), ii) low-protein, high-GI (LP/HGI), iii) high-protein, high-GI (HP/HGI), iv) high-protein, low-GI (HP/LGI) or v) a diet according to healthy eating guidelines. Subjects consuming a high-protein diet regained significantly less weight, and had more favourable changes in body composition, including a lower proportion of weight regain as fat mass and a higher proportion as fat-free mass (Aller *et al.* 2014). These data also confirm previous observations that weight maintenance after weight loss is greater with higher-protein *ad libitum* diets (Westerterp-Plantenga *et al.* 2004; Lejeune *et al.* 2005).

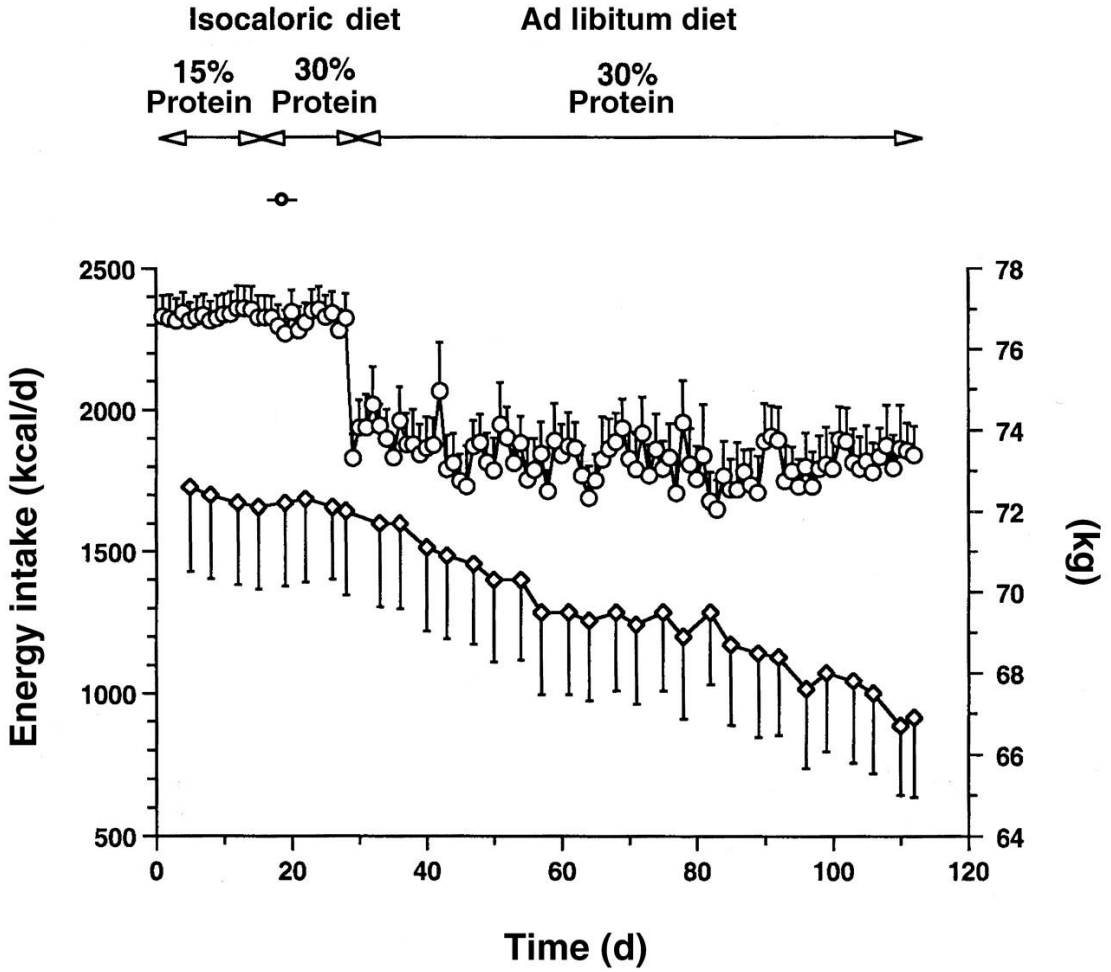

Figure 3.3: Mean total daily energy intake (○) and body weight (⋄) for 19 healthy subjects plotted against day of study. Subjects undertook a 2 week run-in diet comprised of 15 % protein, 35 % fat and 50 % carbohydrate, followed by a 2 week isocaloric diet where energy from protein was increased from 15 to 30 %, and fat decreased to 20 %. Finally, the subjects were admitted to a 12 week ad libitum phase, where the composition of the diet remained the same, but subjects were instructed to consume only as much of the diet as they wished, and foods were supplied to provide 15 % more energy than required for weight maintenance. The bars represent SEs (Weigle et al. 2005).

3.4.2.2 Effects of high-protein energy-restricted diets

During diet-induced weight loss, loss of fat free mass (FFM) typically accounts for approximately 20 % of total weight loss (i.e. 200 g /1 kg) (Krieger *et al.* 2006). FFM, particularly skeletal muscle, is a major contributor to the basal metabolic rate (BMR) and resting energy expenditure (REE), and is integral in protein metabolism. In overweight and obese individuals trying to lose weight, the loss of FFM as a result of acute energy restriction may down-regulate a number of metabolic processes, including protein turnover and

metabolism, and basal metabolic rate (BMR), which may influence weight management (Stein *et al.* 1991; Melissas *et al.* 2012). Generally, the extent to which these processes are diminished is dependent on the degree of energy restriction, and result in an increase in whole-body proteolysis, amino acid oxidation and nitrogen excretion (Pasiakos *et al.* 2010). This effect decreases and plateaus as the body adapts to conserve energy and protein stores (Stein *et al.* 1991; Pasiakos *et al.* 2010).

Thus, in the context of weight loss, protein appears to be an integral component of an energy-restricted diet to offset the demand for metabolic substrates, maintain energy expenditure through the retention of FFM, and optimise protein utilisation (Pasiakos *et al.* 2010). There is an abundance of studies to suggest that high-protein diets, with an intake above the recommended intake of 0.8 g/kg/d may offer the advantage of increased body fat mass loss, and retention of FFM, when compared with a moderate- or low-protein diet (Skov *et al.* 1999b; Farnsworth *et al.* 2003; Layman *et al.* 2003; Krieger *et al.* 2006; Leidy *et al.* 2007; Wycherley *et al.* 2012a; Wycherley *et al.* 2012b; Soenen *et al.* 2013). For example, in the 52 week study by Wycherley *et al* (2012), subjects on a high-protein diet (31-33 % protein) lost approximately 1.5 kg more body weight than those on a standard-protein (20-21 % protein) diet. In addition, the subjects on the high-protein diet lost more body fat (-27 % vs. -22 %), and less lean mass (-4 % vs. -6 %), than those on the standard protein diet (Wycherley *et al.* 2012a). A recent meta-analysis, which selected studies with energy-matched high- and standard-protein arms, conducted for up to 21 weeks, reported a significant additional weight loss of 0.79 kg following a high-protein, compared with low-protein diet (Wycherley *et al.* 2012b).

In a meta-regression analysis by Krieger *et al*, the authors reported that the degree of FFM retention during weight loss through energy restriction tended to increase with successive quartiles of protein intake. In studies of less than 12 weeks duration, high-protein diets (>1.05g/kg/d) were associated with retention of an additional 0.6 kg FFM, while in studies >12 weeks, this was increased to retention of an additional 1.21 kg (Krieger *et al.* 2006). Taken together, these studies suggest that dietary protein, over and above the RDA of 0.8g/kg/d may exert a protective effect on the retention of FFM during energy restriction, and that this protective effect is generally increased with increased amounts of protein in the diet. Studies examining the effects of high-protein diets on fat loss, and total weight loss, under hypoenergetic, isoenergetic and *ad libitum* conditions are reviewed in **Table 3.2**.

In contrast, a number of studies have reported no difference in weight loss between high-protein and adequate or low-protein diets (Piatti *et al.* 1994; Luscombe *et al.* 2002; Farnsworth *et al.* 2003; Layman *et al.* 2003) (**Table 3.2**). A recent review of diets manipulating the amount of protein has put forward two alternate explanations for the discrepancies of the success of diets, in the theories of protein spread and protein change (Bosse and Dixon 2012). In their protein spread theory, Bosse *et al* proposed that, to be classed as "successful", there must be sufficient spread, or difference, in g/kg/day of protein intake between "high-protein" intervention groups and controls. Specifically, a high-protein diet should have 58.4 % more protein than a control diet to observe significant changes in body composition (i.e. loss of fat mass and maintenance or gain of lean mass) and anthropometry (i.e. weight loss), compared to ~39 % spread in "unsuccessful" studies. For example, in the study by Brehm *et al* (**Table 3.2**) protein intake in the high-protein diet (1.19 g/kg/d) was ~74 % greater than that of the standard protein diet (0.88 g/kg/d), and resulted in significantly greater fat and total weight loss in response to the high protein diet. In contrast,

the study by Larsen *et al* had a spread of ~ 16 % between the high-and standard- protein diets (1.32 g/kg/d, 1.11 g/kg/d respectively), and fat and total weight loss did not differ between the two diets.

In addition, the protein change theory postulates that for a study to be "successful" there must be an increase in protein intake, as g/kg/day, from baseline to intervention of ~29 % (i.e. from 1 g/kg/d to >1.3 g/kg/d), compared with the ~5 % increase shown in unsuccessful studies. Of importance to note, is that, in the "unsuccessful" studies included in this review (i.e. those that did not observe changes in anthropometry), groups allocated to the high-protein diet did consume more protein than control; however during the intervention period they actually consumed less protein than their habitual intake, which may explain the lack of any observable change. Further research is required to establish whether there are thresholds for protein change and spread, and how baseline protein levels may affect outcomes of dietary interventions.

Table 3.2: Effects of high-protein diets on weight loss and body composition

Author	Subjects	Duration	Diet	Baseline diet Energy (kJ/d)	Baseline diet Protein g/kg/d	Baseline diet Protein %	Baseline diet CHO	Baseline diet Fat	Study diet Energy (kJ/d)	Study diet Protein g/kg/d	Study diet Protein %	Study diet CHO	Study diet Fat	Mean weight loss (kg) ± SEM	Mean fat loss (kg) ± SEM
Baba, 1999	OB, HI	**4 weeks,** ER (80 % of REE)	HP	-	-	-	-	-	7495	1.78	45	25	30	-8.3 ± 1.9	-7.1 ± 0.7
			SP	-	-	-	-	-	7377	0.50	12	58	30	-6.0 ± 1.5	-6.3 ± 0.1
Brehm, 2005	OB	**16 weeks,** *Ad libitum* HP diet vs. SP ER diet (25 % ER)	HP	9069	1.00	16	48	36	6410	1.19	24	52	24	-9.79 ± 0.7	-6.1 ± 0.4
			SP	9110	0.88	14	51	35	5953	0.88	20	48	32	-6.14 ± 0.9	-3.2 ± 0.3
Claessens, 2009	OB	**17-18 weeks** 6 ER (2100 kJ/d); 12 *ad libitum*	HP	-	-	-	-	-	2100	-	-	-	-		
			SP	-	-	-	-	-	2100	-	-	-	-		
			HP	2161	~1	17	44	35	1828	~1.9	35	42	21	-1.09 ± 0.6*	-2.0 ± 0.5*
			SP	2398	~1	16	48	35	1868	~0.9	16	63	24	+1.19 ± 0.9*	+0.2 ± 0.7*
Farnsworth, 2003	OW/OB, HI	**16 weeks** 12 (-30 % ER); 4 energy balance	HP	-	-	-	-	-	*6300*	*~1.05*	*28*	*45*	*27*	**HP:** M:-11.4 ± 2.1 W:-6.6 ±0.5	**HP:** M:-9.0 ± 2.7 W:-6.6 ±1.4
			SP	-	-	-	-	-	*6500*	*~0.53*	*16*	*57*	*27*		
			HP	-	-	-	-	-	*8000*	*~1.26*	*27*	*45*	*27*	**SP:** M:-9.6 ± 1.7; W:-7.4 ± 0.5	**SP:** M:-7.6 ± 3.1; W:-7.1 ± 2.0
			SP	-	-	-	-	-	*8200*	*~0.66*	*15*	*57*	*28*		
Johnston, 2004	OW/OB	**6 weeks,** (25-30 % ER)	HP	-	-	-	-	-	7080	1.63	32	41	27	-4.6 ± 1.6	-2.5 ± 0.6
			SP	-	-	-	-	-	7080	0.82	15	66	19	-4.8 ± 2.1	-3.0 ± 0.6
Keogh, 2007	OB,HI	**52 weeks** 16 ER (6000 kJ/d), counselled; 36 free-living	HP	-	-	-	-	-	*6000*	*1.37*	*40*	*30*	*30*	-7.6 ± 8.1**	*NR*
			SP	-	-	-	-	-	*6000*	*0.72*	*20*	*30*	*50*	-4.8 ± 6.6**	*NR*
			HP	-	-	-	-	-	7352	1.17	22	44	32	+2.6 ± 4.4**	*NR*
			SP	-	-	-	-	-	6936	1.00	20	47	31	+4.9 ± 4.4**	*NR*
Krebs, 2012	OW/OB, T2DM	**52 weeks,** (25 % ER)	HP	7860	0.85	19	47	32	7258	0.91	21	45	33	-3.2 ± 0.5	-2.8 ± 0.4
			SP	7850	0.86	19	46	33	6784	0.82	20	48	30	-2.4 ± 0.5	-1.7 ± 0.4
Larsen, 2011	OW/OB T2DM	**52 weeks** 12 (30 % ER); 40 energy balance	HP	8897	1.31	21	44	32	6449	1.32	30	40	30	-2.79	*NR*
			SP	9175	1.29	21	46	33	6029	1.11	15	55	30	-3.08	*NR*
			HP						6664	1.27	27	42	31	-2.23	*NR*
			SP						6628	1.12	19	48	32	-2.17	*NR*

Table 3.2 continued: Effects of high-protein diets on weight loss and body composition

Author	Subjects	Duration	Diet	Baseline diet Energy (kJ/d)	Baseline diet Protein g/kg/d	Baseline diet Protein %	Baseline diet CHO	Baseline diet Fat	Study diet Energy (kJ/d)	Study diet Protein g/kg/d	Study diet Protein %	Study diet CHO	Study diet Fat	Mean weight loss (kg) ± SEM	Mean fat loss (kg) ± SEM
Lasker, 2008	OB	**16 weeks,** ER (-2100kJ/d)	HP	9952	0.98	16	49	34	*7100*	*1.60*	*30*	*40*	*30*	-9.1 ± 0.9	-6.0 ±0.6
			SP	9147	0.93	16	49	34	*7100*	*0.71*	*15*	*55*	*30*	-6.9 ± 0.8	-4.4 ± 0.5
Layman, 2003	OB	**10 weeks** ER (7112 kJ/d)	HP	8196	0.88	15	50	35	6987	1.47	30	41	29	-7.5 ± 4.9	-5.6 ± 0.5
			SP	8196	0.88	15	50	35	6941	0.79	16	58	26	-7.0 ± 4.7	-4.7 ± 0.7
Layman, 2005	OW	**16 weeks** ER (7112 kJ/d)	HP	8888	0.87	15	49	36	6062	1.20	29	39	32	-8.7 ± 1.7	-5.9 ± 0.7
			SP	8479	0.87	16	49	35	5377	0.60	17	61	22	-7.8 ± 1.4	-5.0 ± 0.6
Layman, 2009	OW/OB	**52 weeks**													
		16 ER (-2100 kJ/d); 36 W	HP	10060	1.05	16	49	35	6730	1.38	27	40	33	-8.2 ± 0.5	-5.6 ± 0.4
			SP	8780	0.88	15	50	35	6200	0.79	18	59	23	-7.0 ± 0.5	-4.6 ± 0.3
			HP						7180	1.42	26	39	35	-10.4 ± 1.2	-7.3 ± 0.9
			SP						6800	0.82	16	57	27	-8.4 ± 0.9	-5.3 ± 0.6
Leidy, 2007	OW/OB	**12 weeks** ER (-3140 kJ/d)	HP	-	-	-	-	-	6363	1.41	30	45	25	-8.1 ± 1.8	-6.6 ± 0.4
			SP	-	-	-	-	-	6510	0.82	18	57	25	-9.5 ± 5.0	-6.6 ± 0.6
Luscombe, 2002	OB, T2DM	**12 weeks**													
		8 ER (6700 kJ/d); 4 energy balance	HP	-	-	-	-	-	6657	1.16	28	42	30	*NR*	*NR*
			SP	-	-	-	-	-	6648	0.66	16	55	29	*NR*	*NR*
			HP	-	-	-	-	-	7744	~1.5	28	42	30	-4.9 ± 0.4	*NR*
			SP	-	-	-	-	-	7463	~0.8	16	55	29	-4.3 ± 0.7	*NR*
Luscombe, 2003	HI	**16 weeks**													
		12 ER (6500 kJ/d); 4 energy balance	HP	-	-	-	-	-	6358	~1.15	27	45	27	*NR*	*NR*
			SP	-	-	-	-	-	6663	~0.7	16	57	27	*NR*	*NR*
			HP						8068	*NR*	27	45	27	-7.9 ± 4.5	-7.0 ± 0.7
			SP						8235	*NR*	15	57	28	-8.0 ± 2.9	-6.5 ± 0.7
McAuley, 2005	OW/OB	**16 weeks**													
		8 supervised *Ad libitum;*	HP	7847	0.89	17	47	31	5663	1.13	28	35	35	-5.4 ± 1.0	-3.1 ± 0.9
			HPHF	8397	0.99	18	44	34	6222	1.26	29	11	57	-6.6 ± 0.8	-4.4 ± 0.7
			SP	7585	0.87	18	45	31	5745	0.69	21	45	24	-4.3 ± 1.0	-3.4 ± 0.8
		8 supervised WM *ad libitum*	HP	-	-	-	-	-	6397	1.21	26	35	34	-7.0 ± 1.0	-3.9 ± 0.8
			HPHF	-	-	-	-	-	6787	1.14	24	26	46	-7.0 ± 0.8	-4.4 ± 0.7
			SP	-	-	-	-	-	6147	0.90	22	45	28	-4.4 ± 1.0	-3.5 ± 0.8
Mojtahedi, 2011^	OW/OB	**24 weeks** ER (5860 kJ/d)	HP	7063	0.91	19	52	29	5112	0.76	19	56	25		*NR*
			SP	7298	0.98	19	44	37	6214	0.87	20	49	31		*NR*
			HP^	-	-	-	-	-	5732	1.21	27	50	23	-7.7 ± 6.2	*NR*
			SP^	-	-	-	-	-	6812	0.87	18	54	28	-3.6 ± 3.1	*NR*

Table 3.2 continued: Effects of high-protein diets on weight loss and body composition

Author	Subjects	Duration	Diet	Baseline diet Energy (kJ/d)	Baseline diet Protein g/kg/d	Baseline diet Protein %	Baseline diet CHO	Baseline diet Fat	Study diet Energy (kJ/d)	Study diet Protein g/kg/d	Study diet Protein %	Study diet CHO	Study diet Fat	Mean weight loss (kg) ± SEM	Mean fat loss (kg) ± SEM
Noakes, 2005	OB	**12 weeks**	HP	-	-	-	-	-	*5600*	*1.3*	*34*	*46*	*20*	-7.6 ± 3.9	-5.7 ± 0.6
		ER (5600 kJ/d)	SP	-	-	-	-	-	*5600*	*0.66*	*17*	*64*	*20*	-6.9 ± 4.3	-4.5 ± 0.5
Parker, 2002	T2DM	**12 weeks**													**HP:**
		8 ER	HP	-	-	-	-	-	6348	1.20	28	42	28	-4.5 ± 2.0	M:-3.2 ±0.9
		(6700 kJ/d);	SP	-	-	-	-	-	6172	0.73	16	55	26	-4.5 ± 2.0	W:-5.3 ± 0.5;
		4 energy	HP	-	-	-	-	-	8116	1.54	28	42	28	-1.0 ± 2.0	**SP:**
		balance	SP	-	-	-	-	-	7140	0.86	16	55	27	-0.3 ± 2.0	M:-4.4 ± 0.9
															W:-2.9 ± 0.6
Skov, 1999	OW/OB	**24 weeks**													
		0-12 weeks	HP	-	-	-	-	-	8600	1.05	25	46	29		*NR*
		Ad libitum	SP	-	-	-	-	-	10600	1.03	12	59	29		*NR*
		13-24 weeks	HP	-	-	-	-	-	9300	1.38	24	47	29	-8.7± 1.4	-7.6 ± 1.4
		Ad libitum	SP	-	-	-	-	-	11200	0.84	12	59	29	-5.0 ± 1.4	-4.3 ± 1.2
Tang, 2013	OW/OB	**13 weeks**													
		1 baseline	HP	-	-	-	-	-	9354	1.39	24	51	25		
			SP	-	-	-	-	-	9475	0.79	13	62	25		
		12 ER	HP	-	-	-	-	-	9437	1.54	24	51	25	-10.6 ± 0.6	-7.2 ± 0.5
		(-3140 kJ/d)	SP	-	-	-	-	-	9517	0.89	13	62	25	-9.1 ± 0.7	-3.0 ± 0.4
Wycherley, 2010	OB, T2DM	**16 weeks**	HP	-	-	-	-	-	6321	1.16	32	47	18	-9.0 ± 4.8	-7.1 ± 4.0
		ER, (-6000 kJ/d [W]; -7000 kJ/d [M])	SP	-	-	-	-	-	6278	0.71	19	54	23	-8.6 ± 4.6	-6.5 ± 3.7
Wycherley, 2012	OW/OB	**52 weeks**													
		12 supervised, ER	HP	-	-	-	-	-	7134	1.24	33	37	27	-10.2 ± 4.9	-8.1 ± 0.5
		(7000 kJ/d)	SP	-	-	-	-	-	7189	0.81	21	51	25	-9.4 ± 4.4	-5.7 ± 0.5
		40 free living, ER	HP	-	-	-	-	-	7629	1.38	31	36	30	-12.3 ± 8.0	-9.9 ± 1.0
		(7000 kJ/d)	SP	-	-	-	-	-	7249	0.90	20	47	28	-10.9 ± 8.6	-7.3 ± 1.0

ER, energy restricted; HI, hyperinsulinemic; HP, high-protein; NR, not reported; OB, obese; OW, overweight; PHF, high-protein high-fat; SP, standard protein, WM, weight maintenance; Values are means ± SEM (unless otherwise stated). *weight regain during weight maintenance period; **diet recorded at end of 52 weeks, values are means ± SD. *Italicised data* indicate prescribed diet (where no actual diet data was available);^^ HP diet supplemented with 50 g whey protein, SP with 50g maltodextrin.

3.5 Metabolic effects of dietary protein

Dietary protein also has positive effects on physiological mechanisms that modulate body weight, glycaemic control and energy expenditure (particularly diet-induced thermogenesis) which may also affect satiety. The effects of high-protein diets on these outcomes, and the mechanisms underlying them, are discussed in the following sections.

3.5.1 Effects of protein on glycaemic control

Dietary protein exerts unique effects on blood glucose profile both acutely, and improves glycaemic control over the long term. Acutely, protein stimulates the release of both insulin and glucagon, without concomitant changes in peripheral blood glucose; however the mechanisms underlying this phenomenon are complex. Originally, it was hypothesised that the stimulation of insulin by protein would induce cellular uptake of glucose, thereby reducing circulating levels of glucose following a meal. However, protein also stimulates glucagon, which promotes an increase in glycogenolysis and gluconeogenesis to offset the effects of insulin. In addition, augmented secretion of GI peptides, in particular the incretin hormones GLP-1 and GIP, as well as the slowing of gastric emptying, play a role in the glycaemic response to protein (Lan-Pidhainy and Wolever 2010). Whey protein is also unique in that it has potent effects on inhibiting the peptidase dipeptidyl peptidase IV (DPP-IV). DPP-IV hydrolyses incretin hormones to their inactive forms, which results in elevated blood glucose (Tulipano *et al.* 2011a). Whey has been shown to stimulate GLP-1 release and to inhibit DPP-IV in both rodents and humans, resulting in an increased and prolonged insulin response (Drucker 2006b; Gunnarsson *et al.* 2006; Tulipano *et al.* 2011a). A detailed discussion of GLP-1 and GIP will be provided in Chapter 4.

In both healthy and diabetic subjects, protein has been shown to induce a potent insulin response, which may be dependent on the insulin sensitivity of the individual. For example, in healthy individuals, Brand-Miller *et al* reported that, following ingestion of a lean beef steak providing ~75g protein, insulinaemia was induced, while blood glucose remained relatively stable (Brand-Miller *et al.* 2000). Krezowski *et al* have shown that in healthy subjects the insulin response to 50 g of protein (beef) was modest compared with 50 g of glucose, while co-ingestion of protein and glucose had an additive effect on insulin release (Krezowski *et al.* 1986). In contrast, the same group has shown that in type 2 diabetics, a 50 g load of animal protein produced an equivalent insulin response, with no increase in blood glucose, compared with a 50 g load of glucose. When co-ingested, protein and glucose had a synergistic effect on insulin release, and actually reduced the blood glucose response (Nuttall *et al.* 1984). The synergistic effect of protein when co-ingested with 50 g of carbohydrate has also been replicated in type II diabetics in response to 25 g of cottage cheese, turkey, gelatine, fish and soy, but not egg white (Gannon *et al.* 1988). In contrast, others have reported no attenuation of the hypoglycaemic effects of protein in insulin resistant, non-diabetic subjects (Lan-Pidhainy and Wolever 2010), however these differences may be attributed to the sources of protein (i.e. soy vs. lean beef vs. whey protein), and the degree of insulin resistance in different study populations.

Over the longer term (i.e. up to 12 months), high-protein diets also appear to have beneficial effects on glycaemic control. A number of studies have reported favourable changes in markers of glycaemia, including blood glucose and glycated haemoglobin (HbA1c) levels (as a measure of long term blood glucose control), oral glucose tolerance and insulin sensitivity (Piatti *et al.* 1994; McAuley *et al.* 2005). For example, a study by McAuley *et al* (described in **Table 3.2**), reported that after 8 weeks on a high-protein or high-protein/high-

fat diet, insulin sensitivity was improved compared with a high-carbohydrate diet in overweight, insulin resistant women (McAuley *et al.* 2005). Piatti *et al* also reported that, in obese, glucose tolerant women, 3 weeks on a very low calorie (800kcal/d) high-protein diet, resulted in improved glucose disposal and oxidation (measured during a euglycaemic clamp) compared with a high-carbohydrate diet (Piatti *et al.* 1994). In elderly T2DM populations, the addition of essential amino acids to the diet has also been shown to improve metabolic control, including lowering fasting blood glucose and serum insulin levels, and lowering levels of HbA1c, presumably as a result of the insulinotropic effect of the amino acids (Solerte *et al.* 2004; Solerte *et al.* 2008). Collectively, this evidence suggests that increased protein in the diet, both acutely (i.e. in meals) and in the long term has beneficial effects on glycaemia, and can improve glycaemic control in both insulin resistant and type 2 diabetics.

3.5.2 Effects of protein on energy expenditure

Total daily energy expenditure (TDEE) comprises of three main processes: basal metabolic rate (i.e. the energy cost of maintaining bodily function at rest (BMR), active metabolic rate (i.e. the energy expenditure resulting from daily activity (AMR) and diet-induced thermogenesis (DIT). DIT is the increase in energy expenditure above baseline following consumption of food, and is associated with digestion, absorption, storage and disposal of the nutrients ingested (Halton and Hu 2004). Overall, DIT is responsible for ~10 % of total daily energy expenditure in humans; however this varies dependent on the macronutrient ingested (Westerterp 2004). For example, DIT from protein results in an energy cost of 25-30 %, compared with carbohydrate, which has been reported to be between 5 and 15 % of energy consumed (Westerterp *et al.* 1999). DIT from fat remains under contention, with some studies reporting a lower effect compared to carbohydrate (i.e. 2-3 % of energy consumed) (Swaminathan *et al.* 1985), while others report no difference (Nair *et al.* 1983).

A considerable amount of literature exists for the effects of protein, in both very short-term (i.e. 1-10 hours) and short-term (i.e. 12-36 hours) settings on DIT. For example, in subjects fed isocaloric meals high in fat, carbohydrate or protein, DIT has been shown to be higher after 120 min (Swaminathan *et al.* 1985) and up to 7 hours (Crovetti *et al.* 1998) following the high-protein meals compared with the high-carbohydrate and high-fat meals. The DIT that results from increased protein in the diet may increase TDEE, while the fall in TDEE that is normally observed with weight loss can be blunted by the DIT of protein (Baba *et al.* 1999; Luscombe-Marsh *et al.* 2005; Lejeune *et al.* 2006).

Importantly, the higher DIT of protein has also been associated with the satiating capacity of protein. In a 36-h study conducted in a respiratory chamber, women fed in energy balance had a higher DIT (14.6 ±2.9 %) with a high-protein diet (29 % protein, 61 % carbohydrate, 10 % fat), compared with DIT (10.5 ±3.8 %) with a high-fat diet (9 % protein, 30 % carbohydrate, 61 % fat). The high-protein diet was also associated with higher satiety both during meals, and across a 24 hour period (Westerterp-Plantenga *et al.* 1999). While it is important to recognise the effects of protein on inducing energy expenditure, and the relationship between DIT and satiety, the thermic effect of protein alone is not sufficient to promote significant weight loss and improvement in obesity outcomes, without additional dietary and lifestyle changes.

3.6 Risks and adverse effects of high-protein diets

Despite the considerable evidence for high-protein diets in promoting positive changes in body composition, appetite regulation and weight loss, a number of undesirable side effects and metabolic risks have also been reported. For example, a high protein intake, particularly from red meat, has been associated with high total and saturated fat intakes. In addition some

epidemiological studies have shown associations between high intakes of protein, namely animal proteins, with increased risks of osteoporosis and renal disease, while other studies report no association or a negative association; hence, there remains substantial controversy surrounding such associations.

Dietary protein and bone

While there is a consensus that moderate levels of protein intake (i.e. 1.0 – 1.5 g/kg/d) do not adversely affect calcium levels and skeletal metabolism (Spencer *et al.* 1983; Heaney 2000; Dawson-Hughes and Harris 2002; Kerstetter *et al.* 2003), some data have suggested that high-protein diets, especially with a large proportion of animal proteins, may induce a low-grade metabolic-acidosis, resulting in the degradation of bone to neutralise this effect (Sebastian *et al.* 1994; Frassetto *et al.* 1998), which in turn may increase renal calcium excretion (Hegsted and Linkswiler 1981; Kerstetter *et al.* 1997). However, using stable dual-isotopes for calcium, a number of studies have identified that the observed rise in urinary calcium is concomitant with increased intestinal calcium absorption (Cao *et al.* 2011). That is, the increased appearance of calcium in the urine does not necessarily indicate increased resorption from bone, but likely reflects increased turnover from several sources.

In their review, Eisenstein *et al* reported that dietary protein appears to have a calciuretic effect at loads of 2 g/kg/d, when compared with control diets of 0.7-1.0 g/kg/d (Eisenstein *et al.* 2002). A recent meta-analysis has confirmed that there are beneficial effects, albeit small, of both animal and vegetable proteins on bone health (Darling *et al.* 2009). Moreover, there is considerable epidemiological evidence that suggests that higher total, and animal, protein intakes are associated with increased bone mineral density, and lowered risk of osteoporosis and fractures in both normal subjects, and those supplemented with calcium and vitamin D

(Hannan *et al.* 2000; Dawson-Hughes and Harris 2002). Protein intakes of up to 1.5 g/kg/d appear to maintain calcium homeostasis (Spencer *et al.* 1983; Heaney 2000; Dawson-Hughes and Harris 2002; Kerstetter *et al.* 2003), while protein intakes of up to 2.0 g/kg/d do not appear to have deleterious effects on bone. Conversely, low to moderate protein diets appear to have adverse effects on calcium metabolism and skeletal health, and studies have now identified impaired intestinal calcium absorption as a factor in inducing secondary hyperparathyroidism and hypocalciuria when protein intake is restricted to <0.8 g/kg/d (Kerstetter *et al.* 1998; Kerstetter *et al.* 2000). Collectively, these data suggest that moderate- to high-protein diets do not have deleterious effects on bone, and may in fact enhance bone health, while low-protein intakes may result in impaired calcium metabolism.

Dietary protein and renal function

Consumption of amounts of protein, particularly animal protein at up to 2 g/kg/d, have been associated with an increased renal acid load, which may lead to the development of kidney stones (Reddy *et al.* 2002), compensatory increases in renal acid and ammonia excretion, metabolic acidosis and an increased risk of tubule injury (van den Berg *et al.* 2011). In a prospective study of ~45,000 men, aged between 40 and 75 years who were followed for 4 years, animal protein intake was associated with risks of symptomatic kidney stone formation (Curhan *et al.* 1993). In contrast, a review by Eisenstein and Roberts established that in normal, healthy individuals without pre-existing renal disease, there is minimal evidence for adverse effects of high-protein diets on renal function (Eisenstein *et al.* 2002). While some short-term studies have reported that high-protein intakes result in hyperfiltration (Hegsted and Linkswiler 1981; Brandle *et al.* 1996), this can be explained by structural changes in the healthy kidney, including glomerular and tubular changes (Brandle *et al.* 1996), and increases in renal mass, that result in increased glomerular filtration rate (Skov *et al.* 1999a). In

healthy, obese individuals without history of renal disease, no deleterious effects have been observed on renal function following high-protein weight loss diets of up to 2 years (Brinkworth *et al.* 2010; Friedman *et al.* 2012). In addition, in the 11 year follow-up period of the Nurse's Health Study, during which protein intake was accounted for over 4 years, average protein intakes of up to 1.1 g/kg/d were not associated with reduced renal function in healthy women without pre-existing renal disease (Knight *et al.* 2003). In women with mild renal insufficiency, an average protein intake of up to 1.0 g/kg/d was significantly associated with increases in estimated glomerular filtration rate and an accelerated decline in renal function (Knight *et al.* 2003). In people with pre-existing renal disease, or at risk of renal disease (i.e. type II diabetics), limiting dietary protein to 0.6-0.8 g/kg/d is thought to slow progression of the disease, particularly in end-stage chronic kidney disease (Chauveau and Aparicio 2011), and may lower mortality rate (Pedrini *et al.* 1996). In conclusion, the data suggests that in healthy individuals, protein intakes of up to 2.2 g/kg/d have minimal negative effects on renal function for periods of up to 2 years. In individuals with renal insufficiency or disease, high-protein diets should be undertaken with extreme caution, and under close medical supervision.

3.7 Concluding remarks

Protein is a critical component of the diet of humans, and has a number of physiological roles. Considerable evidence suggests that protein is important in regulating total energy intake, glycaemic control and body weight. It is well established that protein has potent effects on appetite, where, kcal for kcal, protein is more satiating than fat or carbohydrate. This satiating effect of protein has generated considerable interest in the value of protein in weight-loss diets, to inhibit hunger and improve weight loss and diet compliance. Also of significance to human health, the evidence largely indicates that high-protein diets may also

improve a number of health-related outcomes, retention of fat free mass and changes in body composition. Despite the considerable evidence for superior effects of protein on appetite sensation and energy intake, the mechanisms underlying these effects are not well described. While it has been demonstrated that DIT from protein contributes to some extent, the pre-and post-absorptive satiety signals generated, in particular, by the GI tract, have not been well explored. Chapter 4 will examine the current evidence for the role of the GI tract in determining appetitive responses, and will highlight the current paucity of information surrounding the GI responses to protein, and how these may relate to the satiating capacity of protein.

Chapter 4: The Role of The Gastrointestinal Tract In The Regulation of Glycaemia, Appetite And Energy Intake

4.1 Introduction

Human eating patterns are influenced by a number of factors. Multiple physiological mechanisms interact to maintain a balance between energy intake and energy expenditure. GI factors are being increasingly recognised as a key part of this complex interplay. Nutrients interact with receptors along the length of the GI tract, as well as in the liver and other organs to produce neural and hormonal feedback signals, that are relayed to the brain. These signals determine a number of outcomes, including how the ingested nutrients are stored and utilised, the postprandial glycaemic response, initiation and termination of meals, and the amount of food consumed (Woods *et al.* 1998).

A number of inter-related GI motor and hormonal functions are critical to the regulation of food intake. Specifically, GI motor factors include antropyloroduodenal (APD) motility (Azpiroz and Malagelada 1987b; Heddle *et al.* 1988a), which regulates gastric emptying (Sepple and Read 1989) and intragastric meal distribution, through changes in proximal and distal gastric motility. GI hormones include the orexigenic hormone, ghrelin (released from the stomach) (Overduin *et al.* 2005), and the anorexigenic hormones, including, but not limited to, CCK (Lilja *et al.* 1984; Cook *et al.* 1997), GLP-1 (Herrmann *et al.* 1995; Little *et al.* 2005; Little *et al.* 2006a), PYY (MacIntosh *et al.* 1999; Onaga *et al.* 2002) and GIP (Thor *et al.* 1987), released from various regions of the small intestine. The postprandial glycaemic response is also modulated by changes in gastric emptying and GI hormone release, along with the release of insulin and glucagon. This chapter will first describe the anatomy of the upper GI tract. It will then focus on the role of inter-related GI motor and hormonal factors

that are critical in the regulation of glycaemia and energy intake, in response to ingestion of fat, carbohydrate and protein, both in isolation, and in mixed form. This chapter will also present the limited evidence regarding how upper GI function may be altered in obesity.

