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# **WHEY PROTEIN AND SATIETY IN HUMANS**

A thesis presented in partial fulfilment of the requirements for the degree of

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# Abstract

Protein is the most satiating macronutrient and there is an effect of dietary protein source, with dairy whey protein being particularly effective in promoting satiety in adult humans. The underlying cause for this remains to be elucidated. The objectives were to confirm that whey protein is more satiating than maltodextrin carbohydrate in adult humans, to understand the potential mediating factors and to investigate which characteristic of whey protein gives rise to its satiating effect. *Ad libitum* food intake at a subsequent test meal after administration of a preload, subjective feelings of appetite (using visual analogue scales) and plasma concentrations of satiety-related hormones and metabolites were determined. Preload diets enriched with whey protein induced a greater reduction in subsequent food intake and suppression in rated feelings of appetite compared with maltodextrin carbohydrate ( $p < 0.05$ ). The time of consumption of the whey protein preload did not influence the satiety response. Plasma concentrations of pancreatic polypeptide hormone, total amino acids, and the branched-chain amino acids appear to play an important role in mediating the satiating effect of whey protein (sustained increases from 15 to 120 min following preload consumption,  $p < 0.05$ ). To determine the underlying characteristic of whey protein causing the satiating effect, the effects on satiety of whey protein components (glycomacropeptide, alpha-lactalbumin, or beta-lactoglobulin) and a free amino acid mixture simulating the amino acid composition of the whey protein were compared with that of the intact whey protein. The amino acid composition of whey protein *per se* appears to be important in the regulation of food intake and the induction of satiety. The individual constituent proteins or whey protein itself did not promote higher satiety than that found based on providing the free amino acids. The absorbed amino acid profile would appear to play an important role in mediating the satiating effect of whey protein.

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## List of Abbreviations

~	Approximately
$\alpha$	Alpha
$\alpha$ -la	Alpha-lactalbumin
$\beta$	Beta
$\beta$ -lg	Beta-lactoglobulin
$\gamma$	Gamma
$\kappa$	Kappa
5-HT	Serotonin
A-LA	Alpha-lactalbumin
AA	Amino acid
ADA	American Diabetes Association
AgRP	Agouti-related peptide
AMPK	Adenosine monophosphate-activated protein kinase
ANOVA	Analysis of variance
AOAC	Association of Official Chemists
ARN	Arcuate nucleus
ATP	Adenosine triphosphate
AUC	Area under the curve
B-LG	Beta-lactoglobulin
BCAA	Branched-chain amino acid
BMI	Body mass index
BSA	Bovine serum albumin
C	Carbohydrate
Carb	Carbohydrate

CART	Cocaine- and amphetamine-regulated transcript
CCK	Cholecystokinin
CMP	Caseinomacropeptide
CNS	Central nervous system
CRF	Corticotrophin releasing factor
DIT	Diet-induced thermogenesis
DPP-IV	Dipeptidyl-peptidase-IV
E	Energy
EAA	Essential amino acid
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
F	Fat
FAO	Food and Agricultural Organisation
FFA	Free fatty acids
GE	Gross energy
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GMP	Glycomacropeptide
HC	High-Carbohydrate
HF	High-Fat
HP	High-Protein
HPLC	High-performance liquid chromatography
iAUC	incremental area under the curve
IFNHH	Institute of Food, Nutrition and Human Health
Ig	Immunoglobulin
J	joule

kcal	kilocalorie
kJ	kilojoule
MC	Melanocortin
ME	Metabolisable energy
MJ	megajoules
mTOR	rapamycin
NP	Normal-Protein
NPY	Neuropeptide Y
NTS	Nucleus tractus solitaries
NW	Normal-weight
OPA	o-phthalaldehyde
OW	Overweight
P	Protein
PKU	Phenylketonuria
POMC	Pro-opiomelanocortin
PP	Pancreatic polypeptide
Pro	Protein
PYY	Peptide tyrosine-tyrosine
SE	Standard error of mean
sem	Standard error of mean
T/LNAA	Tryptophan Large neutral amino acids ratio
UNU	United Nations University
VAS	Visual analogue scales
WHO	World Health Organisation
WP	Whey protein
WPI	Whey protein isolate

# Preface

In recent years, the prevalence of obesity in humans and the increased risk for associated disorders, such as type 2 diabetes, cardiovascular disease, and the Metabolic Syndrome, have increased rapidly. In simple terms, obesity results from an imbalance between food (energy) intake and energy expenditure. There is growing interest in the regulation of food intake as a strategy to combat obesity. Satiety, induced by food consumption, is the state in which an individual has a feeling of fullness and reduced feelings of appetite, and results in a suppression of further food intake. The induction of satiety may help to improve compliance to an energy-restricted diet.

The consumption of high-protein diets has been found to exert a beneficial effect on the factors influencing energy balance. Consumption of high-protein diets has been shown to promote body weight loss and to assist to maintain body weight compared with diets high in either carbohydrate or fat. The association between increased protein consumption and lower body weight has been attributed to a higher energy cost of protein metabolism and a higher satiating effect of protein. It is widely believed that, isoenergetically, dietary protein promotes satiety more than carbohydrate, fat, or alcohol. Moreover, the effect on satiety appears to be dependent on the source of protein. Dairy whey protein has generally been found to induce satiety to a greater extent than proteins from other sources. However, the mechanisms of action of the satiating effect of whey protein remain unclear. The contribution of the whey protein components (namely beta-lactoglobulin, alpha-lactalbumin, and glycomacropeptide) and the plasma amino acid balance consequent upon digestion of whey protein, to the satiety effect are poorly understood. Additive and potentially synergistic effects between these different dairy protein components also require further investigation. The overall aim of the work described in this dissertation was to provide a better understanding of the role of whey protein in promoting satiety in adult humans. An understanding of the

nature of the higher satiating effect of dairy whey protein in comparison with other protein sources or macronutrients is important for its application as a dietary intervention in the control of obesity.

# **Chapter 1**

## **Review of literature**

## 1.1. Introduction

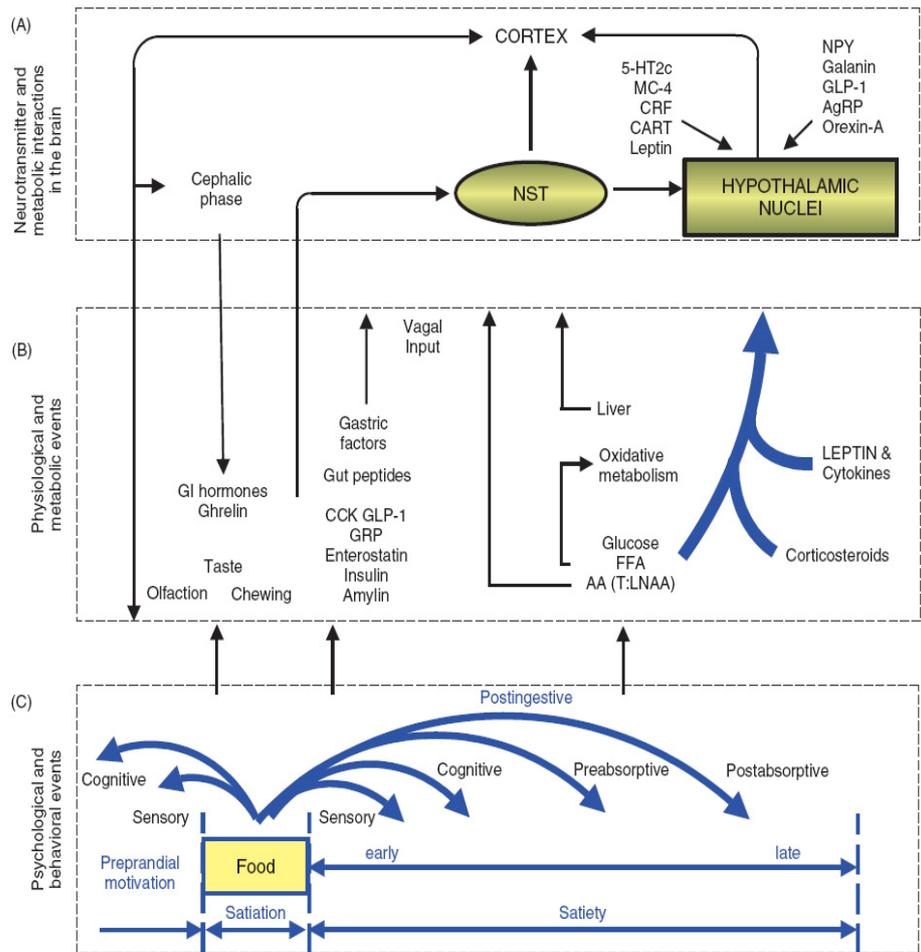
The rising prevalence of overweightness and obesity (*WHO, 2012*), and the increased risk for associated disorders, such as type 2 diabetes, cardiovascular disease, and the Metabolic Syndrome (*Bjorntorp, 2001; Grundy, 2004; Kopelman, 2000; WHO, 2000*), is a growing health problem. The common strategy to prevent obesity and control body weight is to achieve a negative energy balance that results in weight loss by restricting excessive dietary intake coupled with an increase in energy expenditure (*Rosenbaum et al., 1997; WHO, 2000*). Several dietary strategies for the management of obesity have been proposed with the focus being on the energy density and fat content of the diet (*Jebb, 2005*). Another recent strategy involves the consumption of energy-restricted high-protein diets (> 15% of energy from protein), which has been found to result in a reduction of body weight and body fat mass, and help to sustain body weight (*Eisenstein et al., 2002; Gilbert et al., 2011; Halton & Hu, 2004; Noakes, 2008; Paddon-Jones et al., 2008; St Jeor et al., 2001; Te Morenga & Mann, 2012; Westerterp-Plantenga, 2008; Westerterp-Plantenga et al., 2009, 2012; Wycherley et al., 2012*). This effect may be partly attributed to the higher energy expenditure related to protein intake and/or the greater satiating effect of dietary protein in comparison with other macronutrients.

This review evaluates the effect of dietary protein on satiety and food intake. The possible mechanisms involved in the increased satiety following consumption of protein are also addressed. Some general aspects of dairy whey protein and glycomacropeptide are introduced, followed by an overview of experimental studies with humans on the effect of dairy whey protein and glycomacropeptide on satiety, with particular emphasis on the underlying mechanisms.

## **1.2. Satiety**

### **1.2.1. Terminology**

The processes involved in eating behaviour can be considered before (preprandial), during (prandial) and after (postprandial) the consumption of a meal (*Halford & Blundell, 2005*). In general terms, appetite has been defined as a series of psychological and physiological feelings (hunger, desire to eat, and fullness) related to the drive to find and eat food (*Blundell et al., 2010; Kissileff & van Itallie, 1982; Westerterp-Plantenga et al., 1994*). Satiating is the process of meal termination and is influenced by the size of the eating episode (*Blundell et al., 2010*). Satiety is defined as the suppression in further food intake and appetite and a feeling of fullness in the postprandial state. Satiety, induced by food consumption, determines the time interval between two meals (the inter-meal interval) and the amount of food consumed at the next eating event (*Blundell et al., 2010*). The satiety cascade (Figure 1.1.) is the concept that distinguishes four mediating processes (sensory, cognitive, pre-absorptive and post-absorptive) involved in the inhibition of hunger during satiety (*Blundell et al., 2010; Halford & Blundell, 2005*). However, Booth and Thibault (2000) have argued that satiation and satiety are not distinct processes but rather two different aspects of a continuous process of satiety influenced by the size and attributes (energy content and macronutrient composition) of a meal and the dietary habits of the individual. The state of satiety is thought to start as soon as food is eaten and to increase after food consumption.



**Figure 1.1.** Overview of the satiety cascade (reproduced with permission from *Halford & Blundell, 2005*), including the three levels of operations: (A) neurotransmitter and metabolic interactions in the brain, (B) physiological and metabolic events, and (C) psychological and behavioural events.

5-HT, serotonin; AA, amino acid; AgRP, agouti-related peptide; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin, CRF, corticotrophin releasing factor; FFA, free fatty acids; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; GRP, gastric releasing peptide; MC, melanocortin; NPY, neuropeptide Y; NST, nucleus tractus solitarius; T/LNAA, tryptophan large neutral amino acids ratio.

## **1.2.2. Measurement of satiety**

Satiety studies are difficult to perform as many experimental variables, such as subject characteristics, physical state and formulation of the test food, and *ad libitum* or fixed food consumption conditions (*Blundell et al., 2010; Livingstone et al., 2000; Reid & Hetherington, 1997; Rolls & Hammer, 1995; Stubbs et al., 1998*), have to be taken into consideration. Currently, there is no “Gold Standard” method for the measurement of satiety but there are generally accepted measures or biomarkers (*De Graaf et al., 2004*), including rating scales to measure subjective feelings of appetite, measures of food intake, and measurement of satiety-related hormones. It appears that no single assessment tool is sensitive and specific enough to be used as the sole indicator of satiety. In addition, there is no standardised experimental design that provides a robust and valid method to measure satiety.

### **1.2.2.1. Rating scales**

In satiety studies, the most common method to assess subjective perceived feelings of appetite is the visual analogue scales (VAS). VAS are usually 100 mm long lines labelled at each end with extremes of the feeling to be quantified. For example, in the assessment of hunger, the phrases ‘not at all hungry’ (0 mm) and ‘as hungry as I have ever felt’ (100 mm) would be used. Subjects are instructed to rate themselves by marking the scale at the point most appropriate to their feeling at that time. Quantification of the measurement is done by measuring the distance (mm) between 0 m and the mark (*Livingstone et al., 2000; Stubbs et al., 2000*).

In short-term appetite studies, it is common to relate rated subjective feelings of appetite with subsequent food intake to validate the rating method (*De Graaf, 1993; Flint et al., 2000*). Indeed, several studies have shown strong correlations between VAS ratings for appetite and subsequent food intake in young adult subjects (*Barkeling et al., 1995; De Castro & Elmore, 1988*;

*Hulshof et al., 1993; Porrini et al., 1995*). Although appetite VAS ratings may be influenced by several factors, including age, gender, and physical activity (*Gregersen et al., 2011*), the use of VAS to measure feelings of appetite has been found to be reproducible and predict subsequent food intake in young and old adult subjects (*Flint et al., 2000; Parker et al., 2004; Stubbs et al., 2000*).

Appetite can be thought of as a complex motivational state to search for and ingest food. Ratings scales have been commonly used to measure feelings of hunger, desire to eat, prospective food consumption and fullness in satiety studies but these do not encompass the full range of appetite feelings related to food intake (*Hill & Blundell, 1982*). It is possible that further questions may be able to differentiate better between a sensation for meal initiation and satiety (fullness). A collective measure of appetite based on an average score (hunger + desire to eat + prospective food consumption – fullness) may also be helpful when different questions are asked in different satiety studies. It is also important to emphasise that rated appetite scores are usually collected at different time intervals and as such, a temporal relationship between appetite ratings and subsequent food intake should be taken into account for validity.

#### **1.2.2.2. Food intake**

It is debatable whether food intake serves as a proxy for satiety, as the feeling of satiety is a determining factor of food intake (*Wiessing et al., 2012*). Efforts have been made to measure food intake in free-living individuals and in individuals under controlled laboratory conditions. The typical way of assessing food intake in a real life situation is through self-reporting, where the individual either keeps records, or estimates by recall the type and amount of food eaten (*Bingham et al., 1994; Block, 1982*). While these techniques are frequently used, they lack both accuracy and precision (*Block, 1982; Livingstone et al., 1990; Schoeller, 1990*). Methods used in the laboratory for a more precise and accurate assessment of food intake include continuous weighing of foods and laboratory weighing. With respect to continuous

weighing of food, Kissileff *et al.* (1980) developed the University Eating Monitor to measure the amount and rate of food intake with the use of a concealed electronic balance. Alternatively, electronic scales are used to weigh the number of grams of food consumed in a laboratory setting. Subjects are provided with foods that are pre-weighed and of a known composition to allow measurement of the amount of food eaten and nutrient intake. After the meal occasion, the foods are weighed and recorded by the investigator.

The primary food sources of energy are carbohydrates, proteins and fats, termed the “macronutrients” as they are needed in large (“macro”) amounts. Another organic compound that can provide energy is alcohol although it has no required bodily function (*Westerterp-Plantenga et al., 1994*). At the end of the 19<sup>th</sup> century, Atwater devised energy conversion factors for the major nutrients and these values are referred to as the ‘Atwater factors’ (*FAO, 2003*). The Atwater factors assign approximately 17 kJ (4 kcal) per gram to carbohydrate and protein, 37 kJ (9 kcal) per gram for fat and 29 kJ (7 kcal) per gram for alcohol. The energy content of a food largely depends on the amounts of carbohydrate, fat, protein and alcohol in that food, and the metabolisable energy (ME) content of a food can be predicted by multiplying the carbohydrate, protein, fat and alcohol contents with the Atwater factors (*Westerterp-Plantenga et al., 1994*). However, the Atwater factor for carbohydrate does not take into account the differentiation between available carbohydrate and dietary fibre. Total carbohydrate is calculated by the difference between 100 and the sum of the weight of crude protein, crude fat, water and ash in 100 g of food. Southgate and Durnin (1970) introduced a factor of 16 kJ (3.75 kcal) per gram for available carbohydrate expressed as monosaccharide. An energy value of 8 kJ (2 kcal) per gram dietary fibre has been recommended (*FAO, 1973; Livesey, 2001*). Nevertheless, it is important to remember that the energy values calculated using the Atwater factors are average ones as natural foods vary in composition from sample to sample and this is especially applicable for animal sources in which the fat content may be variable (*Garrow, 1978*). There are also other situations where ME values from food tables may be misleading such as when the diets are high in non-digestible carbohydrates or when foods have a variable water content

(*Buchholz & Schoeller, 2004; Zou et al., 2007*). Atwater factors are approximations and are influenced by a number of factors (*Moughan et al., 2010*). Nowadays, the Atwater factors and modified versions of them form the basis for food composition tables that usually list the energy content of foods not as gross energy (GE), the energy contained in food as ingested energy, but as metabolisable energy (ME), the energy available to the human body for metabolism (*Buchholz & Schoeller, 2004*).