4.2 Anatomy and function of the upper gastrointestinal tract

4.2.1 Oral cavity

The oral cavity is the site of initial nutrient detection, whereby ingested material, including food and potential toxins, interact with receptors and is immediately "tasted". From an evolutionary point of view, tasting of ingested matter is vital for health and survival. For example, sweet and savoury tastes promote the intake of energy-rich foods, which provide a nutritional benefit. In contrast, bitterness is closely associated with the presence of toxins, which should be avoided (Lan-Pidhainy and Wolever 2010).

The perception of taste begins on the tongue, where epithelial-derived taste receptor cells detect chemical cues from ingested matter (Lindemann 1996; Gardner *et al.* 2007). Taste buds are onion-shaped clusters of ~50 to 150 taste receptor cells that are distributed across the surface of the tongue and the soft palate. Each taste bud features a taste pore -projections at the apical surface of the taste bud- through which the tastant, makes contact with, and stimulates, taste cell receptors (**Figure 4.1**). These signals are transmitted from the basal nerve endings via the facial nerve to the nucleus tractus solitarius in the brainstem where taste is perceived. These signals also participate in the cephalic phase of digestion, by initiating anticipatory release of GI enzymes and hormones, and stimulating gall bladder contraction, relaxation of the Sphincter of Oddi and receptive gastric relaxation (Cuomo and Sarnelli 2004).

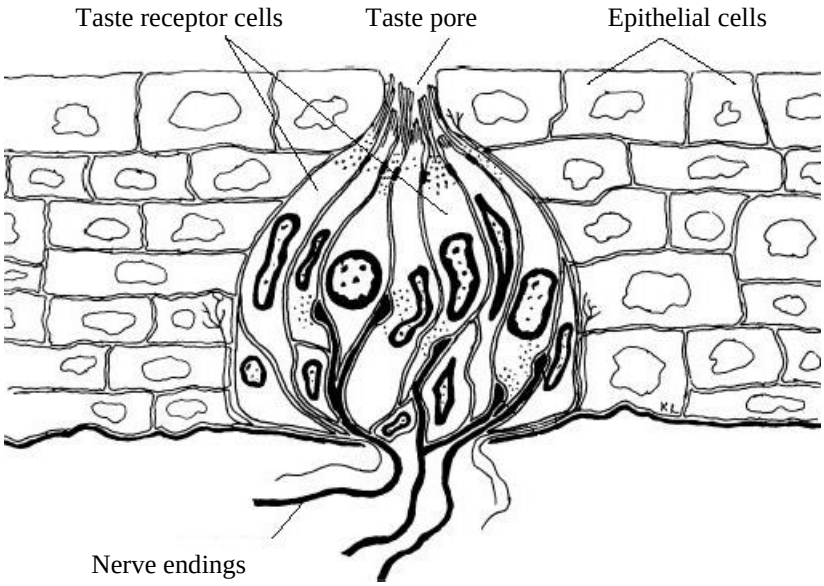

Figure 4.1: Basic anatomy of a taste bud

4.2.2 Stomach

Once food is masticated in the oral cavity and swallowed, the ingested nutrients pass through the oesophagus, a muscular tube lined with stratified squamous epithelium, into the stomach, by peristalsis. The stomach is a J-shaped, sac-like chamber connecting the oesophagus and duodenum, the first section of the small intestine (**Figure 4.2**). The stomach is divided anatomically into three distinct regions, the fundus, the corpus, and the antrum.

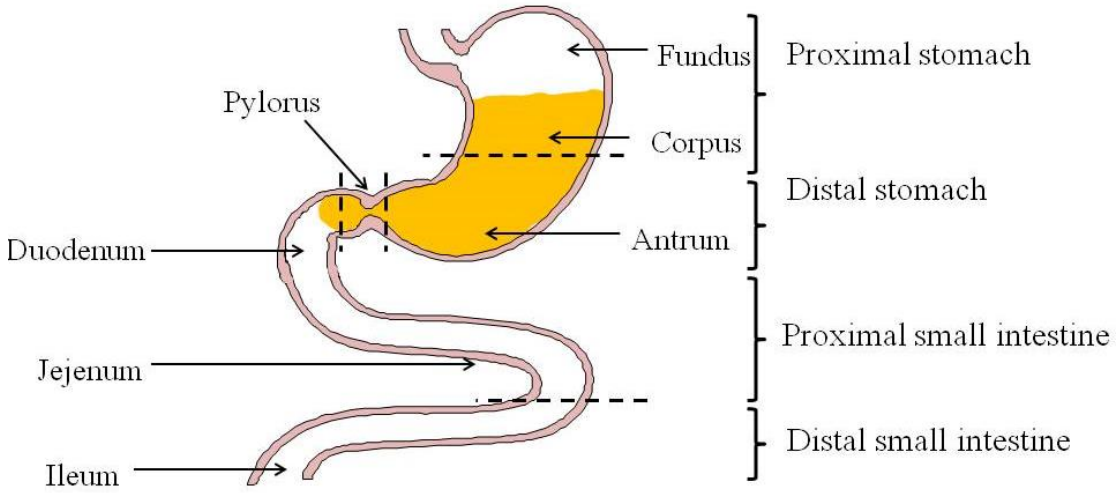

Figure 4.2: Basic anatomy of the stomach and small intestine in the human

The stomach is comprised of two muscular layers, the inner, circular muscle layer, and the outer, longitudinal muscle layer. A third oblique muscle layer covers the fundus. Functionally, the stomach can be divided into two regions, i) the proximal region (comprised of the fundus and corpus) and ii) the distal compartment, which includes the antrum (**Figure 4.2**). The thin, smooth, oblique muscle layer of the proximal stomach allows this area to act as a reservoir for ingested food. The proximal region also generates low frequency, sustained contractions to maintain a basal pressure within the stomach, and to propel food towards the distal stomach and the small intestine, thus contributing to gastric emptying. The distal area of the stomach includes the antrum and the pylorus, and is characterised by a thickening of the circular muscle towards the pylorus, which aids in the mixing and grinding of solid food into smaller particles. The distal region is also characterised by peristaltic patterns of muscular contraction that increase in amplitude as they migrate towards the pylorus, propelling chyme into the duodenum (Burks *et al.* 1985; Cullen and Kelly 1993).

4.2.3 Pylorus

The pylorus is a short, 2-cm region, which connects the antrum of the stomach to the duodenum. The pylorus is a muscular sphincter, that is integral in regulating the flow of gastric digesta from the stomach into the small intestine; transpyloric flow can only occur when the pylorus is open (Horowitz *et al.* 1994). Two types of motility patterns are characteristic of the pylorus. Tonic contractions, known as basal pyloric pressure (BPP), maintain the closure of the pylorus against flow of digesta from the antrum. In contrast, isolated pyloric pressure waves, which intermittently close the gastroduodenal junction, regulate the pulsatile flow of gastric contents from the stomach into the small intestine in response to intestinal feedback (Keinke *et al.* 1984; Horowitz *et al.* 1994).

4.2.4 Small intestine

The small intestine is a muscular tube of approximately 5 m in length, and is divided into three distinct regions; the most proximal region, the duodenum (~25 cm long), the middle region, the jejunum (~2m long), and the most distal region, the ileum (~3m long) (**Figure 4.2**). The small intestine is the primary site for absorption of nutrients and the origin of a number of feedback signals that interact to regulate the transit of food through the gut. These signals modulate gastric emptying by relaxing the proximal stomach, inhibiting antral contractions and stimulating tonic and phasic pyloric contractions.

4.3 Motor function of the upper gastrointestinal tract

Upper GI motility alternates between two distinct patterns: i) the interdigestive migrating motor complex (MMC), which occurs in the fasted state; and ii) the fed motility pattern which characterises the postprandial state and is initiated following food ingestion. The following sections describe patterns of gastric emptying, as well as motility patterns in the stomach, pylorus and small intestine during both the fasted and postprandial states.

4.3.1 Fasting motor patterns

During fasting, the MMC represents the cyclical pattern of motility exhibited by the GI tract. The MMC can be divided into specific phases of activity; i) phase I is a period of quiescence, characterised by few, or no, contraction and lasts for approximately 40-60 min. Phase I is followed by ii) phase II, during which intermittent contractions increase progressively in frequency, with the latent periods between contractions gradually shortening over the 20-40 min of phase II activity; until occurrence of iii) phase III, characterised by a period of contractions occurring at maximum frequency and amplitude, i.e. 3 contractions/min in the stomach and ~12 contractions/min in the duodenum that migrate through the GI tract to the

terminal ileum (Code and Marlett 1975). The phase III period lasts between 5 and 10 min, and is followed by a short transition period (phase IV) (Takahashi 2012), before the activity returns to phase I (Dooley *et al.* 1992). The MMC propagates aborally through the GI tract, ensuring that any food remaining in the stomach or small intestine is propelled to more distal regions, or expelled from the body (Dooley *et al.* 1992).

4.3.2 Postprandial motor patterns

The arrival of nutrients in the stomach and small intestine triggers a number of interrelated changes in GI motor function, which convert fasting motility to the 'fed' or postprandial motor pattern. Following a meal, two motor responses occur in the proximal stomach. First, receptive relaxation occurs, whereby, after swallowing, intragastric pressure is transiently decreased. Secondly, the proximal stomach relaxes to accommodate and store the ingested food, without an increase in intragastric pressure, termed adaptive relaxation (Azpiroz and Malagelada 1987a). Gastric distension interrupts MMC activity in both the stomach and proximal small intestine, while fluid and/or nutrient in the small intestine interrupt MMC activity across the entire small intestine (Code and Marlett 1975). In the antrum, ingested food is mixed with gastric secretions and broken down into chyme by chemical and mechanical digestion in the distal stomach. These antral contractions are propulsive, moving the chyme from the stomach into the duodenum between phasic and tonic pyloric contractions. Gastric emptying occurs only when the pylorus is open; in the fed state the pylorus remains closed for prolonged periods to promote gastric mixing and trituration (Horowitz *et al.* 1994). The chyme is ultimately delivered into the small intestine at an overall rate of 1-4 kcal/min in healthy humans, (Hunt *et al.* 1985), which is optimal for digestion and absorption (Horowitz *et al.* 1994).

4.3.3 Gastric emptying

The rate, and pattern, of gastric emptying of ingested nutrients is modulated by a number of factors, including the characteristics of the ingested meal, and the activity of other regions of the GI tract. Gastric emptying is predominantly pulsatile, rather than continuous, and individual flow pulses are varied, so that the resultant flow can be retrograde or antegrade. Patterns of transpyloric flow are regulated by coordinated motor activity in the proximal and distal stomach, pylorus, and small intestine. The coordinated activity of the stomach and pylorus act as a brake to slow gastric emptying, ensuring food is delivered at a rate that allows optimal digestion and absorption of the ingested food.

The physical state and macronutrient composition of the meal affect rates of gastric emptying. Nutritive liquids and liquefied solids empty from the stomach more slowly, and in a linear fashion, while non-nutritive liquids empty rapidly in a non-linear, mono-exponential fashion (Horowitz *et al.* 1994). The emptying of digestible solids is characterised by a lag phase of between 10 and 30 min before the first substantial emptying occurs. During this lag phase, mechanical trituration reduces particles to < 2 mm diameter (Meyer 1980), and the food is gradually redistributed from the proximal to distal stomach (Horowitz *et al.* 1994). The duration of the lag is influenced by the rate of redistribution of the solid matter, which results in an emptying pattern of the solid portion of a meal that resembles that of a high-calorie meal, i.e. in a linear manner (Park and Camilleri 2005).

4.4 Effects of macronutrients on gastrointestinal motor function

Administration of "pure" carbohydrate or fat directly into the small intestine affects gastric emptying, intragastric meal distribution and APD motility (Chapman *et al.* 1999; Feinle *et al.*

2000; Pilichiewicz *et al.* 2007a; Pilichiewicz *et al.* 2007b), while the effects of protein on GI motility have, until recently, been unclear.

4.4.1 Effects of macronutrients on gastric emptying

It has been established that fat and carbohydrate, when consumed orally, empty from the stomach at a rate of 1-4 kcal/ minute (Brener *et al.* 1983; Horowitz *et al.* 1993). While the rate of emptying of protein has been examined, information remains limited. While some studies report that the gastric emptying rate of protein is similar to that of fat and carbohydrate (Goetze *et al.* 2007), others have suggested that gastric emptying of protein is slowed in a load-dependent manner, so that increased loads of protein are emptied at a similar overall rate (Calbet and MacLean 1997; Khoshoo and Brown 2002; Maughan *et al.* 2004). Further research is necessary to elucidate the overall gastric emptying rate of protein, and to establish the relationship between gastric emptying of protein, GI hormone release, glycaemia and energy intake.

4.4.2 Effects of macronutrients on antropyloroduodenal motility

The infusion of intraduodenal lipid has been shown to stimulate isolated pyloric pressure waves (IPPWs), and suppress antral and duodenal activity (Pilichiewicz *et al.* 2006; Pilichiewicz *et al.* 2007b), in a load-dependent manner. Similarly, intraduodenal glucose stimulates IPPWs and suppresses antral and duodenal motility (Heddle *et al.* 1988d) with the degree of stimulation or suppression dependent on load (Pilichiewicz *et al.* 2007a). Intraduodenal lipid appears to be more potent than glucose, since administration of fat has been shown to stimulate pyloric contractile activity (Cook *et al.* 1997) and slow gastric emptying (Heddle *et al.* 1989) more strongly than an isocaloric amount of glucose (Cook *et al.* 1997; Andrews *et al.* 1998). Direct comparison of equicaloric loads of intraduodenal

lipid, or glucose (3 kcal/min), and combinations of the two (2 kcal/min lipid + 1 kcal/min maltodextrin or 1kcal/min lipid + 2 kcal/min maltodextrin) has confirmed this (Seimon *et al.* 2009a). Pure lipid more potently modified antropyloroduodenal motility and GI hormone release, while reducing the proportion of lipid in the infusion resulted in comparatively diminished gastrointestinal motor and hormonal responses.

In contrast, the effects of protein on GI motility are poorly defined, with a few studies hitherto examining only the effects of single amino acids and amino acid mixtures on APD motility. Edelbroek *et al* observed that in healthy men, intraduodenal L-tryptophan, compared to D-tryptophan, had a greater effect on the stimulation of basal pyloric and duodenal motility (Edelbroek *et al.* 1994). In addition, Gielkens *et al* administered intravenous amino acid mixtures, and observed a greater suppression of antral motility and slowed duodenocaecal transit in healthy men, compared with an IV saline infusion (Gielkens *et al.* 1999). More recently, Steinert *et al* have reported differential effects of small doses of L-glutamine, L-phenylalanine and leucine on GI motility. In the first study, they infused L-glutamine and L-phenylalanine separately for 90 minutes at rates of 0.15 and 0.45 kcal/min. Interestingly, only L-phenylalanine affected GI motility (as antral and pyloric pressures), and only at the highest dose, however both doses stimulated CCK release. In contrast, L-glutamine had no effects on GI motility or hormone release (Steinert *et al.* 2015a). In the second study, the reported that leucine, an abundant branched chain amino acid, infused at 0.45 kcal/min suppresses antral motility, and increased plasma CCK (Steinert *et al.* 2015b). In contrast, 0.15 kcal/min only slightly increased plasma CCK concentrations. Interestingly, the 0.45kcal/min load reduced energy intake at a buffet meal by ~ 13%. Taken together, these data suggest that individual amino acids (as part of whole proteins typically consumed

in the diet) may exert different and specific effects on aspects of GI function. However, it remains unclear how whole proteins may modulate GI motility.

4.4.3 The role of gastrointestinal motor function in determining the glycaemic response and energy intake

The rate of arrival of ingested nutrient to the small intestine, through co-ordinated APD motility to regulate gastric emptying, plays a substantial role in mediating the postprandial glycaemic response. The rate of gastric emptying of carbohydrate is a well-established determinant of postprandial glycaemia (Horowitz *et al.* 1993; Jones *et al.* 1996; Rayner *et al.* 2001; O'Donovan *et al.* 2004), in both healthy individuals (Horowitz *et al.* 1993) and patients with type 2 diabetes (Jones *et al.* 1996). In fact, gastric emptying accounts for approximately 35 % of the variation in peak blood glucose concentrations after a standardised 75 g glucose load. The rate of gastric emptying also determines the magnitude of the response of a number of GI hormones released from the small intestine, which in turn provide feedback to further modulate gastric emptying, and the release of other GI hormones.

GI motility also plays a key role in determining appetite responses and energy intake. For example, the slowing of gastric emptying prolongs gastric distension, and is associated with increased feelings of "fullness" and reduced energy intake. A close, indirect relationship between antral distension and feelings of fullness has been described (Jones *et al.* 1997b), while food intake at a buffet meal has been shown to be inversely related to antral filling from the previous meal (Sturm *et al.* 2004). In humans, distension of the proximal and distal stomach results in slowed gastric emptying, and has been shown to increase perceptions of fullness (Feinle *et al.* 1997; Jones *et al.* 1997a) and suppress energy intake (Kissileff *et al.* 2003; Sturm *et al.* 2004). Furthermore, a recent pooled data analysis of data generated within

our department has identified that, following intraduodenal infusions of lipid and carbohydrate; IPPWs are an independent predictor of subsequent energy intake in healthy, lean males. Thus, GI motility plays a key role in determining glycaemic responses to nutrient, as well as appetite and energy responses, which are also driven by the release of a number of GI hormones, as detailed in the following sections.

4.5 Gastrointestinal hormone release

The release of a number of GI peptides is modulated by the presence of nutrients in the GI tract. The anorexigenic hormone, ghrelin, which is released primarily from the stomach (Kojima *et al.* 1999) to initiate eating, is suppressed by feeding (Cummings *et al.* 2001; Wren *et al.* 2001a). In contrast, the release of orexigenic hormones, including the release of CCK (Lilja *et al.* 1984; Matzinger *et al.* 2000) from the proximal, and the "incretin" hormones GLP-1 (Flint *et al.* 1998; Gutzwiller *et al.* 1999), and GIP, as well as PYY and glucagon from the distal, small intestine, is stimulated by nutrients. Collectively, these hormones have relatively well-defined, and interrelated, effects on GI motility, glycaemia and appetite, including initiation of a meal and meal size; these effects are also dependent on the macronutrient composition of a single meal. The individual GI hormones, their effects on GI motility, the release of other hormones, glycaemia, and appetite and energy intake are described in detail in the subsequent sections, and in **Table 4.1**.

Table 4.1: GI hormones and their described roles within the body

Hormone	Released from	In response to	Stomach	Small intestine	Liver	Pancreas	Adipose stores	Brain	Additional effects	Suppressed by
Ghrelin	stomach	↓ blood glucose	↓ antral phase III activity ↑ GE		↑ plasma glucose	↓ serum insulin		↑ appetite ↑ hunger	↑ meal ingestion	Meal ingestion, CCK, PYY, insulin
CCK	I cells Duodenum Jejunum	Digestive products of fat, protein, CHO*	↓ antral motility, GE ↑ pyloric motility; ↓ ghrelin	↑ PYY ↓ duodenal motility ↓ jejunal motility		↑ enzyme release		↓ appetite	↑ gall bladder contraction; ↓ energy intake	
PYY	L cells Ileum, large intestine	Fat, protein*, CCK	↓ gastric emptying	Mediates ileal brake					May ↓ energy intake	DPP-IV
GLP-1	L cells Distal small intestine	Fat Protein CHO*	↓ antral motility ↓ GE ↑ pyloric motility	↓ duodenal motility		↓glucagon release ↑ insulin release		↓ appetite		DPP-IV
GIP	K cells Duodenum	CHO, protein, fat*	↓ gastric secretions			↑ insulin synthesis and release, ↑ glucagon release	↑ fatty acid synthesis ↑ fatty acid to triglyceride conversion			DPP-IV
Glucagon	Pancreatic α cells	Fasting Exercise ↓ blood glucose Protein	↓ GE ↓ antral contractility	↓ duodenal activity ↓ frequency, ↑ length MMC	↑ glycogenolysis ↑ gluconeogenesis ↓ glycolysis	May stimulate insulin release	↑ lipolysis		May ↓ energy intake	GLP-1 GIP insulin
Insulin	Pancreatic β cells	↑ blood glucose ↑ plasma amino acids GLP-1, GIP	↓ GE (modest effect) ↓ antral pressure waves ↓ antral PIII	↓ duodenal PIII	↑ glycogen production		↑ nutrient storage	Crosses blood brain barrier, ↓energy intake	↑s cell proliferation ↑ uptake of glucose, amino acids, fatty acids	↓ blood glucose

*macronutrients listed in respective order of potency of effect; CCK, cholecystokinin; CHO, carbohydrate; DPP-IV, dipeptidyl peptidase IV; GE, gastric emptying; GIP glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; PIII, phase 3 activity; PYY, peptide-YY.

4.5.1 Ghrelin

Ghrelin is a 28-amino acid peptide, secreted from the fundic region of the stomach by oxyntic cells (Kojima *et al.* 1999). The major active human isoform of ghrelin, acyl ghrelin, is characterised by an n-octanoyl side-chain at the serine 3 position. This side chain is integral for the biological action of ghrelin as an endogenous ligand for the growth-hormone secretagogue receptor type 1a (GHS-R1a) (Kojima *et al.* 1999; Date *et al.* 2000). Other ghrelin isoforms include octanoyl ghrelin-(1-27), decanoyl ghrelin, decanoyl ghrelin-(1-27), as well as the non-active moieties, des-acyl ghrelin and des-acyl ghrelin-(1-27) (Hosoda *et al.* 2003). Ghrelin-secreting P/D_1 cells are the second-most prolific cells in the gastric mucosa, suggesting an integral role in gastric physiology (Dixit *et al.* 2004; Locatelli *et al.* 2005). Ghrelin has also been shown to be secreted in smaller amounts from the islets of Langerhans in the pancreas, and from both alpha- and beta-cells (Wierup *et al.* 2002). Ghrelin has no relevant homology to any other circulating GI peptides (Locatelli *et al.* 2005), and, in contrast to other gut peptides, ghrelin concentrations are high during fasting, and suppressed rather than stimulated, by food (Cummings *et al.* 2001). Studies using pyloric occlusion and small intestinal infusion models in animals, and intraduodenal infusion in humans, have established that the suppression of ghrelin is dependent on feedback arising from the interaction of nutrients with the small intestine, rather than the stomach (Williams *et al.* 2003; Overduin *et al.* 2005; Parker *et al.* 2005). Critically, the magnitude of the post-meal inhibition of ghrelin release has been shown to be directly proportional to the energy content of the meal, so that a more calorific meal suppresses ghrelin release for longer (Callahan *et al.* 2004).

4.5.1.1 Effects of ghrelin on gastrointestinal motility and hormone release

Exogenous ghrelin has been shown to have a prokinetic effect on gastric emptying in rodents (Dornonville de la Cour *et al.* 2004), and in both healthy humans (Levin *et al.* 2006) and

patients with diabetic gastroparesis (Murray *et al.* 2005). Specifically, intravenous administration of ghrelin induces phase III activity in the stomach (Tack *et al.* 2006), increases proximal stomach tone (Tack *et al.* 2006), accelerates gastric emptying, and stimulates hunger (Levin *et al.* 2006). In rodents, intraperitoneal administration of the ghrelin receptor (GHS-R1a) antagonist, "14f", dose-dependently slowed gastric emptying, suggesting that endogenous ghrelin plays a role in regulating gastric emptying, at least in the rat (Puleo *et al.* 2012). Since ghrelin is suppressed, rather than stimulated following meal ingestion (Monteleone *et al.* 2003), and comparative ghrelin receptor antagonist studies in humans have not been conducted, the relevance of the effect of ghrelin on the rate of gastric emptying and GI motility remains unclear.

4.5.1.2 Effects of ghrelin on postprandial glycaemia

Data on the effects of ghrelin on insulin and glycaemic control remain inconsistent. Negative correlations have been reported between ghrelin and body fatness, and ghrelin and insulin concentrations (Tschop *et al.* 2001; Poykko *et al.* 2003), with a positive correlation between ghrelin and insulin sensitivity (Poykko *et al.* 2003). Given that T2DM is characterised by insulin resistance, these data suggest that lower ghrelin levels may play a role in the aetiology of diabetes (Poykko *et al.* 2003). Under experimental conditions, "14f" has been shown to improve intraperitoneal glucose tolerance in rats, to a similar magnitude as a common insulin secretagogue (Puleo *et al.* 2012). In humans, intravenous infusion of ghrelin at rates up to 1.5 nmol/kg/hour has been shown to impair glucose clearance rates and insulin release acutely (Broglio *et al.* 2001; Tong *et al.* 2010). Overall, these data suggest that ghrelin may be involved in glycaemic control through negative-feedback regulation of insulin secretion and glucose disposal, however, further research is required, particularly since the effects of endogenous ghrelin on the blood glucose and insulin response are not established in humans.

4.5.1.3 Effects of ghrelin on appetite and energy intake

Exogenous ghrelin has been shown to increase energy intake in both rodents and humans (Wren *et al.* 2001a; Wren *et al.* 2001b). For example, in rats, 7 days of chronic ghrelin administration resulted in increased weight and adiposity (Wren *et al.* 2001b), while in humans, a 260-min infusion of ghrelin at 5 pmol/kg/min decreased fullness, increased hunger and increased energy intake at a subsequent meal by ~ 28 %, compared with a saline control (Wren *et al.* 2001a). Ghrelin exerts its action by activating the hypothalamic neuropeptide Y-Y1 (NPY-Y1) and agouti-related protein (AGRP) pathways which provide central stimuli for increasing energy intake and reducing energy expenditure (Dixit *et al.* 2004).

In healthy humans, it has been demonstrated that pre-prandial increases in ghrelin are related to hunger scores when meals are initiated voluntarily, suggesting a role for ghrelin in meal initiation in the absence of time- and food-specific cues (Cummings *et al.* 2004). Moreover, ghrelin release is typically upregulated during chronic energy restriction (i.e. anorexia nervosa, weight loss) and down-regulated in positive energy balance (i.e. obesity) (Ariyasu *et al.* 2001). In obese humans, fasting ghrelin concentrations have been shown to be reduced, and the postprandial decline of plasma ghrelin concentrations diminished, or absent (Tschop *et al.* 2001). In diet-induced obese mice, daily administration of a GHS-R1a antagonist reduced food intake and resulted in ~15 % weight loss compared with pair-fed controls, while in healthy normal weight and *ob/ob* mice GHS-R antagonists decreased energy intake, reduced gastric emptying and decreased body weight gain in *ob/ob* mice (Asakawa *et al.* 2003). Collectively, these data suggest that, in both rodents and humans, ghrelin plays a key role in driving eating behaviour, and weight regain. The GHS- receptor is also a primary target of growth hormone in humans. While the role of GHS-R agonists in relation to aging have been explored (Smith *et al.* 2007), the integral relationship between the GHS-R receptor

and growth hormone function means evidence for the effects of GHS-R receptor agonists on feeding behaviour is limited. Inverse agonists of the ghrelin receptor are also currently being explored as potential interventions in obesity, however evidence for these is limited (Holst *et al.* 2006; Bhattacharya *et al.* 2014). Since human responses to ghrelin receptor antagonism may differ compared with rodent models, further research is required to establish the exact physiological role of ghrelin in feeding behaviour in humans.

4.5.2 Cholecystokinin (CCK)

Cholecystokinin is synthesised in the "I" cells of the small intestinal mucosa, primarily in the duodenum and to some extent, the jejunum (**Table 4.1**). Of the multiple forms of CCK, CCK-8, CCK-22, CCK-58 and CCK-33/39 are the primary biologically active forms of CCK found in the human brain, intestine and circulation (Eysselein *et al.* 1990; Wang and Cui 2007). CCK peaks after 15–30 min of meal ingestion in response to the presence of digestive products of fat and protein (Larsson and Rehfeld 1978; Liddle *et al.* 1985; Lieverse *et al.* 1994a; Foltz *et al.* 2008), and to a lesser extent carbohydrate (Parker *et al.* 2005), in the small intestinal lumen. CCK releasing cells are also present in enteric vagal afferent neurones, and sections of the central nervous system, including the thalamus, hypothalamus, basal ganglia and dorsal hindbrain, where CCK acts as a neurotransmitter (Crawley and Beinfeld 1983). Studies administering exogenous CCK have shown effects on a number of functions within the GI tract, including the regulation of gastric emptying (Liddle *et al.* 1986) and GI motility (Meyer *et al.* 1989; Brennan *et al.* 2008), stimulation of gall bladder contraction (Liddle *et al.* 1985; Beglinger *et al.* 1992), stimulation of pancreatic enzyme release (Jansen and Lamers 1983; Beglinger *et al.* 1992), and suppression of energy intake (Kissileff *et al.* 1981; Kissileff *et al.* 2003).

4.5.2.1 Effects of CCK on gastrointestinal motility and hormone release

In both animals (Moran and McHugh 1982; Moran and McHugh 1988) and humans (Liddle *et al.* 1986; Kleibeuker *et al.* 1988), exogenous administration of CCK slows gastric emptying (Liddle *et al.* 1986; McHugh and Moran 1986), suppresses antral and duodenal motility (Rayner *et al.* 2000; Brennan *et al.* 2005) stimulates pyloric pressures (Rayner *et al.* 2000; Brennan *et al.* 2005) and jejunal motility, slows gastric emptying (Fried *et al.* 1991), and suppresses colonic motility (Kellow *et al.* 1987).

Studies using loxiglumide, a CCK_1 receptor antagonist, have established a key role for CCK in slowing gastric emptying and GI transit (Fried *et al.* 1991; Katschinski *et al.* 1996). For example, administration of loxiglumide results in an acceleration of gastric emptying and stimulation of antral motility following a 500-mL liquid fat meal (Intralipid) (Schwizer *et al.* 1997), and the inhibitory effects of duodenal fat on gastric motility (Feinle *et al.* 1996) have been shown to be abolished by the administration of loxiglumide. Moreover, administration of loxiglumide during a 150-min duodenal infusion of a mixed-nutrient liquid meal decreased total numbers of antral, pyloric and duodenal contractions by 44 %, 74 % and 41 % respectively (Katschinski *et al.* 1996).

CCK also appears to play a role in modulating the release of some GI hormones. For example, intraduodenal infusion of a long-chain fatty acid suppressed ghrelin release and stimulated PYY and CCK release. When loxiglumide was administered concurrently to the ID infusion, the suppression of ghrelin secretion was reversed and the PYY response abolished (Degen *et al.* 2007). In addition, IV administration of CCK-8 stimulated PYY release, and suppressed ghrelin release, when compared with a saline control (Brennan *et al.* 2007; Degen *et al.* 2007). Finally, both IV infusion of CCK-8, and consumption of a mixed

meal, stimulated pancreatic polypeptide release; when loxiglumide was administered, both the meal- and CCK-8 induced effects on pancreatic polypeptide were abolished. Taken together, these data suggest that all of these effects are mediated by CCK via the CCK_1 receptor.

4.5.2.2 Effects of CCK on postprandial glycaemia

CCK does not appear to have direct effects on the modulation of postprandial glycaemia. In humans, intravenous administration of CCK at levels normally circulating postprandially has no effect on glucose-stimulated insulin secretion (Fieseler *et al.* 1995a). In studies where exogenous CCK was administered following an orally-administered mixed meal or glucose load (Liddle *et al.* 1988; Schick *et al.* 1991; MacIntosh *et al.* 2001), CCK blunted the insulin response, and lowered blood glucose, however this was probably the result of slowing of gastric emptying. In this respect, CCK appears to indirectly regulate blood glucose concentrations, by modulating gastric emptying into the small intestine, which, in the case of carbohydrate is associated with reduced glucose delivery.

4.5.2.3 Effects of CCK on appetite and energy intake

It is well established that exogenous administration of CCK suppresses appetite and energy intake. Early investigations in rodents using intraperitoneal administration of the biologically active, sulphated octapeptide of CCK (CCK-8) reported a dose-dependent suppression of energy intake (Gibbs *et al.* 1973). More recently, intraperitoneal injection of both CCK-8 and CCK-58 has been shown to reduce meal size similarly, while only CCK-58 increased the inter-meal interval (Overduin *et al.* 2014; Sayegh *et al.* 2014). This suggests that, at least in rats, CCK isomers do not have equal bioactivity; however it is unclear whether the same

holds true for humans. The mechanism by which CCK induces these effects in rodents is thought to be through liberation of gastric stores of leptin (Bado *et al.* 1998).

In humans, intravenous CCK-8 and CCK-33 both increased fullness, decreased hunger and suppressed subsequent energy intake in healthy young (Kissileff *et al.* 1981; Brennan *et al.* 2005), older (MacIntosh *et al.* 2001) and obese (Pi-Sunyer *et al.* 1982) individuals. These changes likely result from the concurrent changes in GI motor and hormone function that mimic the effects of intraduodenal nutrient (i.e. suppression of APD motility). While some studies have reported that exogenous administration of CCK can induce nausea at high doses, which may impact the energy intake reported, others have shown a reduction in energy intake following exogenous CCK, in the absence of nausea (Brennan *et al.* 2008).

Limited studies suggest that endogenous CCK plays a role in the regulation of appetite and energy intake. For example in one study, infusion of the CCK_1 receptor antagonist, loxiglumide, attenuated the inhibitory effects of fat on subsequent energy intake, resulting in increased food intake (Lieverse *et al.* 1994a; Matzinger *et al.* 1999). In another, loxiglumide administered for one hour prior to, and during, ingestion of a meal increased energy intake, albeit modestly, by around 10 %, and increased feelings of hunger compared with a saline infusion (Beglinger *et al.* 2001). These observations demonstrate that endogenous CCK is involved in appetite regulation through CCK_1 receptor mechanisms.

4.5.3 Peptide YY (PYY)

PYY is a 36-amino acid peptide, and part of the pancreatic polypeptide family. PYY is secreted by endocrine "L" cells, predominantly in the ileum and large intestine as $PYY_{(3\text{-}36)}$ and $PYY_{(1\text{-}36)}$ (Adrian *et al.* 1985), the latter of which is rapidly degraded by DPP-IV to

$PYY_{(3\text{-}36)}$. $PYY_{(3\text{-}36)}$ is the active, and most abundant, isoform in the human circulation (Grandt *et al.* 1994; Ueno *et al.* 2008) (**Table 4.1**). $PYY_{(1\text{-}36)}$ is a known agonist of the Y1 and Y2 receptors of the NPY receptor family, and is reported to have orexigenic effects (Corp *et al.* 1990), while $PYY_{(3\text{-}36)}$ is an agonist of the Y2 receptor, with anorexigenic effects (Batterham *et al.* 2002). Release of PYY in the GI tract is dependent on the caloric load of the ingested meal (Ekblad and Sundler 2002), and occurs in response to fat (Onaga *et al.* 2002; Pilichiewicz *et al.* 2006), particularly fatty acids (Pappas *et al.* 1986), and protein (Fu-Cheng *et al.* 1997; Veldhorst *et al.* 2008), but not carbohydrate (Groger *et al.* 1997). The release of PYY has also been shown to occur in response to neurohumoral signals arising from the small intestine, including CCK (Lin *et al.* 2000; Brennan *et al.* 2007; Brennan *et al.* 2008).

4.5.3.1 Effects of PYY on gastrointestinal motility and hormone release

Exogenous administration of PYY in both humans and animals modulates GI motility. In rhesus monkeys, intramuscular injection of $PYY_{(3\text{-}36)}$ has been shown to slow gastric emptying of saline in a dose-dependent manner (Moran *et al.* 2005b). In humans, intravenous infusion of $PYY_{(3\text{-}36)}$ at 0.18 or 0.51 pmol/kg/min also slows gastric emptying and mouth-to-caecum transit dose-dependently (Savage *et al.* 1987). Because PYY-releasing cells are located primarily in the distal small intestine, and the secretion of PYY is related to the fat-induced inhibition of distal gut motility, it has been suggested that PYY is a primary mediator of the ileal brake – the slowing of gastric emptying induced by distal small intestinal feedback in response to fat (Lin *et al.* 1997). In addition to inhibiting GI motility, endogenous PYY release has been shown to inhibit colonic chloride secretion, as well as pancreatic exocrine and insulin release, (Ballantyne 2006) and neuropeptide Y (NPY), release (Lluis *et al.* 1987a; Lluis *et al.* 1987b; Ueno *et al.* 2008).

4.5.3.2 Effects of PYY on postprandial glycaemia

Knowledge of the effects of PYY on postprandial glycaemia is limited. Since PYY is released in proportion to the amount of calories, as well as the amount of fat or protein (but not carbohydrate) present in a meal, it does not appear to have direct effects on glycaemic control. A number of *in vivo* studies have shown that PYY administered at supraphysiological concentrations inhibits insulin secretion in rat, mouse and dog pancreas. In humans, intravenous infusion of $PYY_{(3-36)}$ at 0.8 pmol/kg/min has been found to increase plasma free fatty acids, as well as postprandial, but not pre-prandial, insulin and glucose concentrations (Sloth *et al.* 2007b). This effect was likely due to increased lipolysis increasing circulating free fatty acids, and thus acutely increased insulin resistance.

4.5.3.3 Effects of PYY on appetite and energy intake

A number of studies have examined the effects of PYY on appetite and energy intake, however the results are conflicting. For example, central administration of PYY has been shown to stimulate feeding in rats (Hagan 2002). In humans, subcutaneous administration of $PYY_{(1-36)}$ at up to 200 pmol/kg lean body mass, or of $PYY_{(3-36)}$ at up to 100pmol/kg lean body mass had no effect on energy intake, although $PYY_{(3-36)}$ did increase perceptions of satiety and decreased perceptions of hunger and prospective consumption (Sloth *et al.* 2007a). In contrast, peripheral administration of $PYY_{(3-36)}$ has been shown by others to reduce energy intake in both rodents, and lean and obese humans (Batterham *et al.* 2002; Batterham and Bloom 2003; Batterham *et al.* 2003). Batterham *et al* have reported that PYY, administered at 2 nmol per m^2 of body surface area (which resulted in peak concentrations of ~53 pmol/L in lean, and 57 pmol/L in obese men) reduced energy intake by ~ 30% in both groups (Batterham *et al.* 2003). A more recent study infusing a range of doses of $PYY_{(3-36)}$ (0.2, 0.4 and 0.8 pmol/kg/min [resulting in peak concentrations of ~ 63, 88 and 150 pmol/L]) also

reported a dose-dependent suppression of energy intake. In response to the highest dose (0.8 pmol/kg/min), which resulted in supraphysiological concentrations of plasma PYY, suppression of energy intake was ~32 %, however nausea was a considerable side effect (Degen *et al.* 2005). There is evidence that a threshold for plasma PYY concentrations to suppress energy intake exists; and it appears that shortly beyond this threshold, adverse effects (i.e. nausea) occur (Degen *et al.* 2005; le Roux *et al.* 2006). It is possible that the lack of effects Sloth *et al* observed on energy intake occurred as a result of administering a single, subcutaneous bolus of $PYY_{(1-36)}$ and $PYY_{(3-36)}$, which resulted in a longer, slower appearance of PYY in the plasma, rather than the continuous IV infusion utilised by others. They also used a dose-escalation design, whereby the dose of PYY administered was increased at subsequent study visits, which may have influenced their findings.

Studies using specific PYY receptor antagonists to evaluate the effects of endogenous PYY on appetite and energy intake have not been performed in humans due to a lack of availability and, thus, the role of endogenous PYY in appetite and energy intake regulation in humans remains unknown.

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

(GLP-1 is a 33-amino acid peptide product of the glucagon gene. The glucagon gene is expressed in the pancreas and the small intestinal mucosal endocrine cells. The glucagon gene displays two additional glucagon-like sequences at its carboxyl terminus, and its primary translations product, proglucagon, is cleaved to release two peptides, GLP-1 and GLP-2, which have a high sequence homology to glucagon (Bell *et al.* 1983) (**Table 4.1**). which GLP-1 is released from L cells in the distal small intestinal mucosa in response to fat (Feinle *et al.* 2003), protein (Herrmann *et al.* 1995) and carbohydrate (Feinle *et al.* 2002a;

Pilichiewicz *et al.* 2007a). Bioactive GLP-1 is generated from GLP-$1_{(1-37)}$, and exists most abundantly as GLP-$1_{(7-36)}$ amide, and GLP-$1_{(7-37)}$. Both forms of GLP-1 are rapidly biodegraded by DPP-IV to the inactive forms GLP-$1_{(9-36)}$amide and GLP-$1_{(9–37)}$, which are unable to access the GLP-1 receptor, and lack insulinotropic activity (Mentlein *et al.* 1993; Drucker 2006a). As such, only ~10 % of the GLP-1 that is released reaches the systemic circulation (Deacon 2004). GLP-1 functions as an incretin hormone, stimulating insulin, and suppressing glucagon, release, which increases insulin-dependent glucose clearance in the periphery to maintain normoglycaemia (Gutniak *et al.* 1992; D'Alessio *et al.* 1994).

4.5.4.1 Effects of GLP-1 on gastrointestinal motility and hormone release

GLP-1 has been shown to slow gastric emptying in healthy (Nauck *et al.* 1997; Delgado-Aros *et al.* 2002; Little *et al.* 2006b), type 2 diabetic (Meier *et al.* 2003), and obese subjects (Naslund *et al.* 1998b; Flint *et al.* 2001). Exogenous administration of GLP-1 results in relaxation of the proximal stomach, inhibition of antral and duodenal motility and the stimulation of tonic and phasic pyloric pressure waves, in a load-dependent manner. A study conducted in our department reported that GLP-1 administered at 0.3 and 0.9 pmol/kg/min slowed gastric emptying of both liquids and solids (Little *et al.* 2006b). Others have demonstrated that intravenous administration of GLP-1 at 0.4 and 1.2 pmol/kg/min significantly inhibited the number and amplitude of antral and duodenal contractions, dose-dependently increased pyloric tone and significantly stimulated isolated pyloric pressure waves (Schirra *et al.* 2000). In addition, intravenous GLP-1 also enhanced the pyloric tone stimulated by duodenal infusion of lipid (Schirra *et al.* 2000).

A limited number of studies have examined the effects of endogenous GLP-1 on GI motor function, using the specific GLP-1 receptor antagonist, exendin(9-39). The administration of

exendin(9-39) has been shown to block the effects of GLP-1 on the slowing of gastric emptying in rodents (Tolessa *et al.* 1998), and to attenuate tonic and phasic pyloric motility in response to intraduodenal glucose in humans (Schirra *et al.* 2006).

4.5.4.2 Effect of GLP-1 on postprandial glycaemia

GLP-1 plays a significant role in regulating postprandial blood glucose. The effect of GLP-1 on blood glucose, insulin and glucagon release has generated considerable interest in the potential of GLP-1 (Schirra *et al.* 1998a; Schirra *et al.* 1998b), and specific GLP-1 analogues and receptor agonists, to improve the regulation of blood glucose concentrations in type II diabetes patients (Nauck 1998; Meier *et al.* 2003). This may be particularly important, as the insulinotropic effects of GIP are not maintained in type II diabetes (Nauck *et al.* 1993), and GIP has no effect on gastric emptying (Meier *et al.* 2004). Exogenous administration of GLP-1 lowers glycaemia by stimulating the release of insulin and inhibiting the release of glucagon (Schirra *et al.* 2006). Using exendin(9-39), Schirra *et al* were able to demonstrate the key role of GLP-1 in incretin activity and glycaemia. When subjects were administered exendin(9-39) under fasting conditions blood glucose concentrations were increased, insulin concentrations remained unchanged, and plasma glucagon concentrations were reduced (Schirra *et al.* 2006). Moreover, when endogenous GLP-1 was stimulated by duodenal glucose, exendin(9-39) reduced plasma insulin concentrations, and resulted in elevated blood glucose concentrations.

While in fasted healthy individuals, exogenous GLP-1 stimulates insulin secretion (Kreymann *et al.* 1987), when GLP-1 is co-administered with a meal insulin levels appear to decrease (Nauck *et al.* 1997). Additional observations that postprandial administration of GLP-1 results in reduced, rather than augmented, insulin release, in both healthy (Nauck *et*

al. 1997) and type II diabetic subjects (Meier *et al.* 2003) suggest that GLP-1 may exert its glucose lowering effects by slowing gastric emptying (Nauck *et al.* 1997). A recent study, whereby the inhibitory effect of GLP-1 on gastric emptying was reversed using the prokinetic drug erythromycin, supports this notion, in that, despite increased insulin and GIP responses, erythromycin administration was associated with attenuation of the hypoglycaemic effects of GLP-1 (Meier *et al.* 2005).

4.5.4.3 Effects of GLP-1 on appetite and energy intake

The role of GLP-1 in the regulation of appetite and energy intake is somewhat inconsistent. For example, intravenous administration of GLP-1 at doses up 1.2 pmol/kg/min has been shown to reduce energy intake in humans in some studies (Gutzwiller *et al.* 1999; Naslund *et al.* 1999), but not others (Long *et al.* 1999; Brennan *et al.* 2005). Rodent studies have shown that intravenous GLP-1 decreases food intake (Turton *et al.* 1996), and the effects of endogenous GLP-1 food intake are attenuated by the GLP-1 receptor antagonist exendin(9-39) (Turton *et al.* 1996). When the actions of GLP-1 were blocked by exendin(9-39), food intake did not change in fasted rats, but was doubled in fed rats, and the feeding response to neuropeptide Y, an appetite-stimulant, was augmented (Turton *et al.* 1996). In humans, recent studies have demonstrated that GLP-1 receptor blockade fails to suppress satiety following a standardised breakfast (Melhorn *et al.* 2014), but has modest effects following ingestion of a liquid glucose preload (Steinert *et al.* 2014). Moreover, in both studies, the expected increase in voluntary food intake did not occur following GLP-1 receptor blockade. While the evidence in rats supports the notion that GLP-1 is a physiological regulator of satiety, these recent studies suggest that GLP-1 may not have a major physiological role in food intake in humans (Melhorn *et al.* 2014; Steinert *et al.* 2014). It cannot be ruled out however, that in these studies additional mechanisms, including GI hormones, may have

overridden the possible effect of GLP-1 receptor antagonism on satiation, and further work is required to elucidate the role of GLP-1 in the regulation of energy intake in humans.

4.5.5 Glucose-dependent insulinotropic peptide (GIP)

GIP was first identified for its ability to inhibit gastric secretions (Pederson *et al.* 1975), hence its classical name, 'gastric inhibitory peptide'. GIP is encoded by the GIP gene, and circulates as a 42-amino acid biologically active peptide, secreted from K cells in the proximal small intestine, particularly the duodenum, in response to nutrients, especially carbohydrate (Ebert and Creutzfeldt 1980) and protein (Carr *et al.* 2008). The primary role of GIP in the body is as an incretin hormone, stimulating pancreatic insulin release in the presence of increased glucose concentrations, thus, regulating the glycaemic response (Tseng *et al.* 1996). GIP release is also stimulated by fat, but only weakly, and not sufficiently to stimulate insulin release (Ross and Dupre 1978). GIP exerts a greater proportion of the incretin effect than GLP-1 (Ahren 2013), since GLP-1 is rapidly degraded by DPP-IV and only ~10 % of what is released reaches the systemic circulation (Deacon 2004). GIP also has direct insulinotropic effects on pancreatic beta cells, but does not exhibit the same effects on gastric emptying as GLP-1 (Yamada and Seino 2004). GIP release in response to a meal is proportional to both the caloric value, and rate of delivery of the nutrient to the small intestine, with the rate of nutrient absorption playing a key role in the modulation of GIP release (Creutzfeldt 1979).

4.5.5.1 Effects of GIP on gastrointestinal motility and hormone release

The effects of GIP on GI motility, particularly in humans, remain unclear. In rats, exogenous GIP has been shown to increase intestinal transit time (Ogawa *et al.* 2011). In dogs, exogenous GIP infused at supraphysiological doses suppressed MMC activity, which

reappeared shortly after cessation of the infusion (Thor *et al.* 1987). In humans, exogenous GIP administered at 2 pmol/kg/min (which resulted in supraphysiological GIP concentrations) has been found to have no effect on gastric emptying (Meier *et al.* 2004), while others have shown that exogenous GIP administered at 2 and 5 pmol/kg/min modestly accelerates gastric emptying (Edholm *et al.* 2010). That such high doses of exogenous GIP are required to produce only small effects on gastric emptying suggests GIP is not integral in modulating gastric motility.

4.5.5.2 Effects of GIP on postprandial glycaemia

GIP stimulates pancreatic beta-cell proliferation and postprandial insulin release. Intravenous GIP, when administered simultaneously with glucose, has been shown to stimulate insulin release, suggesting that the insulinotropic effect of GIP is dependent on the presence of glucose (Dupre *et al.* 1973). In the rat, administration of the GIP receptor antagonist, ANT-GIP (GIP (7–30)-NH_2), reduced insulin secretion by ~72 % without affecting the insulinotropic effects of GLP-1 or glucose, suggesting that GIP plays a major role in modulating the postprandial glycaemic response (Tseng *et al.* 1996).

GIP, administered at supraphysiological doses (20ng/kg/min) intravenously for 180 min immediately after a meal, has been shown to override the glucagonostatic effects of GLP-1 in patients with type II diabetes (Chia *et al.* 2009). During the infusion, glucagon release was increased, and postprandial blood glucose increased under already hyperglycaemic conditions (Chia *et al.* 2009). In a second study in patients with type 2 diabetes, co-administration of GIP and GLP-1 also showed that GIP antagonises the normal glucagon-lowering effect of GLP-1 (Mentis *et al.* 2011), however, the mechanisms underlying this remain unknown. Exogenous GIP administration has also shown that the insulinotropic effect of GIP is reduced

in type 2 diabetes (Nauck *et al.* 2004). This demonstrates the critical role of GIP in the regulation of glycaemia, as the loss of the insulinotropic effect of GIP characterises type II diabetes, while the effects of GLP-1 are preserved (Nauck *et al.* 2004).

4.5.5.3 Effects of GIP on appetite and intake

The effects of GIP on appetite and energy intake are unclear. GIP has been implicated in lipid metabolism, including the regulation of insulin-stimulated incorporation of fatty acid into triglycerides, stimulation of lipoprotein lipase activity and the stimulation of fatty acid synthesis (Gautier *et al.* 2008). In rats, GIP receptor blockade using a daily injection of the receptor antagonist (Pro^3GIP) had no effect on body weight or energy intake over 14 days (Parker *et al.* 2007), suggesting that the effects of GIP on appetite are limited.

4.5.6 Glucagon

Glucagon is a 29-amino acid peptide, secreted from the pancreas in response to increases in blood glucose (Bromer *et al.* 1957). Glucagon is synthesised primarily in the A-cells located on the edges of the Islets of Langerhans (Baum *et al.* 1962), from the 160-amino acid precursor, proglucagon.

4.5.6.1 Effects of glucagon on gastrointestinal motility and hormone release

The evidence describing the effects of glucagon on GI motility and hormone release are inconsistent, both between, and within, species. For example, in dogs, intravenous administration of glucagon suppressed antral contractility, with no effect on duodenal motility (Shibata *et al.* 2001). In humans, intravenous exogenous glucagon has been shown in some studies, but not others, to delay gastric emptying of both a glucose (Chernish *et al.* 1978), and mixed nutrient (Jonderko *et al.* 1988; Jonderko *et al.* 1989) meal. Studies have

also shown that injection of a bolus dose of glucagon suppresses antral and duodenal contractility (Gregersen *et al.* 1988), abolishes jejunal contractility (Patel *et al.* 1979) and increases the duration of the MMC, which is associated with a decreased frequency of MMC cycles (Larsen *et al.* 1986).

4.5.6.2 Effects of glucagon on postprandial glycaemia

The primary role of glucagon in the body is to raise blood glucose during fasting, exercise and hypoglycaemia, by stimulating glycogenolysis and gluconeogenesis from lactate, pyruvate, glycerol and specific amino acids (Claus *et al.* 1983). Changes in the ratio of circulating insulin to glucagon shifts lipid metabolism from storage to release (Steinberg *et al.* 1959). Glucagon also acts to oppose the action of insulin in the liver to regulate glucose metabolism postprandially (Stalmans 1983) and may also directly stimulate insulin release (Samols *et al.* 1966) through its own receptors expressed on pancreatic β-cells, at least in non-diabetic individuals (Kawai *et al.* 1995; Moens *et al.* 1998). Hyperglucagonaemia is implicated in hyperglycaemia, and thus may play a role in the pathophysiology of diabetes.

4.5.6.3 Effects of glucagon on appetite and energy intake

Evidence for the effects of glucagon on appetite and energy intake is limited. In rats, intraperitoneal injection of glucagon suppressed food intake in a dose-dependent manner (Geary *et al.* 1986; Le Sauter and Geary 1991), while another study has shown that glucagon suppresses food intake in orally-fed, but not sham-fed rats, suggesting that a gastric, or post-gastric stimulus is required for glucagon to suppress energy intake (Le Sauter and Geary 1987). Using a specific antibody for glucagon, evidence in rats suggests that endogenous glucagon released in response to meal-related nutrients may account for up to 50 % of the meals satiating capacity (Langhans *et al.* 1982; Le Sauter *et al.* 1991). In humans evidence is

scarcer. Glucagon or CCK infused alone intravenously reduced the size of a subsequent meal. Conversely, when glucagon and CCK were infused concurrently, no additive effect on energy intake suppression was seen, suggesting some degree of antagonism (Geary *et al.* 1992).

4.5.7 Insulin

Insulin is a pancreatic peptide, synthesised in β-cells within the Islets of Langerhans, and insulin's primary role within the body is to maintain glucose homeostasis. Insulin crosses the blood-brain barrier to directly access hypothalamic neurons, and thus regulate energy homeostasis (Pardridge *et al.* 1985; Banks *et al.* 1997). Insulin is released in response to an increase in plasma glucose concentrations, as well as by the presence of plasma amino acids (Floyd *et al.* 1966b; Floyd *et al.* 1966a; Knopf *et al.* 1966). The magnitude of insulin release in response to carbohydrate is critically dependent on the release of the incretin hormones, GLP-1 and GIP (Lavin *et al.* 1998; Pilichiewicz *et al.* 2007a). The understanding of the role of insulin in the regulation of GI motility and energy intake in humans is limited, since studies have not been able to distinguish between the effects of insulin, and the effects of hypoglycaemia, on these outcomes.

4.5.7.1 Effects of insulin on gastrointestinal motility and hormone release

In dogs, intravenous infusion of insulin has been shown to stimulate gastric motility (Regan and Barnes 1933; Nelsen *et al.* 1966), and this effect is thought to be due to stimulation of the vagus nerve by hypoglycaemia (Walker *et al.* 1974). In humans, the effects of insulin on gastric emptying and GI motility are far more variable. For example, under euglycaemic conditions, hyperinsulinaemia has been shown to slightly, but significantly, slow gastric emptying of both solids and liquids in healthy humans (Eliasson *et al.* 1995; Kong *et al.*

1998). In contrast, hyperinsulinaemia has little to no effect on gastric emptying in people with type–I and II diabetes (Kong *et al.* 1999). Hyperinsulinaemia has also been shown to inhibit antral phase III activity, to shorten periods of duodenal phase III activity (Bjornsson *et al.* 1995), and increase MMC frequency under fasting conditions (Gielkens *et al.* 1997). In addition, in healthy individuals, hyperinsulinaemia appears to inhibit jejunal motility in the fed state, and slow small intestinal transit time in one study (Kong *et al.* 1998), but not another (Hasler *et al.* 1995). No significant changes in the number of antral, pyloric or duodenal pressure waves, or basal pyloric pressures, were observed in a 45-minute period after insulin was administered (i.e. hypoglycaemia) when compared with a euglycaemic state (saline infusion) (Fraser *et al.* 1991). As such, the effects of insulin appear to depend on both the insulin sensitivity of the individual, as well as the absolute amount of insulin in the circulation. Hyperinsulinaemic euglycaemic clamp studies may help to elucidate this.

4.5.7.2 Effects of insulin on postprandial glycaemia

An initial rise in blood glucose resulting from intestinal absorption triggers insulin release. Insulin acts to remove glucose from the blood to peripheral tissues at a dynamic rate, which maintains blood glucose levels within an acceptable physiological range of ~4-7 mM. Insulin stimulates cell growth and proliferation, the uptake of glucose, amino acids and fatty acids from the blood into cells and the processes that regulate glycogen, protein and lipid synthesis, and inhibits hepatic gluconeogenesis, glycogenolysis, lipolysis and protein degradation (Saltiel and Kahn 2001). Insulin resistance or deficiency results in dysregulation of these processes, and produces elevations in both fasting and postprandial glucose and lipid levels (Saltiel and Kahn 2001).

4.5.7.3 Effects of insulin on appetite and energy intake

The role of insulin in the regulation of appetite and food intake remains unclear. In animals, in the absence of hypoglycaemia, increased levels of circulating insulin appear to suppress energy intake (Nicolaidis and Rowland 1976; Woods *et al.* 1984). In humans, insulin infusion during euglycaemia had no effect on appetite sensations, suggesting that insulin does not enhance satiety, at least under these conditions (Chapman *et al.* 1998). In contrast, endogenous increases in insulin, occurring in response to a meal, have been demonstrated to be related inversely to energy intake at a subsequent meal, at least in lean subjects (Speechly and Buffenstein 2000; Verdich *et al.* 2001), and in response to a protein meal in lean and overweight, but not obese, subjects (Bowen *et al.* 2006).

4.5.8 Effects of macronutrients on gastrointestinal hormone release

Fat, carbohydrate and protein exert macronutrient-specific effects on the magnitude and pattern of secretion of GI hormones after a meal. This section will explore the comparative effects of fat, carbohydrate and protein on ghrelin, CCK, GLP-1, GIP and PYY release.

In both acute (i.e. single meal) (Monteleone *et al.* 2003) and longer-term (up to 2 weeks) (Weigle *et al.* 2003) studies, the meal-related suppression of ghrelin release has been shown to be greater in response to high-carbohydrate and high-protein, compared with high-fat meals/diets. A high-protein breakfast decreased postprandial ghrelin secretion more than a high-carbohydrate breakfast (Blom *et al.* 2006), however, no effects on *ad libitum* energy intake were observed. Others have reported that a high-protein meal led to lower ghrelin levels at 180 minutes compared with high-carbohydrate and high-fat meals in healthy adults, suggesting that dietary protein might enhance longer-term postprandial ghrelin suppression and satiety (Tannous dit El Khoury *et al.* 2006). In contrast, several studies have shown that

while carbohydrate and fat ingestion suppressed ghrelin release, ingestion of a protein-rich meal stimulated ghrelin levels (Erdmann *et al.* 2003; Erdmann *et al.* 2004). Others have found no relationship between ghrelin and energy intake in response to protein (Moran *et al.* 2005a), thus the relationship between ghrelin release, protein and energy intake remains unclear.

Concentrations of plasma CCK are more potently stimulated by intraduodenal lipid compared with carbohydrate in both healthy young and older men (Chapman *et al.* 1999; Feinle *et al.* 2002c). In addition, intraduodenal (Mearadji *et al.* 2001; Seimon *et al.* 2009b) and oral (Liddle *et al.* 1985) fat and protein have been shown to be significantly more potent than carbohydrate in stimulating the secretion of CCK. Intraduodenal leucine and L-phenylalanine have also been shown to stimulate CCK release (Steinert *et al.* 2015a; Steinert *et al.* 2015b). Oral administration of liquid protein preloads to lean and overweight men stimulated and prolonged CCK elevation more than glucose. In obese men, whey and casein milkshakes compared to glucose-or lactose-based drinks, increased plasma CCK by 71%, for up to 90 minutes after consumption of the meal (Bowen *et al.* 2006). In another study, a whey protein milkshake, as well as glucose and fructose drinks, increased CCK to a similar peak at 30 minutes. However while the CCK response to the glucose and fructose drinks gradually declined following this, the whey protein drink produced a second peak at t= 120 min (Bowen *et al.* 2007). This result is congruent with other studies, which have reported a unique CCK response to milk proteins, such that levels reach an initial peak around 15-20 minutes following ingestion, before falling slightly and peaking again at approximately 90 minutes (Fernstrom *et al.* 1979; Bowen *et al.* 2006; Brennan *et al.* 2012); the physiological relevance of this dual peak is not clear.

GLP-1 release is comparably stimulated by both oral and intraduodenal lipid and glucose (Feinle *et al.* 2002b; Adam and Westerterp-Plantenga 2005). Raben *et al* reported that GLP-1 responses were greatest after a high-protein meal, compared to meals rich in fat, carbohydrate or alcohol (Raben *et al.* 2003). In addition, Blom *et al* showed that a whey protein isolate enriched meal stimulated GLP-1 secretion more than a high-carbohydrate meal (Blom *et al.* 2006). Bowen *et al* also reported that 50g of whey protein stimulated GLP-1 (and CCK) release more than glucose and fructose, however the increases in GLP-1 and CCK did not translate to a greater suppression of energy intake at a subsequent *ad libitum* meal, possibly due to the size of the liquid preload (~250 kcal), and the duration between the preload and the *ad libitum* meal overriding the effects of appetite hormones (Bowen *et al.* 2007).

Oral ingestion of carbohydrate or fat has been shown to potently increase postprandial GIP release (Elliott *et al.* 1993), while protein appears to have lesser effects (Karamanlis *et al.* 2007). Co-ingestion of fat with carbohydrate or protein has been shown to augment the GIP response, with GIP responses more potent following carbohydrate+fat compared with protein+fat (Collier and O'Dea 1983), while co-ingestion of protein with glucose has been shown to blunt the GIP response to glucose (Karamanlis *et al.* 2007). In dogs, an intraduodenal infusion of mixed amino acids (i.e. digestion products of protein) or medium chain triglyceride stimulated GIP release, but not as potently as a long chain triglyceride, or glucose, infusion (O'Dorisio *et al.* 1976). In humans, some studies have reported a stimulatory effect of single amino acids, particularly glutamine, on GIP release (Greenfield *et al.* 2009; Chang *et al.* 2013). For example, both oral (Greenfield *et al.* 2009) and intraduodenal (Chang *et al.* 2013) administration of the amino acid glutamine has been shown to stimulate GIP release in both healthy and type 2 diabetic individuals, however the effect of oral glutamine on GIP is modest, compared with glucose (Greenfield *et al.* 2009).

Fat has been shown to be a more potent stimulus for PYY secretion compared with protein or carbohydrate respectively in an oral administration study (Adrian *et al.* 1985) and compared with glucose, when administered intraduodenally (Seimon *et al.* 2009b). Layer *et al* (1995) reported that the ileal infusion of lipid resulted in a significant increase in PYY release, while infusion of carbohydrate or protein produced no change in plasma PYY concentrations (Layer *et al.* 1995). In contrast, others have reported that protein and carbohydrate meals more potently stimulate PYY release than fat (Pedersen-Bjergaard *et al.* 1996). For example, Batterham *et al* have reported that a high-protein mixed-meal stimulated the largest increase in PYY when compared with a high-fat and high-carbohydrate meal. Brennan *et al* reported similar effects, whereby in lean subjects, the magnitude of the increase in PYY from baseline was greater in response to a high-protein and tended to be higher in response to a high-carbohydrate/low-protein, compared with a high-fat, mixed meal (Brennan *et al.* 2012).

Collectively, the above studies identify a number of inconsistencies in the evidence for the effects of protein on individual, yet interrelated, GI hormones. These may be partially attributable to methodological differences, including oral vs. intragastric, intraduodenal and ileal administration of nutrient, differences in composition of meals and different study populations. As the magnitude and secretory patterns of GI hormones in response to protein have not yet been clearly established, investigation is required to further characterise these, to determine their relationship to changes in appetite and energy intake, and to establish how these may be altered in obesity. In particular, this provides an opportunity to undertake novel research in the effects of modes of administration (oral versus small intestinal) of protein on these hormones and the relationships with energy intake and glycaemic control. This will aid in establishing the mechanisms by which protein exerts its appetite-suppressive effects, to ultimately assist in developing robust nutritional strategies to target obesity.

4.6 Perturbations of gastrointestinal function in obesity

Recent, albeit limited, research has suggested that GI motility and hormone release are disturbed in overweight and obese individuals. Given that gut motility and hormone release play a critical role in determining glycaemic regulation, appetite and energy intake, it is crucial to understand how GI function differs between lean and obese populations, and how these factors may contribute to the aetiology and maintenance of obesity.

4.6.1 Changes in gastric emptying in obesity

A number of studies have evaluated gastric emptying in obese humans, however the data remain inconclusive. For example, rates of gastric emptying in obese individuals have been reported to be slower (Horowitz *et al.* 1983; Maddox *et al.* 1989; Jackson *et al.* 2004), similar to (Zahorska-Markiewicz *et al.* 1986; Wisen and Johansson 1992; French *et al.* 1993; Glasbrenner *et al.* 1993; Hutson and Wald 1993; Verdich *et al.* 2000; Hellmig *et al.* 2006; Buchholz *et al.* 2013; Seimon *et al.* 2013) or faster (Wright *et al.* 1983; Gryback *et al.* 1996; Tosetti *et al.* 1996b; Näslund *et al.* 1998; Cardoso-Junior *et al.* 2007) than in lean individuals. The reasons for these differences are unclear, however, it is possible that they may be partly attributable to differences in methodologies between studies, including small numbers, which may have reduced the power to detect statistical differences beyond inter-individual variability (Wisen and Johansson 1992), differences in meal composition or meal consistency (e.g. solid (Tosetti *et al.* 1996a) compared with semi-solid (Maddox *et al.* 1989; French *et al.* 1993; Buchholz *et al.* 2013) or liquid (Wisen and Johansson 1992; Seimon *et al.* 2013) meals), types of food to which radionuclides were added (i.e. egg omelette (Tosetti *et al.* 1996a) eggs, toast and orange juice (Jackson *et al.* 2004), Ensure (a liquid mixed nutrient drink) (Seimon *et al.* 2013) or soups with covertly manipulated composition (French *et al.* 1993)), and the techniques used to assess gastric emptying (e.g. scintigraphy (Wright *et al.*

1983; Maddox *et al.* 1989), breath testing (Jackson *et al.* 2004) or multiple-marker dilution method (Wisen and Johansson 1992). Different studies have also used different selection criteria for "obese" individuals, i.e. moderately (BMI < 40 kg/m^2) (Wright *et al.* 1983; Jackson *et al.* 2004) compared with morbidly (BMI > 40kg/m^2) obese (Naslund *et al.* 1998a; Cardoso-Junior *et al.* 2007). Finally, age, gender, the habitual diet of individuals (Cunningham *et al.* 1991a; Cunningham *et al.* 1991b), the presence of additional co-morbities, including diabetes, and weight stability (Park and Camilleri 2005) can also influence gastric emptying rates.

The rate of gastric emptying is dynamic, and is modulated in response to both the macronutrient composition, and size, of the meal. Under experimental settings, when intragastric pressure is increased, simulating a large meal, the pressure gradient between the stomach and small intestine is increased, which accelerates gastric emptying (Strunz and Grossman 1978; Geliebter *et al.* 1986). In a stomach with increased gastric capacity, a set meal volume may result in a smaller increase in intragastric pressure, slowing gastric emptying (Geliebter *et al.* 1992). Gastric distension stimulates gastric stretch receptors which activate hypothalamic neurons via vagal signalling (Paintal 1953), and induces feelings of satiety (Iggo 1955). In the case of a larger gastric capacity, a larger volume of food may be required to activate this signalling pathway, resulting in overconsumption.

It has been demonstrated that gastric capacity and maximal tolerable volume are greater in obese than lean subjects when tested with a water-filled intragastric latex balloon (Geliebter 1988; Geliebter *et al.* 1988), without differences in subsequent energy intake (Geliebter *et al.* 1988). In contrast, studies using a barostat have reported that basal gastric tone, postprandial gastric accommodation and perceptions of distension do not differ between lean and obese

individuals (Klatt *et al.* 1997). It is important to note that there are differences in compliance between a latex balloon, and heavier, less-compliant polyethylene bags, and the use of an electronically controlled barostat for pressure and volume distension, compared with manual inflation to a set volume, and these methodological differences may partially explain the differences in outcomes (Park and Camilleri 2005). Using the minimally invasive technique of single-photon emission computed tomography imaging, studies have reported no differences in fasting or postprandial gastric volume in response to a liquid mixed-nutrient meal, between obese and lean, however these authors did report a greater fasting volume of the distal stomach in obese, compared with lean subjects (Kim *et al.* 2001). While one study has reported no association between an increased BMI and fasting gastric volume (Delgado-Aros *et al.* 2004), another has shown an association between increased BMI and maximal tolerable volume of a liquid, so that overweight and obese subjects consumed ~225 kcal more at maximal satiation than lean individuals (Park and Camilleri 2005). Collectively, these data demonstrate the paucity of conclusive literature describing the changes in gastric capacity and gastric volume in obesity. If, indeed, these are increased in obesity, it may provide a potential target for improving meal-related satiety, and aiding in weight loss.

4.6.2 Changes in gastrointestinal motor function in obesity

To date, studies examining whether interdigestive motility differs between obese and healthy, lean individuals, and the clinical relevance of these differences, are limited. One study has reported disturbed fasting motility, including a shortened phase I, increased duration of phase II, and less frequent occurrence of phase IIIs of the MMC, with a greater proportion of phase III contractions occurring more distally in the obese, when compared with lean individuals (Pieramico *et al.* 1992). It is unclear whether these changes are involved in the perpetuation of obesity, or if they occur as a result of being overweight. Some authors have suggested that

changes in GI motility may occur as a result of reduced sensitivity to GI hormones that promote motility, including CCK (Gallagher *et al.* 2009), however this notion requires further investigation.

Few studies have evaluated the potential differences in postprandial GI motor responses to macronutrients in the obese, compared with lean individuals. Moreover, if such differences do exist, limited data is available to establish whether they may be implicated in obesity, or if they can be modified. A recent study from our department assessed oral and GI sensitivities to oleic acid in lean and overweight/obese men. The authors reported a diminished number of IPPWs in response to a 90 min intraduodenal infusion of oleic acid (0.78 kcal/min) in the obese compared with lean participants (Stewart *et al.* 2011), suggesting that GI sensing of oleic acid, at least at this concentration, may be compromised in obesity. In another study, following a 120-min intraduodenal infusion of a long-chain triglyceride emulsion (Intralipid®, 2.86 kcal/min) BPP was greater in lean compared with obese individuals at baseline, and after 5 days of 30 % energy restriction (Seimon *et al.* 2014). Interestingly, after 12, but not 4 weeks, of 30 % energy restriction, BPP was significantly increased in the obese participants compared with baseline, and after 5 days of energy restriction (Seimon *et al.* 2014). This suggests that long-term energy restriction may at least partially restore GI sensitivity to intraluminal fat in obese individuals. In light of this evidence, it appears that obese individuals may be less sensitive to fat or lipid. In contrast, there is very limited evidence examining whether the GI motor responses to protein are maintained, or compromised in obesity, and hence further investigation is required.

4.6.3 Changes in the release of gastrointestinal hormones in obesity

There is considerable evidence that the secretion of GI hormones, including ghrelin, CCK, PYY, the incretin hormones, GLP-1 and GIP, as well as the secretion of insulin, may be altered both in the fasted state, and postprandially, in the obese. For example, fasting ghrelin concentrations have been reported to be inversely related to BMI (Tschop *et al.* 2001; Shiiya *et al.* 2002). In addition, since fasting ghrelin concentrations are lower in obese individuals, they cannot be suppressed by a meal to the same extent as in lean individuals (English *et al.* 2002). Interestingly, circulating ghrelin levels have been shown to increase during weight loss (Cummings *et al.* 2002), possibly as a compensatory mechanism to stimulate food intake and maintain the body's energy stores.

When compared with lean individuals, fasting CCK concentrations do not appear to differ in obese individuals (French *et al.* 1993; Lieverse *et al.* 1994b; Seimon *et al.* 2014), while postprandial plasma CCK concentrations have been shown to be greater in obese individuals by some (French *et al.* 1993), but not others (Lieverse *et al.* 1994b; Seimon *et al.* 2014). Since CCK is a known anorexigenic hormone, 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 a recent study, CCK concentrations were significantly reduced in response to 10 weeks of a ~500 kcal VLCD, and these changes were maintained for 1 year after weight loss (Sumithran *et al.* 2011), which provides a potential mechanistic explanation for the difficulties involved with maintaining weight loss long term.

Available data suggest that both fasting (Batterham *et al.* 2003; Paddon-Jones *et al.* 2006) and postprandial (Paddon-Jones *et al.* 2006) plasma PYY concentrations are reduced in obese individuals. Batterham *et* al also reported that the diminished fasting and postprandial PYY

concentrations in obese occur in the presence of an increased energy intake (Batterham *et al.* 2003). This suggests an impaired release of PYY in the obese, which has implications in that the energy content of a meal required to stimulate sufficient PYY to induce an anorectic response may be higher, resulting in overconsumption.