### **1.2.3. Experimental designs**

One of the obstacles in satiety research has been uncertainty about which laboratory method to use. Methods currently being used include the test meal design, the request for a meal and the preload-test meal method. The test meal design or concurrent evaluation method relates to satiation and measures the amount of food consumed when participants are provided with unrestricted access (*ad libitum*) to a large amount of the manipulated test meal (*Berti et al., 2008; Kissileff, 1985; Porrini et al., 1997*). The request for a meal following consumption of a test meal, when subjects are deprived of time cues, and the amount consumed at the next meal is used as a measure of satiety (*Himaya et al., 1997; Marmonier et al., 2000*). The preload-test meal paradigm may serve as a reference experimental technique for assessing subjective ratings of appetite, objective measures of food intake and responses of hormonal factors related to satiety (*Blundell et al., 2010*). This method is based on the premise that a fixed amount of a specific food (preload) is provided at a predetermined time interval prior to a test meal. Although simple in concept, preload studies have differed with respect to subject characteristics (gender, age, BMI, feeding behaviour), preload characteristics (weight/volume, energy content and composition), time interval between preload and test meal, and composition of the test meal (*Blundell et al., 2010; Hellström et al., 2004; Livingstone et al., 2000; Mela, 2001; Reid & Hetherington, 1997; Rolls & Hammer, 1995; Stubbs et al., 1998*) and need to be carefully scrutinised. The preload-test meal method appears to be sensitive specifically to study the influence of a preload on measures of satiety during the time interval between preload and test meal and

on food intake at a subsequent *ad libitum* test meal (Gregersen *et al.*, 2008; Lara *et al.*, 2010; Speechly *et al.*, 1999; Wiessing *et al.*, 2012).

#### **1.2.4. Satiating capacity of foods**

One area of particular interest is the satiating power of foods and there are several measures used to compare the ability of a food to induce satiety, including the Satiating Efficiency measure, the Satiety Quotient and the Satiety Index. Kissileff (1948) proposed the Satiating Efficiency measure and used the preload method to compare the effectiveness of various preloads at decreasing food intake at the subsequent test meal per unit of preload studied (Kissileff *et al.*, 1984). The Satiety Quotient measures the extent to which food consumed during an eating episode reduces feelings of appetite per unit of food intake. The Satiety Quotient is calculated by dividing the difference between ratings of appetite before and after an eating episode (pre minus post) by the food (g) or energy (kJ or kcal) of intake during the episode. If calculated at various time points, the Satiety Quotient can indicate a temporal pattern of the satiating effect of the food consumed (Green *et al.*, 1997). Holt *et al.* (1995) put forward the Satiety Index to measure the capacity of foods of equal energy portions to lower ratings of appetite over 2 h in comparison with white bread as the reference food. The state of satiety is determined by the magnitude and direction of change in subjective feelings of appetite following consumption of a meal and by food intake at the next eating episode. Therefore, the concept of a metric test combining all three distinct measures may have merit. The satiating effect of a food may be represented by an overall score derived from the suppressive effect on subsequent food intake (Satiating Efficiency), and a temporal profile of the reduction in rated subjective feeling of appetite, before and after preload consumption (Satiety Quotient) and/or over a predetermined time period (Satiety Index) per unit of preload. There is widespread agreement that foods differ in their ability to enhance or reduce satiety but much depends upon the measures of satiety used and the design of the studies.

## 1.3. Protein and satiety

### 1.3.1. Effects of protein intake on satiety

In many human studies, protein appears to be more satiating than the other macronutrients (carbohydrate, fat or alcohol). Marmonier *et al.* (2000) convincingly demonstrated that a high-protein meal delayed the request for food for 60 min compared with 34 min for carbohydrate and 25 min for fat. Several scientific reviews have indicated that protein intake results in increased satiety and reduction in food intake (Anderson & Moore, 2004; Eisenstein *et al.*, 2002; Halton & Hu, 2004; Westerterp-Plantenga *et al.*, 2009). Eisenstein *et al.* (2002) reviewed ten preload studies that included measurement of food intake as well as feelings of appetite and found that six showed a lower energy intake at a subsequent meal and increased satiety with a high-protein preload compared with a control preload. Halton and Hu (2004) reviewed short-term studies on dietary protein and satiety and found that eleven out of fourteen studies showed a decrease in subjective ratings of appetite and eight out of fifteen studies showed that food energy intake was lower after consumption of a high-protein diet compared with a control or at least one other macronutrient.

Consumption of preload meals high in protein has been shown to reduce food intake at a subsequent meal and decrease subjective ratings of appetite to a greater extent than available carbohydrate, fat, or alcohol (Table 1.1.). In a study by Poppitt *et al.* (1998) where the satiating effect of four dietary components (fat, carbohydrate, protein, and alcohol) was investigated, ten normal-weight women reported feeling less hungry and more satiated throughout the study period and ate less food at lunch 90 min following a protein preload relative to the other macronutrients. In the Latner and Schwartz (1999) study, twelve normal-weight women consumed 31% more energy at a dinner meal 4.5 hours after a high-carbohydrate lunch and 20% more food energy after a mixed carbohydrate and protein lunch compared to an isoenergetic (450 kcal) high-protein lunch. The latter condition suppressed feelings of hunger more relative to the conditions on the other two diets.

Regarding food intake at a subsequent meal, some studies have found that a smaller amount of food and less food energy were consumed at a test meal after ingestion of a high-protein meal compared with other macronutrients. In a study of nine subjects, Booth *et al.* (1970) found that energy intake at a meal 2 to 3 h later was lower after consumption of the protein-rich lunch compared to the low-protein lunch. This is in line with the study of Barkeling *et al.* (1990) who showed a 12% decrease in food intake at a subsequent meal 4 h later when twenty normal-weight women ate a high-protein (43% of energy derived from protein, 21% fat, 36% carbohydrate) meat casserole compared to when they ate a high-carbohydrate (10% of energy derived from protein, 21% fat, 69% carbohydrate) vegetarian casserole. Similarly, Johnson and Vickers (1993) showed that a smaller amount of food and fewer calories were consumed at a test meal 90 min after ingestion of a high-protein meal compared with a high-carbohydrate or high-fat meal in fourteen normal-weight subjects.

With respect to subjective ratings of appetite, there is some evidence that dietary protein is rated as being more satiating than carbohydrate or fat. Porrini *et al.* (1997) observed that ratings for desire to eat decreased and ratings for fullness and satiety increased immediately after consumption of a high-protein breakfast omelette than after a high-fat omelette. Vandewater and Vickers (1996) found that VAS ratings for hunger and prospective consumption were lower and ratings for stomach fullness were greater 2 min following consumption of a high-protein yoghurt compared to the high-carbohydrate yoghurt version. In addition, ratings for satiety or fullness were shown to be higher after consumption of a high-protein meal compared to the low-protein meal counterparts within a short time frame (4-7 h) (Crovetti *et al.*, 1998; Smeets *et al.*, 2008). Over a whole day test period, three studies reported a greater suppression of appetite after a high-protein meal compared to a meal high in carbohydrate or fat (Johnstone *et al.*, 1996; Lejeune *et al.*, 2006; Stubbs *et al.*, 1996).

However, other studies have failed to find an effect of protein on subsequent food intake and subjective feelings of appetite in comparison with other macronutrients (*De Graaf et al., 1992; Geliebter, 1979; Griffioen-Roose et al., 2011; Johnstone et al., 2000; Potier et al., 2010; Raben et al., 2003; Vozzo et al., 2003*). For instance, in a study by Raben *et al.* (2003), energy intake at a test meal 5 h later and rated feelings of appetite did not differ following ingestion of breakfast meals rich in one of the four macronutrients (protein, carbohydrate, fat, and alcohol). Interestingly, Rolls *et al.* (1988) found that when ten normal-weight women consumed a protein-rich meal, their food intake at a subsequent test meal given 2 h later and ratings of hunger decreased while their ratings of stomach fullness increased versus consuming a meal high in sucrose, fat or sucrose and fat, but to a similar extent as ingestion of a meal high in starch. Similarly, Johnson and Vickers (1993) reported no difference between a high-protein and high-carbohydrate (predominantly starch) preload meal on feelings of appetite, as both preload meals were rated as being more satiating (lower hunger and prospective food consumption scores, and greater stomach fullness scores) compared to a meal high in fat.

**Table 1.1.** Effects of dietary protein on food intake and subjective ratings of appetite in normal-weight adult humans.

Reference	Subjects	Diets	Time interval	Effect on food intake	Effect on appetite
Crovetti <i>et al.</i> (1998)	10 women	Preload meals: HP (2373 kJ/567 kcal, 68.1% P, 19.2% F, 12.6% C) HC (2310 kJ/552 kcal, 10.1% P, 20.7% F, 69.1% C) HF (2310 kJ/552 kcal, 8.6% P, 70.1% F, 21.3% C)	7 h	No difference on test meal intake.	Ratings for fullness increased over the 7 h test period and ratings for desire to eat were lower over 3 h before administration of the test meal after the HP meal than after the HC or HF meals, but ratings for satiety did not differ.
De Graaf <i>et al.</i> (1992)	29 women	550 ml preload breakfasts: A zero condition (0.03 MJ, 8 kcal) and nine other preloads varied in energy levels (0.42 MJ/100 kcal, 1.05MJ/250 kcal, and 1.67 MJ/400 kcal) and macronutrient content: HP (70% P, 3% F, 27% C) HC (1% P, 0% F, 99% C) HF (3% P, 92% F, 5% C)	Food intake at lunch 3.75 h later and for the remainder of the day was recorded in a diary.	No difference in energy intake at lunch, for the remainder of the day and over the whole 24 h test period.	The rated responses to appetite and fullness did not differ in the time interval between preload breakfast and subsequent meal (3.75 h).

<p>Geliebter (1979)</p>	<p>12 men</p>	<p>283 kcal nutrient loads: Protein (egg albumen) Carbohydrate (corn starch) Fat (corn oil)</p>	<p>70 min</p>	<p>No difference in test meal intake.</p>	<p>Ratings for hunger and fullness did not differ.</p>
<p>Griffioen-Roose <i>et al.</i> (2011)</p>	<p>37 women 23 men</p>	<p>Rice meal preloads varying in taste and macronutrient composition: Sweet HP (1170 kJ/280 kcal, 26% P, 25% F, 49% C) Sweet HC (1149 kJ/275 kcal, 6% P, 24% F, 70% C) Savoury HP (1176 kJ/281 kcal, 25% P, 23% F, 52% C) Savoury HC (1162 kJ/278 kcal, 7% P, 24% F, 69% C)</p>	<p>30 min</p>	<p>Test meal intake 30 min later did not differ by taste and macronutrient content.</p>	<p>No change in subjective ratings of appetite between treatments.</p>
<p>Johnson &amp; Vickers (1993)</p>	<p>8 women 6 men</p>	<p>150 and 300 kcal preload meal: HP (80.3% P, 19.7% F, 0% C) HC (13.2% P, 3.5% F, 83.3% C) HF (5.7% P, 84.7% F, 9.6% C)</p>	<p>90 min</p>	<p>A smaller amount of food (about 13%) and fewer calories (about 15%) were consumed after the HP meal compared to the HC or HF meals.</p>	<p>The HC and HP meals decreased hunger and prospective food consumption ratings and increased stomach fullness ratings more than the HF meal.</p>

Johnstone <i>et al.</i> (1996)	6 men	Total meal intakes over 24-h: HP (15.28 MJ, 37.5% P, 28.5% F, 34.0% C) HC (15.12 MJ, 10.4% P, 29.0% F, 60.6% C) HF (15.58 MJ, 10.1% P, 56.5% F, 33.4% C)	Amount (g), energy and nutrient intakes (MJ) were measured throughout the day of overfeeding one macronutrient and on the subsequent day.	No effect on subsequent day intake.	The HP meal decreased ratings for hunger, desire to eat, urge to eat, and prospective consumption and increased fullness ratings over 24 h to a greater extent than the HC or HF meals.
Johnstone <i>et al.</i> (2000)	8 men	Snacks provided during whole day: HP (2.56 MJ, 73.4% P, 12.9% F, 13.7% C) HC (2.61 MJ, 12.6% P, 13.4% F, 74.0% C) HF (2.60 MJ, 12.7% P, 73.8% F, 13.5% C)	Intake of an <i>ad libitum</i> test meal (550 kJ/100 g, 13% P, 40% F, 47% C) was measured throughout the day.	No effect on intake of test meal following consumption of snacks and over the whole day (including snack intake).	Snack macronutrient composition did not affect mean daily ratings for hunger, urge to eat, prospective consumption, thoughts of food and fullness.
Latner & Schwartz (1999)	12 women	450 kcal preload lunch: HP (71.5% P, 19.2% F, 9.5% C) HC (0% P, 0% F, 99% C) Mixed (35.7% P, 9.6% F, 55.1% C)	4.5 h	31% more energy was consumed at dinner following the HC lunch and 20% more following the mixed lunch than following the HP lunch.	Hunger was less in the HP condition than for the HC and mixed treatments. The mixed lunch also reduced hunger scores more than the HC lunch.

Lejeune <i>et al.</i> (2006)	12 women	Diets to be consumed over four days: HP (4.1 kJ/g, 30% P, 30% F, 40% C) HC (4.3 kJ/g, 10% P, 30% F, 60% C)	Total energy intake (20% for breakfast, 40% for lunch and 40% for dinner) was measured on each of the four days.	No difference in total energy intake per day.	The HP diet decreased 24-h AUC for hunger and increased 24-h AUC for satiety (“How satiated are you?”) more than the HC diet condition. On day four, subjects reported feeling less hungry and more satiated before and after dinner following consumption of the HP diet relative to the HC diet.
Poppitt <i>et al.</i> (1998)	12 women	1 MJ baseline meal and drink to which 1MJ of each macronutrient was added: Protein (59.5% P, 20.1% F, 21.3% C) Carbohydrate (11.0% P, 19.1% F, 65.9% C) Fat (11.3% P, 67.0% F, 20.7% C) Alcohol (11.4% P, 20.1% F, 21.3% C, 46.7% Alcohol)	90 min	Energy intake was about 16 % lower after the protein treatment relative to the other macronutrients	Subjects reported feeling less hungry and more satiated ( “How satisfying did you find the meal?”) throughout the test period (120 min) on the protein meal compared to the other macronutrients.

Porrini <i>et al.</i> (1995)	10 men	HP meal (880 kcal, 56% P, 25% F, 19% C) HC meal (960 kcal, 17% P, 27% F, 56% C)	120 min	Subsequent food intake was lower (about 46%) for the HP meal compared with the HC meal.	No difference in ratings for desire to eat, fullness, and satiety.
Porrini <i>et al.</i> (1997)	14 men	Breakfast omelettes: HP (273.5 kcal, 53.5% P, 45.2% F, 1.3% C) HF (284.3 kcal, 14.8% P, 79.3% F, 5.9% C)	120 min	No difference in intake at the subsequent test meal.	Immediately after consuming the HP omelette, ratings for desire to eat decreased and ratings for fullness and satiety (“How sated do you feel?”) increased more than after the HF omelette.
Potier <i>et al.</i> (2010)	22 women 6 men	250 ml preload drinks containing: Protein (1019.1 kJ, 50 g of protein: 90% whey protein and 10% casein) Carbohydrate (1029.1 kJ, 50 g of maltodextrins) Fat (1016.2 kJ, 22.3 g of oil: 50% palm oil and 50% soya bean oil)	60 min	Carbohydrate reduced subsequent energy intake more than fat, while protein had an intermediate effect.	No difference in rated appetite sensations.

Raben <i>et al.</i> (2003)	9 women 10 men	Breakfast meals (2500 kJ for women and 3000 kJ for men) high in: Protein (31.8% P, 31.1% F, 37.2% C) Carbohydrate (12.2% P, 23.7% F, 65.4% C) Fat (11.6% P, 64.6% F, 23.9% C) Alcohol (12.1% P, 24.3% F, 42.9% C, 23.0% Alcohol)	5 h	No difference in subsequent energy intake.	No difference in ratings for hunger, prospective consumption, fullness and satiety after the four breakfast meals.
Rolls <i>et al.</i> (1988)	10 women	Preload meals high in: Protein (298.2 kcal, 74.6% P, 25.4% F, 0% C) Starch (300.3 kcal, 10.1% P, 9.3% F, 80.6% C) Sucrose (299.8 kcal, 2.6% P, 0% F, 97.4% C) Fat (289.7 kcal, 2.8% P, 97.2% F, 0% C) Sucrose and Fat (299.9 kcal, 4.6% P, 37.1% F, 58.3% C)	120 min	Food intake at test meal 120 min later was lower after consumption of the preload meals rich in protein and starch compared to the sucrose, fat and mixed (sucrose and fat) preload meals.	The high-protein and high-starch preload meals decreased hunger and increased stomach fullness over 120 min more than the other three preload meals.

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Smeets <i>et al.</i> (2008)	19 women 11 men	Preload lunches: HP (25% P, 30% F, 45% C) HC (10% P, 30% F, 60% C)	Appetite ratings were measured immediately, at 30, 60, 120, 180 and 240 min following the preload lunch.	Not measured	The ratings for hunger and satiety were higher 30 and 120 min after the HP lunch than after the LP lunch. The area under the curve of the satiety rating score (“How satiated are you?”) was greater after the HP meal than after the HC meal.
Stubbs <i>et al.</i> (1996)	6 men	Breakfast meals: HP (5.29 MJ, 58.8% P, 19.5% F, 21.7% C) HC (5.18 MJ, 18.5% P, 20.9% F, 60.6% C) HF (5.24 MJ, 22.3% P, 57.1% F, 22.3% C)	Energy intake at a test meal lunch 5 h later and throughout the rest of the day was assessed.	Lunch test meal and 24-h energy intakes were similar following all the breakfast meals.	The HP breakfast produced greater suppression of hunger (mean hourly scores) over 24 h than the HC or HF breakfasts, but no difference in ratings of fullness, desire to eat, urge to eat, prospective consumption, and preoccupation with thoughts of food.

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Vandewater & Vickers (1996)	29 women 11 men	676 kcal preload yoghurts: HP (43% P, 6% F, 51% C) HC (20% P, 6% F, 74% C)	Appetite ratings were measured 2 min following consumption of the preload yoghurts.	Not measured	Greater decrease in hunger and prospective consumption ratings and increase in stomach fullness ratings after the HP preload yoghurt than the HC preload.
Vozzo <i>et al.</i> (2003)	15 men	3 MJ preload yoghurts: HP (706 kcal, 29% P, 24% F, 44% C) HC (714 kcal, 14% P, 24% F, 60% C) HF (702 kcal, 14% P, 40% F, 42% C)	Spontaneous food intake of a buffet style test meal from 30 until 435 min after preload ingestion (7 h) was measured.	Subsequent food intake over 7 h was suppressed by the HP and HF preload yoghurts, but not the HC yoghurt, compared with the no preload condition.	No difference in ratings for hunger and fullness.

HP, High-Protein; HC, High-Carbohydrate; HF, High-Fat; P, protein; C, carbohydrate; F, fat; AUC, area under the curve.

There is strong evidence to suggest that in normal-weight adult subjects, dietary protein induces satiety more strongly than available carbohydrate, fat, or alcohol, on an isoenergetic basis, as indicated by food intake at a subsequent meal and subjective ratings of appetite (Table 1.1). The discrepancies in observations from preload studies may be related to the time interval between preload and test meal and the composition of the preload (*Blundell et al., 2010; Livingstone et al., 2000; Rolls & Hammer, 1995; Stubbs et al., 1998*).