The outcomes of studies investigating concentrations of incretin hormones in obesity are inconclusive. Fasting GLP-1 levels have been shown to be reduced (Lugari *et al.* 2004) or similar (Mersebach *et al.* 2003), in obese compared with lean. In addition, plasma GLP-1 responses to oral carbohydrate, or a mixed-nutrient meal, but not fat, were reported to be attenuated in obese, by some (Ranganath *et al.* 1996; Verdich *et al.* 2001), but not others (Seimon *et al.* 2013). It may be that the differences in GLP-1 release observed between studies reflects differences in gastric emptying between lean and obese, rather than an impaired stimulation of GLP-1 release, since gastric emptying rate is critical in determining the magnitude of the incretin response. Using an intraduodenal infusion of fat and carbohydrate, to deliver nutrient directly to the small intestine at a pre-determined rate and thus eliminate the effects of gastric emptying, it has been shown that plasma GLP-1 concentrations between healthy lean and obese subjects did not differ (Feinle *et al.* 2002a).

Changes in the release of plasma GIP concentrations in obese, compared with lean individuals is also inconclusive. For example, some studies have shown plasma GIP concentration to be greater during fasting, and in response to meal ingestion, in obese compared with lean (Stubbs 1996; Vilsboll *et al.* 2003), while other studies have found GIP concentrations to be similar (Verdich *et al.* 2001) or reduced (Wikarek *et al.* 2014).

Both fasting and postprandial concentrations of glucagon have been shown to be similar in lean and obese non-diabetic patients in some studies (Gossain *et al.* 1974), but not others (Arafat *et al.* 2013). Postprandial glucagon concentrations were also observed to be higher in obese, non-diabetic participants, compared with obese, type II diabetics (Kozawa *et al.* 2013) In contrast, despite fasting hyperglycaemia, fasting glucagon levels were higher, and were not suppressed, during a glucose infusion in diabetic subjects (Gossain *et al.* 1974).

Fasting insulin concentrations have consistently been shown to be elevated in non-diabetic obese, compared with healthy lean individuals (Prager *et al.* 1987; Korek *et al.* 2013). In addition, the magnitude of the insulin response to both a high-fat and high-carbohydrate meal is augmented in obesity, inducing hyperinsulinaemia (Korek *et al.* 2013). This hyperinsulinaemia has been associated by some (Flanagan *et al.* 2003), but not others (Caixas *et al.* 2002), with the attenuation of the postprandial reduction of ghrelin levels in obese individuals, which may perpetuate hyperphagia, and thus, weight gain, however the mechanisms underlying this remain unclear.

In summary, it appears that both fasting levels, and the release of GI hormones in response to nutrient are perturbed, albeit to varying degrees, in obese, compared with lean, individuals. The differences in the patterns of secretion, or the sensitivity to, GI hormones following meal ingestion may play a role in the attenuated satiety, increased energy intake and changes in glycaemic control that are commonly observed in obesity. These changes in GI 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. While the changes in GI motor and hormone responses to fat in obesity have been explored, evidence for changes in the GI responses to protein is scarce. Despite the known potent effects of protein on appetite and energy intake,

the physiological mechanisms, specifically in the GI tract are not well known. Moreover, the GI responses to protein, and how these differ between lean and obese individuals has not been thoroughly examined. Hence, the physiological mechanisms through which protein exerts its superior effects on appetite, how these mechanisms may be affected by obesity, and their role in modulating weight loss during a high-protein diet, are unclear.

4.7 Concluding remarks

This chapter has reviewed the anatomy of the GI tract and the role of GI factors, and the interactions between these parameters with the regulation of glycaemia and appetite and energy intake, in both lean and obese humans. It is well-known that the modulation of GI hormone function plays a key role in determining postprandial glycaemia, and in combination with GI motility, is involved in modulating appetite and energy intake responses. While the GI responses to fat and carbohydrate are well established, limited research to date has characterised the effects of protein on these GI mechanisms, both in health and obesity.

Examining the GI motor and hormonal responses to protein, and how these influence appetite and energy intake may help to explain the superior appetite-suppressive effects of protein. Importantly, an understanding of the GI mechanisms underlying the effects of protein on appetite and energy intake may aid in the development of targeted nutritional interventions for the treatment and prevention of obesity.

The studies described in subsequent chapters of this thesis (5-9), address the following aims:

i) To evaluate gastric emptying, GI hormone release and/or suppression, glycaemic responses, appetite and energy intake in response to oral protein loads in lean males (**Chapter 6**).

ii) To evaluate the effects of loads of intraduodenal protein on antropyloroduodenal motility, hormone release/suppression, glycaemic responses, appetite and acute energy intake in lean (**Chapter 7**) and obese (**Chapter 8**) males, and

iii) To compare the effects of intraduodenal protein, and lipid, alone and in combination on antropyloroduodenal motility, hormone release and/or suppression, glycaemic responses, appetite and acute energy intake in lean males (**Chapter 9**).

Chapter 5: Subjects And Methodologies

5.1 Introduction

This chapter describes the techniques that were utilised in the studies described in **Chapter 6-9**. All of the techniques are state-of-the-art, and well established in our laboratory, including high resolution manometry for the measurement of APD motility (Heddle *et al.* 1988c), 3-dimensional ultrasound for the measurement of gastric emptying (Gentilcore *et al.* 2006; Vanis *et al.* 2011), radioimmunoassay for the analysis of plasma concentrations of GI and glucoregulatory hormones (Feltrin *et al.* 2004; Feltrin *et al.* 2006), visual analogue scale (VAS) questionnaires for the assessment of appetite perceptions (Parker *et al.* 2004) , and a standardised, cold, buffet-style meal for assessment of acute energy intake (Feltrin *et al.* 2004).

5.2 Subjects

5.2.1 Study participants

For all studies, healthy male subjects, aged 18–60 years, were enrolled. Lean subjects were of normal body weight for their height, with a BMI of 19–25 kg/m^2. Obese subjects, included in the study described in **Chapters 8**, had a BMI of 30.0–35 kg/m^2. Lean and obese 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.

5.2.2 Subject recruitment

Subjects were recruited through advertisement in local newspapers, flyers throughout the Universities of Adelaide and South Australia, Flinders University, the Royal Adelaide

Hospital, and from an existing database of volunteers who had previously participated in research studies within our Department.

5.2.3 Common exclusion criteria

Prior to enrolment in a study, all subjects underwent a screening visit in our laboratory to determine their eligibility for the study. Subjects were questioned to exclude individuals who: had a history of GI surgery or disease, took medication which affected GI motor function, appetite or body weight, had significant illnesses (i.e. diabetes, cardiovascular disease, epilepsy), evidence of drug use or abuse, consumption of > 2 standard alcoholic drinks (20 g alcohol) per day, or > 5 standard alcoholic drinks per week, were smokers, had chronic food allergies or were lactose-intolerant. Healthy, lean subjects were also required to be unrestrained eaters (i.e. score ≤ 12 in the eating restraint component (Factor 1) of the "Three-Factor Eating Questionnaire" (**Appendix I**) (Stunkard and Messick 1985). While the degree of eating restraint was assessed and recorded in the overweight and obese subjects, it was not used as an exclusion, as overweight and obese subjects were expected to have some degree of eating restraint. A 10-mL blood sample was also taken at screening, and subjects excluded if their iron stores were outside the following ranges: *Ferritin 20-300 µg/L; Iron 8-30 µmol/L; Transferrin 2.0-4.0 g/L; Transferrin Saturation 10-55 %.*

Subjects in the study described in **Chapter 8** were also excluded when their fasting blood glucose and/or glycated haemoglobin (HbA1c), which is a marker of long term (3 month) blood glucose control, were outside the following ranges: *Blood glucose ≥ 6.0 mmol/L; HbA1c ≥ 6.0 %.*

5.2.4 Ethics committee approval

All protocols were approved by the Royal Adelaide Hospital Research Ethics Committee. Each subject provided written, informed consent prior to their enrolment in the study and all experiments were carried out in accordance with the Declaration of Helsinki. Each trial was registered on the Australia and New Zealand Clinical Trial Registry, and their registration numbers are provided in their respective chapters. Written, informed consent was obtained from each subject prior to participation in a study. All subjects understood that their participation in the research was voluntary, and that they were free to withdraw from participation in the study at any time. All subjects were offered an honorarium of $15 per hour for participation in the study assessing gastric emptying of protein (**Chapter 6**) and $18 per hour for participation in all studies involving an intraduodenal catheter (**Chapter 7-9**), as approved by the Royal Adelaide Hospital Ethics Committee.

5.3 Assessment of gastrointestinal motor function

5.3.1 Three-dimensional ultrasonography

3D ultrasonography measurements (**Chapter 6**) were performed using a Logiq™ 9 ultrasonography system (GE Healthcare Technologies, Sydney, NSW, Australia) with TruScan Architecture (i.e. in-built magnetically sensed 3D). For 3D positioning and orientation measurement (POM), a transmitter was placed immediately behind, and to the left of the subject, at the level of the stomach, so that the subject was positioned between the transmitter and the ultrasound scanner, and a snap-on sensor attached to a 3.5C broad spectrum 2.5-4 MHz convex transducer (Tefera *et al.* 2002). As the transmitter produces a spatially varying magnetic field, which is easily distorted by ferrous and conductive metals, all metal objects were removed from the subject and from the area immediately between the POM transmitter and sensor (Liao *et al.* 2004).

5.3.1.1 Three-dimensional image acquisition

For 3D data acquisition, 3D sweeps of the total stomach were taken to evaluate total gastric volume. Subjects were instructed to hold their breath at the end of inspiration, not move, while the stomach was scanned using a continuous translational movement along its long axis. Scans started proximally at the left subcostal margin, where the transducer was tilted cranially to image the superior stomach, then moved distally to the gastroduodenal junction to produce transverse sections of the entire stomach (Gilja *et al.* 1997). Total scanning time for each image was ~10 seconds, and two scans were taken at each time point. When a gastric contraction was observed, the acquisition was paused until the contractile wave passed.

5.3.1.2 Image analysis

The raw data, as original scan planes, were copied to CD-ROM and transferred to a Windows workstation. The images were used for 3D reconstructions of the stomach using EchoPAC-3D software® (GE Vingmed Sound, Horten, Norway) (**Figure 5.1**). Total gastric volumes were derived at each time point and expressed as percentages of volumes at t= 0 min (volume immediately following drink consumption) with total gastric volume at t= 0 defined as 100 %. Gastric emptying profiles were constructed, and the time at which 50 % of the meal had emptied from the stomach (50 % emptying time, T50) was derived.

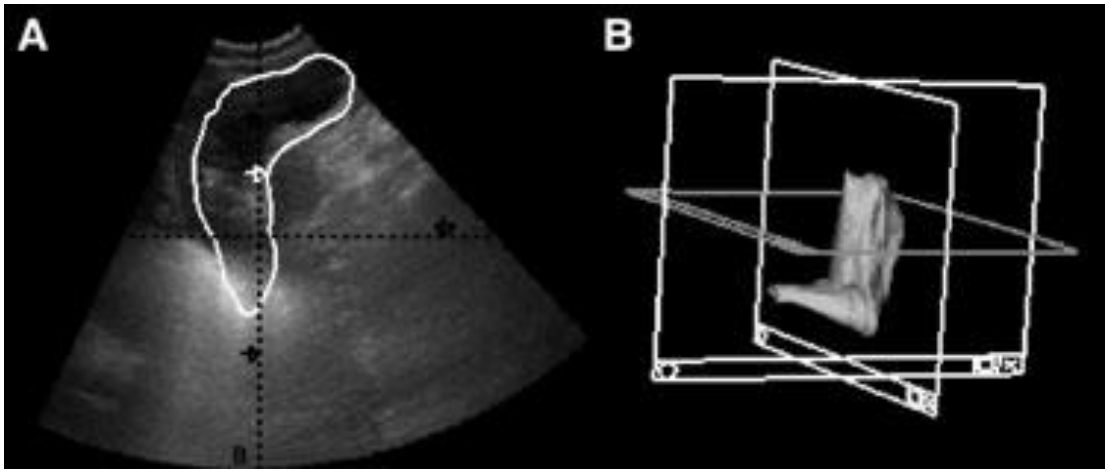

Figure 5.1: Ultrasonic image of the stomach, demonstrating A) region-of-interest; and B) 3D reconstructed volumetric image of the stomach (Gentilcore et al. 2006).

5.3.2 High-resolution manometry

High-resolution perfusion manometry is a well-established technique, used to measure changes in pressure in the GI lumen (Dent 1976; Heddle *et al.* 1988b), and relies on the occurrence of contractions that occlude the lumen around a multi-lumen manometric catheter. The catheter is perfused with degassed water through side-holes, and changes in pressure within regions of interest in the GI tract are transmitted via the water column within the catheter, to external transducers, where they are digitised and relayed to a software program.

5.3.2.1 Catheter design

The silicon rubber manometric catheter (100 cm total length, 3.5 mm outer diameter, Dentsleeve International Ltd, Mui Scientific, Ontario, Canada) consisted of 16 side-holes (0.1 mm in diameter) separated by 1.5 cm intervals. 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 side-hole

(1 mm in diameter) was positioned 11.75 cm distal to the pylorus, and this was used for the administration of the intraduodenal infusions.

5.3.2.2 Nasoduodenal intubation and manometry

Subjects were intubated with the catheter via an anaesthetised nostril (Lignocaine 5 %, Orion Laboratories Pty Ltd, Calcatta, WA, Australia) into the stomach, and the catheter allowed to pass into the duodenum by peristalsis (Heddle *et al.* 1989). The maximum intubation length depended on the height of the individual, but did not exceed 75 cm. A sterile saline-filled reference electrode (20 gauge intravenous cannula) was inserted subcutaneously in the subject's forearm to continuously measure the transmucosal potential difference (TMPD) at the most distal antral (channel 6) and the most proximal duodenal (channel 10) channels to maintain correct positioning of the catheter. The catheter was positioned with the sleeve sensor straddling the pylorus, registered by known TMPDs (channel 6 equal to, or more negative than -20 mV; channel 10 equal to, or more positive than -15 mV), with a difference between the two channels of at least 15 mV (Heddle *et al.* 1988a). All channels were perfused with degassed, distilled water at a rate of 0.15 mL/min, except for the two TMPD channels, which were perfused with degassed 0.9% saline (Heddle *et al.* 1989). 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.

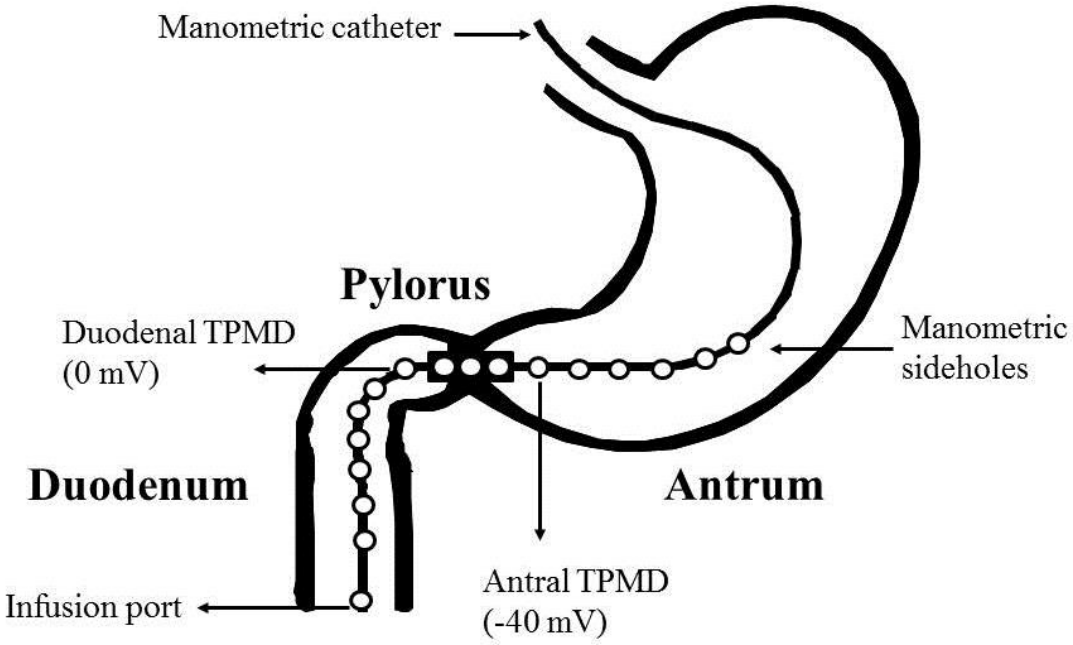

Figure 5.2: Schematic representation of the silicone-rubber manometric catheter used for intraduodenal nutrient infusion, incorporating six antral and seven duodenal side-holes, spaced 1.5 cm apart, a pyloric sleeve sensor and infusion port (Chapters 7-9).

5.3.2.3 Data acquisition and analyses

Manometric pressures were digitised and recorded on a computer-based system running commercially available software (Flexisoft®, Version 3, Oakfield Instruments, A/Prof GS Hebbard, Melbourne, Australia, written in Labview 3.1.1 (National Instruments)) and stored for subsequent analysis. Antropyloroduodenal (APD) pressures were analysed for the i) number and amplitude of antral and duodenal pressure waves (PWs), ii) basal pyloric pressure and iii) number and amplitude of IPPWs. Phasic PWs were defined by an amplitude of ≥ 10 mmHg, with a minimum interval between peaks of 15 seconds for antral and pyloric PWs, and 3 seconds for duodenal waves (Samsom *et al.* 1998). Basal pyloric pressure ("tone", or tonic pyloric pressure) was calculated for each minute by subtracting the pressure (excluding phasic pressures) recorded at the most distal antral channel, from the mean basal pyloric pressure recorded at the sleeve (Heddle *et al.* 1988b) using custom-written software (Prof. A Smout, University Medical Centre, Amsterdam, Netherlands). Numbers and

amplitudes of antral and duodenal pressures were used to calculate antral and duodenal motility indices (MIs) over 60 min, using the following equation: MI = *ln*[(sum of amplitudes x no. phasic PWs) + 1] (Camilleri and Malagelada 1984).

5.4 Evaluation of gastrointestinal and appetite responses to oral and intraduodenal nutrient

Orally administered nutrients were used to assess gastric emptying, intragastric meal distribution, GI hormones, appetite and energy intake. Intraduodenal infusion allowed assessment of GI function, appetite and energy intake without orosensory and gastric influences such as gastric distension and gastric emptying.

5.4.1 Oral protein preloads

Three protein preloads, matched for sensory aspects, were used in the study described in **Chapter 6**; a saline control, and two containing a moderate (30 g), or high (70 g), amount of protein. The preloads were prepared in the Departmental research kitchen on the morning of the study. The base ingredients were distilled water, sodium chloride, lime flavoured low-calorie cordial (Bickford's Australia Pty Ltd, South Australia) and whey protein isolate (ClearPro®, Fonterra Research Centre, Palmerston North, New Zealand). The amount of whey protein was modified to provide the three different loads, and osmolarity of the drinks was matched with the addition of sodium chloride to the solutions. The preloads were served in a wrapped cup with a straw to eliminate visual assessment of the different drinks.

5.4.2 Intraduodenal infusions

In **Chapters 7-9,** intraduodenal infusions were administered using a manometric catheter as described (**5.3.2.1**). All infusion solutions were delivered into the small intestine at a rate of

4 mL/min (3kcal/min) for 90 minutes in **Chapter 9**, so the total volume infused in all study conditions was 360 mL, with a total energy content of 270 kcal. In **Chapters 7 and 8**, different protein loads were infused at a rate of 4 mL/min, for 60 minutes, so the total volume infused was 240 mL.

5.4.2.1 Lipid infusions

A commercially available lipid emulsion, Intralipid® (20 %, 300 mOsm/kg, 2 kcal/mL, Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia), which consists of long-chain triglycerides 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 lipid infusion in **Chapter 9.** Intralipid® was diluted with distilled water to achieve the load required (3 kcal/min), and sodium chloride was added to match the osmolarity of the lipid solutions to the other infusions, as specified. Intralipid® was selected as the lipid emulsion, since the fatty acids comprising it, specifically linoleic, oleic and palmitic acids, resemble fatty acids that are common in the diet. Intralipid® has been used extensively in previous studies by our group, and others, evaluating the effects of lipid on GI function, appetite and energy intake, and is generally well tolerated (Chapman *et al.* 1999; Feinle *et al.* 2000; Pilichiewicz *et al.* 2006; Seimon *et al.* 2009a).

5.4.2.2 Protein infusions

A whey protein hydrolysate, (WPH 821 [18.1 % hydrolysate]; Fonterra Research Centre, Palmerston North, New Zealand) was kindly donated by Fonterra to be used as the protein source in **Chapters 7 and 8,** while a commercially available whey protein hydrolysate (Hydrolysed Whey Protein Isolate DH17 Ultra [18.5 % hydrolysate], MyoPure, Muscle Brand Pty Ltd, NSW, Australia) was used in **Chapter 9**. In all studies, dissolved in distilled

water, and sodium chloride added to match solutions for osmolarity. Accordingly, solutions in **Chapters 7 and 8** had an osmolarity of 680 mOsm/L, and 480 mOsm/L in **Chapter 9**. Whey protein was selected as it is thought to have the most potent appetite-suppressive effects of dietary protein sources (Luhovyy *et al.* 2007). Hydrolysed whey was selected as it is more likely to resemble partially digested protein entering the duodenum after oral ingestion (Silk *et al.* 1985).

5.4.2.3 Lipid :protein combination infusions

Protein stock solutions were prepared by dissolving 28 (1 kcal/min for L2P1 infusion) or 56 (2 kcal/min for L1P2 infusion) g whey protein hydrolysate powder in distilled water, to a volume of 140 or 280 mL, respectively. 105 mL 20% Intralipid, and 87.5 mL 1.8% NaCl solution were added to the P1, and 52.5 mL 20 % Intralipid and 175 mL 1.7% NaCl solution to the P2, stock solutions respectively, to give the L2P1 and L1P2 solutions a total load of 3 kcal/min.

5.5 Assessment of plasma hormone, blood glucose and total amino acid concentrations

For continuous blood sampling, an intravenous cannula was inserted into an antecubital vein. Venous blood samples (10-15 mL) were collected in ice-chilled ethylenediaminetetraacetic acid (EDTA)-treated tubes and centrifuged immediately at 3200 rpm for 15 min at 4◦C to obtain plasma. Plasma samples were then frozen at –80 °C for later analysis. For the studies described in **Chapters 7 and 9**, 4 mL venous blood samples for the measurement of serum insulin were collected in serum-Z tubes containing clotting beads. Radioimmunoassays were used to measure plasma concentrations of ghrelin, CCK, PYY, GLP-1, GIP, and glucagon,

and serum/plasma insulin. Samples from individual subjects were measured in the same run. Intra- and inter-assay coefficients of variation (CVs) are specified within the chapters.

5.5.1 Plasma ghrelin

For the studies described in **Chapters 6 and 7,** plasma ghrelin concentrations (pmol/L) were measured by radioimmunoassay, using a method modified from that previously published (Parker *et al.* 2005). The radiolabel (NEX388) was purchased from Perkin Elmer. Briefly, the sensitivity of the curve was improved by modification to a disequilibrium assay. The standard and samples were incubated with the antibody for 3-4 days prior to incubation with the radiolabel for a further 24 hours at 4^{o}C. The minimum detectable concentration was 11.8 pmol/L.

5.5.2 Plasma peptide tyrosine tyrosine (PYY)

Plasma PYY (pmol/L) 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), thus, the assay measures both PYY(1-36) and PYY(3-36). The antiserum showed <0.001 % cross-reactivity with human pancreatic polypeptide or sulphated 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 $NaPO_4$, 10 mM EDTA, 2 g/L gelatine, 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 minimum detectable concentration was 1.5 pmol/L.

5.5.3 Plasma cholecystokinin (CCK)

Plasma CCK-8 concentrations (pmol/L) were measured after ethanol extraction using an adapted radioimmunoassay (Santangelo *et al.* 1998). Standards were prepared using synthetic sulphated CCK-8 (Sigma-Aldrich, St Louis, MO, USA) and an antibody (C2581, Lot 105H4852, Sigma-Aldrich). Sulphated CCK-8 ^{125}I, labelled with Bolton and Hunter reagent (Perkin Elmer, Boston, MA, USA) was used as tracer. The antibody-bound fraction was separated by the addition of dextran-coated charcoal containing gelatine and the radioactivity determined in the supernatants following centrifugation. The minimum detectable concentration was 1 pmol/L.

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

Plasma GLP-1 concentrations (pmol/L) were determined after ethanol extraction, using a radioimmunoassay kit (GLPIT-36HK, Millipore, Billerica, MA) (Pilichiewicz *et al.* 2007a). The antibody used does not cross-react with glucagon, gastric inhibitory peptide, or other gut or pancreatic peptides, and it measures intact GLP-1$_{(7–36)}$ amide as well as the degraded form, GLP-1$_{(9–36)}$ amide. The minimum detectable concentration was 3 pmol/L.

5.5.5 Plasma glucose-dependent insulinotropic peptide (GIP)

Total GIP (pmol/L) was measured by radioimmunoassay with the use of modifications of a previously published method (Wishart *et al.* 1992). Specifically, the standard curve was prepared in buffer, rather than extracted, charcoal-stripped serum, and the radio-iodinated label was supplied by Perkin Elmer. The minimum detectable concentration was 2 pmol/L.

5.5.6 Plasma glucagon

Plasma glucagon concentrations (pmol/L) were measured by RIA (GL-32K, Millipore, Billerica, MA). The antibody used does not cross-react with insulin, proinsulin, C-peptide, somatostatin, or pancreatic polypeptide and has <0.1% cross-reactivity with oxyntomodulin. The minimum detectable concentration was 5.7 pmol/L.

5.5.7 Plasma/serum insulin

For **Chapters 7 and 9**, insulin concentrations were obtained from serum. For Chapters **6 and 8**, insulin concentrations were obtained from plasma. Serum and plasma insulin concentrations (mU/L) were measured by ELISA (10-1113, Mercodia, Uppsala, Sweden) (Pilichiewicz *et al.* 2007a). The minimum detectable concentration was 1.0 mU/L.

5.5.8 Blood glucose concentrations

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

5.5.9 Total amino acid concentrations

Plasma total amino acid (TAA) concentrations (mmol/L) were analysed in **Chapter 6** using precolumn derivatisation with 6-aminoquinolyl-N hydroxysuccmimidyl carbamate (AQC). The derivatives were then separated and quantified by reversed-phase high-performance liquid chromatography (HPLC). The AAs were detected by fluorescence. Before derivatisation, 100 µl of plasma samples were diluted 1:1 with internal standard solution (Norvaline) and deproteinised by ultra-filtration through a membrane with 10 kDa nominal

molecular weight cut-off (Ultrafree MC with PL-10 membrane, Millipore, MA, USA). AAs contained in the filtrate (100 µl) were labelled using the Waters AccQTag™ chemistry and analysed using a Waters Acquity™ UPLC system (Waters Corporation, MA, USA). The analysis was performed at the Australian Proteome Analysis's Facility established under the Australian Government's National Collaborative Research Infrastructure Strategy (NCRIS). All analyses were performed by the same technician.

5.6 Assessment of appetite and eating behaviour

5.6.1 Visual analogue scale questionnaire

Validated visual analogue scale questionnaires (VAS) are the most commonly used tool to measure subjective appetite perceptions and GI symptoms during or after a feeding challenge (Flint *et al.* 2000; Parker *et al.* 2004). Perceptions of appetite, including hunger, fullness, desire-to-eat and prospective consumption, as well as GI symptoms, including nausea and bloating, were measured. Other perceptions, including drowsiness, tiredness, happiness and anxiety, were included to distract subjects from the main purpose of the questionnaire, but were not evaluated. The VAS comprised of 100 mm horizontal lines with strengths of perceptions anchored at each end, describing the extremes of each sensation (i.e. 0 mm "I am not hungry at all", 100-mm "I am extremely hungry"). Subjects were asked to place a vertical mark along the scale, corresponding with the strength of the sensation they felt at the time point at which the VAS was administered (**Appendix II**). VAS questionnaires were administered at baseline, and at 15 minute intervals throughout the studies.

5.6.2 Buffet meal

A commonly used method for quantifying the amount of food a subject consumes after a specific treatment, meal or preload is *ad libitum* consumption from a buffet meal (Feltrin *et*

al. 2004). The buffet meal contained a variety of food items, as detailed in **Table 5.1**, and the amount of food offered was in excess of what the subject was expected to consume (Feltrin *et al.* 2004). The buffet meal was presented at a fixed time point, immediately after the final ultrasound image was taken (3 hours after the preload) (**Chapter 6**), or immediately after the cessation of intraduodenal infusions (**Chapters 7-9**). Subjects were instructed to consume freely from it until they were comfortably full; the buffet was removed after 30 minutes. All food items were weighed before and after presentation to the subject, and energy consumption (kcal), amount eaten (g) and macronutrient intake of fat, carbohydrate and protein (absolute (g) and % of total) were calculated using commercially available software (Foodworks Version 3.01, Xyris Software (Australia) Pty Ltd, Highgate Hill, QLD, Australia) (Feltrin *et al.* 2004).

Table 5.1: Foods offered in buffet meals

Food items	*Amount served*	*Energy content,*	*Fat*	*Carbohydrate*	*Protein*
	g	*kcal*	*g*	*g*	*g*
Wholemeal bread, 4 slices[a]	125	307.6	4.4	50.0	12.6
White bread, 4 slices[a]	125	304.6	2.5	57.8	10.5
Ham, sliced[b]	100	108.2	3.6	0.0	18.8
Chicken, sliced[c]	100	161.7	7.0	0.0	24.6
Cheese, sliced[d]	100	403.5	33.3	1.0	25.8
Tomato, sliced	100	13.4	0.1	1.9	1.0
Cucumber, sliced	100	10.5	0.1	1.9	0.5
Lettuce	100	6.5	0.1	0.4	0.9
Strawberry yoghurt[e]	200	184.0	3.8	26.9	10.2
Fruit salad[f]	150	89.2	0.1	30.9	0.8
Chocolate custard[g]	150	158.0	5.2	22.7	4.8
Apple	200	100.8	0.2	25.0	0.6
Banana	200	171.0	0.2	39.8	3.4
Orange juice, unsweetened[h]	300	118.2	1.0	25.5	2.2
Iced coffee[i]	500	355.9	8.5	51.5	17.5
Water	600	0.0	0.0	0.0	0.0
Margarine[j]	21	129.5	14.6	0.1	0.1
Mayonnaise[k]	21	77.8	6.8	4.2	0.2
Milky Way®, 1 bar[l]	13	58.2	1.2	9.8	0.4
Total	**3205**	**2758**	**93.5**	**349.3**	**134.7**

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

5.6.3 Percentage compensation

The percent compensation (% compensation) describes the accuracy with which an individual is able to adjust their subsequent energy intake following a preload/infusion to maintain energy balance. % compensation for a given preload/infusion was calculated as the difference between energy intake from the *ad libitum* buffet meal on the control day and in response to the test infusant/meal, divided by the difference in the energy content of the test infusant and the control, multiplied by 100 % (i.e. % compensation = [(EI $_{\text{(buffet; control infusant)}}$ − EI $_{\text{(buffet; test infusant)}}$ / (Energy content $_{\text{(test infusant)}}$ − Energy content $_{\text{(control infusant)}}$] x 100) (Johnson and Birch 1994), so that an index of 100 % represents full compensation of the caloric load of the infusion.

5.6.4 Three-factor eating questionnaire

Since particular eating behaviours may influence the response of individuals to treatments, in terms of energy intake, it was important to identify, and exclude, individuals with restrictive eating behaviour. The Three Factor Eating Questionnaire, devised by Stunkard and Messick (1985) (Stunkard and Messick 1985) assesses three factors of human eating behaviour; i) cognitive restraint of eating, ii) disinhibition of eating, and iii) hunger. The questionnaire contains fifty one items; twenty one of which assess Factor I, sixteen assessing Factor II and Fourteen assessing factor III (see **Appendix I** for scoring and distribution of factors of this questionnaire). The factor used as an exclusion criterion in this thesis is Factor I, which characterises "restrained eating", and describes the tendency of individuals to restrict their food intake as a means of controlling body weight. A score of ≤ 12 for Factor I in the Three Factor Eating Questionnaire was used as a cut-off point – healthy subjects were excluded from participating in these studies if their score was > 12, as this classified them as restrictive

eaters. As described earlier, in obese subjects eating restraint was assessed, but not used as an exclusion, as they were expected to exhibit some degree of eating restraint.

5.7 Statistical analysis

Statistical tests used in each study are described in individual chapters. Data were analysed using commercially available statistical software (SPSS Statistics version 19 (SPSS Inc., Chicago, IL, USA). In accordance with statistical hierarchies, significant effects have been reported as treatment*time interactions, treatment and/or time effects, in that order. Thus, when a treatment or time effect is reported, no treatment*time interaction was observed. In all studies, data are presented as mean values ± standard error of the mean (SEM), and a P value <0.05 was considered statistically significant in all analyses. Relationships between parameters of choice were assessed using linear within-subject correlations (r) where appropriate (Bland and Altman 1995b).

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Overall percentage (%)	30%
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Chapter 10: Conclusions

The studies reported in this thesis have examined key aspects of the inter-related gastrointestinal mechanisms involved in the regulation of glycaemia, appetite and energy intake in response to protein in lean and obese individuals. Three broad aims of the research were: i) to assess gastrointestinal motor and hormone, glycaemic, appetite and energy intake responses, to increasing loads of protein administered both orally and intraduodenally in lean individuals, ii) to determine the comparative effects of equicaloric loads of protein and lipid, and combinations of protein and lipid on gastrointestinal motility, hormone release, glycaemia, appetite and energy intake in healthy, lean individuals and iii) to establish whether obese individuals remain sensitive to the effects of intraduodenal protein, and to explore the comparative effects of protein on gastrointestinal function, blood glucose control, appetite and energy intake in obese, compared with lean, individuals.

The study in **Chapter 6** was carried out to establish the effects of increasing loads of protein, consumed orally, on gastric emptying, gastrointestinal hormone release, appetite and energy intake. We demonstrated that the gastric emptying rate of protein was comparable between 30 g and 70 g loads, when expressed as kilocalories per minute. Furthermore, the study showed that protein empties from the stomach at rates within the range of 1-4 kcal/min, as has been previously established for gastric emptying of fat and carbohydrate. Congruent with this, we observed similar patterns and magnitudes of release of CCK, GLP-1, GIP, and the suppression of ghrelin, in the first ~45 min following both protein loads. However, beyond this, the 70 g load was associated with a sustained GI hormone release, and reduced blood glucose, concomitant with a prolonged exposure to nutrient, compared with the 30 g load. Despite this, energy intake at the buffet meal was similar. Collectively, these data suggest

that the gastric emptying rate of protein is independent of the amount of protein ingested, and importantly, that a threshold amount of protein of ~30 g is sufficient to modify GI hormone, glycaemic and appetitive responses, with no further benefit conferred by higher doses; at least in lean healthy individuals.

Chapter 7 investigated the effects of increasing loads of protein, infused intraduodenally to standardise the rate of delivery to the small intestine, eliminating orosensory and gastric influences, in lean, healthy individuals. The study demonstrated that a 60 min infusion of whey protein had load-dependent effects on the suppression of antral motility, and the stimulation of isolated pyloric pressure waves, plasma CCK and GLP-1 and insulin. We also demonstrated that the highest load of intraduodenal protein, 3 kcal/min (delivering 48 g of protein), reduced blood glucose, while maintaining normoglycaemia. Importantly, we found that whey protein, administered intraduodenally, had very potent effects on the suppression of energy intake, in that the magnitude of the reduction of energy intake at the buffet meal exceeded the caloric content of the infusion. Moreover, when considered in light of the findings from Chapter 6, these data add weight to the notion that ~ 30-48 g of protein is probably optimal to reduce energy intake at the next meal. These findings are of interest for the development of nutrition-based interventions for appetite control and weight loss, in that a moderate amount of protein may be able to be incorporated into a snack, or "pre-load", to reduce subsequent energy intake.

The study in **Chapter 8** explored the hypothesis that, in the obese, gastrointestinal sensitivity to protein remains intact, and obese individuals would have similar responses to intraduodenal protein, compared with lean individuals. We found that, in obese individuals, protein exerted load-dependent effects on GI motor and hormonal function. When the

responses were compared between lean and obese, following equicaloric protein loads (3 kcal/min), GI motor and CCK and GLP-1 responses were similar. Energy intake was only marginally higher following the infusion in obese individuals. Of interest, we observed that blood glucose was reduced by a similar magnitude in both groups; however, the obese group had significantly greater insulin, and a comparatively blunted GIP, response. This may be indicative of early disruption of the insulin-incretin axis in apparently healthy, obese individuals. Whether this may also be accompanied by changes in gastric emptying, and the long-term implications of the elevated insulin responses, require further investigation. In contrast to responses to intraduodenal lipid, which appear to be blunted in the obese, the gastrointestinal tract appears to remain responsive to protein, which highlights a potential mechanism for targeting obesity with diet-based interventions that contain a moderately increased content of high-quality dairy-based protein.

In light of the potent effects of the 3kcal/min protein load observed in **Chapter 7** and the known effects of lipid on gastrointestinal motility and hormone release, **Chapter 9** evaluated the comparative effects of equicaloric (3kcal/min) loads of lipid and protein, and combinations of the two, on gastrointestinal function, appetite and energy intake in healthy, lean individuals. The study established that protein and lipid infused intraduodenally had differential effects on gastrointestinal motor and hormone function. Specifically, lipid more potently stimulated pyloric pressures, CCK and GLP-1, while protein had significantly greater effects on insulin and glucagon release than lipid. Despite this, protein and lipid similarly suppressed energy intake, suggesting that while GI mechanisms appear to play a key role in the appetite-suppressive effects of lipid, additional mechanisms may be involved with respect to protein. These mechanisms require further investigation. Interestingly, protein and lipid administered in 2:1 and 1:2 combinations did not have additive effects on

any of the outcomes measured. This suggests it is highly probable that there is a critical threshold, or rate of delivery, of each nutrient in the small intestine, to elicit an effect.

Some considerations must be taken into account when interpreting the data presented in this thesis. Only males were studied as they have been reported to be are more sensitive to dietary manipulation than females. Whey protein was selected as the source of protein in all studies, as the literature suggests it has more potent effects on appetite and energy intake than other protein sources, and it is one of the most commonly consumed proteins in Australia and other Western countries; however, the findings cannot necessarily be extended to other protein sources. Finally, while we sought to characterise a number of physiological responses to protein in healthy lean humans, we would expect modest differences in some GI mechanisms between obese compared with lean humans, as was demonstrated in Chapter 8. Future directions should consider the effects of different sources of protein on GI motor and hormone function, glycaemia and appetite, and whether these sources, alone or incorporated (in different ratios) into a mixed-nutrient meal, might be useful in modulating energy intake. The potential for whey protein for modifying blood glucose control, particularly in individuals with type 2 diabetes, also warrants further investigation. Finally, if nutrition-based therapies to modulate appetite for the purposes of weight management are to be developed further, future studies should consider the differences in GI function between lean and overweight individuals, and the implications for this on appetite and energy intake.

Collectively, the data have provided novel insights into the roles that gastrointestinal motor and hormone responses to dietary protein play in the regulation of blood glucose, appetite and energy intake in healthy lean and obese subjects. These observations provide substantial and new information about a number of gastrointestinal mechanisms through which high-protein

diets may be effective in maintaining satiety, and thus reducing body weight over the long-term. The data represent an important contribution to the current knowledge of basic appetite and glucoregulatory physiology, and have established several key gastrointestinal mechanisms underlying the effects of protein in the diet. As such, the findings in this thesis provide a substantial foundation for the development of more tailored dietary interventions as potential treatments for obesity and obesity-related diseases.

Appendices

Appendix I: 3 factor Eating Questionnaire and scoring

Factor 1 (**F1**) = eating restraint
Factor 2 (**F2**) = hunger
Factor 3 (**F3**) = disinhibition

Questions under each factor are denoted at the end of the question; answers that score in that factor are circled.

Appendix I

Name: Date:

Part 1: 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. **(F2)**

(T) (F)

2. I usually eat too much at social occasions, like parties and picnics. **(F2)**

(T) (F)

3. I am usually so hungry that I eat more than three times a day. **(F3)**

(T) (F)

4. When I have eaten my quota of calories/fat, I am usually good about not eating any more. **(F1)**

(T) (F)

5. Dieting is so hard for me because I just get too hungry. **(F3)**

(T) (F)

6. I deliberately take small helpings as a means of controlling my weight. **(F1)**

(T) (F)

7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry. (**F2**)

(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. **(F3)**

(T) (F)

9. When I feel anxious, I find myself eating. **(F2)**

(T) (F)

10. Life is too short to worry about dieting. **(F1)**

(T) (F)

11. Since my weight goes up and down, I have gone on reducing diets more than once. **(F2)**

(T) (F)

12. I often feel so hungry that I just have to eat something. **(F3)**

(T) (F)

13. When I am with someone who is overeating, I usually overeat too. **(F2)**

(T) (F)

14. I have a pretty good idea of the number of calories/grams of fat in common foods. **(F1)**

(T) (F)

15. Sometimes when I start eating, I just can't seem to stop. **(F2)**

(T) (F)

16. It is not difficult for me to leave something on my plate. **(F2)**

(T) (F)

17. At certain times of the day, I get hungry because I have got used to eating then. **(F3)**

(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. **(F1)**

(T) (F)

19. Being with someone who is eating often makes me hungry enough to eat also. **(F3)**

(T) (F)

20. When I feel blue, I often overeat. **(F2)**
(T) (F)

21. I enjoy eating too much to spoil it by counting calories, counting grams of fat or watching my weight. **(F1)**
(T) (F)

22. When I see a real delicacy, I often get so hungry that I have to eat right away. **(F3)**
(T) (F)

23. I often stop eating when I am not really full as a conscious means of limiting the amount I eat. **(F1)**
(T) (F)

24. I get so hungry that my stomach often seems like a bottomless pit. **(F3)**
(T) (F)

25. My weight has hardly changed at all in the last ten years. **(F2)**
(T) (F)

26. I am always hungry, so it is hard for me to stop eating before I finish the food on my plate. **(F3)**
(T) (F)

27. When I feel lonely, I console myself by eating. **(F2)**
(T) (F)

28. I consciously hold back at meals in order to not gain weight. (**F1**)
(T) (F)

29. I sometimes get very hungry late in the evening or at night (**F3**)
(T) (F)

30. I eat anything I want anytime I want. (**F1**)
(T) (F)

31. Without even thinking about it, I take a long time to eat. (**F2**)
(T) (F)

32. I count calories/grams of fat as a conscious means of controlling my weight. (**F1**)
(T) (F)

33. I do not eat some foods because they make me fat. (**F1**)
(T) (F)

34. I am always hungry enough to eat at any time. (**F3**)
(T) (F)

35. I pay a great deal of attention to changes in my figure. (**F1**)
(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. (**F2**)
(T) (F)

Part 2: Each question in this section is followed by a number of options. After reading each question carefully, choose one option which most applies to you, and circle the appropriate answer.

37. How often are you dieting in a conscious effort to control your weight?

1	2	(3)	(4)
rarely	sometimes	usually	always

38. Would a weight fluctuation of 3 kg affect the way you live your life?

1	2	(3)	(4)
not at all	slightly	moderately	very much

39. How often do you feel hungry?

1	2	(3)	(4)
only at meal times	sometimes between meals	often between meals	almost always

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

1	2	(3)	(4)
never	rarely	often	always

41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

1	2	(3)	(4)
easy	slightly difficult	moderately difficult	very difficult

42. How conscious are you of what you are eating?

1	2	(3)	(4)
not at all	slightly	moderately	extremely

43. How frequently do you avoid 'buying large' on tempting foods?

1	2	(3)	(4)
almost 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'

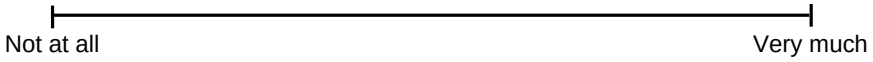
6 constantly limit food intake, never 'give in'

Appendix II: Visual Analog Scale

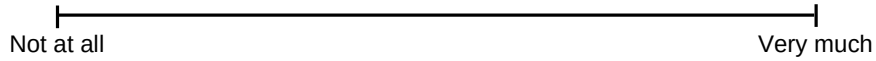
Name (Initials): **Visit:** **Time:**

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

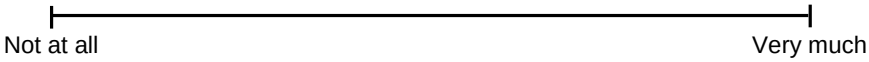
I feel nauseated



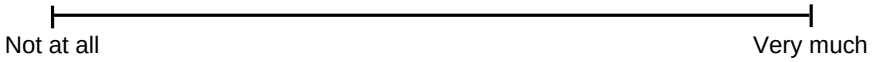
I feel drowsy



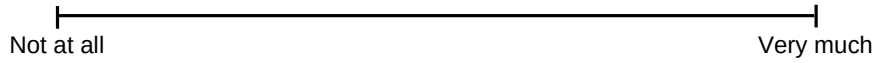
I feel bloated



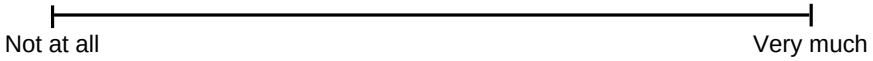
I feel anxious



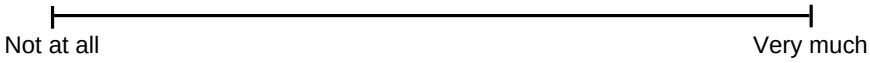
I feel hungry



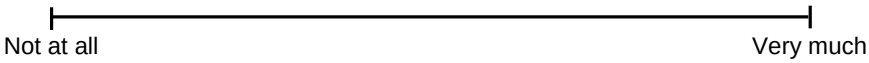
I feel full



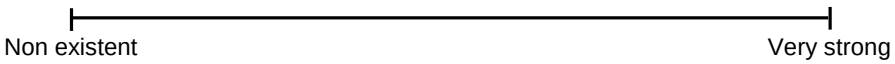
I feel happy



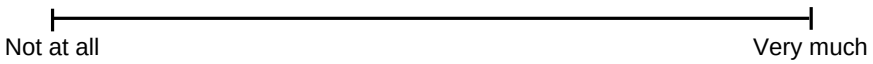
I feel energetic



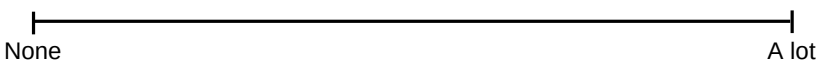
How strong is your desire to eat?



I feel comfortable



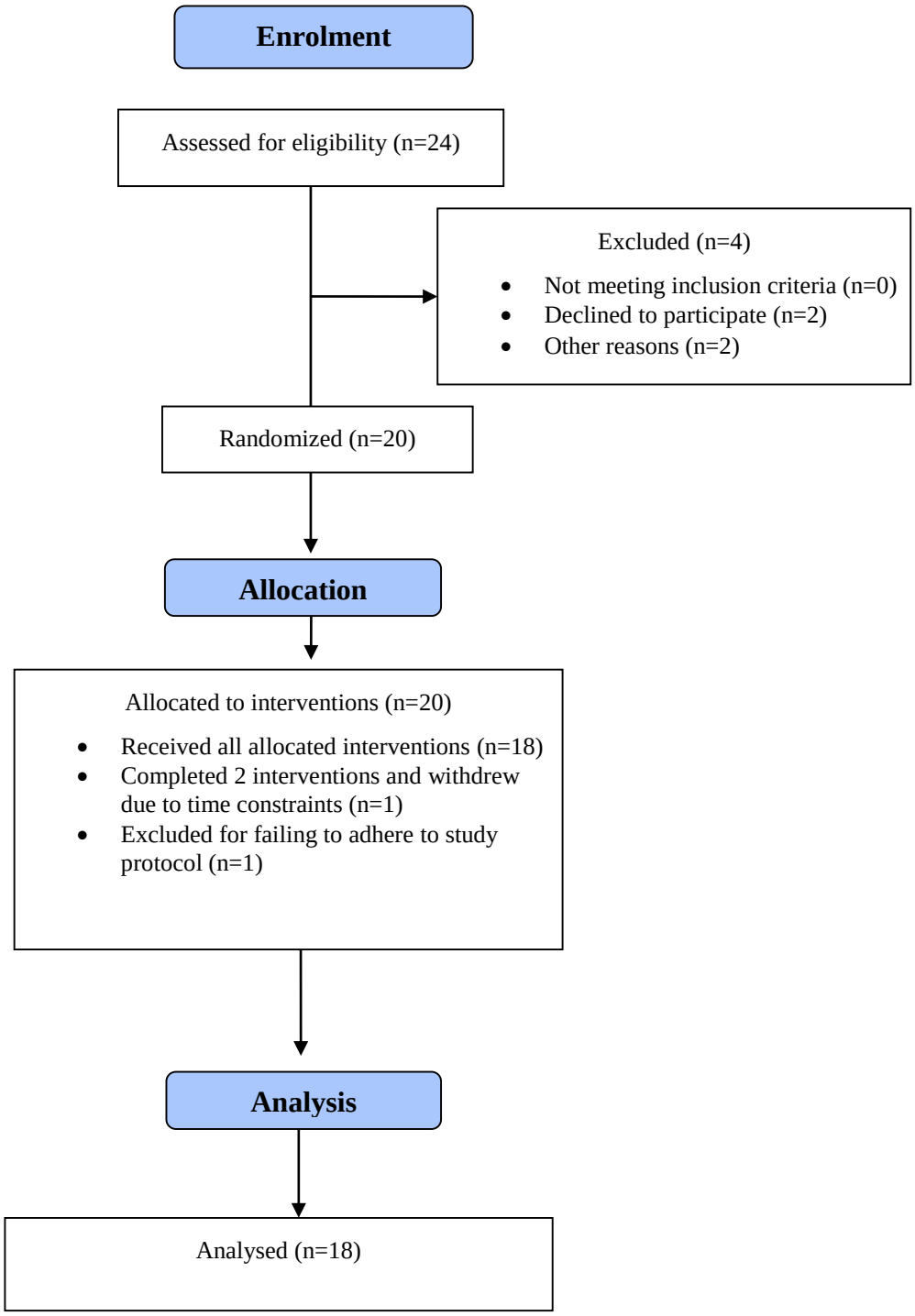
How much food do you think you can eat?



Appendix III: Characteristics of study participants in Chapter 6

ID	Age	Weight (kg)	Height (m)	BMI	Waist (cm)	Restraint score	Exercise:
1	20	67.2	1.87	19.2	74	1	no planned exercise (asthma)
2	22	59.3	1.71	20.2	70	5	weights 3x/wk., running 3x/wk.
3	22	70.8	1.70	24.4	85	7	bike 1 hr 4x wk.
4	20	85.5	1.85	24.9	86	9	No planned exercise
5	21	77.2	1.83	23.0	85	10	Gym 30 min 4x/wk.; soccer 1 hr/wk.
6	18	68.2	1.81	20.8	75	11	powerlifting 1.5 hr 2x/wk.; parkour
7	30	62	1.75	20.2	84	3	no planned exercise
8	20	90.2	1.90	24.9	89	8	football 1 hr 2x/wk.; weights 3x/wk., basketball 2 x/wk.
9	28	81.8	1.81	24.9	92	2	no planned exercise
10	29	67.5	1.80	20.8	87	5	no planned exercise
11	31	66.3	1.80	20.4	72	1	cycle 1 hr 1-2 x/wk.
12	34	71.5	1.83	21.3	75	6	Pilates 1 hr 1-2x/wk.; weights 45 min 3x/wk.
13	37	67.4	1.64	25.0	82	10	no planned exercise
14	25	71	1.89	19.8	75	2	cycling 1 hr 5x/week
15	29	59.3	1.75	19.3	75	4	no exercise
16	19	69	1.75	22.5	70	2	gym 1 hr 3x/week
17	26	62	1.73	20.7	82	8	no exercise
18	23	63	1.75	20.5	72	4	no exercise
19	22	68.3	1.85	19.9	74	2	walk 1 hr daily; tennis 1.5 hr/wk.
20	18	55	1.72	18.5	68	2	No reported

Appendix IV: Flow diagram of participants in Chapter 6



Appendix V: Appetite ratings measured by VAS in Chapter 7

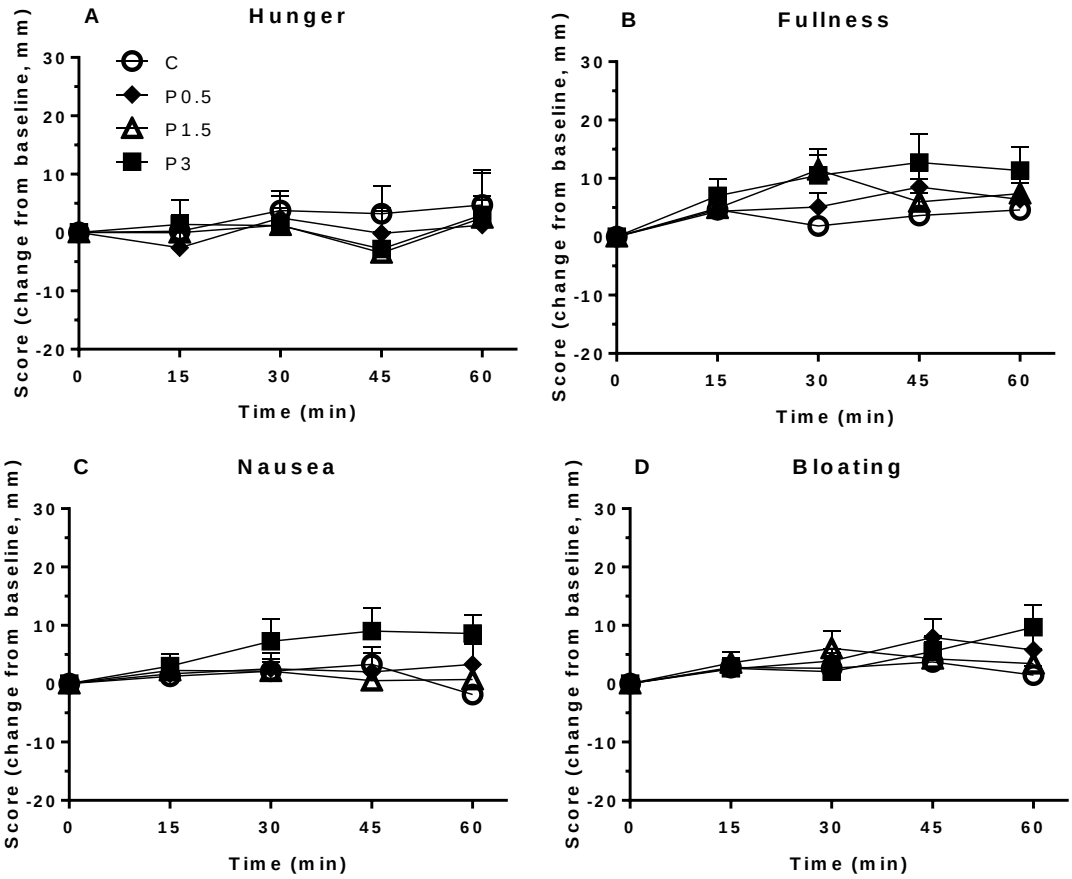
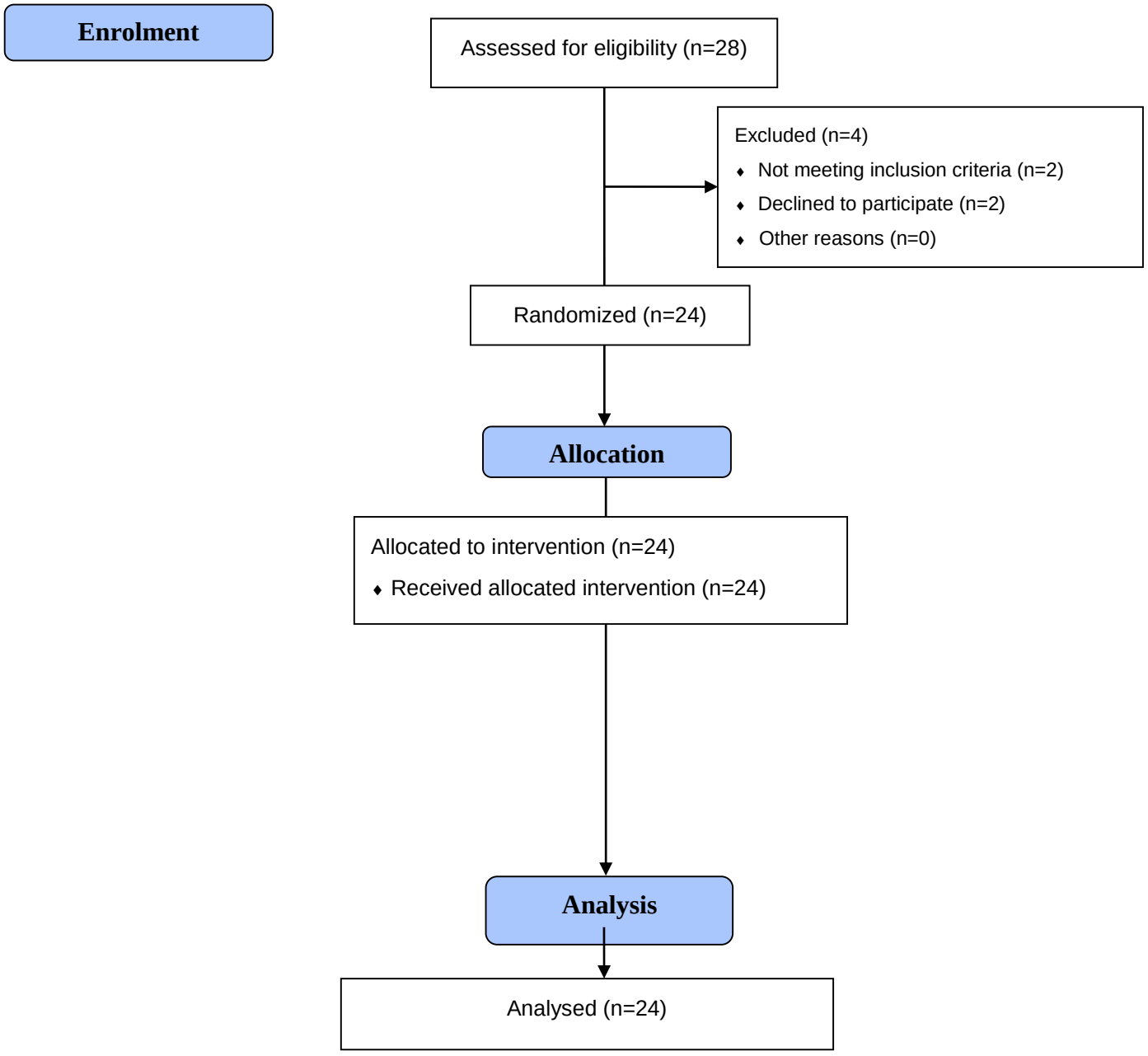
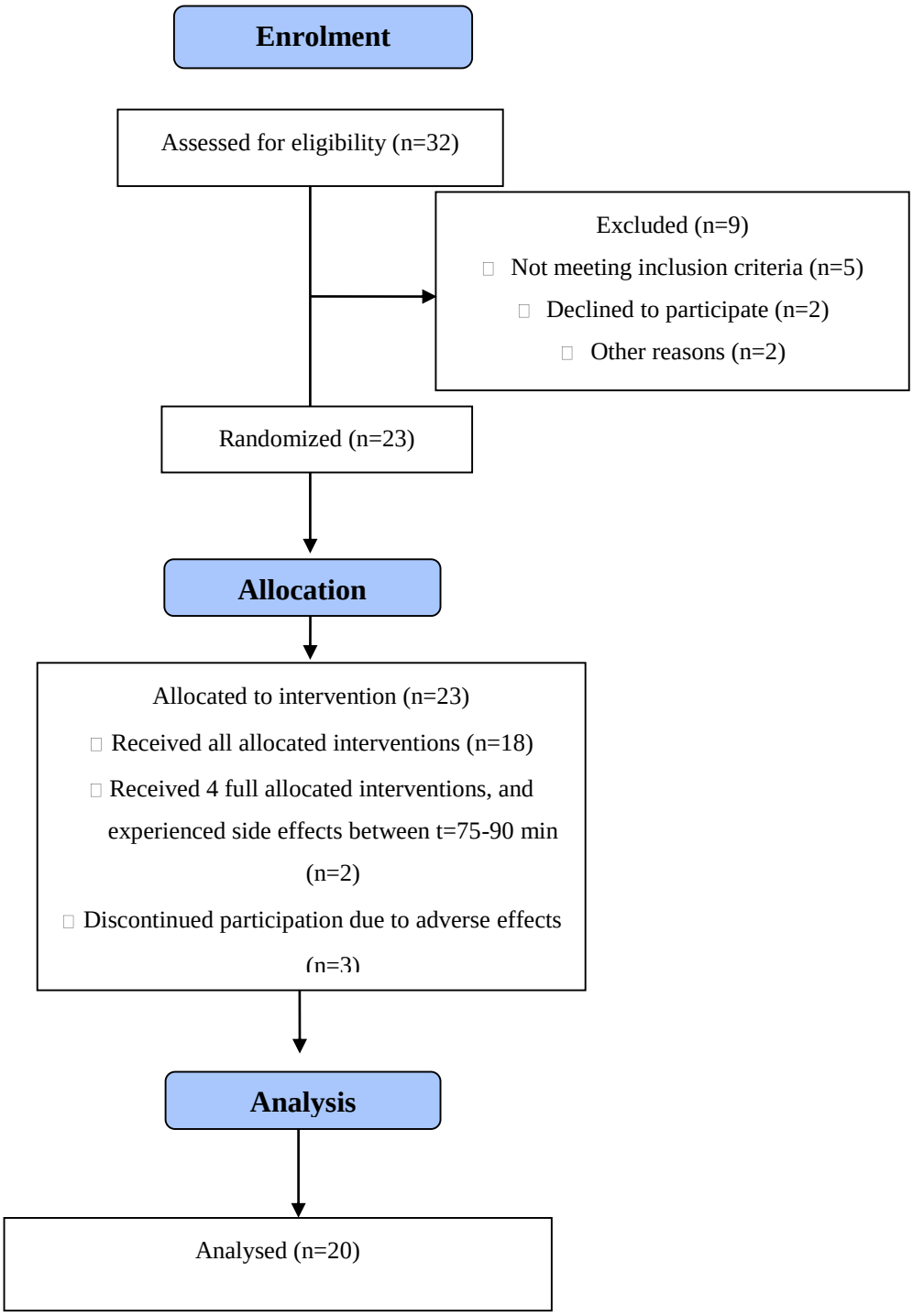


Figure legend: Hunger (**A**), fullness (**B**), nausea (**C**), bloating (**D**) during 60-min infusions of 0.5 kcal/min (P0.5), 1.5 kcal/min (P1.5), 3 kcal/min (P3) or saline control (C). Data are means ± SEM; n=16, treatment*time interaction analysed using repeated measures ANOVA; Significant effects determined by post-hoc comparisons using Bonferroni's correction.

Appendix VI: Flow diagram of participants in Chapter 8



Appendix VII: Flow diagram of participants in Chapter 9



Appendix VIII: Appetite ratings measured by VAS in Chapter 9

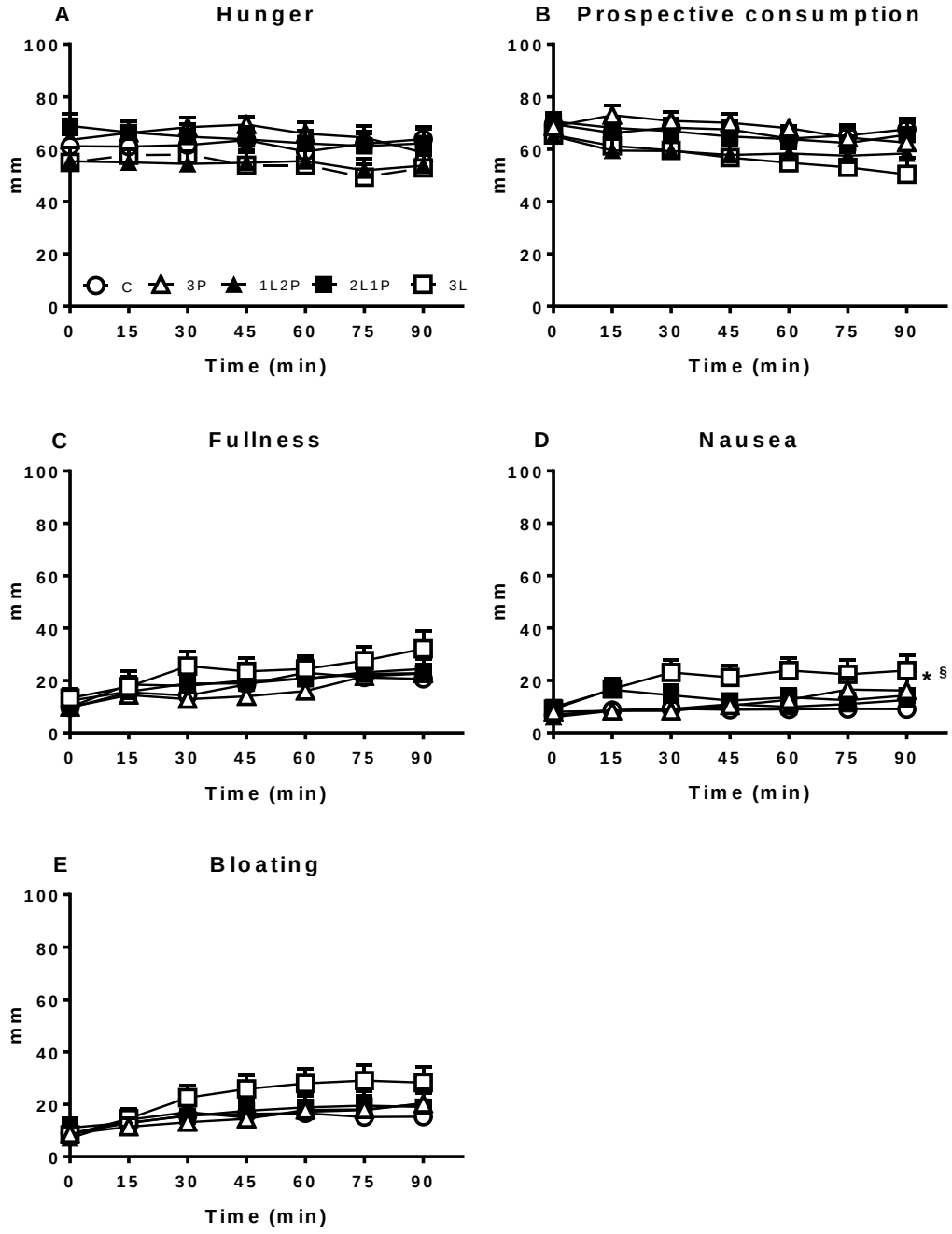


Figure legend: (**A**) Hunger, (**B**) prospective consumption, (**C**) fullness, (**D**) nausea and (**E**) bloating ratings measured using visual analog scales (VAS) during 90-minute, 3 kcal/min intraduodenal infusions of a saline control (C), whey protein hydrolysate (P3), Intralipid and whey protein hydrolysate in 1:2 (L1P2) and 2:1 (L2P1) ratios, or pure lipid (L3). Data are means ± SEM; n=20. Treatment effect by one-way ANOVA for AUCs of the VAS profiles, significant differences ($P<0.05$) determined by post-hoc comparisons using Bonferroni's correction. (A) treatment effect ($P=0.09$); (B) treatment effect ($P<0.05$), no significant differences in post-hoc paired comparisons; (C) treatment effect ($P=0.076$); (D) treatment effect ($P<0.01$), (*) significantly different vs. C and L1P2 ($P<0.05$), (§) trend for significant difference vs. P3 ($P=0.07$); (E) treatment effect ($P=0.07$).

References

Adam, T.C. and Westerterp-Plantenga, M.S. (2005). "Nutrient-stimulated GLP-1 release in normal-weight men and women." Horm Metab Res **37**(2): 111-117.

Adan, R.A. (2013). "Mechanisms underlying current and future anti-obesity drugs." Trends Neurosci **36**(2): 133-140.

Adrian, T.E., Long, R.G., Fuessl, H.S., *et al.* (1985). "Plasma peptide YY (PYY) in dumping syndrome." Dig Dis Sci **30**(12): 1145-1148.

Ahren, B. (2013). "Incretin dysfunction in type 2 diabetes: clinical impact and future perspectives." Diabetes Metab **39**(3): 195-201.

Akhavan, T., Luhovyy, B.L., Brown, P.H., *et al.* (2010). "Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults." Am J Clin Nutr **91**(4): 966-975.

Aller, E.E., Larsen, T.M., Claus, H., *et al.* (2014). "Weight loss maintenance in overweight subjects on ad libitum diets with high or low protein content and glycemic index: the DIOGENES trial 12-month results." Int J Obes (Lond).

Allison, D.B., Gadde, K.M., Garvey, W.T., *et al.* (2012). "Controlled-release phentermine/topiramate in severely obese adults: a randomized controlled trial (EQUIP)." Obesity (Silver Spring) **20**(2): 330-342.

Almiron-Roig, E., Palla, L., Guest, K., *et al.* (2013). "Factors that determine energy compensation: a systematic review of preload studies." Nutr Rev **71**(7): 458-473.

Anderson, G.H., Tecimer, S.N., Shah, D., *et al.* (2004). "Protein source, quantity, and time of consumption determine the effect of proteins on short-term food intake in young men." J Nutr **134**(11): 3011-3015.

Anderson, S.A., Tews, J.K. and Harper, A.E. (1990). "Dietary branched-chain amino acids and protein selection by rats." J Nutr **120**(1): 52-63.

Andrews, J.M., Rayner, C.K., Doran, S., *et al.* (1998). "Physiological changes in blood glucose affect appetite and pyloric motility during intraduodenal lipid infusion." Am J Physiol **275**(4 Pt 1): G797-804.

Appel, L.J., Sacks, F.M., Carey, V.J., *et al.* (2005). "Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial." JAMA **294**(19): 2455-2464.

Arafat, A.M., Weickert, M.O., Adamidou, A., *et al.* (2013). "The impact of insulin-independent, glucagon-induced suppression of total ghrelin on satiety in obesity and type 1 diabetes mellitus." J Clin Endocrinol Metab **98**(10): 4133-4142.

Ariyasu, H., Takaya, K., Tagami, T., *et al.* (2001). "Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans." J Clin Endocrinol Metab **86**(10): 4753-4758.

Asakawa, A., Inui, A., Kaga, T., *et al.* (2003). "Antagonism of ghrelin receptor reduces food intake and body weight gain in mice." Gut **52**(7): 947-952.

Astbury, N.M., Stevenson, E.J., Morris, P., *et al.* (2010). "Dose-response effect of a whey protein preload on within-day energy intake in lean subjects." Br J Nutr **104**(12): 1858-1867.

Astrup, A. and Raben, A. (1995). "Carbohydrate and obesity." Int J Obes Relat Metab Disord **19 Suppl 5**: S27-37.

Australian Bureau of Statistics (2009). National Health Survey: a summary of results 2007-08. ABS. Canberra.

Azpiroz, F. and Malagelada, J.R. (1987a). "Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis." Gastroenterology **92**(4): 934-943.

Azpiroz, F. and Malagelada, J.R. (1987b). "Importance of vagal input in maintaining gastric tone in the dog." J Physiol **384**: 511-524.

Baba, N.H., Sawaya, S., Torbay, N., *et al.* (1999). "High protein vs high carbohydrate hypoenergetic diet for the treatment of obese hyperinsulinemic subjects." Int J Obes Relat Metab Disord **23**(11): 1202-1206.

Bado, A., Levasseur, S., Attoub, S., *et al.* (1998). "The stomach is a source of leptin." Nature **394**(6695): 790-793.

Ballantyne, G.H. (2006). "Peptide YY(1-36) and peptide YY(3-36): Part I. Distribution, release and actions." Obes Surg **16**(5): 651-658.

Banks, W.A., Jaspan, J.B., Huang, W., *et al.* (1997). "Transport of insulin across the blood-brain barrier: saturability at euglycemic doses of insulin." Peptides **18**(9): 1423-1429.

Barkeling, B., Rossner, S. and Bjorvell, H. (1990). "Effects of a high-protein meal (meat) and a high-carbohydrate meal (vegetarian) on satiety measured by automated computerized monitoring of subsequent food intake, motivation to eat and food preferences." Int J Obes **14**(9): 743-751.

Barrows, K. and Snook, J.T. (1987). "Effect of a high-protein, very-low-calorie diet on resting metabolism, thyroid hormones, and energy expenditure of obese middle-aged women." Am J Clin Nutr **45**(2): 391-398.

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., *et al.* (2003). "Inhibition of food intake in obese subjects by peptide YY3-36." N Engl J Med **349**(10): 941-948.

Batterham, R.L., Cowley, M.A., Small, C.J., *et al.* (2002). "Gut hormone PYY(3-36) physiologically inhibits food intake." Nature **418**(6898): 650-654.

Batterham, R.L., Heffron, H., Kapoor, S., *et al.* (2006). "Critical role for peptide YY in protein-mediated satiation and body-weight regulation." Cell Metab **4**(3): 223-233.

Baum, J., Simons, B.E., Jr., Unger, R.H., *et al.* (1962). "Localization of glucagon in the alpha cells in the pancreatic islet by immunofluorescent technics." Diabetes **11**: 371-374.

Beckman, L.M., Beckman, T.R. and Earthman, C.P. (2010). "Changes in gastrointestinal hormones and leptin after Roux-en-Y gastric bypass procedure: a review." J Am Diet Assoc **110**(4): 571-584.

Beglinger, C., Degen, L., Matzinger, D., *et al.* (2001). "Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans." Am J Physiol Regul Integr Comp Physiol **280**(4): R1149-1154.