A wide variation in time delay interval between preload and subsequent meal consumption has been used across preload studies determined by changes in feelings of appetite (*Fischer et al., 2004*) or plasma amino acid profiles (*Luhovyy et al., 2007*) or plasma gastrointestinal hormones (*Veldhorst et al., 2009*). *Rolls et al.* (1991) demonstrated that twenty-eight normal-weight subjects compensated well for the energy intake from a preload yoghurt (357 kcal) by eating less at a subsequent test meal compared to the no preload condition in the 30 min delay condition, but energy compensation became less precise as the time interval increased to 90 and 180 min. Nonetheless, two studies found that subsequent food intake and ratings of appetite did not differ following consumption of preloads high in either carbohydrate or fat, regardless of time interval (30, 90 and 180 min in the *Rolls et al.* (1991) study and 90 and 270 min in the *Blundell et al.* (1993) study). In a recent study of normal-weight boys (average age approximately 12 years) by *Bellissimo et al.* (2007), ingestion of a preload beverage containing whey protein (837 kJ, 1 g/kg body weight) reduced (about 14%) energy intake at a subsequent test meal 60 min later more than a preload containing glucose (837 kJ, 1 g/kg body weight), but this effect was not seen when the time delay interval between preload and test meal was 30 min. Similarly, *Bertenshaw et al.* (2008) observed a lower energy intake at a subsequent test meal following consumption a preload drink enriched with whey protein (1258.6 kJ, 49.6% of energy from protein, 0.5% fat and 49.9% carbohydrate) compared to maltodextrin carbohydrate (1246.5 kJ, 2.3% of energy from protein, 0.1% fat and 97.6% carbohydrate), independently of time delay interval (30 and 120 min). The effect of the time interval between the ingestion of a preload and subsequent

food intake on the measurement of satiety appears to be critical and deserves more investigative attention.

The varying findings regarding the satiating effect of dietary protein may be accounted for by differences in the composition of the preload used. The possibility that the sensory qualities of preloads may alter food intake and satiety has been examined with conflicting results (*Anderson, 2006; Bellisle et al., 2012; Sorensen et al., 2003; Yeomans et al., 2004*). For instance, it is thought that an increase in palatability results in an increase in food intake, but the effect of palatability on subjective ratings of appetite is unclear (*Drewnowski, 1998; Sorensen et al., 2003; Yeomans et al., 2004*). Studies have reported either increased or decreased ratings of hunger following consumption of a more palatable meal than after a less palatable meal or no difference in ratings of appetite (*Sorensen et al., 2003*). The physical form of the preloads is an important factor with some evidence suggesting that liquid preloads are less satiating and elicit lower energy compensatory responses than their solid counterparts (*Almiron-Roig et al., 2003; Drewnowski & Bellisle, 2007; Mattes, 2006*). With respect to dietary protein, a high-protein solid food was shown to sometimes decrease (*Mourao et al., 2007*) and sometimes not influence (*Akhavan et al., 2011*) subsequent food intake compared with a high-protein liquid form. In addition, a solid form of protein was found to suppress feelings of appetite to a greater extent than the liquid protein meal (*Akhavan et al., 2011; Leidy et al., 2011; Martens et al., 2011*). Although these factors were not measured, the mixed findings may have been due to differences in gastric motility, stomach emptying rate or the post-prandial release of satiety-related hormonal signals.

The protein content of the preloads may also play a role. The World Health Organisation (WHO) recommends that a dietary protein intake of on average 10-15% of energy when in energy balance is considered to be normal (*WHO, 2000*). An increase in satiety induced by dietary protein has been demonstrated in the short-term with preload meals containing a relatively high amount of protein (25-80% of energy derived from protein). In a recent study of twenty-eight normal-weight men by *Bertenshaw et al. (2009)*, increasing the

proportion of energy derived from protein in preload drinks from (1155 kJ, 17.3 g protein, 25.1% of energy from protein, 0.3% from fat and 74.6% from carbohydrate) to 50% (1163 kJ, 34.4 g protein, 49.5% of energy from protein, 0.2% from fat and 50% from carbohydrate) was associated with a dose dependent reduction in intake at a subsequent test meal provided 30 min later compared to when a low-energy control preload drink (328 kJ, 2.4 g protein, 12% of energy from protein, 3.4% fat and 84% carbohydrate) was imbibed, with an intermediate effect when the provided energy from protein was 13% (1151 kJ, 8.8 g protein, 12.8% of energy from protein, 0.2% fat and 87.1% carbohydrate) (*Bertenshaw et al., 2009*). The European Food Safety Authority (EFSA) also suggested that a daily serving of 49.15 g of protein would be needed to promote satiety (*EFSA, 2010*). Furthermore, most satiety studies have used preloads with mixed macronutrient composition, which have been suggested to be more acceptable and representative of a real meal, but the concurrent carbohydrate and fat contents may have influence satiety. However, two studies using pure protein, fat or carbohydrate preloads did not show a differential effect on subsequent food intake and appetite ratings (*Fischer et al., 2004; Potier et al., 2010*). This indicates that macronutrient composition when given in pure forms had little effect on measures of satiety and this remains to be further tested. The greater satiating effect of dietary protein in comparison with other macronutrients must be interpreted with caution because of the variations in experimental designs.

### **1.3.2. Mechanisms by which protein intake affects food intake and satiety**

The mechanisms by which dietary protein may influence food intake and satiety are unclear. It has been suggested that intake of a high protein diet may favourably affect energy expenditure, circulating concentrations of amino acids, and hormonal changes related to satiety (*Fromentin et al., 2012; Veldhorst et al., 2008*). Bioactive peptides, peptides with specific amino acid sequences which are active either locally in the gastrointestinal system or

systemically to modulate physiological functions, released during protein digestion may also impact on the regulation of food intake and satiety (*Froetschel, 1996; Jahan-Mihan et al., 2011; Moughan et al., 2007; Rutherford-Markwick & Moughan, 2005*).

### **1.3.2.1. Energy expenditure**

There is a component of energy expenditure associated with the consumption of food known as diet-induced thermogenesis (DIT) (or the thermic effect of food or specific dynamic action) (*Garrow, 1978*) and can be categorised into obligatory and facultative processes (*Jequier, 1983a*). Obligatory thermogenesis is the heat produced during ingestion, digestion, absorption and storage of nutrients. Complex carbohydrates are broken down to simple sugars, fats to fatty acids and monoglycerides, and proteins to amino acids. There are also some energetic costs to convert the excess energy from food into reserve fuel such as glycogen or adipose tissue for storage (*Warwick & Baines, 2000; Westerterp-Plantenga et al., 1994*). Facultative thermogenesis is the energy expended during the above obligatory processes. Facultative thermogenesis is the energy dissipated in response to excess dietary intake (*Jequier, 1983a; Westerterp-Plantenga et al., 1994*) and is thought to be under hormonal control (*Gulick, 1922*). In a healthy individual consuming an average mixed diet and being in energy balance, it has been estimated that DIT accounts for 10% of daily energy expenditure (*Sims & Danforth, 1987; Tappy, 1996*). A reduced DIT is thought to contribute to the pathogenesis of human obesity (*Jequier, 1983b; Jequier & Schutz, 1985*), but it is unclear whether this thermogenic impairment is a cause or consequence of obesity (*Bessard et al., 1983; Jequier & Schutz, 1985*).

DIT relates to the amount of adenosine triphosphate (ATP) macromolecules required for the metabolism and storage of nutrients and is influenced by the energy content and nutritional composition of a meal

(Kinabo & Durnin, 1990). Protein has been shown to have the greatest effect on DIT and energy expenditure compared with other macronutrients (Crovetti et al. 1998, Lejeune et al., 2006; Karst et al., 1984, Miller & Mumford, 1967; Nair et al., 1983; Raben et al., 2003; Robinson et al., 1990; Tappy, 1996; Westerterp, 2004; Westerterp et al., 1999; Westerterp-Plantenga et al., 1999). For instance, Karst et al. (1984) studied the thermic effect of three protein sources (egg albumen, gelatine, and casein), two carbohydrates (starch and hydrolysed starch), and two fats (sunflower oil and butter) in twelve normal-weight men for 6 h. While fat produced no observed thermic effect, the thermic effect of protein was three times larger than that of carbohydrate. In addition, Tappy (1996) reported that the DIT values of the macronutrients are 20 to 30% for protein, 5 to 10% for carbohydrate and 0 to 3% for fat. A review of data from nineteen trials revealed that for each 1% change in energy from protein, the thermic effect of the meal increased by 0.22% (Westerterp, 2004).

Several studies have shown that the higher thermic effect of dietary protein may be related to its satiating effect (Crovetti et al. 1998, Lejeune et al., 2006; Westerterp-Plantenga et al., 1999). As the heat produced from DIT helps to maintain deep body temperature, consumption of high-protein diets results in greater levels of post-prandial DIT and an increase in body temperature. The homeostatic hypothesis put forward by Brobeck (1948) suggests that when body temperature is high, food intake is decreased to reduce heat production and prevent hyperthermia. However, it is debatable whether the maintenance of body temperature is the effect of food intake, most likely through the process of diet-induced thermogenesis, or the cause for the ingestion of food. High-protein diets seem to be effective in sustaining or increasing fat-free body mass. The preservation of a higher lean mass has an added energy cost and appears to have a significant effect on resting energy expenditure (Westerterp-Plantenga et al., 1999). Consumption of dietary protein above the required level will also increase protein turnover by increasing protein synthesis and protein breakdown in the body (Garlick et al., 1991; Robinson et al., 1990; Tessari et al., 2003; Van Milgen, 2002). All of the

processes associated with intake of high-protein diets result in a lower metabolic efficiency of energy utilisation that may be related to satiety.

### **1.3.2.2. Amino acids**

The aminostatic theory put forward by Mellinkoff *et al.* (1956) suggests that amino acids in the circulation influence eating behaviour. An inverse relationship between the serum concentration of amino acids and appetite has been shown. Mellinkoff *et al.* (1956) also suggested that the central nervous system (CNS) may be able to detect changes in circulating amino acids independent of their role as a source of energy. The observation that a deficit of amino acids in the diet results in hyperphagia in rats supports the aminostatic hypothesis (Gietzen, 1993; Gietzen *et al.*, 1998). An increase in circulating amino acids in response to ingestion of a protein diet may be detected by chemoreceptors found in the anterior piriform cortex of the CNS (Gietzen *et al.*, 1998; Rudell *et al.*, 2011) to activate hypothalamic neurones involved in the suppression in food intake and stimulation of satiety (see Section 1.4.3.3. Amino acids).

### **1.3.2.3. Satiety-related hormones**

Several review articles have investigated numerous gastrointestinal hormones that have been implicated in the regulation of food intake and satiety (Cummings & Overduin, 2007; Murphy *et al.*, 2006; Stanley *et al.*, 2005; Strader & Woods, 2005; Woods, 2004, 2005; Wren & Bloom, 2007). They can be classified as orexigenic (appetite-stimulating) (e.g. ghrelin, neuropeptide Y) and anorexigenic (appetite-suppressing or satiety-stimulating) (e.g. cholecystokinin, glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide, peptide tyrosine-tyrosine and pancreatic polypeptide). A comprehensive review article by Karhunen *et al.* (2008) examined the release of a number of gastrointestinal hormones, related to the control of food intake and satiety, in response to consumption of different macronutrients. In

particular, protein intake has been found to be associated with a decrease in circulating ghrelin and an increase in anorexigenic hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY).

#### **1.3.2.3.1. Ghrelin**

Of all of the gastrointestinal hormones involved in the regulation of food intake, the peptide ghrelin appears to have the most potent orexigenic activity. Ghrelin is a 28 amino acid peptide discovered in 1999 (*Kojima et al., 1999*). It is synthesised predominantly in the stomach (*Ariyasu et al., 2001; Date et al., 2000; Kojima et al., 1999*). The major bioactive form of ghrelin is acylated ghrelin where a fatty acid, primarily *n*-octanoic acid, attaches to the third serine residue (*Hosoda et al., 2003*). However, acylated ghrelin accounts for only approximately 10% of total ghrelin found in the blood and the majority (90%) is in the non-acylated form (de-acylated ghrelin) (*Hosoda et al., 2004*). Administration of ghrelin has been shown to potently result in an increase in food intake (*Druce et al., 2005; Nakazato et al., 2001; Neary et al., 2004; Wren et al., 2001*) and ghrelin concentrations rise prior to feeding and decrease after food ingestion (*Ariyasu et al., 2001; Cummings et al., 2001, 2004*). Ghrelin activates the neuropeptide Y (NPY) neurones present in the hypothalamus to lead to an increase in food intake, which may be mediated by either a central secretion of ghrelin (*Cowley et al., 2003; Nakazato et al., 2001*) or activation of vagal neurones by circulating ghrelin (*Date et al., 2002*). A number of studies found an increase (*Erdmann et al., 2003, 2004*) or no change (*Groschl et al., 2003; Lejeune et al., 2006; Smeets et al., 2008*) in postprandial circulating ghrelin levels after ingestion of a protein-rich meal. Other studies showed that protein intake resulted in decreased postprandial circulating ghrelin concentrations (*Al Awar et al., 2005; Blom et al., 2006; Bowen et al., 2006a, 2006b; Tannous dit El Khoury et al., 2006*). Nonetheless, Foster-Schubert *et al.* (2008) compared the response of plasma total and acylated ghrelin concentrations to oral isoenergetic and isovolumetric doses of carbohydrate, fat and protein and reported a decrease in both total and acylated

ghrelin in the initial three hours. Total ghrelin nadir (lowest) levels were lower after ingestion of carbohydrate and protein compared with the consumption of fat. The investigators suggested a hierarchy for the suppression of both total and acylated ghrelin: carbohydrate more than protein more than fat.

#### **1.3.2.3.2. Cholecystokinin**

The most widely documented gastrointestinal hormone related to satiety is cholecystokinin (CCK). In 1973, Gibbs *et al.* (1973) discovered that administration of purified or synthetic CCK led to a reduced food intake in rats. CCK is secreted from the endocrine I cells of the small intestine (Buchan *et al.*, 1978). The precursor of human CCK is a protein of 95 amino acid residues and CCK exists in multiple molecular forms including CCK-8, -22, -33, -39, -58 and -83 (Rehfeld, 2004; Rehfeld *et al.*, 2001). It is generally agreed that CCK-8 is the most abundant form found in the CNS (Beinfeld *et al.*, 1981; Rehfeld *et al.*, 1985) while CCK-33 is the predominant form present in human plasma (Cantor & Rehfeld, 1987; Liddle *et al.*, 1985; Rehfeld, 1998; Rehfeld *et al.*, 2001). Protein intake has been shown to lead to an increase in circulating levels of CCK (Blom *et al.*, 2006; Bowen *et al.*, 2006b; Burton-Freeman, 2008; Hall *et al.*, 2003; Liddle *et al.*, 1985). There is evidence in rats that suggests that bioactive peptides from protein digestion reduces food intake by activating opioid and CCK receptors and a suppression in food intake was also observed when these receptors were blocked with antagonists (Baile *et al.*, 1986; Froetschel *et al.*, 2001; Pupovac & Anderson, 2002). The satiating signal of CCK may be transmitted to the CNS directly either by CCK crossing the blood-brain barrier or attaching to its A receptors in the CNS (Rehfeld, 2004; Wank, 2004), or indirectly by activating vagal afferent nerves (Schwartz & Moran, 1994; Schwartz *et al.*, 1991; Reidelberger *et al.*, 2004). Other mechanisms by which dietary protein may exert its satiating-inducing effect via CCK include increased gastric distension (Kissileff *et al.*, 2003; Melton *et al.*, 1992) and slower gastric emptying rate (French *et al.*, 1993; Moran & McHugh, 1982; Moran *et al.*, 1993; Schwartz *et al.*, 1993).

#### **1.3.2.3.3. Glucagon-like peptide-1**

Glucagon-like peptide-1 (GLP-1) is a 30 amino acid peptide derived from proglucagon and mainly secreted from the intestinal endocrine L cells (Baggio & Drucker, 2007; Kieffer & Habener, 1999). The predominant bioactive forms of GLP-1 present in human plasma are GLP-1 (7-36) amide and glycine-extended GLP-1 (7-37) (Orskov *et al.*, 1994). In a meta-analysis, Verdich *et al.* (2001) concluded that intravenous administration of GLP-1 (7-36) amide results in an average decrease in food energy intake of 11.7%, irrespective of the BMI status of the individual. The effect of meal composition on the secretion of GLP-1 has been reviewed by Kieffer and Habener (1999). Circulating levels of GLP-1 have been found to increase following consumption of a mixed high-protein meal (Blom *et al.*, 2006; Bowen *et al.*, 2006a; Kieffer & Habener, 1999; Lejeune *et al.*, 2006), but not in the Smeets *et al.* (2008) study. The satiating effect of GLP-1 may be mediated via activation of GLP-1 receptor located in the hypothalamic arcuate nucleus (ARN) region and on vagal afferent pathways to the CNS (Baggio & Drucker, 2007; Kieffer & Habener, 1999), stimulation of glucose-dependent insulin secretion (incretin effect) (Hansotia & Drucker, 2005; Kreymann *et al.*, 1987; Nauck, 1999; Salehi *et al.*, 2008), and a reduction in gastric emptying rate (Hellström & Näslund, 2001; Meier *et al.*, 2003; Nauck *et al.*, 1997; Salehi *et al.*, 2008; Schirra & Göke, 2005; Schirra *et al.*, 2006).