Beglinger, C., Hildebrand, P., Adler, G., *et al.* (1992). "Postprandial control of gallbladder contraction and exocrine pancreatic secretion in man." Eur J Clin Invest **22**(12): 827-834.

Bell, G.I., Santerre, R.F. and Mullenbach, G.T. (1983). "Hamster preproglucagon contains the sequence of glucagon and two related peptides." Nature **302**(5910): 716-718.

Belza, A., Ritz, C., Sorensen, M.Q., *et al.* (2013). "Contribution of gastroenteropancreatic appetite hormones to protein-induced satiety." Am J Clin Nutr **97**(5): 980-989.

Bhattacharya, S.K., Andrews, K., Beveridge, R., *et al.* (2014). "Discovery of PF-5190457, a Potent, Selective, and Orally Bioavailable Ghrelin Receptor Inverse Agonist Clinical Candidate." ACS Med Chem Lett **5**(5): 474-479.

Bilsborough, S. and Mann, N. (2006). "A review of issues of dietary protein intake in humans." Int J Sport Nutr Exerc Metab **16**(2): 129-152.

Bjornsson, E.S., Urbanavicius, V., Eliasson, B., *et al.* (1995). "Effects of insulin and beta-adrenergic blockade on the migrating motor complex in humans." Scand J Gastroenterol **30**(3): 219-224.

Black, D.W., Goldstein, R.B. and Mason, E.E. (1992). "Prevalence of mental disorder in 88 morbidly obese bariatric clinic patients." Am J Psychiatry **149**(2): 227-234.

Blaine, B.E., Rodman, J. and Newman, J.M. (2007). "Weight loss treatment and psychological well-being: a review and meta-analysis." J Health Psychol **12**(1): 66-82.

Bland, J.M. and Altman, D.G. (1995a). "Calculating correlation coefficients with repeated observations: Part 1--Correlation within subjects." BMJ **310**(6977): 446.

Bland, J.M. and Altman, D.G. (1995b). "Calculating correlation coefficients with repeated observations: Part 2--Correlation between subjects." BMJ **310**(6980): 633.

Blom, W.A., Lluch, A., Stafleu, A., *et al.* (2006). "Effect of a high-protein breakfast on the postprandial ghrelin response." Am J Clin Nutr **83**(2): 211-220.

Blundell, J.E., Lawton, C.L., Cotton, J.R., *et al.* (1996). "Control of human appetite: implications for the intake of dietary fat." Annu Rev Nutr **16**: 285-319.

Blundell, J.E., Lawton, C.L. and Hill, A.J. (1993). "Mechanisms of appetite control and their abnormalities in obese patients." Horm Res **39 Suppl 3**: 72-76.

Boirie, Y., Dangin, M., Gachon, P., *et al.* (1997). "Slow and fast dietary proteins differently modulate postprandial protein accretion." Proceedings of the National Academy of Sciences of the United States of America **94**(26): 14930-14935.

Bosse, J.D. and Dixon, B.M. (2012). "Dietary protein in weight management: a review proposing protein spread and change theories." Nutr Metab (Lond) **9**(1): 81.

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**(8): 2913-2919.

Bowen, J., Noakes, M. and Clifton, P.M. (2007). "Appetite hormones and energy intake in obese men after consumption of fructose, glucose and whey protein beverages." Int J Obes (Lond) **31**(11): 1696-1703.

Boyd, K.A., O'Donovan, D.G., Doran, S., *et al.* (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**(2): G188-196.

Brand-Miller, J.C., Colagiuri, S. and Gan, S.T. (2000). "Insulin sensitivity predicts glycemia after a protein load." Metabolism **49**(1): 1-5.

Brandle, E., Sieberth, H.G. and Hautmann, R.E. (1996). "Effect of chronic dietary protein intake on the renal function in healthy subjects." Eur J Clin Nutr **50**(11): 734-740.

Bray, G.A. (2008). "Lifestyle and pharmacological approaches to weight loss: efficacy and safety." J Clin Endocrinol Metab **93**(11 Suppl 1): S81-88.

Brener, W., Hendrix, T.R. and McHugh, P.R. (1983). "Regulation of the gastric emptying of glucose." Gastroenterology **85**(1): 76-82.

Brennan, I.M., Feltrin, K.L., Horowitz, M., *et al.* (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**(6): R1477-1485.

Brennan, I.M., Feltrin, K.L., Nair, N.S., *et al.* (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**(3): G602-610.

Brennan, I.M., Little, T.J., Feltrin, K.L., *et al.* (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**(6): E1487-1494.

Brennan, I.M., Luscombe-Marsh, N.D., Seimon, R.V., *et al.* (2012). "Effects of fat, protein, and carbohydrate and protein load on appetite, plasma cholecystokinin, peptide YY,

and ghrelin, and energy intake in lean and obese men." Am J Physiol Gastrointest Liver Physiol **303**(1): G129-140.

Brennan, I.M., Otto, B., Feltrin, K.L., *et al.* (2007). "Intravenous CCK-8, but not GLP-1, suppresses ghrelin and stimulates PYY release in healthy men." Peptides **28**(3): 607-611.

Brinkworth, G.D., Buckley, J.D., Noakes, M., *et al.* (2010). "Renal function following long-term weight loss in individuals with abdominal obesity on a very-low-carbohydrate diet vs high-carbohydrate diet." J Am Diet Assoc **110**(4): 633-638.

Brinkworth, G.D., Noakes, M., Keogh, J.B., *et al.* (2004a). "Long-term effects of a high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulinemic subjects." Int J Obes Relat Metab Disord **28**(5): 661-670.

Brinkworth, G.D., Noakes, M., Parker, B., *et al.* (2004b). "Long-term effects of advice to consume a high-protein, low-fat diet, rather than a conventional weight-loss diet, in obese adults with type 2 diabetes: one-year follow-up of a randomised trial." Diabetologia **47**(10): 1677-1686.

Broglio, F., Arvat, E., Benso, A., *et al.* (2001). "Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans." J Clin Endocrinol Metab **86**(10): 5083-5086.

Bromer, W.W., Sinn, L.G., Staub, A., *et al.* (1957). "The amino acid sequence of glucagon." Diabetes **6**(3): 234-238.

Buchholz, V., Berkenstadt, H., Goitein, D., *et al.* (2013). "Gastric emptying is not prolonged in obese patients." Surg Obes Relat Dis **9**(5): 714-717.

Burks, T.F., Galligan, J.J., Porreca, F., *et al.* (1985). "Regulation of gastric emptying." Fed Proc **44**(14): 2897-2901.

Burton, P.R. and Brown, W.A. (2011). "The mechanism of weight loss with laparoscopic adjustable gastric banding: induction of satiety not restriction." Int J Obes (Lond) **35 Suppl 3**: S26-30.

Burton, P.R., Brown, W.A., Laurie, C., *et al.* (2010). "Criteria for assessing esophageal motility in laparoscopic adjustable gastric band patients: the importance of the lower esophageal contractile segment." Obes Surg **20**(3): 316-325.

Butler, R.N., Davies, M., Gehling, N.J., *et al.* (1981). "The effect preloads of amino acid on shortterm satiety." Am J Clin Nutr **34**(10): 2045-2047.

Caixas, A., Bashore, C., Nash, W., *et al.* (2002). "Insulin, unlike food intake, does not suppress ghrelin in human subjects." J Clin Endocrinol Metab **87**(4): 1902.

Calbet, J.A. and MacLean, D.A. (1997). "Role of caloric content on gastric emptying in humans." J Physiol **498 (Pt 2)**(2): 553-559.

Calbet, J.A. and MacLean, D.A. (2002). "Plasma glucagon and insulin responses depend on the rate of appearance of amino acids after ingestion of different protein solutions in humans." J Nutr **132**(8): 2174-2182.

Callahan, H.S., Cummings, D.E., Pepe, M.S., *et al.* (2004). "Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans." J Clin Endocrinol Metab **89**(3): 1319-1324.

Camilleri, M. and Malagelada, J.R. (1984). "Abnormal intestinal motility in diabetics with the gastroparesis syndrome." Eur J Clin Invest **14**(6): 420-427.

Cao, J.J., Johnson, L.K. and Hunt, J.R. (2011). "A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption or formation in postmenopausal women." J Nutr **141**(3): 391-397.

Cardoso-Junior, A., Coelho, L.G., Savassi-Rocha, P.R., *et al.* (2007). "Gastric emptying of solids and semi-solids in morbidly obese and non-obese subjects: an assessment using the 13C-octanoic acid and 13C-acetic acid breath tests." Obes Surg **17**(2): 236-241.

Carr, R.D., Larsen, M.O., Winzell, M.S., *et al.* (2008). "Incretin and islet hormonal responses to fat and protein ingestion in healthy men." Am J Physiol Endocrinol Metab **295**(4): E779-784.

Chaikomin, R., Jones, K.L., Feinle-Bisset, C., *et al.* (2009). "Effects of intraluminal local anesthetic on upper gastrointestinal motor, sensory, and peptide hormone responses to intraduodenal glucose." Eur J Gastroenterol Hepatol **21**(3): 258-265.

Chaikomin, R., Wu, K.L., Doran, S., *et al.* (2008). "Effects of mid-jejunal compared to duodenal glucose infusion on peptide hormone release and appetite in healthy men." Regul Pept **150**(1-3): 38-42.

Chang, J., Wu, T., Greenfield, J.R., *et al.* (2013). "Effects of intraduodenal glutamine on incretin hormone and insulin release, the glycemic response to an intraduodenal glucose infusion, and antropyloroduodenal motility in health and type 2 diabetes." Diabetes Care **36**(8): 2262-2265.

Chapman, I.M., Goble, E.A., Wittert, G.A., *et al.* (1999). "Effects of small-intestinal fat and carbohydrate infusions on appetite and food intake in obese and nonobese men." Am J Clin Nutr **69**(1): 6-12.

Chapman, I.M., Goble, E.A., Wittert, G.A., *et al.* (1998). "Effect of intravenous glucose and euglycemic insulin infusions on short-term appetite and food intake." Am J Physiol **274**(3 Pt 2): R596-603.

Chaudhari, N., Landin, A.M. and Roper, S.D. (2000). "A metabotropic glutamate receptor variant functions as a taste receptor." Nat Neurosci **3**(2): 113-119.

Chauveau, P. and Aparicio, M. (2011). "Benefits in nutritional interventions in patients with CKD stage 3-4." J Ren Nutr **21**(1): 20-22.

Chernish, S.M., Brunelle, R.R., Rosenak, B.D., *et al.* (1978). "Comparison of the effects of glucagon and atropine sulfate on gastric emptying." Am J Gastroenterol **70**(6): 581-586.

Chia, C.W., Carlson, O.D., Kim, W., *et al.* (2009). "Exogenous glucose-dependent insulinotropic polypeptide worsens post prandial hyperglycemia in type 2 diabetes." Diabetes **58**(6): 1342-1349.

Claus, T.H., Park, C.R. and Pilkis, S.J. (1983). Glucagon and gluconeogenesis. Glucagon I. L. PJ. Berlin, Springer-Verlag**:** 315-360.

Clegg, A.J., Colquitt, J., Sidhu, M.K., *et al.* (2002). "The clinical effectiveness and cost-effectiveness of surgery for people with morbid obesity: a systematic review and economic evaluation." Health Technol Assess **6**(12): 1-153.

Clifton, P.M., Keogh, J.B. and Noakes, M. (2008). "Long-term effects of a high-protein weight-loss diet." Am J Clin Nutr **87**(1): 23-29.

Code, C.F. and Marlett, J.A. (1975). "The interdigestive myo-electric complex of the stomach and small bowel of dogs." J Physiol **246**(2): 289-309.

Colagiuri, S., Lee, C.M., Colagiuri, R., *et al.* (2010). "The cost of overweight and obesity in Australia." Med J Aust **192**(5): 260-264.

Collier, G. and O'Dea, K. (1983). "The effect of coingestion of fat on the glucose, insulin, and gastric inhibitory polypeptide responses to carbohydrate and protein." Am J Clin Nutr **37**(6): 941-944.

Collins, P.J., Horowitz, M., Cook, D.J., *et al.* (1983). "Gastric emptying in normal subjects--a reproducible technique using a single scintillation camera and computer system." Gut **24**(12): 1117-1125.

Collins, P.J., Houghton, L.A., Read, N.W., *et al.* (1991). "Role of the proximal and distal stomach in mixed solid and liquid meal emptying." Gut **32**(6): 615-619.

Connolly, H.M., Crary, J.L., McGoon, M.D., *et al.* (1997). "Valvular heart disease associated with fenfluramine-phentermine." N Engl J Med **337**(9): 581-588.

Cook, C.G., Andrews, J.M., Jones, K.L., *et al.* (1997). "Effects of small intestinal nutrient infusion on appetite and pyloric motility are modified by age." Am J Physiol **273**(2 Pt 2): R755-761.

Corp, E.S., Melville, L.D., Greenberg, D., *et al.* (1990). "Effect of fourth ventricular neuropeptide Y and peptide YY on ingestive and other behaviors." Am J Physiol **259**(2 Pt 2): R317-323.

Cottam, D.R., Atkinson, J., Anderson, A., *et al.* (2006). "A case-controlled matched-pair cohort study of laparoscopic Roux-en-Y gastric bypass and Lap-Band patients in a single US center with three-year follow-up." Obes Surg **16**(5): 534-540.

Crawley, J.N. and Beinfeld, M.C. (1983). "Rapid development of tolerance to the behavioural actions of cholecystokinin." Nature **302**(5910): 703-706.

Creutzfeldt, W. (1979). "The incretin concept today." Diabetologia **16**(2): 75-85.

Crovetti, R., Porrini, M., Santangelo, A., *et al.* (1998). "The influence of thermic effect of food on satiety." Eur J Clin Nutr **52**(7): 482-488.

Cukier, K., Pilichiewicz, A.N., Chaikomin, R., *et al.* (2008). "Effect of small intestinal glucose load on plasma ghrelin in healthy men." Am J Physiol Regul Integr Comp Physiol **295**(2): R459-462.

Cullen, J.J. and Kelly, K.A. (1993). "Gastric motor physiology and pathophysiology." Surg Clin North Am **73**(6): 1145-1160.

Cummings, D.E. (2012). "Metabolic surgery for type 2 diabetes." Nat Med **18**(5): 656-658.

Cummings, D.E., Frayo, R.S., Marmonier, C., *et al.* (2004). "Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues." Am J Physiol Endocrinol Metab **287**(2): E297-304.

Cummings, D.E. and Overduin, J. (2007). "Gastrointestinal regulation of food intake." J Clin Invest **117**(1): 13-23.

Cummings, D.E., Purnell, J.Q., Frayo, R.S., *et al.* (2001). "A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans." Diabetes **50**(8): 1714-1719.

Cummings, D.E., Weigle, D.S., Frayo, R.S., *et al.* (2002). "Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery." N Engl J Med **346**(21): 1623-1630.

Cunningham, K.M., Daly, J., Horowitz, M., *et al.* (1991a). "Gastrointestinal adaptation to diets of differing fat composition in human volunteers." Gut **32**(5): 483-486.

Cunningham, K.M., Horowitz, M. and Read, N.W. (1991b). "The effect of short-term dietary supplementation with glucose on gastric emptying in humans." Br J Nutr **65**(1): 15-19.

Cuomo, R. and Sarnelli, G. (2004). "Food intake and gastrointestinal motility. A complex interplay." Nutr Metab Cardiovasc Dis **14**(4): 173-179.

Curhan, G.C., Willett, W.C., Rimm, E.B., *et al.* (1993). "A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones." N Engl J Med **328**(12): 833-838.

Curioni, C.C. and Lourenco, P.M. (2005). "Long-term weight loss after diet and exercise: a systematic review." Int J Obes (Lond) **29**(10): 1168-1174.

D'Alessio, D.A., Kahn, S.E., Leusner, C.R., *et al.* (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**(5): 2263-2266.

Darling, A.L., Millward, D.J., Torgerson, D.J., *et al.* (2009). "Dietary protein and bone health: a systematic review and meta-analysis." Am J Clin Nutr **90**(6): 1674-1692.

Date, Y., Kojima, M., Hosoda, H., *et al.* (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**(11): 4255-4261.

Dawson-Hughes, B. and Harris, S.S. (2002). "Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women." Am J Clin Nutr **75**(4): 773-779.

de Castro, J.M. (2004). "The time of day of food intake influences overall intake in humans." Journal of Nutrition **134**(1): 104-111.

de Graaf, C., Blom, W.A., Smeets, P.A., *et al.* (2004). "Biomarkers of satiation and satiety." Am J Clin Nutr **79**(6): 946-961.

de Jong, J.R., van Ramshorst, B., Gooszen, H.G., *et al.* (2009). "Weight loss after laparoscopic adjustable gastric banding is not caused by altered gastric emptying." Obes Surg **19**(3): 287-292.

de Zwaan, M. (2001). "Binge eating disorder and obesity." Int J Obes Relat Metab Disord **25 Suppl 1**: S51-55.

Deacon, C.F. (2004). "Circulation and degradation of GIP and GLP-1." Horm Metab Res **36**(11-12): 761-765.

Degen, L., Drewe, J., Piccoli, F., *et al.* (2007). "Effect of CCK-1 receptor blockade on ghrelin and PYY secretion in men." Am J Physiol Regul Integr Comp Physiol **292**(4): R1391-1399.

Degen, L., Oesch, S., Casanova, M., *et al.* (2005). "Effect of peptide YY3-36 on food intake in humans." Gastroenterology **129**(5): 1430-1436.

Delgado-Aros, S., Cremonini, F., Castillo, J.E., *et al.* (2004). "Independent influences of body mass and gastric volumes on satiation in humans." Gastroenterology **126**(2): 432-440.

Delgado-Aros, S., Kim, D.Y., Burton, D.D., *et al.* (2002). "Effect of GLP-1 on gastric volume, emptying, maximum volume ingested, and postprandial symptoms in humans." Am J Physiol Gastrointest Liver Physiol **282**(3): G424-431.

Dent, J. (1976). "A new technique for continuous sphincter pressure measurement." Gastroenterology **71**(2): 263-267.

Dirksen, C., Jorgensen, N.B., Bojsen-Moller, K.N., *et al.* (2013). "Gut hormones, early dumping and resting energy expenditure in patients with good and poor weight loss response after Roux-en-Y gastric bypass." Int J Obes (Lond).

Dixit, V.D., Schaffer, E.M., Pyle, R.S., *et al.* (2004). "Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells." J Clin Invest **114**(1): 57-66.

Dixon, A.F., Dixon, J.B. and O'Brien, P.E. (2005). "Laparoscopic adjustable gastric banding induces prolonged satiety: a randomized blind crossover study." J Clin Endocrinol Metab **90**(2): 813-819.

Dooley, C.P., Di Lorenzo, C. and Valenzuela, J.E. (1992). "Variability of migrating motor complex in humans." Dig Dis Sci **37**(5): 723-728.

Dornonville de la Cour, C., Lindstrom, E., Norlen, P., *et al.* (2004). "Ghrelin stimulates gastric emptying but is without effect on acid secretion and gastric endocrine cells." Regul Pept **120**(1-3): 23-32.

Douglas, S.M., Ortinau, L.C., Hoertel, H.A., *et al.* (2013). "Low, moderate, or high protein yogurt snacks on appetite control and subsequent eating in healthy women." Appetite **60**(1): 117-122.

Drewnowski, A. and Specter, S.E. (2004). "Poverty and obesity: the role of energy density and energy costs." Am J Clin Nutr **79**(1): 6-16.

Drucker, D.J. (2006a). "The biology of incretin hormones." Cell Metab **3**(3): 153-165.

Drucker, D.J. (2006b). "Enhancing the action of incretin hormones: a new whey forward?" Endocrinology **147**(7): 3171-3172.

Duca, F.A., Sakar, Y. and Covasa, M. (2013). "The modulatory role of high fat feeding on gastrointestinal signals in obesity." J Nutr Biochem **24**(10): 1663-1677.

Due, A., Toubro, S., Skov, A.R., *et al.* (2004). "Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: a randomised 1-year trial." Int J Obes Relat Metab Disord **28**(10): 1283-1290.

Dupre, J., Ross, S.A., Watson, D., *et al.* (1973). "Stimulation of insulin secretion by gastric inhibitory polypeptide in man." J Clin Endocrinol Metab **37**(5): 826-828.

Ebert, R. and Creutzfeldt, W. (1980). "Reversal of impaired GIP and insulin secretion in patients with pancreatogenic steatorrhea following enzyme substitution." Diabetologia **19**(3): 198-204.

Edelbroek, M., Sun, W.M., Horowitz, M., *et al.* (1994). "Stereospecific effects of intraduodenal tryptophan on pyloric and duodenal motility in humans." Scand J Gastroenterol **29**(12): 1088-1095.

Edholm, T., Degerblad, M., Gryback, P., *et al.* (2010). "Differential incretin effects of GIP and GLP-1 on gastric emptying, appetite, and insulin-glucose homeostasis." Neurogastroenterol Motil **22**(11): 1191-1200, e1315.

Eisenstein, J., Roberts, S.B., Dallal, G., *et al.* (2002). "High-protein weight-loss diets: are they safe and do they work? A review of the experimental and epidemiologic data." Nutr Rev **60**(7 Pt 1): 189-200.

Ekblad, E. and Sundler, F. (2002). "Distribution of pancreatic polypeptide and peptide YY." Peptides **23**(2): 251-261.

Elder, K.A. and Wolfe, B.M. (2007). "Bariatric surgery: a review of procedures and outcomes." Gastroenterology **132**(6): 2253-2271.

Eliasson, B., Bjornsson, E., Urbanavicius, V., *et al.* (1995). "Hyperinsulinaemia impairs gastrointestinal motility and slows carbohydrate absorption." Diabetologia **38**(1): 79-85.

Elliott, R.M., Morgan, L.M., Tredger, J.A., *et al.* (1993). "Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns." J Endocrinol **138**(1): 159-166.

English, P.J., Ghatei, M.A., Malik, I.A., *et al.* (2002). "Food fails to suppress ghrelin levels in obese humans." J Clin Endocrinol Metab **87**(6): 2984.

Erdmann, J., Lippl, F. and Schusdziarra, V. (2003). "Differential effect of protein and fat on plasma ghrelin levels in man." Regul Pept **116**(1-3): 101-107.

Erdmann, J., Topsch, R., Lippl, F., *et al.* (2004). "Postprandial response of plasma ghrelin levels to various test meals in relation to food intake, plasma insulin, and glucose." J Clin Endocrinol Metab **89**(6): 3048-3054.

Eysselein, V.E., Eberlein, G.A., Hesse, W.H., *et al.* (1990). "Molecular variants of cholecystokinin after endogenous stimulation in humans: a time study." Am J Physiol **258**(6 Pt 1): G951-957.

Farnsworth, E., Luscombe, N.D., Noakes, M., *et al.* (2003). "Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women." Am J Clin Nutr **78**(1): 31-39.

Feinle-Bisset, C. and Azpiroz, F. (2013). "Dietary and lifestyle factors in functional dyspepsia." Nat Rev Gastroenterol Hepatol.

Feinle, C., Chapman, I.M., Wishart, J., *et al.* (2002a). "Plasma glucagon-like peptide-1 (GLP-1) responses to duodenal fat and glucose infusions in lean and obese men." Peptides **23**(8): 1491-1495.

Feinle, C., Christen, M., Grundy, D., *et al.* (2002b). "Effects of duodenal fat, protein or mixed-nutrient infusions on epigastric sensations during sustained gastric distension in healthy humans." Neurogastroenterol Motil **14**(2): 205-213.

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**(5): 1379-1385.

Feinle, C., Grundy, D., Otto, B., *et al.* (2000). "Relationship between increasing duodenal lipid doses, gastric perception, and plasma hormone levels in humans." Am J Physiol Regul Integr Comp Physiol **278**(5): R1217-1223.

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 **273**(3 Pt 1): G721-726.

Feinle, C., Meier, O., Otto, B., *et al.* (2001a). "Role of duodenal lipid and cholecystokinin A receptors in the pathophysiology of functional dyspepsia." Gut **48**(3): 347-355.

Feinle, C., O'Donovan, D., Doran, S., *et al.* (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**(5): G798-807.

Feinle, C., O'Donovan, D. and Horowitz, M. (2002c). "Carbohydrate and satiety." Nutr Rev **60**(6): 155-169.

Feinle, C., Rades, T., Otto, B., *et al.* (2001b). "Fat digestion modulates gastrointestinal sensations induced by gastric distention and duodenal lipid in humans." Gastroenterology **120**(5): 1100-1107.

Feinle, C. and Read, N.W. (1996). "Ondansetron reduces nausea induced by gastroduodenal stimulation without changing gastric motility." Am J Physiol **271**(4 Pt 1): G591-597.

Feltrin, K.L., Little, T.J., Meyer, J.H., *et al.* (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**(Pt 2): 767-777.

Feltrin, K.L., Little, T.J., Meyer, J.H., *et al.* (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**(3): R524-533.

Feltrin, K.L., Patterson, M., Ghatei, M.A., *et al.* (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**(7): 1638-1643.

Fernstrom, J.D., Wurtman, R.J., Hammarstrom-Wiklund, B., *et al.* (1979). "Diurnal variations in plasma concentrations of tryptophan, tryosine, and other neutral amino acids: effect of dietary protein intake." Am J Clin Nutr **32**(9): 1912-1922.

Fieseler, P., Bridenbaugh, S., Nustede, R., *et al.* (1995a). "Physiological augmentation of amino acid-induced insulin secretion by GIP and GLP-I but not by CCK-8." Am J Physiol **268**(5 Pt 1): E949-955.

Fieseler, P., Bridenbaugh, S., Nustede, R., *et al.* (1995b). "Physiological augmentation of amino acid-induced insulin secretion by GIP and GLP-I but not by CCK-8." Am J Physiol Endocrinol Metab **268**(5 Pt 1): E949-955.

Flanagan, D.E., Evans, M.L., Monsod, T.P., *et al.* (2003). "The influence of insulin on circulating ghrelin." Am J Physiol Endocrinol Metab **284**(2): E313-316.

Flint, A., Raben, A., Astrup, A., *et al.* (1998). "Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans." J Clin Invest **101**(3): 515-520.

Flint, A., Raben, A., Blundell, J.E., *et al.* (2000). "Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies." International Journal of Obesity & Related Metabolic Disorders **24**(1): 38.

Flint, A., Raben, A., Ersboll, A.K., *et al.* (2001). "The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity." Int J Obes Relat Metab Disord **25**(6): 781-792.

Floyd, J.C., Jr., Fajans, S.S., Conn, J.W., *et al.* (1966a). "Insulin secretion in response to protein ingestion." J Clin Invest **45**(9): 1479-1486.

Floyd, J.C., Jr., Fajans, S.S., Conn, J.W., *et al.* (1966b). "Stimulation of insulin secretion by amino acids." J Clin Invest **45**(9): 1487-1502.

Foltz, M., Ansems, P., Schwarz, J., *et al.* (2008). "Protein hydrolysates induce CCK release from enteroendocrine cells and act as partial agonists of the CCK1 receptor." J Agric Food Chem **56**(3): 837-843.

Fraser, R., Fuller, J., Horowitz, M., *et al.* (1991). "Effect of insulin-induced hypoglycaemia on antral, pyloric and duodenal motility in fasting subjects." Clin Sci (Lond) **81**(2): 281-285.

Frassetto, L.A., Todd, K.M., Morris, R.C., Jr., *et al.* (1998). "Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents." Am J Clin Nutr **68**(3): 576-583.

French, S.J., Murray, B., Rumsey, R.D., *et al.* (1993). "Preliminary studies on the gastrointestinal responses to fatty meals in obese people." Int J Obes Relat Metab Disord **17**(5): 295-300.

Frid, A.H., Nilsson, M., Holst, J.J., *et al.* (2005). "Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects." Am J Clin Nutr **82**(1): 69-75.

Fried, M., Erlacher, U., Schwizer, W., *et al.* (1991). "Role of cholecystokinin in the regulation of gastric emptying and pancreatic enzyme secretion in humans. Studies with the cholecystokinin-receptor antagonist loxiglumide." Gastroenterology **101**(2): 503-511.

Friedman, A.N., Ogden, L.G., Foster, G.D., *et al.* (2012). "Comparative effects of low-carbohydrate high-protein versus low-fat diets on the kidney." Clin J Am Soc Nephrol **7**(7): 1103-1111.

Fu-Cheng, X., Anini, Y., Chariot, J., *et al.* (1997). "Mechanisms of peptide YY release induced by an intraduodenal meal in rats: neural regulation by proximal gut." Pflugers Arch **433**(5): 571-579.

Furst, S., Riba, P., Friedmann, T., *et al.* (2005). "Peripheral versus central antinociceptive actions of 6-amino acid-substituted derivatives of 14-O-methyloxymorphone in acute and inflammatory pain in the rat." J Pharmacol Exp Ther **312**(2): 609-618.

Gallagher, T.K., Baird, A.W. and Winter, D.C. (2009). "Constitutive basal and stimulated human small bowel contractility is enhanced in obesity." Ann Surg Innov Res **3**: 4.

Gannon, M.C. and Nuttall, F.Q. (2010). "Amino acid ingestion and glucose metabolism--a review." IUBMB Life **62**(9): 660-668.

Gannon, M.C., Nuttall, F.Q., Neil, B.J., *et al.* (1988). "The insulin and glucose responses to meals of glucose plus various proteins in type II diabetic subjects." Metabolism **37**(11): 1081-1088.

Gardner, C.D., Kiazand, A., Alhassan, S., *et al.* (2007). "Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among

overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial." JAMA **297**(9): 969-977.

Gautier, J.F., Choukem, S.P. and Girard, J. (2008). "Physiology of incretins (GIP and GLP-1) and abnormalities in type 2 diabetes." Diabetes Metab **34 Suppl 2**: S65-72.

Geary, N., Kissileff, H.R., Pi-Sunyer, F.X., *et al.* (1992). "Individual, but not simultaneous, glucagon and cholecystokinin infusions inhibit feeding in men." Am J Physiol **262**(6 Pt 2): R975-980.

Geary, N., Smith, G.P. and Gibbs, J. (1986). "Pancreatic glucagon and bombesin inhibit meal size in ventromedial hypothalamus-lesioned rats." Regul Pept **15**(3): 261-268.

Geliebter, A. (1988). "Gastric distension and gastric capacity in relation to food intake in humans." Physiol Behav **44**(4-5): 665-668.

Geliebter, A., Melton, P.M., McCray, R.S., *et al.* (1992). "Gastric capacity, gastric emptying, and test-meal intake in normal and bulimic women." Am J Clin Nutr **56**(4): 656-661.

Geliebter, A., Westreich, S. and Gage, D. (1988). "Gastric distention by balloon and test-meal intake in obese and lean subjects." Am J Clin Nutr **48**(3): 592-594.

Geliebter, A., Westreich, S., Pierson, R.N., Jr., *et al.* (1986). "Extra-abdominal pressure alters food intake, intragastric pressure, and gastric emptying rate." Am J Physiol **250**(4 Pt 2): R549-552.

Gentilcore, D., Hausken, T., Horowitz, M., *et al.* (2006). "Measurements of gastric emptying of low- and high-nutrient liquids using 3D ultrasonography and scintigraphy in healthy subjects." Neurogastroenterol Motil **18**(12): 1062-1068.

Gibbs, J., Young, R.C. and Smith, G.P. (1973). "Cholecystokinin elicits satiety in rats with open gastric fistulas." Nature **245**(5424): 323-325.

Gielkens, H.A., Penning, C., van dan Biggelaar, A., *et al.* (1999). "Effect of i.v. amino acids on satiety in humans." JPEN J Parenter Enteral Nutr **23**(2): 56-60.

Gielkens, H.A., Verkijk, M., Frolich, M., *et al.* (1997). "Is the effect of acute hyperglycaemia on interdigestive antroduodenal motility and small-bowel transit mediated by insulin?" Eur J Clin Invest **27**(8): 703-710.

Gilja, O.H., Detmer, P.R., Jong, J.M., *et al.* (1997). "Intragastric distribution and gastric emptying assessed by three-dimensional ultrasonography." Gastroenterology **113**(1): 38-49.

Gill, R.S., Birch, D.W., Shi, X., *et al.* (2010). "Sleeve gastrectomy and type 2 diabetes mellitus: a systematic review." Surg Obes Relat Dis **6**(6): 707-713.

Glasbrenner, B., Pieramico, O., Brecht-Krauss, D., *et al.* (1993). "Gastric emptying of solids and liquids in obesity." Clin Investig **71**(7): 542-546.

Goetze, O., Steingoetter, A., Menne, D., *et al.* (2007). "The effect of macronutrients on gastric volume responses and gastric emptying in humans: A magnetic resonance imaging study." Am J Physiol Gastrointest Liver Physiol **292**(1): G11-17.

Gosby, A.K., Conigrave, A.D., Lau, N.S., *et al.* (2011). "Testing protein leverage in lean humans: a randomised controlled experimental study." PLoS One **6**(10): e25929.

Gosby, A.K., Conigrave, A.D., Raubenheimer, D., *et al.* (2014). "Protein leverage and energy intake." Obes Rev **15**(3): 183-191.

Gossain, V.V., Matute, M.L. and Kalkhoff, R.K. (1974). "Relative influence of obesity and diabetes on plasma alpha-cell glucagon." J Clin Endocrinol Metab **38**(2): 238-243.

Grandt, D., Schimiczek, M., Beglinger, C., *et al.* (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**(2): 151-159.

Greenfield, J.R., Farooqi, I.S., Keogh, J.M., *et al.* (2009). "Oral glutamine increases circulating glucagon-like peptide 1, glucagon, and insulin concentrations in lean, obese, and type 2 diabetic subjects." Am J Clin Nutr **89**(1): 106-113.

Gregersen, H., Rittig, S., Kraglund, K., *et al.* (1988). "The effects of glucagon and glucagon-(1-21)-peptide on antroduodenal motility in healthy volunteers." Scand J Gastroenterol Suppl **152**: 42-47.

Griffioen-Roose, S., Mars, M., Siebelink, E., *et al.* (2012). "Protein status elicits compensatory changes in food intake and food preferences." Am J Clin Nutr **95**(1): 32-38.

Griffith, P.S., Birch, D.W., Sharma, A.M., *et al.* (2012). "Managing complications associated with laparoscopic Roux-en-Y gastric bypass for morbid obesity." Can J Surg **55**(5): 329-336.

Groger, G., Unger, A., Holst, J.J., *et al.* (1997). "Ileal carbohydrates inhibit cholinergically stimulated exocrine pancreatic secretion in humans." Int J Pancreatol **22**(1): 23-29.

Gryback, P., Naslund, E., Hellstrom, P.M., *et al.* (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**(12): 1562-1567.

Guh, D.P., Zhang, W., Bansback, N., *et al.* (2009). "The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis." BMC Public Health **9**: 88.

Gunnarsson, P.T., Winzell, M.S., Deacon, C.F., *et al.* (2006). "Glucose-induced incretin hormone release and inactivation are differently modulated by oral fat and protein in mice." Endocrinology **147**(7): 3173-3180.

Gutniak, M., Orskov, C., Holst, J.J., *et al.* (1992). "Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus." N Engl J Med **326**(20): 1316-1322.

Gutzwiller, J.P., Drewe, J., Goke, B., *et al.* (1999). "Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2." Am J Physiol **276**(5 Pt 2): R1541-1544.

Haby, M.M., Markwick, A., Peeters, A., *et al.* (2012). "Future predictions of body mass index and overweight prevalence in Australia, 2005-2025." Health Promot Int **27**(2): 250-260.

Haddock, C.K., Poston, W.S., Dill, P.L., *et al.* (2002). "Pharmacotherapy for obesity: a quantitative analysis of four decades of published randomized clinical trials." Int J Obes Relat Metab Disord **26**(2): 262-273.

Hagan, M.M. (2002). "Peptide YY: a key mediator of orexigenic behavior." Peptides **23**(2): 377-382.

Hall, W.L., Millward, D.J., Long, S.J., *et al.* (2003). "Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite." Br J Nutr **89**(2): 239-248.

Halton, T.L. and Hu, F.B. (2004). "The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review." J Am Coll Nutr **23**(5): 373-385.

Hannan, M.T., Tucker, K.L., Dawson-Hughes, B., *et al.* (2000). "Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study." J Bone Miner Res **15**(12): 2504-2512.

Hasler, W.L., Soudah, H.C., Dulai, G., *et al.* (1995). "Mediation of hyperglycemia-evoked gastric slow-wave dysrhythmias by endogenous prostaglandins." Gastroenterology **108**(3): 727-736.

Hauptman, J.B., Jeunet, F.S. and Hartmann, D. (1992). "Initial studies in humans with the novel gastrointestinal lipase inhibitor Ro 18-0647 (tetrahydrolipstatin)." Am J Clin Nutr **55**(1 Suppl): 309S-313S.

He, M., Tan, K.C., Li, E.T., *et al.* (2001). "Body fat determination by dual energy X-ray absorptiometry and its relation to body mass index and waist circumference in Hong Kong Chinese." Int J Obes Relat Metab Disord **25**(5): 748-752.

Heaney, R.P. (2000). "Dietary protein and phosphorus do not affect calcium absorption." Am J Clin Nutr **72**(3): 758-761.

Heddle, R., Collins, P.J., Dent, J., *et al.* (1989). "Motor mechanisms associated with slowing of the gastric emptying of a solid meal by an intraduodenal lipid infusion." J Gastroenterol Hepatol **4**(5): 437-447.

Heddle, R., Dent, J., Read, N.W., *et al.* (1988a). "Antropyloroduodenal motor responses to intraduodenal lipid infusion in healthy volunteers." Am J Physiol **254**(5 Pt 1): G671-679.

Heddle, R., Dent, J., Toouli, J., *et al.* (1988b). "Topography and measurement of pyloric pressure waves and tone in humans." Am J Physiol Gastrointest Liver Physiol **255**(4 Pt 1): G490-497.

Heddle, R., Dent, J., Toouli, J., *et al.* (1988c). "Topography and measurement of pyloric pressure waves and tone in humans." Am J Physiol **255**(4 Pt 1): G490-497.

Heddle, R., Fone, D., Dent, J., *et al.* (1988d). "Stimulation of pyloric motility by intraduodenal dextrose in normal subjects." Gut **29**(10): 1349-1357.

Hegsted, M. and Linkswiler, H.M. (1981). "Long-term effects of level of protein intake on calcium metabolism in young adult women." J Nutr **111**(2): 244-251.

Hellmig, S., Von Schoning, F., Gadow, C., *et al.* (2006). "Gastric emptying time of fluids and solids in healthy subjects determined by 13C breath tests: influence of age, sex and body mass index." J Gastroenterol Hepatol **21**(12): 1832-1838.

Herrmann, C., Goke, R., Richter, G., *et al.* (1995). "Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients." Digestion **56**(2): 117-126.

Hill, A.J., Blundell, J.E. (1986). "Macronutrients and satiety: the effects of high carbohydrate and high protein meals on subjective motivation to eat and preferences." Nutr Behav **3**: 133-144.

Holst, B., Lang, M., Brandt, E., *et al.* (2006). "Ghrelin receptor inverse agonists: identification of an active peptide core and its interaction epitopes on the receptor." Mol Pharmacol **70**(3): 936-946.

Horowitz, M., Collins, P.J., Cook, D.J., *et al.* (1983). "Abnormalities of gastric emptying in obese patients." Int J Obes **7**(5): 415-421.

Horowitz, M., Dent, J., Fraser, R., *et al.* (1994). "Role and integration of mechanisms controlling gastric emptying." Dig Dis Sci **39**(12 Suppl): 7S-13S.

Horowitz, M., Edelbroek, M.A., Wishart, J.M., *et al.* (1993). "Relationship between oral glucose tolerance and gastric emptying in normal healthy subjects." Diabetologia **36**(9): 857-862.

Horowitz, M., Maddox, A.F., Wishart, J.M., *et al.* (1991). "Relationships between oesophageal transit and solid and liquid gastric emptying in diabetes mellitus." Eur J Nucl Med **18**(4): 229-234.

Hosoda, H., Kojima, M., Mizushima, T., *et al.* (2003). "Structural Divergence of Human Ghrelin: Identification of multiple ghrelin-derived molecules produced by post-translational processing " Journal of Biological Chemistry **278**(1): 64-70.

Houghton, L.A., Read, N.W., Heddle, R., *et al.* (1988). "Relationship of the motor activity of the antrum, pylorus, and duodenum to gastric emptying of a solid-liquid mixed meal." Gastroenterology **94**(6): 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**(6): 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**(9): 1405-1409.

Iggo, A. (1955). "Tension receptors in the stomach and the urinary bladder." J Physiol **128**(3): 593-607.

Jackson, S.J., Leahy, F.E., McGowan, A.A., *et al.* (2004). "Delayed gastric emptying in the obese: an assessment using the non-invasive (13)C-octanoic acid breath test." Diabetes Obes Metab **6**(4): 264-270.

Jacobsen, S.H., Olesen, S.C., Dirksen, C., *et al.* (2012). "Changes in gastrointestinal hormone responses, insulin sensitivity, and beta-cell function within 2 weeks after gastric bypass in non-diabetic subjects." Obes Surg **22**(7): 1084-1096.

Jakicic, J.M., Clark, K., Coleman, E., *et al.* (2001). "American College of Sports Medicine position stand. Appropriate intervention strategies for weight loss and prevention of weight regain for adults." Med Sci Sports Exerc **33**(12): 2145-2156.

James, W.P., Caterson, I.D., Coutinho, W., *et al.* (2010). "Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects." N Engl J Med **363**(10): 905-917.

Jandacek, R.J. and Woods, S.C. (2004). "Pharmaceutical approaches to the treatment of obesity." Drug Discov Today **9**(20): 874-880.

Jansen, J.B. and Lamers, C.B. (1983). "Radioimmunoassay of cholecystokinin in human tissue and plasma." Clin Chim Acta **131**(3): 305-316.

Johnson, J. and Vickers, Z. (1993). "Effects of flavor and macronutrient composition of food servings on liking, hunger and subsequent intake." Appetite **21**(1): 25-39.

Johnson, S.L. and Birch, L.L. (1994). "Parents' and children's adiposity and eating style." Pediatrics **94**(5): 653-661.

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**(3): 586-591.

Joint WHO FAO UNU Expert Consultation (2007). Protein and amino acid requirements in human nutrition. World Health Organisation Technical Report Series**:** 1-265.

Jonderko, G., Golab, T. and Jonderko, K. (1988). "A pharmacological dose of glucagon suppresses gastric emptying of a radiolabelled solid meal in humans." Scand J Clin Lab Invest **48**(8): 743-746.

Jonderko, G., Jonderko, K. and Golab, T. (1989). "Effect of glucagon on gastric emptying and on postprandial gastrin and insulin release in man." Mater Med Pol **21**(2): 92-96.

Jones, K.L., Doran, S.M., Hveem, K., *et al.* (1997a). "Relation between postprandial satiation and antral area in normal subjects." Am J Clin Nutr **66**(1): 127-132.

Jones, K.L., Doran, S.M., Hveem, K., *et al.* (1997b). "Relation between postprandial satiation and antral area in normal subjects." Am J Clin Nutr **66**(1): 127-132.

Jones, K.L., Horowitz, M., Carney, B.I., *et al.* (1996). "Gastric emptying in early noninsulin-dependent diabetes mellitus." J Nucl Med **37**(10): 1643-1648.

Jones, K.L., O'Donovan, D., Russo, A., *et al.* (2005). "Effects of drink volume and glucose load on gastric emptying and postprandial blood pressure in healthy older subjects." Am J Physiol Gastrointest Liver Physiol **289**(2): G240-248.

Journel, M., Chaumontet, C., Darcel, N., *et al.* (2012). "Brain responses to high-protein diets." Adv Nutr **3**(3): 322-329.

Kagawa, M., Kerr, D., Uchida, H., *et al.* (2006). "Differences in the relationship between BMI and percentage body fat between Japanese and Australian-Caucasian young men." Br J Nutr **95**(5): 1002-1007.

Kang, J.G., Park, C.Y., Kang, J.H., *et al.* (2010). "Randomized controlled trial to investigate the effects of a newly developed formulation of phentermine diffuse-controlled release for obesity." Diabetes Obes Metab **12**(10): 876-882.

Karamanlis, A., Chaikomin, R., Doran, S., *et al.* (2007). "Effects of protein on glycemic and incretin responses and gastric emptying after oral glucose in healthy subjects." Am J Clin Nutr **86**(5): 1364-1368.

Katschinski, M., Schirra, J., Begliner, C., *et al.* (1996). "Intestinal phase of human antro-pyloro-duodenal motility: cholinergic and CCK-mediated regulation." Eur J Clin Invest **26**(7): 574-583.

Kawai, K., Yokota, C., Ohashi, S., *et al.* (1995). "Evidence that glucagon stimulates insulin secretion through its own receptor in rats." Diabetologia **38**(3): 274-276.

Keinke, O., Schemann, M. and Ehrlein, H.J. (1984). "Mechanical factors regulating gastric emptying of viscous nutrient meals in dogs." Q J Exp Physiol **69**(4): 781-795.

Kellow, J.E., Miller, L.J., Phillips, S.F., *et al.* (1987). "Sensitivities of human jejunum, ileum, proximal colon, and gallbladder to cholecystokinin octapeptide." Am J Physiol **252**(3 Pt 1): G345-356.

Kernan, W.N., Viscoli, C.M., Brass, L.M., *et al.* (2000). "Phenylpropanolamine and the risk of hemorrhagic stroke." N Engl J Med **343**(25): 1826-1832.

Kerstetter, J.E., Caseria, D.M., Mitnick, M.E., *et al.* (1997). "Increased circulating concentrations of parathyroid hormone in healthy, young women consuming a protein-restricted diet." Am J Clin Nutr **66**(5): 1188-1196.

Kerstetter, J.E., O'Brien, K.O. and Insogna, K.L. (1998). "Dietary protein affects intestinal calcium absorption." Am J Clin Nutr **68**(4): 859-865.

Kerstetter, J.E., O'Brien, K.O. and Insogna, K.L. (2003). "Dietary protein, calcium metabolism, and skeletal homeostasis revisited." Am J Clin Nutr **78**(3 Suppl): 584S-592S.

Kerstetter, J.E., Svastisalee, C.M., Caseria, D.M., *et al.* (2000). "A threshold for low-protein-diet-induced elevations in parathyroid hormone." Am J Clin Nutr **72**(1): 168-173.

Khoshoo, V. and Brown, S. (2002). "Gastric emptying of two whey-based formulas of different energy density and its clinical implication in children with volume intolerance." Eur J Clin Nutr **56**(7): 656-658.

Kim, D.Y., Camilleri, M., Murray, J.A., *et al.* (2001). "Is there a role for gastric accommodation and satiety in asymptomatic obese people?" Obes Res **9**(11): 655-661.

Kim, K.K., Cho, H.J., Kang, H.C., *et al.* (2006). "Effects on weight reduction and safety of short-term phentermine administration in Korean obese people." Yonsei Med J **47**(5): 614-625.

Kissileff, H.R., Carretta, J.C., Geliebter, A., *et al.* (2003). "Cholecystokinin and stomach distension combine to reduce food intake in humans." Am J Physiol Regul Integr Comp Physiol **285**(5): R992-998.

Kissileff, H.R., Pi-Sunyer, F.X., Thornton, J., *et al.* (1981). "C-terminal octapeptide of cholecystokinin decreases food intake in man." Am J Clin Nutr **34**(2): 154-160.

Klatt, S., Pieramico, O., Guthner, C., *et al.* (1997). "Proximal gastric motility functions are normal in severe obesity." Digestion **58**(2): 115-119.

Kleibeuker, J.H., Beekhuis, H., Jansen, J.B., *et al.* (1988). "Cholecystokinin is a physiological hormonal mediator of fat-induced inhibition of gastric emptying in man." Eur J Clin Invest **18**(2): 173-177.

Knight, E.L., Stampfer, M.J., Hankinson, S.E., *et al.* (2003). "The impact of protein intake on renal function decline in women with normal renal function or mild renal insufficiency." Ann Intern Med **138**(6): 460-467.

Knopf, R.F., Conn, J.W., Floyd, J.C., Jr., *et al.* (1966). "The normal endocrine response to ingestion of protein and infusions of amino acids. Sequential secretion of insulin and growth hormone." Trans Assoc Am Physicians **79**: 312-321.

Kojima, M., Hosoda, H., Date, Y., *et al.* (1999). "Ghrelin is a growth-hormone-releasing acylated peptide from stomach." Nature **402**(6762): 656-660.

Kong, M.F., Chapman, I., Goble, E., *et al.* (1999). "Effects of oral fructose and glucose on plasma GLP-1 and appetite in normal subjects." Peptides **20**(5): 545-551.

Kong, M.F., King, P., Macdonald, I.A., *et al.* (1998). "Effect of euglycaemic hyperinsulinaemia on gastric emptying and gastrointestinal hormone responses in normal subjects." Diabetologia **41**(4): 474-481.

Korek, E., Krauss, H., Gibas-Dorna, M., *et al.* (2013). "Fasting and postprandial levels of ghrelin, leptin and insulin in lean, obese and anorexic subjects." Prz Gastroenterol **8**(6): 383-389.

Korner, J., Inabnet, W., Conwell, I.M., *et al.* (2006). "Differential effects of gastric bypass and banding on circulating gut hormone and leptin levels." Obesity (Silver Spring) **14**(9): 1553-1561.

Kozawa, J., Okita, K., Iwahashi, H., *et al.* (2013). "Early postprandial glucagon surge affects postprandial glucose levels in obese and non-obese patients with type 2 diabetes." Endocr J **60**(6): 813-818.

Kreymann, B., Williams, G., Ghatei, M.A., *et al.* (1987). "Glucagon-like peptide-1 7-36: a physiological incretin in man." Lancet **2**(8571): 1300-1304.

Krezowski, P.A., Nuttall, F.Q., Gannon, M.C., *et al.* (1986). "The effect of protein ingestion on the metabolic response to oral glucose in normal individuals." Am J Clin Nutr **44**(6): 847-856.

Krieger, J.W., Sitren, H.S., Daniels, M.J., *et al.* (2006). "Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1." Am J Clin Nutr **83**(2): 260-274.

L'Heureux-Bouron, D., Tome, D., Rampin, O., *et al.* (2003). "Total subdiaphragmatic vagotomy does not suppress high protein diet-induced food intake depression in rats." J Nutr **133**(8): 2639-2642.

Lan-Pidhainy, X. and Wolever, T.M. (2010). "The hypoglycemic effect of fat and protein is not attenuated by insulin resistance." Am J Clin Nutr **91**(1): 98-105.

Langer, F.B., Reza Hoda, M.A., Bohdjalian, A., *et al.* (2005). "Sleeve gastrectomy and gastric banding: effects on plasma ghrelin levels." Obes Surg **15**(7): 1024-1029.

Langhans, W., Zeiger, U., Scharrer, E., *et al.* (1982). "Stimulation of feeding in rats by intraperitoneal injection of antibodies to glucagon." Science **218**(4575): 894-896.

Larsen, S., Osnes, M. and Christensen, M.S. (1986). "The effect of glucagon, glucagon-(1-21)-peptide, and placebo on duodenal pressure activity in healthy subjects." Scand J Gastroenterol **21**(5): 634-640.

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**(1-2): 23-31.

Lasker, D.A., Evans, E.M. and Layman, D.K. (2008). "Moderate carbohydrate, moderate protein weight loss diet reduces cardiovascular disease risk compared to high carbohydrate, low protein diet in obese adults: A randomized clinical trial." Nutr Metab (Lond) **5**: 30.

Lavin, J.H., Wittert, G.A., Andrews, J., *et al.* (1998). "Interaction of insulin, glucagon-like peptide 1, gastric inhibitory polypeptide, and appetite in response to intraduodenal carbohydrate." Am J Clin Nutr **68**(3): 591-598.

Layer, P., Holst, J.J., Grandt, D., *et al.* (1995). "Ileal release of glucagon-like peptide-1 (GLP-1). Association with inhibition of gastric acid secretion in humans." Dig Dis Sci **40**(5): 1074-1082.

Layman, D.K. (2003). "The role of leucine in weight loss diets and glucose homeostasis." J Nutr **133**(1): 261S-267S.

Layman, D.K. (2009). "Dietary Guidelines should reflect new understandings about adult protein needs." Nutr Metab (Lond) **6**: 12.

Layman, D.K., Boileau, R.A., Erickson, D.J., *et al.* (2003). "A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women." J Nutr **133**(2): 411-417.

Layman, D.K., Evans, E., Baum, J.I., *et al.* (2005). "Dietary protein and exercise have additive effects on body composition during weight loss in adult women." J Nutr **135**(8): 1903-1910.

Layman, D.K., Evans, E.M., Erickson, D., *et al.* (2009). "A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults." J Nutr **139**(3): 514-521.

le Roux, C.W., Batterham, R.L., Aylwin, S.J., *et al.* (2006). "Attenuated peptide YY release in obese subjects is associated with reduced satiety." Endocrinology **147**(1): 3-8.

Le Sauter, J. and Geary, N. (1987). "Pancreatic glucagon and cholecystokinin synergistically inhibit sham feeding in rats." Am J Physiol **253**(5 Pt 2): R719-725.

Le Sauter, J. and Geary, N. (1991). "Hepatic portal glucagon infusion decreases spontaneous meal size in rats." Am J Physiol **261**(1 Pt 2): R154-161.

Le Sauter, J., Noh, U. and Geary, N. (1991). "Hepatic portal infusion of glucagon antibodies increases spontaneous meal size in rats." Am J Physiol **261**(1 Pt 2): R162-165.

Leidy, H.J., Carnell, N.S., Mattes, R.D., *et al.* (2007). "Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women." Obesity (Silver Spring) **15**(2): 421-429.

Lejeune, M.P., Kovacs, E.M. and Westerterp-Plantenga, M.S. (2005). "Additional protein intake limits weight regain after weight loss in humans." Br J Nutr **93**(2): 281-289.

Lejeune, M.P.G.M., Westerterp, K.R., Adam, T.C.M., *et al.* (2006). "Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber." American Journal of Clinical Nutrition **83**(1): 89-94.

Levin, F., Edholm, T., Schmidt, P.T., *et al.* (2006). "Ghrelin stimulates gastric emptying and hunger in normal-weight humans." J Clin Endocrinol Metab **91**(9): 3296-3302.

Liao, D., Gregersen, H., Hausken, T., *et al.* (2004). "Analysis of surface geometry of the human stomach using real-time 3-D ultrasonography in vivo." Neurogastroenterol Motil **16**(3): 315-324.

Liddle, R.A. (2000). "Regulation of cholecystokinin secretion in humans." J Gastroenterol **35**(3): 181-187.

Liddle, R.A., Goldfine, I.D., Rosen, M.S., *et al.* (1985). "Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction." J Clin Invest **75**(4): 1144-1152.

Liddle, R.A., Morita, E.T., Conrad, C.K., *et al.* (1986). "Regulation of gastric emptying in humans by cholecystokinin." J Clin Invest **77**(3): 992-996.

Liddle, R.A., Rushakoff, R.J., Morita, E.T., *et al.* (1988). "Physiological role for cholecystokinin in reducing postprandial hyperglycemia in humans." J Clin Invest **81**(6): 1675-1681.

Lieverse, R.J., Jansen, J.B., Masclee, A.A., *et al.* (1994a). "Effect of a low dose of intraduodenal fat on satiety in humans: studies using the type A cholecystokinin receptor antagonist loxiglumide." Gut **35**(4): 501-505.

Lieverse, R.J., Masclee, A.A., Jansen, J.B., *et al.* (1994b). "Plasma cholecystokinin and pancreatic polypeptide secretion in response to bombesin, meal ingestion and modified sham feeding in lean and obese persons." Int J Obes Relat Metab Disord **18**(2): 123-127.

Lilja, P., Wiener, I., Inoue, K., *et al.* (1984). "Release of cholecystokinin in response to food and intraduodenal fat in pigs, dogs and man." Surg Gynecol Obstet **159**(6): 557-561.

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**(10): 1561-1563.

Lin, H.C., Doty, J.E., Reedy, T.J., *et al.* (1989). "Inhibition of gastric emptying by glucose depends on length of intestine exposed to nutrient." Am J Physiol **256**(2 Pt 1): G404-411.

Lin, H.C., Zhao, X.T. and Wang, L. (1997). "Intestinal transit is more potently inhibited by fat in the distal (ileal brake) than in the proximal (jejunal brake) gut." Dig Dis Sci **42**(1): 19-25.

Lindemann, B. (1996). "Taste reception." Physiol Rev **76**(3): 718-766.

Little, T.J., Doran, S., Meyer, J.H., *et al.* (2006a). "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**(3): E647-655.

Little, T.J., Feltrin, K.L., Horowitz, M., *et al.* (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**(4): R1090-1098.

Little, T.J., Pilichiewicz, A.N., Russo, A., *et al.* (2006b). "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**(5): 1916-1923.

Little, T.J., Russo, A., Meyer, J.H., *et al.* (2007). "Free fatty acids have more potent effects on gastric emptying, gut hormones, and appetite than triacylglycerides." Gastroenterology **133**(4): 1124-1131.

Lluis, F., Gomez, G., Fujimura, M., *et al.* (1987a). "Peptide YY inhibits nutrient-, hormonal-, and vagally-stimulated pancreatic exocrine secretion." Pancreas **2**(4): 454-462.

Lluis, F., Gomez, G., Fujimura, M., *et al.* (1987b). "Peptide YY interacts with secretin and duodenal acidification to inhibit gastric acid secretion." Regul Pept **18**(3-4): 155-163.

Locatelli, V., Bresciani, E., Bulgarelli, I., *et al.* (2005). "Ghrelin in gastroenteric pathophysiology." J Endocrinol Invest **28**(9): 843-848.

Long, S.J., Sutton, J.A., Amaee, W.B., *et al.* (1999). "No effect of glucagon-like peptide-1 on short-term satiety and energy intake in man." Br J Nutr **81**(4): 273-279.

Look Ahead Research Group, Pi-Sunyer, X., Blackburn, G., *et al.* (2007). "Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial." Diabetes Care **30**(6): 1374-1383.

Lugari, R., Dei Cas, A., Ugolotti, D., *et al.* (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**(2): 111-115.

Luhovyy, B.L., Akhavan, T. and Anderson, H. (2007). "Whey proteins in the regulation of food intake and satiety." Journal of the American College of Nutrition **26**(6): 704s-712s.

Lund, A., Vilsbøll, T., Bagger, J.I., *et al.* (2011). The separate and combined impact of the intestinal hormones, GIP, GLP-1, and GLP-2, on glucagon secretion in type 2 diabetes.

Luscombe-Marsh, N.D., Noakes, M., Wittert, G.A., *et al.* (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**(4): 762-772.

Luscombe, N.D., Clifton, P.M., Noakes, M., *et al.* (2003a). "Effect of a high-protein, energy-restricted diet on weight loss and energy expenditure after weight stabilization in hyperinsulinemic subjects." Int J Obes Relat Metab Disord **27**(5): 582-590.

Luscombe, N.D., Clifton, P.M., Noakes, M., *et al.* (2003b). "Effect of a high-protein, energy-restricted diet on weight loss and energy expenditure after weight stabilization in hyperinsulinemic subjects." International Journal of Obesity **27**(5): 582-590.

Luscombe, N.D., Clifton, P.M., Noakes, M., *et al.* (2002). "Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditure, and the thermic effect of feeding in type 2 diabetes." Diabetes Care **25**(4): 652-657.

Ma, J., Stevens, J.E., Cukier, K., *et al.* (2009). "Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes." Diabetes Care **32**(9): 1600-1602.

MacIntosh, C.G., Andrews, J.M., Jones, K.L., *et al.* (1999). "Effects of age on concentrations of plasma cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility." Am J Clin Nutr **69**(5): 999-1006.

MacIntosh, C.G., Morley, J.E., Wishart, J., *et al.* (2001). "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**(12): 5830-5837.

Maddox, A., Horowitz, M., Wishart, J., *et al.* (1989). "Gastric and oesophageal emptying in obesity." Scand J Gastroenterol **24**(5): 593-598.

Marsset-Baglieri, A., Fromentin, G., Airinei, G., *et al.* (2014). "Milk protein fractions moderately extend the duration of satiety compared with carbohydrates independently of their digestive kinetics in overweight subjects." Br J Nutr **112**(4): 557-564.

Martens, E., Lemmens, S. and Westerterp-Plantenga, M. (2013a). "No difference in protein leverage affecting energy intake between soy and whey protein." Faseb Journal **27**.

Martens, E.A., Lemmens, S.G. and Westerterp-Plantenga, M.S. (2013b). "Protein leverage affects energy intake of high-protein diets in humans." Am J Clin Nutr **97**(1): 86-93.

Martens, E.A., Tan, S.Y., Dunlop, M.V., *et al.* (2014). "Protein leverage effects of beef protein on energy intake in humans." Am J Clin Nutr **99**(6): 1397-1406.

Martens, M.J.I., Lemmens, S.G.T., Born, J.M., *et al.* (2011). "A Solid High-Protein Meal Evokes Stronger Hunger Suppression Than a Liquefied High-Protein Meal." Obesity **19**(3): 522-527.

Martens, M.J.I. and Westerterp-Plantenga, M.S. (2012). "Mode of Consumption Plays a Role in Alleviating Hunger and Thirst." Obesity **20**(3): 517-524.

Mathus-Vliegen, E.M. and Balance Study, G. (2005). "Long-term maintenance of weight loss with sibutramine in a GP setting following a specialist guided very-low-calorie diet: a double-blind, placebo-controlled, parallel group study." Eur J Clin Nutr **59 Suppl 1**: S31-38; discussion S39.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., *et al.* (1985). "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man." Diabetologia **28**(7): 412-419.

Matzinger, D., Degen, L., Drewe, J., *et al.* (2000). "The role of long chain fatty acids in regulating food intake and cholecystokinin release in humans." Gut **46**(5): 688-693.

Matzinger, D., Gutzwiller, J.P., Drewe, J., *et al.* (1999). "Inhibition of food intake in response to intestinal lipid is mediated by cholecystokinin in humans." Am J Physiol **277**(6 Pt 2): R1718-1724.

Maughan, R.J., Leiper, J.B. and Vist, G.E. (2004). "Gastric emptying and fluid availability after ingestion of glucose and soy protein hydrolysate solutions in man." Exp Physiol **89**(1): 101-108.

McAuley, K.A., Hopkins, C.M., Smith, K.J., *et al.* (2005). "Comparison of high-fat and high-protein diets with a high-carbohydrate diet in insulin-resistant obese women." Diabetologia **48**(1): 8-16.

McAuley, K.A., Williams, S.M., Mann, J.I., *et al.* (2002). "Increased risk of type 2 diabetes despite same degree of adiposity in different racial groups." Diabetes Care **25**(12): 2360-2361.

McElroy, S.L., Kotwal, R., Malhotra, S., *et al.* (2004). "Are mood disorders and obesity related? A review for the mental health professional." J Clin Psychiatry **65**(5): 634-651, quiz 730.

McHugh, P.R. and Moran, T.H. (1986). "The stomach, cholecystokinin, and satiety." Fed Proc **45**(5): 1384-1390.

McLaughlin, T., Abbasi, F., Lamendola, C., *et al.* (2001). "Metabolic changes following sibutramine-assisted weight loss in obese individuals: role of plasma free fatty acids in the insulin resistance of obesity." Metabolism **50**(7): 819-824.

Mearadji, B., Masclee, A.A., Onkenhout, W., *et al.* (2001). "Effect of intraduodenal and intravenous amino acids on proximal gastric motor function in man." Dig Dis Sci **46**(1): 38-45.

Mehanna, M.J., Birjawi, G., Moukaddam, H.A., *et al.* (2006). "Complications of adjustable gastric banding, a radiological pictorial review." AJR Am J Roentgenol **186**(2): 522-534.

Meier, J.J., Gallwitz, B., Salmen, S., *et al.* (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**(6): 2719-2725.

Meier, J.J., Goetze, O., Anstipp, J., *et al.* (2004). "Gastric inhibitory polypeptide does not inhibit gastric emptying in humans." Am J Physiol Endocrinol Metab **286**(4): E621-625.

Meier, J.J., Kemmeries, G., Holst, J.J., *et al.* (2005). "Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects." Diabetes **54**(7): 2212-2218.

Melhorn, S.J., Tyagi, V., Smeraglio, A., *et al.* (2014). "Initial evidence that GLP-1 receptor blockade fails to suppress postprandial satiety or promote food intake in humans." Appetite **82**: 85-90.

Melissas, J., Daskalakis, M., Koukouraki, S., *et al.* (2008). "Sleeve gastrectomy-a "food limiting" operation." Obes Surg **18**(10): 1251-1256.

Melissas, J., Koukouraki, S., Askoxylakis, J., *et al.* (2007). "Sleeve gastrectomy: a restrictive procedure?" Obes Surg **17**(1): 57-62.

Melissas, J., Leventi, A., Klinaki, I., *et al.* (2012). "Alterations of Global Gastrointestinal Motility After Sleeve Gastrectomy: A Prospective Study." Ann Surg.

Mellinkoff, S.M., Frankland, M., Boyle, D., *et al.* (1956). "Relationship between serum amino acid concentration and fluctuations in appetite." J Appl Physiol **8**(5): 535-538.

Mentis, N., Vardarli, I., Kothe, L.D., *et al.* (2011). "GIP does not potentiate the antidiabetic effects of GLP-1 in hyperglycemic patients with type 2 diabetes." Diabetes **60**(4): 1270-1276.

Mentlein, R., Gallwitz, B. and Schmidt, W.E. (1993). "Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum." Eur J Biochem **214**(3): 829-835.

Mersebach, H., Svendsen, O.L., Holst, J.J., *et al.* (2003). "Comparisons of leptin, incretins and body composition in obese and lean patients with hypopituitarism and healthy individuals." Clin Endocrinol (Oxf) **58**(1): 65-71.

Meyer, B.M., Werth, B.A., Beglinger, C., *et al.* (1989). "Role of cholecystokinin in regulation of gastrointestinal motor functions." Lancet **2**(8653): 12-15.

Meyer, J.H. (1980). "Gastric emptying of ordinary food: effect of antrum on particle size." Am J Physiol **239**(3): G133-135.

Mikkelsen, P.B., Toubro, S. and Astrup, A. (2000). "Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate." Am J Clin Nutr **72**(5): 1135-1141.

Mithieux, G. (2009). "A novel function of intestinal gluconeogenesis: central signaling in glucose and energy homeostasis." Nutrition **25**(9): 881-884.

Mithieux, G., Andreelli, F. and Magnan, C. (2009). "Intestinal gluconeogenesis: key signal of central control of energy and glucose homeostasis." Curr Opin Clin Nutr Metab Care **12**(4): 419-423.

Mithieux, G., Misery, P., Magnan, C., *et al.* (2005). "Portal sensing of intestinal gluconeogenesis is a mechanistic link in the diminution of food intake induced by diet protein." Cell Metab **2**(5): 321-329.

Moayyedi, P. (2008). "The epidemiology of obesity and gastrointestinal and other diseases: an overview." Dig Dis Sci **53**(9): 2293-2299.

Moens, K., Flamez, D., Van Schravendijk, C., *et al.* (1998). "Dual glucagon recognition by pancreatic beta-cells via glucagon and glucagon-like peptide 1 receptors." Diabetes **47**(1): 66-72.

Mognol, P., Chosidow, D. and Marmuse, J.P. (2004). "Laparoscopic conversion of laparoscopic gastric banding to Roux-en-Y gastric bypass: a review of 70 patients." Obes Surg **14**(10): 1349-1353.

Monteleone, P., Bencivenga, R., Longobardi, N., *et al.* (2003). "Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women." J Clin Endocrinol Metab **88**(11): 5510-5514.

Moran, L.J., Luscombe-Marsh, N.D., Noakes, M., *et al.* (2005a). "The satiating effect of dietary protein is unrelated to postprandial ghrelin secretion." J Clin Endocrinol Metab **90**(9): 5205-5211.

Moran, T.H. and McHugh, P.R. (1982). "Cholecystokinin suppresses food intake by inhibiting gastric emptying." Am J Physiol **242**(5): R491-497.

Moran, T.H. and McHugh, P.R. (1988). "Gastric and nongastric mechanisms for satiety action of cholecystokinin." Am J Physiol **254**(4 Pt 2): R628-632.

Moran, T.H., Smedh, U., Kinzig, K.P., *et al.* (2005b). "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**(2): R384-388.

Mourao, D.M., Bressan, J., Campbell, W.W., *et al.* (2007). "Effects of food form on appetite and energy intake in lean and obese young adults." Int J Obes (Lond) **31**(11): 1688-1695.

Murray, C.D., Martin, N.M., Patterson, M., *et al.* (2005). "Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study." Gut **54**(12): 1693-1698.

Nair, K.S., Halliday, D. and Garrow, J.S. (1983). "Thermic response to isoenergetic protein, carbohydrate or fat meals in lean and obese subjects." Clin Sci (Lond) **65**(3): 307-312.

Nair, N.S., Brennan, I.M., Little, T.J., *et al.* (2009). "Reproducibility of energy intake, gastric emptying, blood glucose, plasma insulin and cholecystokinin responses in healthy young males." Br J Nutr **101**(7): 1094-1102.

Naslund, E., Barkeling, B., King, N., *et al.* (1999). "Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men." Int J Obes Relat Metab Disord **23**(3): 304-311.

Naslund, E., Gryback, P., Backman, L., *et al.* (1998a). "Distal small bowel hormones: correlation with fasting antroduodenal motility and gastric emptying." Dig Dis Sci **43**(5): 945-952.

Näslund, E., Grybäck, P., Backman, L., *et al.* (1998). "Distal small bowel hormones: correlation with fasting antroduodenal motility and gastric emptying." Dig Dis Sci **43**(5): 945-952.

Naslund, E., Gutniak, M., Skogar, S., *et al.* (1998b). "Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men." Am J Clin Nutr **68**(3): 525-530.

National Health and Medical Research Council (2013). Clinical Practice Guidelines for the Management of Overweight and Obesity in Adults Commonwealth of Australia. Canberra, Commonwealth of Australia

Nauck, M.A. (1998). "Glucagon-like peptide 1 (GLP-1): a potent gut hormone with a possible therapeutic perspective." Acta Diabetol **35**(3): 117-129.

Nauck, M.A., Baller, B. and Meier, J.J. (2004). "Gastric inhibitory polypeptide and glucagon-like peptide-1 in the pathogenesis of type 2 diabetes." Diabetes **53 Suppl 3**: S190-196.

Nauck, M.A., Heimesaat, M.M., Orskov, C., *et al.* (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**(1): 301-307.

Nauck, M.A., Niedereichholz, U., Ettler, R., *et al.* (1997). "Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans." Am J Physiol **273**(5 Pt 1): E981-988.

Nelsen, T.S., Eigenbrodt, E.H. and Keoshian, L.A. (1966). "Motor responses of the canine stomach to insulin and feeding." Arch Surg **92**(3): 379-385.

Neter, J.E., Stam, B.E., Kok, F.J., *et al.* (2003). "Influence of weight reduction on blood pressure: a meta-analysis of randomized controlled trials." Hypertension **42**(5): 878-884.

Nicolaidis, S. and Rowland, N. (1976). "Metering of intravenous versus oral nutrients and regulation of energy balance." Am J Physiol **231**(3): 661-668.

Nilsson, M., Holst, J.J. and Bjorck, I.M.E. (2007). "Metabolic effects of amino acid mixtures and whey protein in healthy subjects: studies using glucose-equivalent drinks." American Journal of Clinical Nutrition **85**(4): 996-1004.

Noah, J., Smith, A., Birch, D., *et al.* (2013). "The Metabolic Effects of Laparoscopic Sleeve Gastrectomy: A Review." jmiss **2**(1): 3-7.

Noakes, M., Keogh, J.B., Foster, P.R., *et al.* (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**(6): 1298-1306.

Nuttall, F.Q., Mooradian, A.D., Gannon, M.C., *et al.* (1984). "Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load." Diabetes Care **7**(5): 465-470.

O'Donovan, D.G., Doran, S., Feinle-Bisset, C., *et al.* (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**(7): 3431-3435.

O'Dorisio, T.M., Cataland, S., Stevenson, M., *et al.* (1976). "Gastric inhibitory polypeptide (GIP). Intestinal distribution and stimulation by amino acids and medium-chain triglycerides." Am J Dig Dis **21**(9): 761-765.

Ogawa, E., Hosokawa, M., Harada, N., *et al.* (2011). "The effect of gastric inhibitory polypeptide on intestinal glucose absorption and intestinal motility in mice." Biochem Biophys Res Commun **404**(1): 115-120.

Onaga, T., Zabielski, R. and Kato, S. (2002). "Multiple regulation of peptide YY secretion in the digestive tract." Peptides **23**(2): 279-290.

Ortinau, L.C., Hoertel, H.A., Douglas, S.M., *et al.* (2014). "Effects of high-protein vs. high-fat snacks on appetite control, satiety, and eating initiation in healthy women." Nutrition Journal **13**: 97.

Overduin, J., Frayo, R.S., Grill, H.J., *et al.* (2005). "Role of the duodenum and macronutrient type in ghrelin regulation." Endocrinology **146**(2): 845-850.

Overduin, J., Gibbs, J., Cummings, D.E., *et al.* (2014). "CCK-58 elicits both satiety and satiation in rats while CCK-8 elicits only satiation." Peptides **54**: 71-80.

Paddon-Jones, D. and Rasmussen, B.B. (2009). "Dietary protein recommendations and the prevention of sarcopenia." Curr Opin Clin Nutr Metab Care **12**(1): 86-90.

Paddon-Jones, D., Sheffield-Moore, M., Katsanos, C.S., *et al.* (2006). "Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein." Exp Gerontol **41**(2): 215-219.