#### **1.3.2.3.4. Peptide YY**

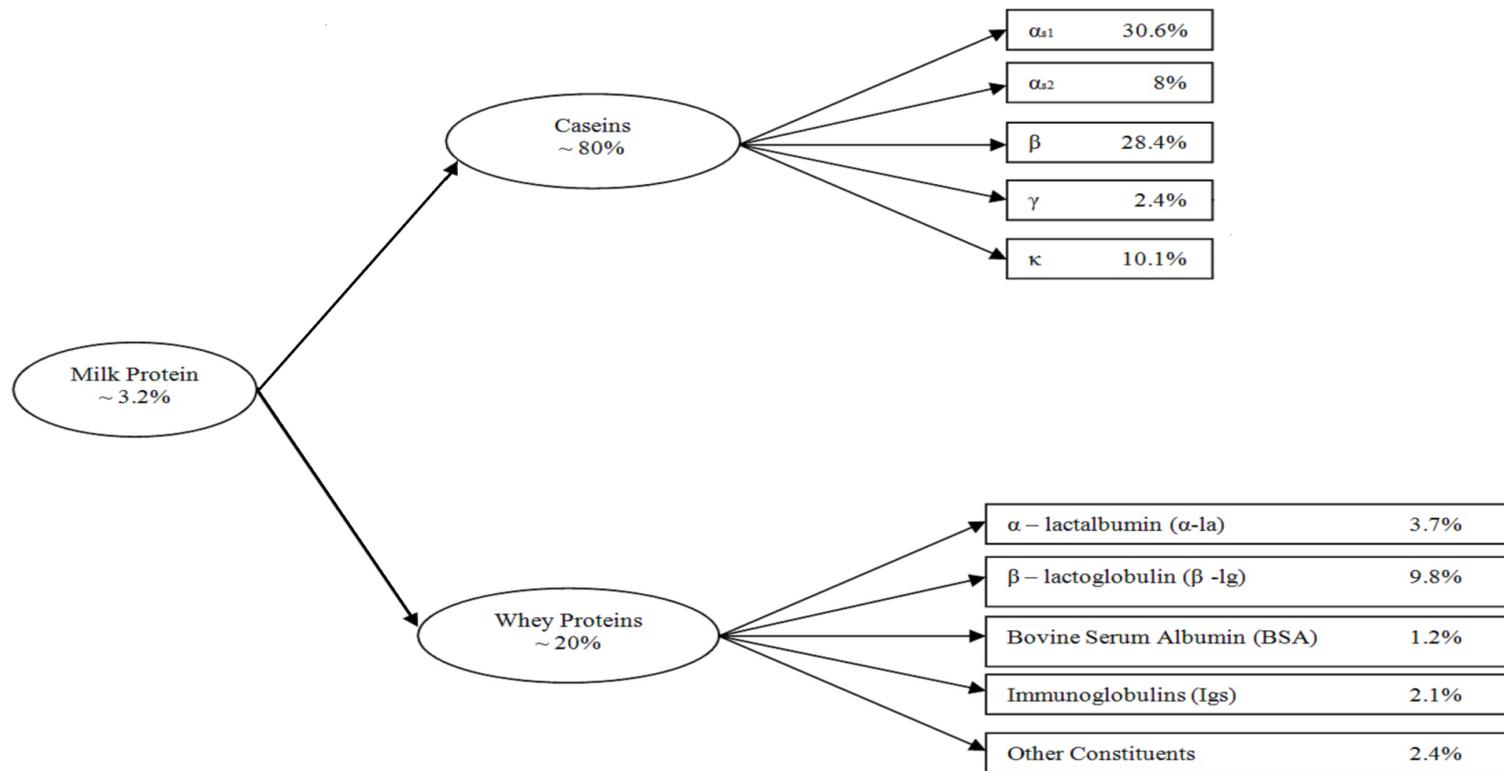
Peptide tyrosine-tyrosine (PYY) is a member of the pancreatic polypeptide-fold family, which also includes the orexigenic neuropeptide Y (NPY) and anorexigenic pancreatic polypeptide (PP) hormones. PYY is made up of 36 amino acid residues (Larhammar, 1996) and is mostly released by the endocrine L cells in the distal ileum (Adrian *et al.*, 1985; Tatemoto *et al.*, 1988). The two main circulating bioactive forms of PYY are PYY (1-36) and

PYY (3-36) (*Grandt et al., 1994*). PYY (3-36) results from the cleavage of the tyrosine-proline residues from the N-terminal by dipeptidyl-peptidase-IV (DPP-IV) (*Ballantyne, 2006; Batterham & Bloom, 2003*). PYY (1-36) may bind to four G-protein-coupled receptors (Y1, Y2, Y4 and Y5) to have an orexigenic effect while the anorexigenic action of PYY (3-36) is mediated via a strong specific affinity to the Y2 receptor (*Ballantyne, 2006*). PYY (3-36) crosses the blood-brain barrier (*Nonaka et al., 2003*) to attach to its Y2 receptors highly expressed on the NPY neurones in the hypothalamic arcuate nucleus (ARN) (*Batterham et al., 2002*). PYY (3-36) inhibits the activation of the orexigenic NPY neurones, which in turn releases the inhibitory action of NPY neurones on the anorexigenic pro-opiomelanocortin (POMC) neurones (*Batterham et al., 2002*). However, recent studies have demonstrated that PYY (3-36) inhibits the POMC neurones and has an orexigenic effect (*Acuna-Goycolea & van del Pol, 2005; Ghamari-Langroudi et al., 2005*). Indeed, administration of PYY (3-36) has been found to result in an acute anorexigenic effect followed by a delayed orexigenic effect in (*Parkinson et al., 2008*). These findings highlight the physiological importance of a hormonal balance of PYY in the regulation of food intake and energy homeostasis. The role of PYY as a modulator of food intake and a regulator of body weight has been extensively reviewed (*Ballantyne, 2006; Batterham & Bloom, 2003; Karra et al., 2009; Le Roux & Bloom, 2005; Ueno et al., 2008*). While *Batterham et al.* (2006) reported that plasma PYY concentrations were higher following consumption of a diet high in protein (65.3% of energy from protein, 17.4% from fat and 17.3% from carbohydrate) compared with carbohydrate (17.5% of energy from protein, 17.7% from fat and 64.6% from carbohydrate) or fat (17.0% of energy from protein, 66.2% from fat and 16.8% from carbohydrate), *Smeets et al.* (2008) found no difference in plasma PYY response between a high-protein (25% of energy from protein, 30% from fat and 45% from carbohydrate) and a high-carbohydrate diet (10% of energy from protein, 30% from fat and 60% from carbohydrate).

## **1.4. Whey protein and satiety**

### **1.4.1. Nutritional profile of whey protein**

Cow's milk contains approximately 3.2% protein, and milk proteins fall into two categories: casein and whey protein (Figure 1.2.). Caseins represent approximately 80% while whey proteins represent the rest (20%) of the total milk proteins (*Walstra et al., 2006*). During cheese making, whey is the soluble component of milk that is separated from the casein curd. For years, whey was considered as a waste product of the cheese making process but recently, whey has become a valuable ingredient in numerous food products, particularly in protein fortified products (*Walzem et al., 2002*). Depending on the extraction method, there are two main types of whey protein: acid whey, which is formed by the removal of casein by acid precipitation at pH 4.6 at room temperature, or the most common sweet whey, which involves coagulation via enzymatic action (*Glass & Hedrick, 1977; Walstra et al., 2006*). Therefore, whey protein comprises a heterogeneous group of proteins varying greatly in composition and amino acid residues. The major components of whey protein are  $\beta$ -lactoglobulin ( $\beta$ -lg) and  $\alpha$ -lactalbumin ( $\alpha$ -la). Whey protein is also abundant in bovine serum albumin (BSA), immunoglobulins (Igs) and other nitrogenous constituents including lactoferrin, lactoperoxidase, and other bioactive compounds and enzymes (*Walstra et al., 2006*).



**Figure 1.2.** Protein composition of bovine milk (Adapted with permission from *Walstra et al., 2006*).

~ = approximately;  $\alpha$  = alpha;  $\beta$  = beta;  $\gamma$  = gamma;  $\kappa$  = kappa.

## **1.4.2. Effect of whey protein intake on satiety**

### **1.4.2.1. Dose dependent effect**

A dose dependent relationship between the amount of whey protein consumed and a reduction in subsequent food intake and an increase in satiety has not been consistently reported. Poppitt *et al.* (2011) did not observe a dose dependent reduction in food energy intake at a test meal 120 min later when fifty overweight women were given low doses of whey protein (5, 10, and 20 g) in comparison with a water control. However, two studies using normal-weight humans showed that whey protein dose dependently suppressed food intake at a subsequent test meal compared with water control (Akhavan *et al.*, 2010; Astbury *et al.*, 2010). Akhavan *et al.* (2010) showed that test meal intake 30 min later decreased in a dose dependent manner when whey protein doses increased from 20 to 30 to 40 g, but not at a low dose of 10 g. Astbury *et al.* (2010) found a linear relationship between whey protein doses (6.8, 13.1, and 25.4 g absolute protein and 12.9, 25.4, and 50.4 g of absolute protein) and a reduction in food intake at a test meal 90 min later. With respect to subjective ratings of appetite, while Akhavan *et al.* (2010) and Astbury *et al.* (2010) did not find any difference in normal-weight subjects, Poppitt *et al.* (2011) reported that overweight women felt less hungry and fuller over 120 min when beverages containing whey protein were ingested compared with the water beverage. The response was, however, not dose dependent and there was no difference between the three whey protein doses on ratings of hunger and fullness. In comparison with a water control, a single dose of ingested whey protein resulted in a greater suppression on subsequent food intake in some (Akhavan *et al.*, 2011, Anderson *et al.*, 2004) but not all (Abou-Samra *et al.*, 2011) studies.

#### 1.4.2.2. Whey protein versus casein

Boirie *et al.* (1997) proposed the terms ‘fast’ and ‘slow’ proteins for whey protein and casein, respectively, based on the premise that whey protein empties from the stomach faster than casein to elicit a rapid and sharp increase in plasma amino acid concentrations while casein causes a slower, lower and more prolonged increase in plasma amino acid concentrations. The latter effect is due to the precipitation of casein as a curd in the acidic condition of the stomach where it undergoes digestion by gastric proteases and results in casein entering the small intestine more slowly in the form of small peptides (Boirie *et al.*, 1997; Boutrou *et al.*, 2013; Chabance *et al.*, 1998; Dangin *et al.*, 2001; Daniel *et al.*, 1990).

The effect of the two major milk protein fractions on food intake and satiety has been addressed. Although Hall *et al.* (2003) reported that ingestion of liquid meals containing whey protein (1695 kJ, 48 g, 60.2% of energy from protein, 20.0% from fat and 19.8% from carbohydrate) led to reduced food intake at a buffet meal 90 min later and increased subjective ratings of appetite more than ingestion of casein liquid meals (1674 kJ, 48 g, 51.3% of energy from protein, 24.7% from fat and 24.0% from carbohydrate) in sixteen normal-weight subjects, other investigators did not find any differential effects on subsequent food intake and subjective ratings of appetite between whey protein and casein (Bowen *et al.*, 2006b) or between casein and a mixture of whey protein and casein (2:1 ratio) (Potier *et al.*, 2009). When other types of protein were tested along with whey protein and casein, the findings on the effect of whey protein versus casein on subsequent food intake and subjective feelings of appetite were also inconsistent. Studies have found either increased (Veldhorst *et al.*, 2009a) or similar effect (Lorenzen *et al.*, 2012; Veldhorst *et al.*, 2009b) of whey protein compared to casein. On the other hand, recent studies have reported that rated feelings of satiety were greater following consumption of casein relative to whey protein in normal-weight subjects (Abou-Samra *et al.*, 2011; Acheson *et al.*, 2011). Similarly, Alfenas *et al.* (2010) comparing ingestion of whey protein versus casein for 7 consecutive days reported that twenty-six normal-weight participants fed the casein diet had

lower daily energy intake than on the whey protein diet, as recorded using dietary diaries.

#### **1.4.2.3. Whey protein versus other protein sources**

Several human studies have investigated whether consumption of whey protein resulted in a stronger effect on satiety than different sources of protein (Table 1.2.). In a study with normal-weight men by Anderson *et al.* (2004), dietary whey protein (50 g) resulted in a greater reduction in energy intake at a test meal 60 min later compared with egg albumen. In another study with twenty-two normal-weight men, ingestion of whey protein (50 g) had a greater effect in lowering food intake at a test meal 4 h later and ratings of hunger than turkey meat, tuna or egg albumen (*Pal & Ellis, 2010*). Although two studies showed no difference in food intake at a test meal provided 180 min following consumption of preloads containing different protein sources, differences in subjective ratings of appetite were observed (*Diepvens et al., 2008; Veldhorst et al., 2009a*). Consumption of whey protein had a greater effect in suppressing feelings of hunger than either casein or soya protein in twenty-five normal-weight participants (*Veldhorst et al., 2009a*). Similarly, thirty-nine overweight subjects reported a greater feeling of satiety and fullness following consumption of a whey protein preload than preloads containing milk protein or a whey protein and pea protein hydrolysate mixture (*Diepvens et al., 2008*). Nonetheless, several other studies have found that dietary whey protein does not affect energy intake at a test meal or subjective feelings of appetite differently from egg albumen (*Abou-Samra et al., 2011*), gluten (*Bowen et al., 2006a*), pea protein hydrolysate (*Diepvens et al., 2008*), or soya protein (*Anderson et al., 2004; Bowen et al., 2006a; Veldhorst et al., 2009b*). In a recent study by Acheson *et al.* (2011), rated feelings of hunger, desire to eat and prospective food consumption were lower, whereas ratings of fullness were higher, following consumption of test meals containing casein and soya protein over the 330 min test period compared with ingestion of a whey protein test meal. While the evidence is not fully conclusive (*EFSA, 2010*), several

studies suggest that the consumption of whey protein is more satiating than other proteins.

#### **1.4.2.4. Whey protein versus carbohydrate**

With regard to the satiating effect of whey protein versus carbohydrate, findings may depend on the type of carbohydrate used. Bowen *et al.* (2006a, 2007) showed no difference between whey protein and fructose or lactose on energy intake at a subsequent meal 180 min later. However, glucose has been shown to lead to increased subsequent food intake more than whey protein (Bellissimo *et al.*, 2007; Zafar *et al.*, 2013), but similar effects have also been reported (Bellissimo *et al.*, 2007; Bowen *et al.*, 2006a, 2006b, 2007). Bellissimo *et al.* (2007) showed that following consumption of a preload drink containing whey protein (1 g/kg body weight), energy intake at a subsequent meal 60 min later was lower (about 13%) compared with a glucose preload drink. Similarly, Zafar *et al.* (2013) found a greater reduction in food energy intake (about 15%) following consumption of a whey protein preload drink (130 kcal, 25 g whey protein) relative to glucose (200 kcal, 50 g glucose). When the effect of the disaccharide sucrose (glucose and fructose) on satiety was compared to whey protein, Anderson *et al.* (2004) demonstrated a greater suppressive effect (about 19%) on food intake 60 min later of a preload drink (833 kJ, 0.65 g/kg body weight) containing whey protein relative to sucrose. With respect to the effect of maltodextrin on satiety compared with whey protein, although no differential effects on subsequent food intake and ratings of appetite were observed by Abou-Samra *et al.* (2011), Bertenshaw *et al.* (2008) reported that energy intake at a subsequent test meal was lower (about 10%) following ingestion of a preload drink enriched with whey protein (1258.6 kJ, 37.7 g protein, and 38.0 g carbohydrate per 300 ml) compared to maltodextrin carbohydrate (1246.5 kJ, 1.7 g protein, and 72.8 g carbohydrate per 300 ml), independent of the time interval between preload drink and test meal (30, 60 or 120 min). The comparison of the satiating effect between whey protein and carbohydrate merits more investigation.

**Table 1.2.** Effects of dietary protein source on food intake and subjective ratings of appetite in adult humans.

Reference	Subjects	Diets	Time interval	Effect on food intake	Effect on appetite
Abou-Samra <i>et al.</i> (2011)	29 NW men	250 ml water control and 200 ml preload drinks (80 kcal) containing either 20 g maltodextrin or 20 g protein: Maltodextrin (21 g added, 80 kcal, 100% C) Whey protein (22.2 g added, 83 kcal, 95.2% P, 4.8% C) Casein (23 g added, 80 kcal, 96.3% P, 3.2% F, 0.5% C) Egg albumen (24 g added, 94 kcal, 94.8% P, 5.2% C) Pea protein (22.2 g added, 78 kcal, 95.2% P, 4.8% C)	30 min	Greater reduction in energy intake after the casein and pea protein preloads compared to water control, but whey protein had an intermediate effect.	Combined satiety score (fullness, hunger, desire to eat, and prospective food consumption) ratings taken every 10 min over 30 min were higher after the casein and pea protein preloads compared to the other four preloads. Ratings were also higher after the whey protein, egg albumen and maltodextrin preloads than the water preload.

Acheson <i>et al.</i> (2011)	23 NW subjects	In addition to a carbohydrate (maltodextrin and dextrose monohydrate, 459 kcal, 0.02 g/kg body weight, 1.2% P, 3.3% F, 95.5% C) test meal, whey protein, casein and soya protein were provided at 0.81 g/kg body weight (459 kcal, 50% P, 10% F, 40% C)	Appetite ratings were collected 15 min before test meal consumption and at different time intervals over 330 min following test meal consumption.	Not measured	When expressed as incremental AUC over 330 min, whey protein led to a lower combined satiety score rating (fullness, hunger, desire to eat and prospective food consumption) than the carbohydrate, casein and soya protein test meals.
Anderson <i>et al.</i> (2004)	13 NW men	In addition to a water control (67 kJ from added maltodextrin), sucrose, whey protein, egg albumen, and soya protein were provided at 0.65 g/kg body weight in 400 ml preload beverages (833 kJ, 50 g)	60 min	Whey and soya proteins decreased food intake relative to water control. No difference between whey and soya proteins but the suppressive effect of whey protein was greater than for egg albumen and sucrose.	Not measured
Anderson <i>et al.</i> (2004)	22 NW men	50 g of whey protein and egg albumen preload beverages (833 kJ in 400 ml) in addition to water control (67 kJ from added maltodextrin in 400 ml)	60 min	Whey protein reduced food intake compared with the water control (about 26%) and egg albumen (about 22%).	Not measured

Bowen <i>et al.</i> (2006a)	25 NW men 47 OW men	Soup preloads: Glucose (65 g added, 1158 kJ, 1.5% P, 11% F, 87% C) Whey protein (55 g added, 1216 kJ, 71% P, 11% F, 18% C) Soya protein (57 g added, 1199 kJ, 71% P, 11% F, 18% C) Gluten (57 g added, 1227 kJ, 71% P, 12% F, 17% C)	180 min	Energy intake after glucose was higher than gluten, with intermediate effect of whey and soya proteins. No effect of BMI status on subsequent food intake.	Ratings of hunger and prospective food consumption did not differ between the four preloads, independently of BMI status.
Diepvens <i>et al.</i> (2008)	20 OW women 19 OW men	300 ml preload drinks (1024 kJ, 25% P, 33% F, 42% C) containing: 15 g milk protein (80% casein and 20% whey protein) 15 g whey protein 15 g pea protein hydrolysate Mixture of 7.5 g whey protein and 7.5 g pea protein hydrolysate	180 min	No difference in food intake at the test meal.	Greater rating of satiety and fullness with whey protein at 30 and 90 min compared to the milk protein and mixed preloads, with a similar effect for pea protein hydrolysate.

Hall <i>et al.</i> (2003)	8 NW women 8 NW men	450 ml preload drinks containing 48 g: Whey protein (1695 kJ, 60.2% P, 20.0% F, 19.8% C) Casein (1674 kJ, 51.3% P, 24.7% F, 24.0% C)	90 min	The whey preload drink reduced energy intake at buffet meal by 829 kJ or 18% compared with the casein preload drink.	No difference in subjective ratings.
Hall <i>et al.</i> (2003)	8 NW women 1 NW man	450 ml preload drinks containing 48 g: Whey protein (1695 kJ, 60.2% P, 20.0% F, 19.8% C) Casein (1674 kJ, 51.3% P, 24.7% F, 24.0% C)	90 min (Fixed lunch 42 g/kg body weight)	Not measured	Greater ratings of fullness and reduced ratings of desire to eat over 180 min following ingestion of the whey protein preload drink compared with casein.
Lorenzen <i>et al.</i> (2012)	17 OW men	1.5 MJ preload drinks containing: Whey protein (36 g, 44.0% P, 1.1% F, 54.9% C) Casein (34 g, 43.5% P, 1.4% F, 55.1% C) Skim milk (28 g casein and 7 g whey protein, 42.3% P, 0.8% F, 56.9% C)	240 min	Subsequent food intake lower (9%) after the whey protein and casein preload meals than the skim milk preload meal.	No effect of preloads on combined appetite score and individual ratings of satiety, fullness, hunger, and prospective food consumption.

Pal & Ellis (2010)	22 NW men	600 g breakfast preload meals containing 50.8 g protein (71% P, 14% F, 15% C): Whey protein (1161 kJ) Turkey meat (1217 kJ) Egg albumen (1218 kJ) Tuna (1213 kJ)	240 min	Greater suppression of food energy intake by the whey protein preload compared with the tuna (about 10%), turkey meat (about 16%) and egg albumen (about 16%) preloads.	When expressed as incremental AUC over 240 min, whey protein induced lower ratings of hunger and prospective food consumption compared to the other three treatments. Fullness ratings were greater with the whey protein and tuna preload meals than with the turkey meat and egg albumen meals.
Veldhorst <i>et al.</i> (2009a)	14 NW women 11 NW men	Custard breakfast preloads (mean energy content $2.52 \pm 0.07$ MJ) with either whey protein, casein or soya protein at two different energy levels: NP (10% P, 35% F, 55% C) HP (25% P, 20% F, 55% C)	180 min	No difference in subsequent food intake among protein source and protein level.	When the protein energy level was low (10%), whey protein decreased ratings of hunger compared to casein (at all time points and incremental AUC) or soya protein (at 20 min).

Veldhorst <i>et al.</i> (2009b)	14 NW women 10 NW men	Custard breakfast preloads (mean energy content $2.39 \pm 0.06$ MJ) with either whey protein with glycomacropeptide, whey protein without glycomacropeptide, casein, soya protein, alpha-lactalbumin, gelatine, or gelatine and tryptophan, at two different energy levels: NP (10% P, 35% F, 55% C) HP (25% P, 20% F, 55% C)	180 min	Independent of protein level, subsequent food intake was lower after preloads containing alpha-lactalbumin, gelatine or gelatine and tryptophan than after preloads with whey protein without glycomacropeptide, casein or soya protein. Food intake after whey protein with glycomacropeptide preload was higher than after preloads with alpha-lactalbumin or gelatine and tryptophan.	When expressed as incremental AUC over 180 min, breakfast preloads containing alpha-lactalbumin, gelatine or gelatin with tryptophan generally decreased ratings of appetite more than the breakfast preloads containing whey protein with glycomacropeptide, whey protein without glycomacropeptide, casein, or soya protein.
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NW, normal-weight; OW, overweight; P, protein; C, carbohydrate; F, fat; NP, Normal-Protein; HP, High-Protein; AUC, area under the curve.

### **1.4.3. Mechanisms of action of whey protein**

Dietary whey protein may influence food intake and satiety via a number of mechanisms (*Jakubowicz & Froy, 2013; Luhovyy et al., 2007*). Firstly, whey protein ingestion results in the release of amino acids and small peptides. The circulating levels of amino acids rapidly increase and this may contribute to satiety through increased amino acid concentrations in the brain and an associated reduction in appetite. Whey protein intake or subsequent amino acids may induce a higher post-prandial release of satiety-inducing hormones, including CCK, GLP-1, and PYY while inhibiting the action of ghrelin. Whey protein may also stimulate a lower glycaemic and higher insulin response than other protein types. The greater satiating effect of whey protein in comparison with other protein types may also be, in part, due to the higher thermic response and increased protein synthesis. Some or all of the above factors may mediate the satiating effect of whey protein, but the relationship needs to be clarified.

The stronger effect of whey protein on satiety compared with other protein types or carbohydrate has been attributed to its protein components, the presence of glycomacropeptide (GMP) and its amino acid composition. In addition, bioactive peptides released during digestion of whey protein may play important roles in regulating gastrointestinal function associated with the control of food intake and satiety (*Froetschel, 1996; Haque et al., 2008; Jahan-Mihan et al., 2011; Korhonen & Pihlanto, 2006; Madureira et al., 2010; Moughan, 2008; Moughan et al., 2007; Rutherfurd-Markwick & Moughan, 2005*).

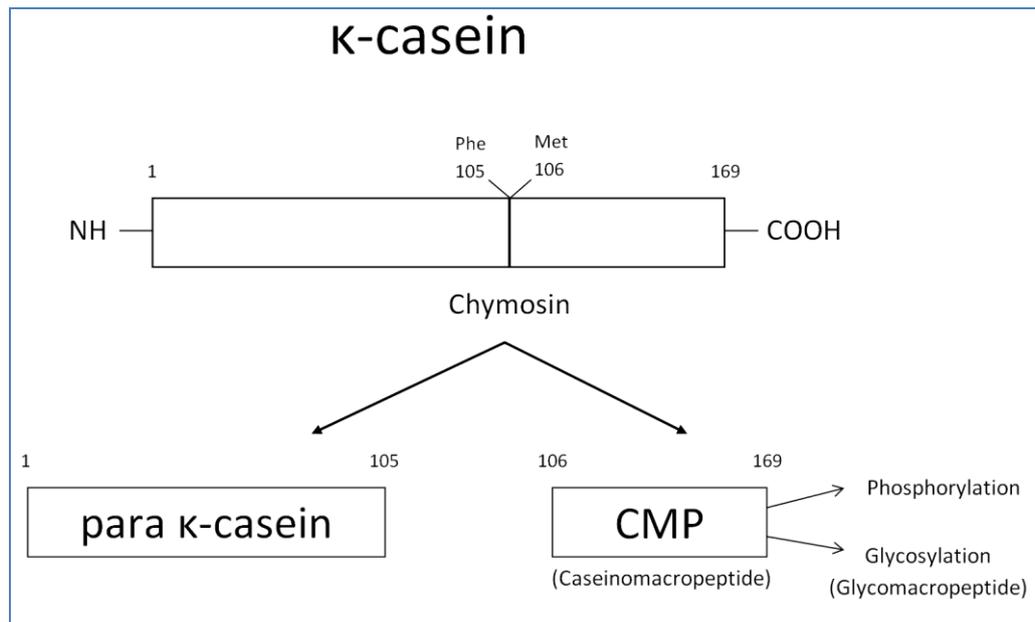
#### **1.4.3.1. Protein components**

Whey protein is constituted predominantly of  $\beta$ -lactoglobulin (51%) and  $\alpha$ -lactalbumin (19%) (Walstra *et al.*, 2006). Studies investigating the effect of these two whey protein fractions individually on food intake and satiety are lacking. A rat study by Pichon *et al.* (2008) showed that consumption of either whey protein concentrate or a whey protein enriched with  $\beta$ -lactoglobulin reduced daily food energy intake compared to whole milk proteins. In addition to this,  $\alpha$ -lactalbumin has been shown to influence food energy intake and appetite suppression in humans. Hursel *et al.* (2010) found that a breakfast diet containing whey protein enriched with  $\alpha$ -lactalbumin (41% of energy derived from protein, 12% from fat and 47% from carbohydrate) decreased rated feelings of hunger and desire to eat compared to a breakfast diet with whey protein (41% of energy derived from protein, 12% from fat and 47% from carbohydrate) in thirty-five normal-weight adult subjects. In a study of twenty-four normal-weight subjects by Veldhorst *et al.* (2009b), consumption of isolated  $\alpha$ -lactalbumin providing 10% or 25% of energy resulted in a lower energy intake at a test meal 180 min later and a greater suppression of rated appetite feelings compared to whey protein. The effects of the individual protein fractions present in whey protein on the regulation of food intake and satiety remain unclear. The additive and potentially synergistic effects of these different whey protein components have also not been tested.

#### **1.4.3.2. Glycomacropeptide**

Whey protein, isolated using enzymes after cheese production (sweet whey also known as cheese or rennet whey), contains caseinomacropeptide (CMP), a 64 amino acid soluble peptide cleaved from the action of chymosin (rennet) on  $\kappa$ -casein (Figure 1.3.). In addition, CMP is the first product released after casein digestion by enzymatic gastric proteases (chymosin and/or pepsin) and emptied from the stomach (Ledoux *et al.*, 1999; Yvon *et al.*, 1994). CMP exists in various forms, differing in the degree of phosphorylation and glycosylation (Beucher *et al.*, 1994; Yvon *et al.*, 1994). Glycomacropeptide

(GMP) refers to the glycosylated form of CMP and contains varying amounts of oligosaccharides, mostly sialic acid (*N*-acetylneuraminic acid), galactosamine, and galactose, attached to threonine by glycosidic linkages (Walstra *et al.*, 2006). There is interest in the development of dietary interventions or functional products involving GMP due to its various biological activities (Brody, 2000). Pure GMP is completely devoid of phenylalanine, tryptophan, tyrosine, arginine, cysteine, and histidine (Etzel, 2004). Phenylketonuria (PKU) is an inborn error of metabolism caused by mutations in the gene encoding the enzyme phenylalanine hydroxylase, which converts the aromatic amino acid phenylalanine to tyrosine (Donlon *et al.*, 2007). Consumption of a diet based on GMP (phenylalanine-free) and supplementary amino acids absent in GMP is therefore advantageous to PKU patients.



**Figure 1.3.** The enzyme chymosin breaks down  $\kappa$ -casein into para- $\kappa$ -casein and caseinomacropeptide (CMP). The latter can undergo glycosylation to form glycomacropeptide (GMP).

Although GMP has been shown to stimulate CCK release in animals (*Beucher et al., 1994; Pedersen et al., 2000; Yvon et al., 1994*), the effects of CMP or GMP on food intake, feelings of appetite, CCK release, and CCK-mediated satiety have not been thoroughly addressed in humans. *Keogh et al. (2010)* investigated the effect of degree of glycosylation of CMP on food intake and CCK release in twenty overweight men. There was no difference observed in plasma CCK response, energy intake at a test meal 180 min or VAS-rated feelings of appetite following consumption of preloads (895 kJ) containing either glucose, GMP-depleted whey protein concentrate (44.4 g protein), glycosylated GMP (42.3 g protein) or minimally glycosylated GMP (41.3 g protein). To test the effects of a preload containing GMP alone on food intake, feelings of appetite and CCK release, *Burton-Freeman (2008)* fed twenty normal-weight adults a milkshake preload (300 ml) containing no added protein (control, 978 kJ, 2% energy from protein, 6% from fat and 94% from carbohydrate), whey protein without GMP (WP-GMP, 29.2 g added, 995 kJ, 44% of energy from protein, 5% from fat and 53% from carbohydrate), whey protein with GMP (WP+GMP, 27.3 g added, 999 kJ, 44% of energy from protein, 3% from fat and 53% from carbohydrate) or GMP isolate (GMP, 0.8 g added, 986 kJ, 3% of energy from protein, 6% from fat and 93% from carbohydrate). Energy intake at a test meal provided 75 min after drinking the milkshake preloads did not differ. Although rated feelings of appetite did not differ in the nine men, the preload drinks containing WP-GMP and WP+GMP induced a greater decrease in rated feelings of appetite over 75 min compared with the control milkshake preload in the ten women. The post-prandial CCK response differed by gender, with the milkshake preload containing WP-GMP showing the greatest CCK response compared with the other three preloads in men, whereas for women, the CCK response was greater after consumption of the WP-GMP and WP+GMP preloads relative to the control and GMP preloads. It is possible that the amount of GMP used in the *Burton-Freeman (2008)* study may have been too low as *Gustafson et al. (2001)* also reported that 0.4 and 2.0 g of caseinomacropeptide (CMP) had no effect on food intake 60 min later or subjective ratings of appetite in fifty-two normal-weight subjects. However, *Veldhorst et al. (2009c)* found that the presence of GMP as part of whey protein in a breakfast meal reduced energy intake at lunch 180

min later more than a breakfast containing whey protein devoid of GMP in twenty-five normal-weight participants, but the amount of GMP present in the whey protein has not been reported.

### 1.4.3.3. Amino acids

The relatively high proportion of essential amino acids (EAA) and branched-chain amino acids (BCAA) in whey protein compared with other protein types is also of great interest (*Etzel, 2004; Glass & Hedrick, 1977; Moughan, 2008*). These amino acids may play a role in the reduction of food intake and stimulation of satiety (*Fromentin et al., 2012*), related to the aminostatic hypothesis (*Bray, 1997; Mellinkoff et al., 1956*) and the varying thermic effects of individual amino acids (*van Milgen, 2002*). An increase in post-prandial circulating levels of EAA has been found to be associated with a reduction in food intake (*Harper & Peters, 1989; Peters & Harper, 1987*). Nonetheless, there was no difference in short-term food intake observed between consumption of either essential amino acids and non-essential amino acids mixture (*Anderson et al., 1994*). An amino acid of interest is the EAA tryptophan (*Latham & Blundell, 1979; Morris et al., 1987*) as it is a precursor of serotonin, a neurotransmitter known to suppress food intake and appetite (*Halford et al., 2011*). Etzel (2004) reported that isolated whey protein contain 13% more BCAA (isoleucine, leucine and valine) and 51% more leucine than an average for proteins in terms of amino acid sequence. This is reflected by a rapid higher circulating BCAA response (*Hall et al., 2003; Veldhorst et al., 2009a, 2009c*). It should be noted that BCAA are metabolised for energy in the muscles rather than the liver. Therefore, BCAA stimulate protein synthesis in the muscles and are insulinotropic (via insulin signalling pathway) to a greater extent than other amino acids. In addition, BCAA may act directly in the CNS by stimulating the mammalian target of rapamycin (mTOR) and inhibiting the adenosine monophosphate-activated protein kinase (AMPK) neuronal pathways. This in turn activates the anorexigenic POMC neurones to modulate food intake. The BCAA leucine has been proposed as being the most important amino acid involved in the regulation of food intake and body weight (*Garlick,*

2005; Layman, 2003; Layman & Walker, 2006). The relationship between the constituents of whey protein and its satiating effect remains to be clarified.

A comprehensive review of the effect of whey protein and glycomacropeptide on the control of food intake and satiety is covered as a separate addendum to this literature review in the form of a published review (*see Appendix I*).

## **1.5. Conclusions and scope of thesis**

The prevalence of overweightness and obesity has increased rapidly in recent years. Among the factors implicated in the cause and treatment of obesity, dietary energy intake is the primary target. A number of studies have shown that high-protein diets lead to body weight loss and the maintenance of a lower body weight. At least in the short-term, high-protein diets result in suppression of further food intake and appetite, thus promoting satiety in comparison with other macronutrients. The effect of the type of dietary protein on satiety has also been the subject of several investigations, with dairy whey protein thought to elicit a stronger satiating effect than other protein sources. Glycomacropeptide (GMP) has been shown to stimulate the release of the satiety-related gastrointestinal hormone cholecystokinin and may play a role in the satiating effect of whey protein. However, discrepancies in published results and a lack of understanding of a relationship between the satiating effect (subsequent food intake and subjective ratings of appetite) and satiety-related mechanistic factors (orexigenic and anorexigenic hormones and metabolites) requires the need for well-controlled short-term studies testing the effect of dairy whey protein and its components (including glycomacropeptide) on satiety.

Based on the review of the scientific literature, the following set of experimental questions was formulated:

- (1) Does a combination of whey protein and glycomacropeptide, which may maximise the release of the satiety-related hormone cholecystokinin, induce satiety in adult humans?
- (2) Is the satiety response of whey protein influenced by the time interval between ingestion of a preload and the points of measurement?
- (3) What are the underlying mediators by which whey protein induces satiety (satiety-related hormones and/or metabolites)?
- (4) Is the satiating effect of functional protein components of whey protein (including glycomacropeptide) greater when they are consumed as independent entities or in combination?
- (5) Does the unique amino acid composition of whey protein play a role in satiety?

This set of questions guided the conduct of the studies reported in this dissertation.

## **1.6. Literature cited**

- Abou-Samra, R., Keersmaekers, L., Brienza, D., Mukherjee, R., & Macé, K. (2011). Effect of different protein sources on satiation and short-term satiety when consumed as a starter. *Nutr J*, *10*, 139.
- Acheson, K. J., Blondel-Lubrano, A., Oquey-Araymon, S., Beaumont, M., Emady-Azar, S., Ammon-Zufferey, C., Monnard, I., Pinaud, S., Nielsen-Moennoz, C., & Bovetto, L. (2011). Protein choices targeting thermogenesis and metabolism. *Am J Clin Nutr*, *93*(3), 525–534.

- Acuna-Goycolea, C., & van del Pol, A. N. (2005). Peptide YY(3-36) inhibits both anorexigenic proopiomelanocortin and orexigenic neuropeptide Y neurons: implications for hypothalamic regulation of energy homeostasis. *J Neurosci*, *25*(45), 10510–10519.
- Adrian, T. E., Ferri, G. L., Bacarese-Hamilton, A. J., Fuessl, H. S., Polak, J. M., & Bloom, S. R. (1985). Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology*, *89*(5), 1070–1077.
- Akhavan, T., Luhovyy, B. L., Brown, P. H., Cho, C. E., & Anderson, G. H. (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.
- Akhavan, T., Luhovyy, B. L., & Anderson, G. H. (2011). Effect of drinking compared with eating sugars or whey protein on short-term appetite and food intake. *Int J Obes*, *35*(4), 562–569.
- Al Awar, R., Obeid, O., Hwalla, N., & Azar, S. (2005). Postprandial acylated ghrelin status following fat and protein manipulation of meals in healthy young women. *Clin Sci*, *109*(4), 405–411.
- Alfenas, R. de C. G., Bressan, J., & Paiva, A. C. de. (2010). Effects of protein quality on appetite and energy metabolism in normal weight subjects. *Arq Bras Endocrinol Metab*, *54*(1), 45–51.
- Almiron-Roig, E., Chen, Y., & Drewnowski, A. (2003). Liquid calories and the failure of satiety: how good is the evidence? *Obes Rev*, *4*(4), 201–212.
- Anderson, G. H. (2006). Sugars-containing beverages and post-prandial satiety and food intake. *Int J Obes*, *30*(S3), S52–S59.
- Anderson, G. H., & Moore, S. E. (2004). Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr*, *134*(4), 974S–979S.
- Anderson, G. H., Li, E. T., Anthony, S. P., Ng, L. T., & Bialik, R. (1994). Dissociation between plasma and brain amino acid profiles and short-term food intake in the rat. *Am J Physiol*, *266*(5 Pt 2), R1675–R1686.
- Anderson, G. H., Tecimer, S. N., Shah, D., & Zafar, T. A. (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.

- Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K., & Nakao, K. (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.
- Astbury, N. M., Stevenson, E. J., Morris, P., Taylor, M. A., & Macdonald, I. A. (2010). Dose-response effect of a whey protein preload on within-day energy intake in lean subjects. *Br J Nutr*, *104*(12), 1858–1867.
- Baggio, L. L., & Drucker, D. J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology*, *132*(6), 2131–2157.
- Baile, C. A., McLaughlin, C. L., & Della-Fera, M. A. (1986). Role of cholecystokinin and opioid peptides in control of food intake. *Physiol Behav*, *66*(1), 172–234.
- 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.
- Barkeling, B., Rössner, S., & Björvell, 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.
- Barkeling, B., Rössner, S., & Sjoberg, A. (1995). Methodological studies on single meal food intake characteristics in normal weight and obese men and women. *Int J Obes Relat Metab Disord*, *19*(4), 284–290.
- Batterham, R. L., & Bloom, S. M. (2003). The gut hormone peptide YY regulates appetite. *Ann N Y Acad Sci*, *994*, 162–168.
- Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., Wren, A. M., Brynes, A. E., Low, M. J., Ghatei, M. A., Cone, R. D., & Bloom, S. R. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature*, *418*(6898), 650–654.
- Batterham, R. L., Heffron, H., Kapoor, S., Chivers, J. E., Chandarana, K., Herzog, H., Le Roux, C. W., Thomas, E. L., Bell, J. D., & Withers, D. J. (2006). Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab*, *4*(3), 223–233.

- Beinfeld, M. C., Meyer, D. K., Eskay, R. L., Jensen, R. T., & Brownstein, M. J. (1981). The distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by radioimmunoassay. *Brain Res*, 212(1), 51–57.
- Bellisle, F., Drewnowski, A., Anderson, G. H., Westerterp-Plantenga, M., & Martin, C. K. (2012). Sweetness, satiation, and satiety. *Journal of Nutrition*, 142(6), 1149S–1154S.
- Bellissimo, N., Desantadina, M. V., Pencharz, P. B., Berall, G. B., Thomas, S. G., & Anderson, G. H. (2007). A comparison of short-term appetite and energy intakes in normal weight and obese boys following glucose and whey-protein drinks. *Int J Obes*, 32(2), 362–371.
- Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiol Behav*, 93(3), 427–436.
- Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2009). Dose-dependent effects of beverage protein content upon short-term intake. *Appetite*, 52(3), 580–587.
- Berti, C., Riso, P., & Porrini, M. (2008). Satiating properties of meat-preparations: role of protein content and energy density. *J Am Coll Nutr*, 27(2), 244–252.
- Bessard, T., Schutz, Y., & Jequier, E. (1983). Energy expenditure and postprandial thermogenesis in obese women before and after weight loss. *Am J Clin Nutr*, 38(5), 680–693.
- Beucher, S., Levenez, F., Yvon, M., & Corring, T. (1994). Effects of gastric digestive products from casein on CCK release by intestinal cells in rat. *J Nutr Biochem*, 5(12), 578–584.
- Bingham, S. A., Gill, C., Welch, A., Day, K., Cassidy, A., Khaw, K. T., Sneyd, M. J., Key, T. J., Roe, L., & Day, N. E. (1994). Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *Br J Nutr*, 72(4), 619–643.
- Bjorntorp, P. (2001). *International textbook of obesity*. New York: Wiley.

- Block, G. (1982). A review of validations of dietary assessment methods. *Am J Epidemiol*, *115*(4), 492–505.
- Blom, W. A. M., Lluch, A., Stafleu, A., Vinoy, S., Holst, J. J., Schaafsma, G., & Hendriks, H. F. (2006). Effect of a high-protein breakfast on the postprandial ghrelin response. *Am J Clin Nutr*, *83*(2), 211–220.
- Blundell, J. E., Burley, V. J., Cotton, J. R., & Lawton, C. L. (1993). Dietary fat and the control of energy intake: evaluating the effects of fat on meal size and postmeal satiety. *Am J Clin Nutr*, *57*(5 Suppl), 772S–777S.
- Blundell, J., de Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., van der Knaap, H., Westerterp, M. (2010). Appetite control: methodological aspects of the evaluation of foods. *Obes Rev*, *11*(3), 251–270.
- Boirie, Y., Dangin, M., Gachon, P., Vasson, M. P., Maubois, J. L., & Beaufrère, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci U S A*, *94*(26), 14930–14935.
- Booth, D. A., & Thibault, L. (1999). Macronutrient-specific hungers and satieties and their neural bases, learnt from pre- and postingestional effects of eating particular foodstuffs. In: H. R. Berthoud & R. J. Seeley (Eds.), *Neural and metabolic control of macronutrient intake* (pp. 61–91). Boca Raton: CRC Press.
- Booth, D. A., Chase, A., & Campbell, A. T. (1970). Relative effectiveness of protein in the late stages of appetite suppression in man. *Physiol Behav*, *5*(11), 1299–1302.
- Boutrou, R., Gaudichon, C., Dupont, D., Jardin, J., Airinei, G., Marsset-Baglieri, A., Benamouzig, R., Tomé, D., & Leonil, J. (2013). Sequential release of milk protein-derived bioactive peptides in the jejunum in healthy humans. *Am J Clin Nutr*, *97*(6), 1314–1323.
- Bowen, J., Noakes, M., & Clifton, P. M. (2006a). 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., & Clifton, P. M. (2007). Appetite hormones and energy intake in obese men after consumption of fructose, glucose and whey protein beverages. *Int J Obes*, 31(11), 1696–1703.
- Bowen, J., Noakes, M., Trenergy, C., & Clifton, P. M. (2006b). Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *J Clin Endocrinol Metab*, 91(4), 1477–1483.
- Bray, G. (1997). Amino acids, protein, and body weight. *Obes Res*, 5(4), 373–376.
- Brobeck, J. R. (1948). Food intake as a mechanism of temperature regulation. *Yale J Biol Med*, 20(6), 545–552.
- Brody, E. P. (2000). Biological activities of bovine glycomacropptides. *Br J Nutr*, 84(Suppl 1), S39–S46.
- Buchan, A. M., Polak, J. M., Solcia, E., Capella, C., Hudson, D., & Pearse, A. G. (1978). Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK. *Gut*, 19(5), 403–407.
- Buchholz, A. C., & Schoeller, D. A. (2004). Is a calorie a calorie? *Am J Clin Nutr*, 79(5), 899S–906S.
- Burton-Freeman, B. M. (2008). Glycomacropptide (GMP) is not critical to whey-induced satiety, but may have a unique role in energy intake regulation through cholecystokinin (CCK). *Physiol Behav*, 93(1-2), 379–387.
- Cantor, P., & Rehfeld, J. F. (1987). The molecular nature of cholecystokinin in human plasma. *Clin Chim Acta*, 168(2), 153–158.
- Chabance, B., Marteau, P., Rambaud, J. C., Migliore-Samour, D., Boynard, M., Perrotin, P., Guillet, R., Jollès, P., & Fiat, A. M. (1998). Casein peptide release and passage to the blood in humans during digestion of milk or yogurt. *Biochimie*, 80(2), 155–165.
- Cowley, M. A., Smith, R. G., Diano, S., Tschöp, M., Pronchuk, N., Grove, K. L., Strasburger, C. J., Bidlingmaier, M., Esterman, M., Heiman, M. L., Garcia-Segura, L. M., Nillni, E. A., Mendez, P., Low, M. J., Sotonyi, P., Friedman, J. M., Liu, H., Pinto, S., Colmers, W. F., Cone, R. D., & Horvath, T. L. (2003). The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron*, 37(4), 649–661.

- Crovetti, R., Porrini, M., Santangelo, A., & Testolin, G. (1998). The influence of thermic effect of food on satiety. *Eur J Clin Nutr*, *52*(7), 482–488.
- Cummings, D. E., & Overduin, J. (2007). Gastrointestinal regulation of food intake. *J Clin Invest*, *117*(1), 13–23.
- Cummings, D. E., Frayo, R. S., Marmonier, C., Aubert, R., & Chapelot, D. (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–E304.
- Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., & Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, *50*(8), 1714–1719.
- Dangin, M., Boirie, Y., Garcia-Rodenas, C., Gachon, P., Fauquant, J., Callier, P., Ballèvre, O., & Beaufrère, B. (2001). The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab*, *280*(2), E340–E348.
- Daniel, H., Vohwinkel, M., & Rehner, G. (1990). Effect of casein and beta-casomorphins on gastrointestinal motility in rats. *J Nutr*, *120*(3), 252–257.
- Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M. S., Suganuma, T., Matsukura, S., Kangawa, K., & Nakazato, M. (2000). Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesised in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology*, *141*(11), 4255–4261.
- Date, Y., Murakami, N., Toshinai, K., Matsukura, S., Nijijima, A., Matsuo, H., Kangawa, K., & Nakazato, M. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology*, *123*(4), 1120–1128.
- De Castro, J. M., & Elmore, D. K. (1988). Subjective hunger relationships with meal patterns in the spontaneous feeding behavior of humans: evidence for a causal connection. *Physiol Behav*, *43*(2), 159–165.
- De Graaf, C. (1993). The validity of appetite ratings. *Appetite*, *21*(2), 156–160.
- De Graaf, C., Blom, W. A., Smeets, P. A., Stafleu, A., & Hendriks, H. F. (2004). Biomarkers of satiation and satiety. *Am J Clin Nutr*, *79*(6), 946–961.

- De Graaf, C., Hulshof, T., Weststrate, J. A., & Jas, P. (1992). Short-term effects of different amounts of protein, fats, and carbohydrates on satiety. *Am J Clin Nutr*, 55(1), 33–38.
- Diepvens, K., Häberer, D., & Westerterp-Plantenga, M. (2008). Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *Int J Obes*, 32(3), 510–518.
- Donlon, J., Levy, H., & Scriver, C. (2007). Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: C. Scriver, A. Beaudet, W. Sly, & D. Valle (Eds.), *Metabolic and molecular basis of inherited disease* (Chapter 77, pp. 1–150). New York: McGraw-Hill.
- Drewnowski, A. (1998). Energy density, palatability, and satiety: implications for weight control. *Nutr Res*, 56(12), 347–353.
- Drewnowski, A., & Bellisle, F. (2007). Liquid calories, sugar, and body weight. *Am J Clin Nutr*, 85(3), 651–661.
- Druce, M. R., Wren, A. M., Park, A. J., Milton, J. E., Patterson, M., Frost, G., Ghatei, M. A., Small, C., & Bloom, S. R. (2005). Ghrelin increases food intake in obese as well as in lean subjects. *Int J Obes*, 29(9), 1130–1136.
- Eisenstein, J., Roberts, S. B., Dallal, G., & Saltzman, E. (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), 189–200.
- Erdmann, J., Lippl, F., & 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., Gussmann, P., & Schusdziarra, V. (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.
- Etzel, M. R. (2004). Manufacture and use of dairy protein fractions. *J Nutr*, 134(4), 996S–1002S.
- EFSA (European Food Safety Authority). (2010). Scientific Opinion on the substantiation of health claims related to protein and increase in satiety leading to a reduction in energy intake (ID 414, 616, 730), contribution to the maintenance or achievement of a normal body weight (ID 414, 616, 730), maintenance of normal bone (ID 416) and growth and maintenance

of muscle mass (ID 415, 417, 593, 594, 714) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J*, 8(10), 1811.

EFSA (European Food Safety Authority). (2010). Scientific Opinion on the substantiation of health claims related to whey protein and increase in satiety leading to a reduction in energy intake (ID 425), contribution to the maintenance or achievement of a normal body weight (ID 1683), growth or maintenance of muscle mass (ID 418, 419, 423, 426, 427, 429, 4307), increase in lean body mass during energy restriction and resistance training (ID 421), reduction of body fat mass during energy restriction and resistance training (ID 420, 421), increase in muscle strength (ID 422, 429), increase in endurance capacity during the subsequent exercise bout after strenuous exercise (ID 428), skeletal muscle tissue repair (ID 428) and faster recovery from muscle fatigue after exercise (ID 423, 428, 431), pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J*, 8(10), 1818.

FAO (Food and Agriculture Organization). (1973). *Energy and protein requirements. Report of a Joint FAO/WHO Expert Committee*. Rome: Food and Agriculture Organization.

FAO (Food and Agriculture Organization). (2003). *Food energy - methods of analysis and conversion factors*. Rome: Food and Agriculture Organization.

Fischer, K., Colombani, P. C., & Wenk, C. (2004). Metabolic and cognitive coefficients in the development of hunger sensations after pure macronutrient ingestion in the morning. *Appetite*, 42(1), 49–61.

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord*, 24(1), 38–48.

Foster-Schubert, K. E., Overduin, J., Prudom, C. E., Liu, J., Callahan, H. S., Gaylinn, B. D., Thorner, M. O., & Cummings, D. E. (2008). Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. *J Clin Endocrinol Metab*, 93(5), 1971–1979.

- French, S. J., Murray, B., Rumsey, R. D., Sepple, C. P., & Read, N. W. (1993). Is cholecystokinin a satiety hormone? Correlations of plasma cholecystokinin with hunger, satiety and gastric emptying in normal volunteers. *Appetite*, *21*(2), 95–104.
- Froetschel, M. A. (1996). Bioactive peptides in digesta that regulate gastrointestinal function and intake. *J Anim Sci*, *74*(10), 2500–2508.
- Froetschel, M. A., Azain, M. J., Edwards, G. L., Barb, C. R., & Amos, H. E. (2001). Opioid and cholecystokinin antagonists alleviate gastric inhibition of food intake by premeal loads of casein in meal-fed rats. *J Nutr*, *131*(12), 3270–3276.
- Fromentin, G., Darcel, N., Chaumontet, C., Marsset-Baglieri, A., Nadkarni, N., & Tome, D. (2012). Peripheral and central mechanisms involved in the control of food intake by dietary amino acids and proteins. *Nutr Res Rev*, *25*(1), 29–39.
- Garlick, P. J. (2005). The role of leucine in the regulation of protein metabolism. *J Nutr*, *135*(6 Suppl), 1553S–1556S.
- Garlick, P. J., McNurlan, M. A., & Ballmer, P. E. (1991). Influence of dietary protein intake on whole-body protein turnover in humans. *Diabetes Care*, *14*(12), 1189–1198.
- Garrow, J. S. (1978). Energy balance and obesity in man. 2nd ed. New York: North Holland/Elsevier.
- Geliebter, A. A. (1979). Effects of equicaloric loads of protein, fat, and carbohydrate on food intake in the rat and man. *Physiol Behav*, *22*(2), 267–273.
- Ghamari-Langroudi, M., Colmers, W. F., & Cone, R. D. (2005). PYY3-36 inhibits the action potential firing activity of POMC neurons of arcuate nucleus through postsynaptic Y2 receptors. *Cell Metab*, *2*(3), 191–199.
- Gibbs, J., Young, R. C., & Smith, G. P. (1973). Cholecystokinin decreases food intake in rats. *J Comp Physiol Psychol*, *84*(3), 488–495.
- Gietzen, D. W. (1993). Neural mechanisms in the responses to amino acid deficiency. *J Nutr*, *123*(4), 610–625.
- Gietzen, D. W., Erecius, L. F., & Rogers, Q. R. (1998). Neurochemical changes after imbalanced diets suggest a brain circuit mediating anorectic responses to amino acid deficiency in rats. *J Nutr*, *128*(4), 771–781.

- Gilbert, J. A., Bendsen, N. T., Tremblay, A., & Astrup, A. (2011). Effect of proteins from different sources on body composition. *Nutr Metab Cardiovasc Dis*, 21 (Suppl 2), B16–B31.
- Glass, L., & Hedrick, T. I. (1977). Nutritional composition of sweet- and acid-type dry wheys. I. Major factors including amino acids. *J Dairy Sci*, 60(2), 185–189.
- Grandt, D., Schimiczek, M., Beglinger, C., Layer, P., Goebell, H., Eysselein, V. E., & Reeve, J. R. Jr. (1994). Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. *Regul Pept*, 51(2), 151–159.
- Green, S. M., Delargy, H. J., Joanes, D. & Blundell, J. E. (1997). A satiety quotient: a formulation to assess the satiating effect of food. *Appetite*, 29(3), 291–304.
- Gregersen, N. T., Flint, A., Bitz, C., Blundell, J. E., Raben, A., & Astrup, A. (2008). Reproducibility and power of ad libitum energy intake assessed by repeated single meals. *Am J Clin Nutr*, 87(5), 1277–1281.
- Gregersen, N. T., Moller, B. K., Raben, A., Kristensen, S. T., Holm, L., Flint, A., & Astrup, A. (2011). Determinants of appetite ratings: the role of age, gender, BMI, physical activity, smoking habits, and diet/weight concern. *Food Nutr Res*, 55, 7028, doi: 10.3402/fnr.v55i0.7028.
- Griffioen-Roose, S., Mars, M., Finlayson, G., Blundell, J. E., & De Graaf, C. (2011). The effect of within-meal protein content and taste on subsequent food choice and satiety. *Br J Nutr*, 106(5), 779–788.
- Groschl, M., Knerr, I., Topf, H. G., Schmid, P., Rascher, W., & Rauch, M. (2003). Endocrine responses to the oral ingestion of a physiological dose of essential amino acids in humans. *J Endocrinol*, 179(2), 237–244.
- Grundy, S. M. (2004). Obesity, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab*, 89(6), 2595–2600.
- Gulick, A. (1922). A study of weight regulation in the adult human body during over-nutrition. *Am J Physiol*, 60(2), 371–395.
- Gustafson, D. R., McMahon, D. J., Morrey, J., & Nan, R. (2001). Appetite is not influenced by a unique milk peptide: caseinomacropeptide (CMP). *Appetite*, 36(2), 157–163.