Paddon-Jones, D., Westman, E., Mattes, R.D., *et al.* (2008). "Protein, weight management, and satiety." American Journal of Clinical Nutrition **87**(5): 1558s-1561s.

Padwal, R., Klarenbach, S., Wiebe, N., *et al.* (2011). "Bariatric surgery: a systematic review of the clinical and economic evidence." J Gen Intern Med **26**(10): 1183-1194.

Padwal, R., Li, S.K. and Lau, D.C. (2004). "Long-term pharmacotherapy for obesity and overweight." Cochrane Database Syst Rev(3): CD004094.

Padwal, R.S. and Majumdar, S.R. (2007). "Drug treatments for obesity: orlistat, sibutramine, and rimonabant." Lancet **369**(9555): 71-77.

Paintal, A.S. (1953). "Impulses in vagal afferent fibres from stretch receptors in the stomach and their role in the peripheral mechanism of hunger." Nature **172**(4391): 1194-1195.

Pal, S. and Ellis, V. (2010). "The acute effects of four protein meals on insulin, glucose, appetite and energy intake in lean men." Br J Nutr **104**(8): 1241-1248.

Pappas, T.N., Debas, H.T., Chang, A.M., *et al.* (1986). "Peptide YY release by fatty acids is sufficient to inhibit gastric emptying in dogs." Gastroenterology **91**(6): 1386-1389.

Pardridge, W.M., Eisenberg, J. and Yang, J. (1985). "Human blood-brain barrier insulin receptor." J Neurochem **44**(6): 1771-1778.

Park, M.I. and Camilleri, M. (2005). "Gastric motor and sensory functions in obesity." Obes Res **13**(3): 491-500.

Parker, B.A., Doran, S., Wishart, J., *et al.* (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**(5): 539-546.

Parker, B.A., Sturm, K., MacIntosh, C.G., *et al.* (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**(2): 212-218.

Parker, J.C., Irwin, N., Lavery, K.S., *et al.* (2007). "Metabolic effects of sub-chronic ablation of the incretin receptors by daily administration of (Pro3)GIP and exendin(9-39)amide in obese diabetic (ob/ob) mice." Biol Chem **388**(2): 221-226.

Pasiakos, S.M., Vislocky, L.M., Carbone, J.W., *et al.* (2010). "Acute Energy Deprivation Affects Skeletal Muscle Protein Synthesis and Associated Intracellular Signaling Proteins in Physically Active Adults." Journal of Nutrition **140**(4): 745-751.

Patel, G.K., Whalen, G.E., Soergel, K.H., *et al.* (1979). "Glucagon effects on the human small intestine." Dig Dis Sci **24**(7): 501-508.

Pedersen-Bjergaard, U., Host, U., Kelbaek, H., *et al.* (1996). "Influence of meal composition on postprandial peripheral plasma concentrations of vasoactive peptides in man." Scand J Clin Lab Invest **56**(6): 497-503.

Pederson, R.A., Schubert, H.E. and Brown, J.C. (1975). "Gastric inhibitory polypeptide. Its physiologic release and insulinotropic action in the dog." Diabetes **24**(12): 1050-1056.

Pedrini, M.T., Levey, A.S., Lau, J., *et al.* (1996). "The effect of dietary protein restriction on the progression of diabetic and nondiabetic renal diseases: a meta-analysis." Ann Intern Med **124**(7): 627-632.

Penhoat, A., Mutel, E., Amigo-Correig, M., *et al.* (2011). "Protein-induced satiety is abolished in the absence of intestinal gluconeogenesis." Physiol Behav **105**(1): 89-93.

Pepino, M.Y., Finkbeiner, S., Beauchamp, G.K., *et al.* (2010). "Obese women have lower monosodium glutamate taste sensitivity and prefer higher concentrations than do normal-weight women." Obesity (Silver Spring) **18**(5): 959-965.

Peterli, R., Wolnerhanssen, B., Peters, T., *et al.* (2009). "Improvement in glucose metabolism after bariatric surgery: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: a prospective randomized trial." Ann Surg **250**(2): 234-241.

Petersen, B.L., Ward, L.S., Bastian, E.D., *et al.* (2009). "A whey protein supplement decreases post-prandial glycemia." Nutrition Journal **8**: 47.

Pi-Sunyer, X., Kissileff, H.R., Thornton, J., *et al.* (1982). "C-terminal octapeptide of cholecystokinin decreases food intake in obese men." Physiol Behav **29**(4): 627-630.

Piatti, P.M., Monti, F., Fermo, I., *et al.* (1994). "Hypocaloric high-protein diet improves glucose oxidation and spares lean body mass: comparison to hypocaloric high-carbohydrate diet." Metabolism **43**(12): 1481-1487.

Pieramico, O., Malfertheiner, P., Nelson, D.K., *et al.* (1992). "Interdigestive gastroduodenal motility and cycling of putative regulatory hormones in severe obesity." Scand J Gastroenterol **27**(7): 538-544.

Pilichiewicz, A.N., Chaikomin, R., Brennan, I.M., *et al.* (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**(3): E743-753.

Pilichiewicz, A.N., Feltrin, K.L., Horowitz, M., *et al.* (2008). "Functional dyspepsia is associated with a greater symptomatic response to fat but not carbohydrate, increased fasting and postprandial CCK, and diminished PYY." Am J Gastroenterol **103**(10): 2613-2623.

Pilichiewicz, A.N., Little, T.J., Brennan, I.M., *et al.* (2006). "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**(3): R668-677.

Pilichiewicz, A.N., Papadopoulos, P., Brennan, I.M., *et al.* (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**(6): R2170-2178.

Poortmans, J.R. and Dellalieux, O. (2000). "Do regular high protein diets have potential health risks on kidney function in athletes?" Int J Sport Nutr Exerc Metab **10**(1): 28-38.

Poppitt, S.D., McCormack, D. and Buffenstein, R. (1998). "Short-term effects of macronutrient preloads on appetite and energy intake in lean women." Physiol Behav **64**(3): 279-285.

Poppitt, S.D., Proctor, J., McGill, A.T., *et al.* (2011). "Low-dose whey protein-enriched water beverages alter satiety in a study of overweight women." Appetite **56**(2): 456-464.

Porrini, M., Crovetti, R., Testolin, G., *et al.* (1995). "Evaluation of satiety sensations and food intake after different preloads." Appetite **25**(1): 17-30.

Porrini, M., Santangelo, A., Crovetti, R., *et al.* (1997). "Weight, protein, fat, and timing of preloads affect food intake." Physiol Behav **62**(3): 563-570.

Potier, M., Darcel, N. and Tome, D. (2009). "Protein, amino acids and the control of food intake." Curr Opin Clin Nutr Metab Care **12**(1): 54-58.

Poykko, S.M., Kellokoski, E., Horkko, S., *et al.* (2003). "Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes." Diabetes **52**(10): 2546-2553.

Prager, R., Wallace, P. and Olefsky, J.M. (1987). "Hyperinsulinemia does not compensate for peripheral insulin resistance in obesity." Diabetes **36**(3): 327-334.

Puleo, L., Marini, P., Avallone, R., *et al.* (2012). "Synthesis and pharmacological evaluation of indolinone derivatives as novel ghrelin receptor antagonists." Bioorg Med Chem **20**(18): 5623-5636.

Raben, A., Agerholm-Larsen, L., Flint, A., *et al.* (2003). "Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake." Am J Clin Nutr **77**(1): 91-100.

Ramon, J.M., Salvans, S., Crous, X., *et al.* (2012). "Effect of Roux-en-Y gastric bypass vs sleeve gastrectomy on glucose and gut hormones: a prospective randomised trial." J Gastrointest Surg **16**(6): 1116-1122.

Ranganath, L.R., Beety, J.M., Morgan, L.M., *et al.* (1996). "Attenuated GLP-1 secretion in obesity: cause or consequence?" Gut **38**(6): 916-919.

Rasmussen, B.B., Tipton, K.D., Miller, S.L., *et al.* (2000). "An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise." J Appl Physiol **88**(2): 386-392.

Raybould, H.E. (2010). "Gut chemosensing: interactions between gut endocrine cells and visceral afferents." Auton Neurosci **153**(1-2): 41-46.

Rayner, C.K., Park, H.S., Doran, S.M., *et al.* (2000). "Effects of cholecystokinin on appetite and pyloric motility during physiological hyperglycemia." Am J Physiol Gastrointest Liver Physiol **278**(1): G98-G104.

Rayner, C.K., Samsom, M., Jones, K.L., *et al.* (2001). "Relationships of upper gastrointestinal motor and sensory function with glycemic control." Diabetes Care **24**(2): 371-381.

Reaven, G., Segal, K., Hauptman, J., *et al.* (2001). "Effect of orlistat-assisted weight loss in decreasing coronary heart disease risk in patients with syndrome X." Am J Cardiol **87**(7): 827-831.

Reddy, S.T., Wang, C.Y., Sakhaee, K., *et al.* (2002). "Effect of low-carbohydrate high-protein diets on acid-base balance, stone-forming propensity, and calcium metabolism." Am J Kidney Dis **40**(2): 265-274.

Regan, J.F. and Barnes, B.O. (1933). "The Relation of the Hypophysis to Experimental Diabetes." Science **77**(1991): 214.

Rolls, B.J., Hetherington, M. and Burley, V.J. (1988). "The specificity of satiety: the influence of foods of different macronutrient content on the development of satiety." Physiol Behav **43**(2): 145-153.

Rolls, B.J., Kim-Harris, S., Fischman, M.W., *et al.* (1994). "Satiety after preloads with different amounts of fat and carbohydrate: implications for obesity." Am J Clin Nutr **60**(4): 476-487.

Romero, F., Nicolau, J., Flores, L., *et al.* (2012). "Comparable early changes in gastrointestinal hormones after sleeve gastrectomy and Roux-En-Y gastric bypass surgery for morbidly obese type 2 diabetic subjects." Surg Endosc **26**(8): 2231-2239.

Rosenthal, R.J., Szomstein, S., Kennedy, C.I., *et al.* (2006). "Laparoscopic surgery for morbid obesity: 1,001 consecutive bariatric operations performed at The Bariatric Institute, Cleveland Clinic Florida." Obes Surg **16**(2): 119-124.

Ross, S.A. and Dupre, J. (1978). "Effects of ingestion of triglyceride or galactose on secretion of gastric inhibitory polypeptide and on responses to intravenous glucose in normal and diabetic subjects." Diabetes **27**(3): 327-333.

Rothman, R.B. and Baumann, M.H. (2009). "Appetite suppressants, cardiac valve disease and combination pharmacotherapy." Am J Ther **16**(4): 354-364.

Rubino, F., Gagner, M., Gentileschi, P., *et al.* (2004). "The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism." Ann Surg **240**(2): 236-242.

Rush, E.C., Plank, L.D., Laulu, M.S., *et al.* (1997). "Prediction of percentage body fat from anthropometric measurements: comparison of New Zealand European and Polynesian young women." Am J Clin Nutr **66**(1): 2-7.

Ryan, A.T., Feinle-Bisset, C., Kallas, A., *et al.* (2012). "Intraduodenal protein modulates antropyloroduodenal motility, hormone release, glycemia, appetite, and energy intake in lean men." Am J Clin Nutr **96**(3): 474-482.

Ryan, A.T., Luscombe-Marsh, N.D., Saies, A.A., *et al.* (2013). "Effects of intraduodenal lipid and protein on gut motility and hormone release, glycemia, appetite, and energy intake in lean men." Am J Clin Nutr **98**(2): 300-311.

Sacks, F.M., Bray, G.A., Carey, V.J., *et al.* (2009). "Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates." N Engl J Med **360**(9): 859-873.

Salehi, A., Gunnerud, U., Muhammed, S.J., *et al.* (2012). "The insulinogenic effect of whey protein is partially mediated by a direct effect of amino acids and GIP on beta-cells." Nutr Metab (Lond) **9**(1): 48.

Saliba, J., Wattacheril, J. and Abumrad, N.N. (2009). "Endocrine and metabolic response to gastric bypass." Curr Opin Clin Nutr Metab Care **12**(5): 515-521.

Saltiel, A.R. and Kahn, C.R. (2001). "Insulin signalling and the regulation of glucose and lipid metabolism." Nature **414**(6865): 799-806.

Samols, E., Marri, G. and Marks, V. (1966). "Interrelationship of glucagon, insulin and glucose. The insulinogenic effect of glucagon." Diabetes **15**(12): 855-866.

Samsom, M., Roelofs, J.M., Akkermans, L.M., *et al.* (1998). "Proximal gastric motor activity in response to a liquid meal in type I diabetes mellitus with autonomic neuropathy." Dig Dis Sci **43**(3): 491-496.

Santangelo, A., Peracchi, M., Conte, D., *et al.* (1998). "Physical state of meal affects gastric emptying, cholecystokinin release and satiety." Br J Nutr **80**(6): 521-527.

Sarkhosh, K., Birch, D.W., Sharma, A., *et al.* (2013). "Complications associated with laparoscopic sleeve gastrectomy for morbid obesity: a surgeon's guide." Can J Surg **56**(5): 347-352.

Savage, A.P., Adrian, T.E., Carolan, G., *et al.* (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**(2): 166-170.

Sayegh, A.I., Washington, M.C., Raboin, S.J., *et al.* (2014). "CCK-58 prolongs the intermeal interval, whereas CCK-8 reduces this interval: not all forms of cholecystokinin have equal bioactivity." Peptides **55**: 120-125.

Schick, R.R., Schusdziarra, V., Mossner, J., *et al.* (1991). "Effect of CCK on food intake in man: physiological or pharmacological effect?" Z Gastroenterol **29**(2): 53-58.

Schirra, J., Houck, P., Wank, U., *et al.* (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**(5): 622-631.

Schirra, J., Leicht, P., Hildebrand, P., *et al.* (1998a). "Mechanisms of the antidiabetic action of subcutaneous glucagon-like peptide-1(7-36)amide in non-insulin dependent diabetes mellitus." J Endocrinol **156**(1): 177-186.

Schirra, J., Nicolaus, M., Roggel, R., *et al.* (2006). "Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans." Gut **55**(2): 243-251.

Schirra, J., Sturm, K., Leicht, P., *et al.* (1998b). "Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans." J Clin Invest **101**(7): 1421-1430.

Schoeller, D.A. and Buchholz, A.C. (2005). "Energetics of obesity and weight control: does diet composition matter?" J Am Diet Assoc **105**(5 Suppl 1): S24-28.

Schwizer, W., Borovicka, J., Kunz, P., *et al.* (1997). "Role of cholecystokinin in the regulation of liquid gastric emptying and gastric motility in humans: studies with the CCK antagonist loxiglumide." Gut **41**(4): 500-504.

Sebastian, A., Harris, S.T., Ottaway, J.H., *et al.* (1994). "Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate." N Engl J Med **330**(25): 1776-1781.

Seimon, R.V., Brennan, I.M., Russo, A., *et al.* (2013). "Gastric emptying, mouth-to-cecum transit, and glycemic, insulin, incretin, and energy intake responses to a mixed-nutrient liquid in lean, overweight, and obese males." Am J Physiol Endocrinol Metab **304**(3): E294-300.

Seimon, R.V., Feltrin, K.L., Meyer, J.H., *et al.* (2009a). "Effects of varying combinations of intraduodenal lipid and carbohydrate on antropyloroduodenal motility, hormone release, and appetite in healthy males." American Journal of Physiology-Regulatory Integrative and Comparative Physiology **296**(4): R912-R920.

Seimon, R.V., Lange, K., Little, T.J., *et al.* (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**(1): 61-68.

Seimon, R.V., Taylor, P., Little, T.J., *et al.* (2014). "Effects of acute and longer-term dietary restriction on upper gut motility, hormone, appetite, and energy-intake responses to duodenal lipid in lean and obese men." Am J Clin Nutr **99**(1): 24-34.

Seimon, R.V., Wooster, T., Otto, B., *et al.* (2009b). "The droplet size of intraduodenal fat emulsions influences antropyloroduodenal motility, hormone release, and appetite in healthy males." Am J Clin Nutr **89**(6): 1729-1736.

Sepple, C.P. and Read, N.W. (1989). "Gastrointestinal correlates of the development of hunger in man." Appetite **13**(3): 183-191.

Shibata, C., Naito, H., Jin, X.L., *et al.* (2001). "Effect of glucagon, glicentin, glucagon-like peptide-1 and -2 on interdigestive gastroduodenal motility in dogs with a vagally denervated gastric pouch." Scand J Gastroenterol **36**(10): 1049-1055.

Shiiya, T., Nakazato, M., Mizuta, M., *et al.* (2002). "Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion." J Clin Endocrinol Metab **87**(1): 240-244.

Silk, D.B., Grimble, G.K. and Rees, R.G. (1985). "Protein digestion and amino acid and peptide absorption." Proc Nutr Soc **44**(1): 63-72.

Simons, J.P., Schols, A.M., Westerterp, K.R., *et al.* (1999). "Bioelectrical impedance analysis to assess changes in total body water in patients with cancer." Clin Nutr **18**(1): 35-39.

Sjodin, A., Gasteyger, C., Nielsen, A.L., *et al.* (2010). "The effect of the triple monoamine reuptake inhibitor tesofensine on energy metabolism and appetite in overweight and moderately obese men." Int J Obes (Lond) **34**(11): 1634-1643.

Sjostrom, L., Lindroos, A.K., Peltonen, M., *et al.* (2004). "Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery." N Engl J Med **351**(26): 2683-2693.

Skov, A.R., Toubro, S., Bulow, J., *et al.* (1999a). "Changes in renal function during weight loss induced by high vs low-protein low-fat diets in overweight subjects." Int J Obes Relat Metab Disord **23**(11): 1170-1177.

Skov, A.R., Toubro, S., Ronn, B., *et al.* (1999b). "Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity." Int J Obes Relat Metab Disord **23**(5): 528-536.

Sloth, B., Davidsen, L., Holst, J.J., *et al.* (2007a). "Effect of subcutaneous injections of PYY1-36 and PYY3-36 on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males." Am J Physiol Endocrinol Metab **293**(2): E604-609.

Sloth, B., Holst, J.J., Flint, A., *et al.* (2007b). "Effects of PYY1-36 and PYY3-36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects." Am J Physiol Endocrinol Metab **292**(4): E1062-1068.

Smeets, A.J., Soenen, S., Luscombe-Marsh, N.D., *et al.* (2008). "Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch." Journal of Nutrition **138**(4): 698-702.

Smith, D.E., Marcus, M.D., Lewis, C.E., *et al.* (1998). "Prevalence of binge eating disorder, obesity, and depression in a biracial cohort of young adults." Ann Behav Med **20**(3): 227-232.

Smith, R.G., Sun, Y., Jiang, H., *et al.* (2007). "Ghrelin receptor (GHS-R1A) agonists show potential as interventive agents during aging." Ann N Y Acad Sci **1119**: 147-164.

Snow, V., Barry, P., Fitterman, N., *et al.* (2005). "Pharmacologic and surgical management of obesity in primary care: a clinical practice guideline from the American College of Physicians." Ann Intern Med **142**(7): 525-531.

Soenen, S., Martens, E.A., Hochstenbach-Waelen, A., *et al.* (2013). "Normal protein intake is required for body weight loss and weight maintenance, and elevated protein intake for additional preservation of resting energy expenditure and fat free mass." J Nutr **143**(5): 591-596.

Solerte, S.B., Fioravanti, M., Locatelli, E., *et al.* (2008). "Improvement of blood glucose control and insulin sensitivity during a long-term (60 weeks) randomized study with amino acid dietary supplements in elderly subjects with type 2 diabetes mellitus." Am J Cardiol **101**(11A): 82E-88E.

Solerte, S.B., Gazzaruso, C., Schifino, N., *et al.* (2004). "Metabolic effects of orally administered amino acid mixture in elderly subjects with poorly controlled type 2 diabetes mellitus." Am J Cardiol **93**(8A): 23A-29A.

Speechly, D.P. and Buffenstein, R. (2000). "Appetite dysfunction in obese males: evidence for role of hyperinsulinaemia in passive overconsumption with a high fat diet." Eur J Clin Nutr **54**(3): 225-233.

Spencer, H., Kramer, L., DeBartolo, M., *et al.* (1983). "Further studies of the effect of a high protein diet as meat on calcium metabolism." Am J Clin Nutr **37**(6): 924-929.

Stalmans, W. (1983). Glucagon and liver glycogen metabolism. Glucagon I. L. PJ. Berlin, Springer-Verlag**:** 291-314.

Stanstrup, J., Schou, S.S., Holmer-Jensen, J., *et al.* (2014). "Whey protein delays gastric emptying and suppresses plasma fatty acids and their metabolites compared to casein, gluten, and fish protein." J Proteome Res **13**(5): 2396-2408.

Stein, T.P., Rumpler, W.V., Leskiw, M.J., *et al.* (1991). "Effect of Reduced Dietary-Intake on Energy-Expenditure, Protein-Turnover, and Glucose Cycling in Man." Metabolism-Clinical and Experimental **40**(5): 478-483.

Steinberg, D., Shafrir, E. and Vaughan, M. (1959). "Direct effect of glucagon on release of unesterified fatty acids (UFA) from adipose tissue." Clin. Res **7**: 220.

Steinert, R.E., Landrock, M.F., Horowitz, M., *et al.* (2015a). "Effects of Intraduodenal Infusions of L-phenylalanine and L-glutamine on Antropyloroduodenal Motility and Plasma Cholecystokinin in Healthy Men." J Neurogastroenterol Motil **21**(3): 404-413.

Steinert, R.E., Landrock, M.F., Ullrich, S.S., *et al.* (2015b). "Effects of intraduodenal infusion of the branched-chain amino acid leucine on ad libitum eating, gut motor and hormone functions, and glycemia in healthy men." Am J Clin Nutr **102**(4): 820-827.

Steinert, R.E., Meyer-Gerspach, A.C. and Beglinger, C. (2012). "The role of the stomach in the control of appetite and the secretion of satiation peptides." Am J Physiol Endocrinol Metab **302**(6): E666-673.

Steinert, R.E., Schirra, J., Meyer-Gerspach, A.C., *et al.* (2014). "Effect of glucagon-like peptide-1 receptor antagonism on appetite and food intake in healthy men." Am J Clin Nutr **100**(2): 514-523.

Stevens, J.E., Gilja, O.H., Gentilcore, D., *et al.* (2011). "Measurement of gastric emptying of a high-nutrient liquid by 3D ultrasonography in diabetic gastroparesis." Neurogastroenterol Motil **23**(3): 220-225, e113-224.

Stewart, J.E., Seimon, R.V., Otto, B., *et al.* (2011). "Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men." Am J Clin Nutr **93**(4): 703-711.

Strunz, U.T. and Grossman, M.I. (1978). "Effect of intragastric pressure on gastric emptying and secretion." Am J Physiol **235**(5): E552-555.

Stubbs, R.J. (1996). "Dietary macronutrients and glucostatic control of feeding." Proc Nutr Soc **55**(1B): 467-483.

Stubbs, R.J., van Wyk, M.C., Johnstone, A.M., *et al.* (1996). "Breakfasts high in protein, fat or carbohydrate: effect on within-day appetite and energy balance." Eur J Clin Nutr **50**(7): 409-417.

Stunkard, A.J. and Messick, S. (1985). "The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger." J Psychosom Res **29**(1): 71-83.

Sturm, K., MacIntosh, C.G., Parker, B.A., *et al.* (2003). "Appetite, food intake, and plasma concentrations of cholecystokinin, ghrelin, and other gastrointestinal hormones in undernourished older women and well-nourished young and older women." J Clin Endocrinol Metab **88**(8): 3747-3755.

Sturm, K., Parker, B., Wishart, J., *et al.* (2004). "Energy intake and appetite are related to antral area in healthy young and older subjects." Am J Clin Nutr **80**(3): 656-667.

Sumithran, P., Prendergast, L.A., Delbridge, E., *et al.* (2011). "Long-term persistence of hormonal adaptations to weight loss." N Engl J Med **365**(17): 1597-1604.

Swaminathan, R., King, R.F., Holmfield, J., *et al.* (1985). "Thermic effect of feeding carbohydrate, fat, protein and mixed meal in lean and obese subjects." Am J Clin Nutr **42**(2): 177-181.

Swinburn, B.A., Ley, S.J., Carmichael, H.E., *et al.* (1999). "Body size and composition in Polynesians." Int J Obes Relat Metab Disord **23**(11): 1178-1183.

Tack, J., Depoortere, I., Bisschops, R., *et al.* (2006). "Influence of ghrelin on interdigestive gastrointestinal motility in humans." Gut **55**(3): 327-333.

Takahashi, T. (2012). "Mechanism of interdigestive migrating motor complex." J Neurogastroenterol Motil **18**(3): 246-257.

Tannous dit El Khoury, D., Obeid, O., Azar, S.T., *et al.* (2006). "Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males." Annals of Nutrition and Metabolism **50**(3): 260-269.

Tappy, L. (1996). "Thermic effect of food and sympathetic nervous system activity in humans." Reprod Nutr Dev **36**(4): 391-397.

Tefera, S., Gilja, O.H., Olafsdottir, E., *et al.* (2002). "Intragastric maldistribution of a liquid meal in patients with reflux oesophagitis assessed by three dimensional ultrasonography." Gut **50**(2): 153-158.

Thimister, P.W., Hopman, W.P., Sloots, C.E., *et al.* (1996). "Role of intraduodenal proteases in plasma cholecystokinin and pancreaticobiliary responses to protein and amino acids." Gastroenterology **110**(2): 567-575.

Thompson, D., Edelsberg, J., Colditz, G.A., *et al.* (1999). "Lifetime health and economic consequences of obesity." Arch Intern Med **159**(18): 2177-2183.

Thor, P., Laskiewicz, J., Konturek, J.W., *et al.* (1987). "Role of GIP and insulin in glucose-induced changes in intestinal motility patterns." Am J Physiol **252**(1 Pt 1): G8-12.

Toh, S.Y., Zarshenas, N. and Jorgensen, J. (2009). "Prevalence of nutrient deficiencies in bariatric patients." Nutrition **25**(11-12): 1150-1156.

Tolessa, T., Gutniak, M., Holst, J.J., *et al.* (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**(10): 2284-2290.

Tome, D. (2004). "Protein, amino acids and the control of food intake." Br J Nutr **92 Suppl 1**: S27-30.

Tome, D., Schwarz, J., Darcel, N., *et al.* (2009). "Protein, amino acids, vagus nerve signaling, and the brain." Am J Clin Nutr **90**(3): 838S-843S.

Tong, J., Prigeon, R.L., Davis, H.W., *et al.* (2010). "Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans." Diabetes **59**(9): 2145-2151.

Tosetti, C., Corinaldesi, R., Stanghellini, V., *et al.* (1996a). "Gastric emptying of solids in morbid obesity." Int J Obes Relat Metab Disord **20**(3): 200-205.

Tosetti, C., Corinaldesi, R., Stanghellini, V., *et al.* (1996b). "Gastric emptying of solids in morbid obesity." Int J Obes Relat Metab Disord **20**(3): 200-205.

Tschop, M., Weyer, C., Tataranni, P.A., *et al.* (2001). "Circulating ghrelin levels are decreased in human obesity." Diabetes **50**(4): 707-709.

Tseng, C.C., Kieffer, T.J., Jarboe, L.A., *et al.* (1996). "Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide (GIP). Effect of a specific glucose-dependent insulinotropic polypeptide receptor antagonist in the rat." J Clin Invest **98**(11): 2440-2445.

Tulipano, G., Sibilia, V., Caroli, A.M., *et al.* (2011a). "Whey proteins as source of dipeptidyl dipeptidase IV (dipeptidyl peptidase-4) inhibitors." Peptides **32**(4): 835-838.

Tulipano, G., Sibilia, V., Caroli, A.M., *et al.* (2011b). "Whey proteins as source of dipeptidyl dipeptidase IV (dipeptidyl peptidase-4) inhibitors." Peptides **32**(4): 835-838.

Turton, M.D., O'Shea, D., Gunn, I., *et al.* (1996). "A role for glucagon-like peptide-1 in the central regulation of feeding." Nature **379**(6560): 69-72.

Ueno, H., Yamaguchi, H., Mizuta, M., *et al.* (2008). "The role of PYY in feeding regulation." Regul Pept **145**(1-3): 12-16.

Uneyama, H., Niijima, A., San Gabriel, A., *et al.* (2006). "Luminal amino acid sensing in the rat gastric mucosa." Am J Physiol Gastrointest Liver Physiol **291**(6): G1163-1170.

Valette, M., Bellisle, F., Carette, C., *et al.* (2012). "Eating behaviour in obese patients with melanocortin-4 receptor mutations: a literature review." Int J Obes (Lond).

van den Berg, E., Hospers, F.A., Navis, G., *et al.* (2011). "Dietary acid load and rapid progression to end-stage renal disease of diabetic nephropathy in Westernized South Asian people." J Nephrol **24**(1): 11-17.

Vanis, L., Hausken, T., Gentilcore, D., *et al.* (2011). "Comparative effects of glucose and xylose on blood pressure, gastric emptying and incretin hormones in healthy older subjects." Br J Nutr **105**(11): 1644-1651.

Veldhorst, M., Smeets, A., Soenen, S., *et al.* (2008). "Protein-induced satiety: Effects and mechanisms of different proteins." Physiology & Behavior **94**(2): 300-307.

Veldhorst, M.A., Nieuwenhuizen, A.G., Hochstenbach-Waelen, A., *et al.* (2009a). "Dose-dependent satiating effect of whey relative to casein or soy." Physiol Behav **96**(4-5): 675-682.

Veldhorst, M.A.B., Nieuwenhuizen, A.G., Hochstenbach-Waelen, A., *et al.* (2009b). "Comparison of the effects of a high- and normal-casein breakfast on satiety, 'satiety' hormones, plasma amino acids and subsequent energy intake." British Journal of Nutrition **101**(2): 295-303.

Veldhorst, M.A.B., Nieuwenhuizen, A.G., Hochstenbach-Waelen, A., *et al.* (2009c). "Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety." Appetite **52**(2): 388-395.

Verdich, C., Madsen, J.L., Toubro, S., *et al.* (2000). "Effect of obesity and major weight reduction on gastric emptying." Int J Obes Relat Metab Disord **24**(7): 899-905.

Verdich, C., Toubro, S., Buemann, B., *et al.* (2001). "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**(8): 1206-1214.

Vilsboll, T., Agerso, H., Krarup, T., *et al.* (2003). "Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects." J Clin Endocrinol Metab **88**(1): 220-224.

Walker, G.D., Stewart, J.J. and Bass, P. (1974). "The effect of parietal cell and truncal vagotomy on gastric and duodenal contractile activity of the unanesthetized dog." Ann Surg **179**(6): 853-858.

Walls, E.K. and Koopmans, H.S. (1992). "Differential effects of intravenous glucose, amino acids, and lipid on daily food intake in rats." Am J Physiol **262**(2 Pt 2): R225-234.

Walls, H.L., Magliano, D.J., Stevenson, C.E., *et al.* (2012). "Projected progression of the prevalence of obesity in Australia." Obesity (Silver Spring) **20**(4): 872-878.

Wang, B.J. and Cui, Z.J. (2007). "How does cholecystokinin stimulate exocrine pancreatic secretion? From birds, rodents, to humans." Am J Physiol Regul Integr Comp Physiol **292**(2): R666-678.

Weigle, D.S., Breen, P.A., Matthys, C.C., *et al.* (2005). "A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations." Am J Clin Nutr **82**(1): 41-48.

Weigle, D.S., Cummings, D.E., Newby, P.D., *et al.* (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**(4): 1577-1586.

Weintraub, M., Sundaresan, P.R., Madan, M., *et al.* (1992). "Long-term weight control study. I (weeks 0 to 34). The enhancement of behavior modification, caloric restriction, and exercise by fenfluramine plus phentermine versus placebo." Clin Pharmacol Ther **51**(5): 586-594.

Westerterp-Plantenga, M.S., Lejeune, M.P.G.M., Nijs, I., *et al.* (2004). "High protein intake sustains weight maintenance after body weight loss in humans." International Journal of Obesity **28**(1): 57-64.

Westerterp-Plantenga, M.S., Lejeune, M.P.G.M., Smeets, A.J.P.G., *et al.* (2009a). "Sex differences in energy homeostatis following a diet relatively high in protein exchanged with carbohydrate, assessed in a respiration chamber in humans." Physiology & Behavior **97**(3-4): 414-419.

Westerterp-Plantenga, M.S., Luscombe-Marsh, N., Lejeune, M.P.G.M., *et al.* (2006). "Dietary protein, metabolism, and body-weight regulation: dose-response effects." International Journal of Obesity **30**: S16-S23.

Westerterp-Plantenga, M.S., Nieuwenhuizen, A., Tome, D., *et al.* (2009b). "Dietary protein, weight loss, and weight maintenance." Annu Rev Nutr **29**: 21-41.

Westerterp-Plantenga, M.S., Rolland, V., Wilson, S.A., *et al.* (1999). "Satiety related to 24 h diet-induced thermogenesis during high protein/carbohydrate vs high fat diets measured in a respiration chamber." Eur J Clin Nutr **53**(6): 495-502.

Westerterp, K.R. (2004). "Diet induced thermogenesis." Nutr Metab (Lond) **1**(1): 5.

Westerterp, K.R., Wilson, S.A. and Rolland, V. (1999). "Diet induced thermogenesis measured over 24h in a respiration chamber: effect of diet composition." Int J Obes Relat Metab Disord **23**(3): 287-292.

Wierup, N., Svensson, H., Mulder, H., *et al.* (2002). "The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas." Regul Pept **107**(1-3): 63-69.

Wikarek, T., Chudek, J., Owczarek, A., *et al.* (2014). "Effect of dietary macronutrients on postprandial incretin hormone release and satiety in obese and normal-weight women." Br J Nutr **111**(2): 236-246.

Williams, D.L., Cummings, D.E., Grill, H.J., *et al.* (2003). "Meal-related ghrelin suppression requires postgastric feedback." Endocrinology **144**(7): 2765-2767.

Wing, R.R., Lang, W., Wadden, T.A., *et al.* (2011). "Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes." Diabetes Care **34**(7): 1481-1486.

Wisen, O. and Johansson, C. (1992). "Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal." Metabolism **41**(4): 390-395.

Wishart, J., Morris, H.A. and Horowitz, M. (1992). "Radioimmunoassay of gastric inhibitory polypeptide in plasma." Clin Chem **38**(10): 2156-2157.

Withrow, D. and Alter, D.A. (2011). "The economic burden of obesity worldwide: a systematic review of the direct costs of obesity." Obes Rev **12**(2): 131-141.

Woerle, H.J., Carneiro, L., Derani, A., *et al.* (2012). "The Role of Endogenous Incretin Secretion as Amplifier of Glucose-Stimulated Insulin Secretion in Healthy Subjects and Patients With Type 2 Diabetes." Diabetes **61**(9): 2349-2358.

Woods, S.C., Seeley, R.J., Porte, D., Jr., *et al.* (1998). "Signals that regulate food intake and energy homeostasis." Science **280**(5368): 1378-1383.

Woods, S.C., Stein, L.J., McKay, L.D., *et al.* (1984). "Suppression of food intake by intravenous nutrients and insulin in the baboon." Am J Physiol **247**(2 Pt 2): R393-401.

World Health Organisation. (2015). "Obesity and Overweight - Fact sheet 311." Retrieved 16/1/2013.

Wren, A.M., Seal, L.J., Cohen, M.A., *et al.* (2001a). "Ghrelin enhances appetite and increases food intake in humans." J Clin Endocrinol Metab **86**(12): 5992.

Wren, A.M., Small, C.J., Abbott, C.R., *et al.* (2001b). "Ghrelin causes hyperphagia and obesity in rats." Diabetes **50**(11): 2540-2547.

Wright, R.A., Krinsky, S., Fleeman, C., *et al.* (1983). "Gastric emptying and obesity." Gastroenterology **84**(4): 747-751.

Wycherley, T.P., Brinkworth, G.D., Clifton, P.M., *et al.* (2012a). "Comparison of the effects of 52 weeks weight loss with either a high-protein or high-carbohydrate diet on body composition and cardiometabolic risk factors in overweight and obese males." Nutr Diabetes **2**: e40.

Wycherley, T.P., Moran, L.J., Clifton, P.M., *et al.* (2012b). "Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials." Am J Clin Nutr **96**(6): 1281-1298.

Yamada, Y. and Seino, Y. (2004). "Physiology of GIP--a lesson from GIP receptor knockout mice." Horm Metab Res **36**(11-12): 771-774.

Yanovski, S.Z., Yanovski, J.A., Gwirtsman, H.E., *et al.* (1993). "Normal dexamethasone suppression in obese binge and nonbinge eaters with rapid weight loss." J Clin Endocrinol Metab **76**(3): 675-679.

Zahorska-Markiewicz, B., Jonderko, K., Lelek, A., *et al.* (1986). "Gastric emptying in obesity." Hum Nutr Clin Nutr **40**(4): 309-313.