- Halford, J. C., & Blundell, J. E. (2005). APPETITE | Physiological and neurobiological aspects. In: C. Benjamin (Ed.), *Encyclopedia of Human Nutrition* (2nd ed. pp. 147–154). Oxford: Elsevier.
- Halford, J. C., Boyland, E. J., Lawton, C. L., Blundell, J. E., & Harrold, J. A. (2011). Serotonergic anti-obesity agents: past experience and future prospects. *Drugs*, *71*(17), 2247–2255.
- Hall, W. L., Millward, D. J., Long, S. J., & Morgan, L. M. (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., & 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.
- Hansotia, T., & Drucker, D. J. (2005). GIP and GLP-1 as incretin hormones: lessons from single and double incretin receptor knockout mice. *Regul Pept*, *128*(2), 125–134.
- Haque, E., Chand, R., Kapila, S. (2008). Biofunctional properties of bioactive peptides of milk origin. *Food Rev Int*, *25*(1), 28–43.
- Hellström, P. M., & Näslund, E. (2001). Interactions between gastric emptying and satiety, with special reference to glucagon-like peptide-1. *Physiol Behav*, *74*(4-5), 735–741.
- Hellström, P. M., Geliebter, A., Näslund, E., Schmidt, P. T., Yahav, E. K., Hashim, S. A., & Yeomans, M. R. (2004). Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. *Br J Nutr*, *92*(Suppl 1), S47–S57.
- Hill, A. J., & Blundell, J. E. (1982). Nutrients and behavior: research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *J Psychiatr Res*, *17*(2), 203–212.
- Himaya, A., Fantino, M., Antoine, J. M., Brondel, L., & Louis-Sylvestre, J. (1997). Satiety power of dietary fat: a new appraisal. *Am J Clin Nutr*, *65*(5), 1410–1418.
- Holt, S. H., Miller, J. C., Petocz, P. & Farmakalidis, E. (1995). A satiety index of common foods. *Eur J Clin Nutr*, *49*(9), 675–690.

- Hosoda, H., Doi, K., Nagaya, N., Okumura, H., Nakagawa, E., Enomoto, M., Ono, F., & Kangawa, K. (2004). Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolysed to desacyl ghrelin in blood samples. *Clin Chem*, *50*(6), 1077–1080.
- Hosoda, H., Kojima, M., Mizushima, T., Shimizu, S., & Kangawa, K. (2003). Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing. *J Biol Chem*, *278*(1), 64–70.
- Hulshof, T., de Graaf, C., & Weststrate, J. A. (1993). The effects of preloads varying in physical state and fat content on satiety and energy intake. *Appetite*, *21*(3), 273–286.
- Hursel, R., van der Zee, L., & Westerterp-Plantenga, M. S. (2010). Effects of a breakfast yoghurt, with additional total whey protein or caseinomacropptide-depleted alpha-lactalbumin-enriched whey protein, on diet-induced thermogenesis and appetite suppression. *Br J Nutr*, *103*(5), 775–780.
- Jahan-Mihan, A., Luhovyy, B. L., El Khoury, D., & Anderson, G. H. (2011). Dietary proteins as determinants of metabolic and physiological functions of the gastrointestinal tract. *Nutrients*, *3*(5), 574–603.
- Jebb, S. A. (2005). Dietary strategies for the prevention of obesity. *Proc Nutr Soc*, *64*(2), 217–227.
- Jequier, E. (1983a). Thermogenic responses induced by nutrients in man: their importance in energy balance regulation. *Experientia Suppl*, *44*, 26–44.
- Jequier, E. (1983b). Does a thermogenic defect play a role in the pathogenesis of human obesity? *Clin Physiol*, *3*(1), 1–7.
- Jequier, E., & Schutz, Y. (1985). New evidence for a thermogenic defect in human obesity. *Int J Obes*, *9*(Suppl 2), 1–7.
- Johnson, J., & Vickers, Z. (1993). Effect of flavor and macronutrient composition of food servings on liking, hunger and subsequent intake. *Appetite*, *21*(1), 25–39.
- Johnstone, A. M., Shannon, E., Whybrow, S., Reid, C. A., & Stubbs, R. J. (2000). Altering the temporal distribution of energy intake with

- isoenergetically dense foods given as snacks does not affect total daily energy intake in normal-weight men. *Br J Nutr*, 83(1), 7–14.
- Johnstone, A. M., Stubbs, R. J., & Harbron, C. G. (1996). Effect of overfeeding macronutrients on day-to-day food intake in man. *Eur J Clin Nutr*, 50(7), 418–430.
- Jakubowicz, D., & Froy, O. (2013). Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and Type 2 diabetes. *J Nutr Biochem*, 24(1), 1–5.
- Karhunen, L. J., Juvonen, K. R., Huotari, A., Purhonen, A. K., & Herzig, K. H. (2008). Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regul Pept*, 149(1-3), 70–78.
- Karra, E., Chandarana, K., & Batterham, R. L. (2009). The role of peptide YY in appetite regulation and obesity. *J Physiol*, 587(Pt 1), 19–25.
- Karst, H., Steiniger, J., Noack, R., & Steglich, H. D. (1984). Diet-induced thermogenesis in man: thermic effects of single proteins, carbohydrates and fats depending on their energy amount. *Ann Nutr Metab*, 28(4), 245–252.
- Keogh, J. B., Woonton, B. W., Taylor, C. M., Janakievski, F., Desilva, K., & Clifton, P. M. (2010). Effect of glycomacropeptide fractions on cholecystokinin and food intake. *Br J Nutr*, 104(2), 286–290.
- Kieffer, T. J., & Habener, J. F. (1999). The glucagon-like peptides. *Endocr Rev*, 20(6), 876–913.
- Kinabo, J. L., & Durnin, J. V. G. A. (1990). Thermic effect of food in man: Effect of meal composition, and energy content. *Br J Nutr*, 64(1), 37–44.
- Kissileff, H. R. (1984). Satiating efficiency and a strategy for conducting food loading experiments. *Neurosci Biobehav Rev*, 8(1), 129–135.
- Kissileff, H. R. (1985). Effects of physical state (liquid-solid) of foods on food intake: procedural and substantive contributions. *Am J Clin Nutr*, 42(5 Suppl), 956–965.
- Kissileff, H. R., & van Itallie, T. B. (1982). Physiology of the control of food intake. *Annu Rev Nutr*, 2, 371–418.
- Kissileff, H. R., Carretta, J. C., Geliebter, A., & Pi-Sunyer, F. X. (2003). Cholecystokinin and stomach distension combine to reduce food intake

- in humans. *Am J Physiol Regul Integr Comp Physiol*, 285(5), R992–R998.
- Kissileff, H. R., Gruss, L. P., Thornton, J., & Jordan, H. A. (1984). The satiating efficiency of foods. *Physiol Behav*, 32(2), 319–332.
- Kissileff, H. R., Klingsberg, G., & Van Itallie, T. B. (1980). Universal eating monitor for continuous recording of solid or liquid consumption in man. *Am J Physiol*, 238(1), R14–R22.
- Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402(6762), 656–660.
- Kopelman, P. G. (2000). Obesity as a medical problem. *Nature*, 404(6778), 635–643.
- Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: production and functionality. *Int Dairy J*, 16(9), 945–960.
- Kreymann, B., Williams, G., Ghatei, M. A., & Bloom, S. R. (1987). Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet*, 2(8571), 1300–1304.
- Lara, J., Taylor, M. A., & Macdonald, I. A. (2010). Is ad libitum energy intake in overweight subjects reproducible in laboratory studies using the preload paradigm? *Eur J Clin Nutr*, 64(9), 1028–1031.
- Larhammar, D. (1996). Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. *Regul Pept*, 62(1), 1–11.
- Latham, C. J., & Blundell, J. E. (1979). Evidence for the effect of tryptophan on the pattern of food consumption in free feeding and food deprived rats. *Life Sci*, 24(21), 1971–1978.
- Latner, J. D., & Schwartz, M. (1999). The effects of a high-carbohydrate, high-protein or balanced lunch upon later food intake and hunger ratings. *Appetite*, 33(1), 119–128.
- Layman, D. K. (2003). The role of leucine in weight loss diets and glucose homeostasis. *J Nutr*, 133(1), 261S–267S.
- Layman, D. K., & Walker, D. A. (2006). Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr*, 136(1 Suppl), 319S–323S.

- Le Roux, C. W., & Bloom, S. R. (2005). Peptide YY, appetite and food intake. *Proc Nutr Soc*, *64*(2), 213–216.
- Ledoux, N., Mahé, S., Duarry, M., Bourras, M., Benamouzig, R., & Tomé, D. (1999). Intraluminal immunoreactive caseinomacropeptide after milk protein ingestion in humans. *Nahrung*, *43*(3), 196–200.
- Leidy, H. J., Bales-Voelker, L. I., & Harris, C. T. (2011). A protein-rich beverage consumed as a breakfast meal leads to weaker appetitive and dietary responses v. a protein-rich solid breakfast meal in adolescents. *Br J Nutr*, *106*(1), 37–41.
- Lejeune, M. P., Westerterp, K. R., Adam, T. C., Luscombe-Marsh, N. D., Westerterp-Plantenga, M. S. (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. *Am J Clin Nutr*, *83*(1), 89–94.
- Liddle, R.A., Goldfine, I. D., Rosen, M. S., Taplitz, R. A., & Williams, J. A. (1985). Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. *J Clin Invest*, *75*(4), 1144–1152.
- Livesey, G. (2001). A perspective on food energy standards for nutrition labelling. *Br J Nutr*, *85*(3), 271–287.
- Livingstone, M. B., Prentice, A. M., Strain, J. J., Coward, W. A., Black, A. E., Barker, M. E., McKenna, P. G., & Whitehead, R. G. (1990). Accuracy of weighed dietary records in studies of diet and health. *BMJ*, *300*(6726), 708–712.
- Livingstone, M. B., Robson, P. J., Welch, R. W., Burns, A. A., Burrows, M. S., & McCormack, C. (2000). Methodological issues in the assessment of satiety. *Scand J Nutr*, *44*(3), 98–103.
- Lorenzen, J., Frederiksen, R., Hoppe, C., Hvid, R., & Astrup, A. (2012). The effect of milk proteins on appetite regulation and diet-induced thermogenesis. *Eur J Clin Nutr*, *66*(5), 622–627.
- Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr*, *26*(6), S704–S712.

- Madureira, A. R., Tavares, T., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2010). Invited review: physiological properties of bioactive peptides obtained from whey proteins. *J Dairy Sci*, *93*(2), 437–455.
- Marmonier, C., Chapelot, D., & Louis-Sylvestre, J. (2000). Effects of macronutrient content and energy density of snacks consumed in a satiety state on the onset of the next meal. *Appetite*, *34*(2), 161–168.
- Martens, M. J., Lemmens, S. G., Born, J. M., & Westerterp-Plantenga, M. S. (2011). A solid high-protein meal evokes stronger hunger suppression than a liquefied high-protein meal. *Obesity*, *19*(3), 522–527.
- Mattes, R. D. (2006). Beverages and positive energy balance: the menace is the menace. *Int J Obes*, *30*(S3), S60–S65.
- Meier, J. J., Gallwitz, B., Salmen, S., Goetze, O., Holst, J. J., Schmidt, W. E., & Nauck, M. A. (2003). Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab*, *88*(6), 719–725.
- Mela, D. J. (2001). Determinants of food choice: relationships with obesity and weight control. *Obes Res*, *9*(Suppl 4), 249S–255S.
- Mellinkoff, S. M., Frankland, M., Boyle, D., & Greipel, M. (1956). Relationship between serum amino acid concentration and fluctuations in appetite. *J Appl Physiol*, *8*(5), 535–538.
- Melton, P. M., Kissileff, H. R., & Pi-Sunyer, F. X. (1992). Cholecystokinin (CCK-8) affects gastric pressure and ratings of hunger and fullness in women. *Am J Physiol*, *263*(2 Pt 2), R452–R456.
- Miller, D. S., & Mumford, P. (1967). Gluttony. 1. An experimental study of overeating low- or high-protein diets. *Am J Clin Nutr*, *20*(11), 1212–1222.
- Moran, T. H., & McHugh, P. R. (1982). Cholecystokinin suppresses food intake by inhibiting gastric emptying. *Am J Physiol*, *242*(5), R491–R497.
- Moran, T. H., Ameglio, P. J., Schwartz, G. J., Peyton, H. J., & McHugh, P. R. (1993). Endogenous cholecystokinin in the control of gastric emptying of liquid nutrient loads in rhesus monkeys. *Am J Physiol*, *265*(2 Pt 2), R371–R375.

- Morris, P., Li, E. T., MacMillan, M. L., & Anderson, G. H. (1987). Food intake and selection after peripheral tryptophan. *Physiol Behav*, *40*(2), 155–163.
- Moughan, P. J. (2008). Milk proteins: a cornucopia for developing functional foods. In: A. Thompson, M. Boland, & H. Singh (Eds.), *Milk proteins: from expression to food* (pp. 483–499). London: Academic Press.
- Moughan, P. J., Coles, L. T., Rutherford, S. M., & Zou, L. (2010). Describing dietary energy - do we need a new approach? [Conference Abstract Nutrition Society of Australia and Nutrition Society of New Zealand: IV]. *Australas Med J*, *1*(1), 97–98.
- Moughan, P. J., Fuller, M. F., Han, K. S., Kies, A. K., & Miner-Williams, W. (2007). Food-derived bioactive peptides influence gut function. *Int J Sport Nutr Exerc Metab*, *17* Suppl, S5–S22.
- Mourao, D. M., Bressan, J., Campbell, W. W., & Mattes, R. D. (2007). Effects of food form on appetite and energy intake in lean and obese young adults. *Int J Obes*, *31*(11), 1688–1695.
- Murphy, K. G., Dhillon, W. S., & Bloom, S. R. (2006). Gut peptides in the regulation of food intake and energy homeostasis. *Endocr Rev*, *27*(7), 719–727.
- Nair, K. S., Halliday, D., & Garrow, J. S. (1983). Thermic response to isoenergetic protein, carbohydrate or fat meals in lean and obese subjects. *Clin Sci*, *65*(3), 307–312.
- Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., & Matsukura, S. (2001). A role for ghrelin in the central regulation of feeding. *Nature*, *409*(6817), 194–198.
- Nauck, M. A. (1999). Is glucagon-like peptide 1 an incretin hormone? *Diabetologia*, *42*(3), 373–379.
- Nauck, M. A., Niedereichholz, U., Ettl, R., Holst, J. J., Orskov, C., Ritzel, R., & Schmiegel, W. H. (1997). Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol*, *273*(5 Pt 1), E981–E988.
- Neary, N. M., Small, C. J., Wren, A. M., Lee, J. L., Druce, M. R., Palmieri, C., Frost, G. S., Ghatei, M. A., Coombes, R. C., & Bloom, S. R. (2004). Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin Endocrinol Metab*, *89*(6), 2832–2836.

- Noakes, M. (2008). The role of protein in weight management. *Asia Pac J Clin Nutr*, 17(Suppl 1), 169–171.
- Nonaka, N., Shioda, S., Niehoff, M. L., & Banks, W. A. (2003). Characterization of blood-brain barrier permeability to PYY3-36 in the mouse. *J Pharmacol Exp Ther*, 306(3), 948–953.
- Orskov, C., Rabenhøj, L., Wettergren, A., Kofod, H., & Holst, J. J. (1994). Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide 1 in humans. *Diabetes*, 43(4), 535–539.
- Pal, S., & 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.
- Parker, B. A., Strum, K., MacIntosh, C. G., Feinle, C., Horowitz, M., & Chapman, I. M. (2004). Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr*, 58(2), 212–218.
- Parkinson, J. R., Dhillon, W. S., Small, C. J., Chaudhri, O. B., Bewick, G. A., Pritchard, I., Moore, S., Ghatei, M. A., & Bloom, S. R. (2008). PYY3-36 injection in mice produces an acute anorexigenic effect followed by a delayed orexigenic effect not observed with other anorexigenic gut hormones. *Am J Physiol Endocrinol Meta*, 294(4), E698–E708.
- Paddon-Jones, D., Westman, E., Mattes, R. D., Wolfe, R. R., Astrup, A., & Westerterp-Plantenga, M. (2008). Protein, weight management, and satiety. *Am J Clin Nutr*, 87(5), 1558S–1561S.
- Pedersen, N. L., Nagain-Domaine, C., Mahe, S., Chariot, J., Roze, C., & Tome, D. (2000). Caseinomacropptide specifically stimulates exocrine pancreatic secretion in the anesthetized rat. *Peptides*, 21(10), 1527–1535.
- Peters, J. C., & Harper, A. E. (1987). Acute effects of dietary protein on food intake tissue amino acids, and brain serotonin. *Am J Physiol*, 252(5 Pt 2), R902–R914.
- Pichon, L., Potier, M., Tome, D., Mikogami, T., Laplaize, B., Martin-Rouas, C., & Fromentin, G. (2008). High-protein diets containing different milk protein fractions differently influence energy intake and adiposity in the rat. *Br J Nutr*, 99(4), 739–748.

- Poppitt, S. D., McCormack, D., & 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., Wiessing, K. R., Falk, S., Xin, L., Budgett, S. C., Darragh, A., & Hall, R. S. (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., & Silva, S. (1995) Evaluation of satiety sensations and food intake after different preloads. *Appetite*, *25*(1), 17–30.
- Porrini, M., Santangelo, A., Crovetti, R., Riso, P., Testolin, G., & Blundell, J. E. (1997). Weight, protein, fat, and timing of preloads affect food intake. *Physiol Behav*, *62*(3), 563–570.
- Potier, M., Fromentin, G., Calvez, J., Benamouzig, R., Martin-Rouas, C., Pichon, L., Tomé, D., & Marsset-Baglieri, A. (2009). A high-protein, moderate-energy, regular cheesy snack is energetically compensated in human subjects. *Br J Nutr*, *102*(4), 625–631.
- Potier, M., Fromentin, G., Lesdema, A., Benamouzig, R., Tomé, D., & Marsset-Baglieri, A. (2010). The satiety effect of disguised liquid preloads administered acutely and differing only in their nutrient content tended to be weaker for lipids but did not differ between proteins and carbohydrates in human subjects. *Br J Nutr*, *104*(9), 1406–1414.
- Pupovac, J., & Anderson, G. H. (2002). Dietary peptides induce satiety via cholecystokinin-A and peripheral opioid receptors in rats. *J Nutr*, *132*(9), 2775–2780.
- Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J. J., & Astrup, A. (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.
- Rehfeld, J. F. (1998). Accurate measurement of cholecystokinin in plasma. *Clin Chem*, *44*(5), 991–1001.
- Rehfeld, J. F. (2004). Clinical endocrinology and metabolism. Cholecystokinin. *Best Pract Res Clin Endocrinol Metab*, *18*(4), 569–586.

- Rehfeld, J. F., Hansen, H. F., Marley, P. D., & Stengaard-Pedersen, K. (1985). Molecular forms of cholecystokinin in the brain and the relationship to neuronal gastrins. *Ann N Y Acad Sci*, *448*, 11–23.
- Rehfeld, J. F., Sun, G., Christensen, T., & Hillingsø, J. G. (2001). The predominant cholecystokinin in human plasma and intestine is cholecystokinin-33. *J Clin Endocrinol Metab*, *86*(1), 251–258.
- Reid, M., & Hetherington, M. (1997). Relative effects of carbohydrates and protein on satiety – a review of methodology. *Neurosci Biobehav Rev*, *21*(3), 295–308.
- Reidelberger, R. D., Hernandez, J., Fritsch, B., & Hulce, M. (2004). Abdominal vagal mediation of the satiating effects of CCK in rats. *Am J Physiol Regul Integr Comp Physiol*, *286*(6), R1005–R1012.
- Robinson, S. M., Jaccard, C., Persaud, C., Jackson, A. A., Jequier, E., & Schutz, Y. (1990). Protein turnover and thermogenesis in response to high-protein and high-carbohydrate feeding in men. *Am J Clin Nutr*, *52*(1), 72–80.
- Rolls, B. J., & Hammer, V. A. (1995). Fat, carbohydrate, and the regulation of energy intake. *Am J Clin Nutr*, *62*(5 Suppl), 1086S–1095S.
- Rolls, B. J., Hetherington, M., & 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, S., McNelis, A. L., Fischman, M. W., Foltin, R. W., & Moran, T. H. (1991). Time course of effects of preloads high in fat or carbohydrate on food intake and hunger ratings in humans. *Am J Physiol*, *260*(4 Pt 2), 756–763.
- Rosenbaum, M., Leibel, R. L., & Hirsch, J. (1997). Obesity. *N Engl J Med*, *337*(6), 396–407.
- Rudell, J. B., Rechs, A. J., Kelman, T. J., Ross-Inta, C. M., Hao, S., & Gietzen, D. W. (2011). The anterior piriform cortex is sufficient for detecting depletion of an indispensable amino acid, showing independent cortical sensory function. *J Neurosci*, *31*(5), 1583–1590.
- Rutherford-Markwick, K. J., & Moughan, P. J. (2005). Bioactive peptides derived from food. *JAOAC Int*, *88*(3), 955–966.

- Salehi, M., Vahl, T. P., & D'Alessio, D. A. (2008). Regulation of islet hormone release and gastric emptying by endogenous glucagon-like peptide 1 after glucose ingestion. *J Clin Endocrinol Metab*, *93*(12), 4909–4916.
- Schirra, J., & Göke, B. (2005). The physiological role of GLP-1 in human: incretin, ileal brake or more? *Regul Pept*, *128*(2), 109–115.
- Schirra, J., Nicolaus, M., Roggel, R., Katschinski, M., Storr, M., Woerle, H. J., & Göke, B. (2006). Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. *Gut*, *55*(2), 243–251.
- Schoeller, D. A. (1990). How accurate is self-reported dietary energy intake? *Nutr Rev*, *48*(10), 373–379.
- Schwartz, G. J., & Moran, T. H. (1994). CCK elicits and modulates vagal afferent activity arising from gastric and duodenal sites. *Ann N Y Acad Sci*, *23*, 121–128.
- Schwartz, G. J., & Moran, T. H. (1998). Duodenal nutrient exposure elicits nutrient-specific gut motility and vagal afferent signals in rats. *Am J Physiol*, *274*(5 Pt 2), R1236–R1242.
- Schwartz, G. J., Berkow, G., McHugh, P. R., & Moran, T. H. (1993). Gastric branch vagotomy blocks nutrient and cholecystokinin-induced suppression of gastric emptying. *Am J Physiol*, *264*(3 Pt 2), R630–R637.
- Schwartz, G. J., McHugh, P. R., & Moran, T. H. (1991). Integration of vagal afferent responses to gastric loads and cholecystokinin in rats. *Am J Physiol*, *261*(1 Pt 2), R64–R69.
- Sims, E. A., & Danforth, E. Jr. (1987). Expenditure and storage of energy in man. *J Clin Invest*, *79*(4), 1019–1025.
- Smeets, A. J., Soenen, S., Luscombe-Marsh, N. D., Ueland, O., & Westerterp-Plantenga, M. S. (2008). Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. *J Nutr*, *138*(4), 698–702.
- Sorensen, L. B., Moller, P., Flint, A., Martens, M., & Raben, A. (2012). Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. *Int J Nutr*, *27*(10), 1152–1166.

- Southgate, D. A., & Durnin, J. V. (1970). Calorie conversion factors. An experimental reassessment of the factors used in the calculation of the energy value of human diets. *Br J Nutr*, 24(2), 517–535.
- Speechly, D. P., Rogers, G. G., & Buffenstein, R. (1999). Acute appetite reduction associated with an increased frequency of eating in obese males. *Int J Obes Relat Metab Disord*, 23(11), 1151–1159.
- St Jeor, S. T., Howard, B. V., Prewitt, T. E., Bovee, V., Bazzarre, T., & Eckel, R. H. (2001). Dietary protein and weight reduction: a statement for healthcare professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. *Circulation*, 104(15), 1869–1874.
- Stanley, S., Wynne, K., McGowan, B., & Bloom, S. (2005). Hormonal regulation of food intake. *Physiol Rev*, 85(4), 1131–1158.
- Strader, A. D., & Woods, S. C. (2005). Gastrointestinal hormones and food intake. *Gastroenterology*, 128(1), 175–191.
- Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Rowley, E., Reid, C., Elia, M., Stratton, R., Delargy, H., King, N., & Blundell, J. E. (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr*, 84(04), 405–415.
- Stubbs, R. J., Johnstone, A. M., O'Reilly, L. M., & Poppitt, S. D. (1998). Methodological issues relating to the measurement of food, energy and nutrient intake in human laboratory-based studies. *Proc Nutr Soc*, 57(3), 357–372.
- Stubbs, R. J., Van Wyk, M. C., Johnstone, A. M., & Harbron, C. G. (1996). Breakfasts high in protein, fat or carbohydrate: effect on within-day appetite and energy balance. *Eur J Clin Nutr*, 50(7), 409–417.
- Tannous dit El Khoury, D., Obeid, O., Azar, S. T., Hwalla, N. (2006). Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. *Ann Nutr Metab*, 50(3), 260–269.

- Tatemoto, K., Nakano, I., Makk, G., Angwin, P., Mann, M., Schilling, J., & Go, V. L. (1988). Isolation and primary structure of human peptide YY. *Biochem Biophys Res Commun*, *157*(2), 713–717.
- Tappy, L. (1996). Thermic effect of food and sympathetic nervous system activity in humans. *Reprod Nutr Dev*, *36*(4), 391–397.
- Te Morenga, L., & Mann, J. (2012). The role of high-protein diets in body weight management and health. *Br J Nutr*, *108*(Suppl 2), S130–S138.
- Tessari, P., Kiwanuka, E., Zanetti, M., & Barazzoni, R. (2003). Postprandial body protein synthesis and amino acid catabolism measured with leucine and phenylalanine-tyrosine tracers. *Am J Physiol Endocrinol Metab*, *284*(5), E1037–E1042.
- Ueno, H., Yamaguchi, H., Mizuta, M., & Nakazato, M. (2008). The role of PYY in feeding regulation. *Regul Pept*, *145*(1-3), 12–16.
- Van Milgen, J. (2002). Modeling biochemical aspects of energy metabolism in mammals. *J Nutr*, *132*(10), 3195–3202.
- Vandewater, K., & Vickers, Z. (1996). Higher-protein foods produce greater sensory-specific satiety. *Physiol Behav*, *59*(3), 579–583.
- Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N., & Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. *Physiol Behav*, *94*(2), 300–307.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., van Vught, A. J., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009a). Dose-dependent satiating effect of whey relative to casein or soy. *Physiol Behav*, *96*(4-5), 675–682.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009b). A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. *Clin Nutr*, *28*(2), 147–155.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009c). Effects of complete whey-protein

- breakfasts versus whey without GMP-breakfasts on energy intake and satiety. *Appetite*, 52(2), 388–395.
- Verdich, C., Flint, A., Gutzwiller, P., Näslund, E., Beglinger, C, Hellström, P. M., Long, S. J., Morgan, L. M., Holst, J. J., & Astrup, A. (2001). A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on *ad libitum* energy intake in humans. *J Clin Endocrinol Metab*, 86(9), 4382–4389.
- Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006) *Dairy science and technology*. 2nd ed. Boca Raton: CRC/Taylor & Francis.
- Walzem, R. L., Dillard, C. J., & German, J. B. (2002). Whey components: millennia of evolution create functionalities for mammalian nutrition: what we know and what we may be overlooking. *Crit Rev Food Sci Nutr*, 42(4), 353–375.
- Wank, S. A. (1995). Cholecystokinin receptors. *Am J Physiol*, 269(5 Pt 1), G628–G646.
- Warwick, P. M., & Baines, J. (2000). Point of view: energy factors for food labelling and other purposes should be derived in a consistent fashion for all food components. *Br J Nutr*, 84(6), 897–902.
- Westerterp, K. R. (2004). Diet induced thermogenesis. *Nutr Metab*, 18(1), 1–5.
- Westerterp, K. R., Wilson, S. A., & 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.
- Westerterp-Plantenga, M. S. (2009). Protein intake and energy balance. *Regul Pept*, 149(1-3), 67–69.
- Westerterp-Plantenga, M. S., Fredrix, E. W. H. M., Steffens, A. B., & Kissileff, H. R. (1994). *Food intake and energy expenditure*. Boca Raton: CRC Press.
- Westerterp-Plantenga, M. S., Lemmens, S. G., & Westerterp, K. R. (2009). Dietary protein - its role in satiety, energetic, weight loss and health. *Br J Nutr*, 108(Suppl 2), S105–S112.
- Westerterp-Plantenga, M. S., Nieuwenhuizen, A. Tomé, D., Soenen, S., & Westerterp, K. R. (2009). Dietary protein, weight loss, and weight maintenance. *Annu Rev Nutr*, 29(7), 21–41.

- Westerterp-Plantenga, M. S., Rolland, V., Wilson, S. A., & Westerterp, K. R. (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.
- WHO (World Health Organization). (2000). *Obesity: preventing and managing the global epidemic (WHO technical report series no. 894.)*. Geneva: World Health Organization.
- WHO. (2012). *Obesity and overweight (WHO fact sheet no. 311.)*: World Health Organization.
- Wiessing, K. R., Xin, L., McGill, A. T., Budgett, S. C., Strik, C. M., & Poppitt, S. D. (2012). Sensitivity of ad libitum meals to detect changes in hunger. Restricted-item or multi-item test meals in the design of preload appetite studies. *Appetite*, 58(3), 1076–1082.
- Woods, S. C. (2004). Gastrointestinal satiety signals I. An overview of gastrointestinal signals that influence food intake. *Am J Physiol Gastrointest Liver Physiol*, 286(1), G7–G13.
- Woods, S. C. (2005). Signals that influence food intake and body weight. *Physiol Behav*, 86(5), 709–716.
- Wren, A. M., & Bloom, S. C. (2007). Gut hormones and appetite control. *Gastroenterology*, 132(6), 2116–2130.
- Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., Dhillo, W. S., Ghatei, M. A., & Bloom, S. R. (2001). Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab*, 86(12), 5992.
- Wycherley, T. P., Moran, L. J., Clifton, P. M., Noakes, M., & Brinkworth, G. D. (2012). 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.
- Yeomans, M. R., Blundell, J. E., & Leshem, M. (2004). Palatability: response to nutritional need or need-free stimulation of appetite? *Br J Nutr*, 92(Suppl 1), S3–S14.
- Yvon, M., Beucher, S., Guilloteau, P., Le Hueron-Luron, I., & Corring, T. (1994). Effects of caseinomacropptide (CMP) on digestion regulation. *Reprod Nutr Dev*, 34(6), 527–537.

- Zafar, T. A., Waslien, C., AlRaefaei, A., Alrashidi, N., & AlMahmoud, E. (2013). Whey protein sweetened beverages reduce glycemic and appetite responses and food intake in young females. *Nutr Res*, *33*(4), 303–310.
- Zou, M. L., Moughan, P. J., Awati, A., & Livesey, G. (2007). Accuracy of the Atwater factors and related food energy conversion factors with low-fat, high-fiber diets when energy intake is reduced spontaneously. *Am J Clin Nutr*, *86*(6), 1649–1656.

## Chapter 2

### **The influence of whey protein and glycomacropeptide on satiety in adult humans**

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Human studies have shown that dairy whey protein is more satiating than other protein sources. Glycomacropeptide (GMP), present in whey protein manufactured during cheese production, may induce satiety due to its potent ability to stimulate the release of satiety-related hormone cholecystokinin. The aim of the present study was to investigate whether the consumption of whey protein and glycomacropeptide administered together has an effect on subsequent food intake and subjective ratings of appetite in adult humans.

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## 2.1. Abstract

Protein is often considered the most satiating macronutrient. The objective was to determine the short-term effect of mixtures of whey protein and glycomacropeptide (GMP) versus a carbohydrate control on satiety in healthy adult humans. The study was a randomised crossover Latin Square design. On 4 separate days, fifty healthy subjects (19 males and 31 females) received a subject-specific breakfast (08:00 h), a preload drink (12:00 h) and lunch (12:30 h). The preload drink was presented as a milkshake with either maltodextrin carbohydrate (control), whey protein isolate (WPI) with no GMP, WPI with naturally present 21% GMP or WPI with naturally present 21% GMP plus added GMP. Satiety was assessed using visual analogue scales (VAS) and by determining *ad libitum* food intake during a cafeteria style meal offered 30 min after the preload. The VAS indicated that the lower GMP treatment induced a greater feeling of fullness immediately after consumption of the preload compared with the other treatments. Energy and macronutrient intake at lunch did not differ significantly ( $p>0.05$ ) between treatments although subjects chose to eat foods higher in carbohydrate and lower in protein after the protein preloads. Women consumed the least amount of protein after the protein preloads whereas no difference was found in men. There was some evidence that whey proteins and their components enhance satiety over a short-term period compared to carbohydrate but there was no consistent effect of either whey protein alone or glycomacropeptide.

## 2.2. Introduction

The manner in which dietary chemical composition influences food intake and energy balance is central to an understanding of obesity and body weight control (*Blundell et al., 1996*). Dietary manipulations that maximise satiety are promising as they improve compliance with energy restricted diets (*Westerterp-Plantenga et al., 2004*). The aim is to provide foods shortly before

a meal (preload) that increase the feeling of fullness and thus help to reduce the amount of food consumed at the next meal (*Kissileff & Van Itallie, 1982*) and the total (preload plus meal) amount of food consumed.

Various satiety-inducing food ingredients have been investigated (*Kirkmeyer & Mattes, 2000*) and it is considered that in humans, protein is the most satiating of the three macronutrients (*Anderson & Moore, 2004; Eisenstein et al., 2002; Halton & Hu, 2004*). Marmonier *et al.* (2000) showed that protein delays the return of hunger more than fat or carbohydrate. Eisenstein *et al.* (2002) reviewed ten preload studies and found that in eight out of the ten studies, energy intake in the subsequent meal was lower after a higher-protein preload than after a lower-protein preload. With respect to subjective ratings, a number of studies have shown that protein is more satiating than carbohydrate or fat (*Hill & Blundell, 1986, 1990; Latner & Schwartz, 1999; Porrini et al., 1997; Rolls et al., 1988; Vandewater & Vickers, 1996*). However, Geliebter (1979), Driver (1988), and Barkeling *et al.* (1990) found that protein did not affect subjective responses to satiety. In general, short-term studies to date have found an increased satiating capacity of protein-rich foods over foods rich in either fat or carbohydrate (*Eisenstein et al., 2002*).

Protein source has also been evaluated for its effect on satiety and food intake in humans. In one study (*Anderson et al., 2004*), isolates of whey, soya protein, or egg albumen were fed to young men in flavoured beverages and subjects were provided with a pizza meal 1–2 h later. The whey preload resulted in the greatest reduction in food intake compared with an energy-free water control. Soya protein also led to a decreased food intake, but no effect was found for egg albumen. Within milk protein types, Hall *et al.* (2003) reported that whey is more satiating than casein. Food energy intake from a buffet meal consumed 90 min after ingestion of a whey preload was significantly less compared with a casein preload. Also, subjective measures of satiety were greater following consumption of the whey preload.

A possible explanation for the effect of whey protein on food intake may reside in peptides present in whey and their physiological actions relevant

to food intake regulation (*Anderson & Moore, 2004*). Whey protein is high in beta-lactoglobulin, alpha-lactalbumin, branched chain amino acids (BCAAs) (especially leucine) (*Luhovyy et al., 2007*), and when prepared by ultrafiltration (*Brody, 2000*), the peptide, glycomacropeptide (GMP).

Interest in the intake regulatory effect of caseinomacropeptide (CMP), the unglycosylated form of GMP, arises from its known actions on gastrointestinal function and the release of satiety gut hormones, specifically cholecystokinin (CCK). CMP inhibits gastric acid secretions in calves (*Guilloteau et al., 1994*) while a fraction of CMP, variant A, with slight glycosylation, has been shown to stimulate CCK release in rats (*Beucher et al., 1994*). In humans, the effects of GMP on food intake, CCK release and CCK-mediated satiety have not yet been thoroughly addressed. A pre-meal beverage containing whey protein enriched with GMP, oleic acid, calcium and specific fibres reduced hunger and subsequent meal intake in overweight females (*Portman et al., 2000*). To the contrary, *Gustafson et al. (2001)* found no effect of GMP on objective or subjective measures of satiety when human participants consumed 0.4 g or 2.0 g of GMP in a 100ml preload beverage of 34 J. However, the doses may have been too small to exert any effect and studies at higher doses are required.

It was postulated that whey protein with naturally present GMP and with added GMP would have a greater effect on satiety and satiety ratings than whey protein devoid of GMP which in turn would have a greater effect than a simple carbohydrate source. The objective was to ascertain short-term effects on satiety in adult humans consequent upon the ingestion of whey protein or whey protein plus glycomacropeptide preloads versus a carbohydrate control. Satiety was assessed using visual analogue scales (VAS) and by determining *ad libitum* food energy intake during a free-choice cafeteria style meal offered 30 min after the preloads.

## **2.3. Subjects and methods**

### **2.3.1. Subjects**

Study subjects aged 18–40 years were recruited by public advertisement. All individuals were required to complete a health checklist to assess their suitability for the study. Inclusion criteria were: nonsmoker, no known food allergies, no known current medical problems, not taking medications known to affect appetite such as antidepressants, regular breakfast consumers, not following a diet to lose or gain weight, not pregnant or breast-feeding. Potential subjects completed the Three Factor Eating Questionnaire (*Stunkard & Messick, 1985*), which measures cognitive dietary restraint, disinhibition and hunger. Eligible volunteers provided written informed consent. The study protocol was approved by the Massey University Human Ethics Committee (Application no. 06/59).

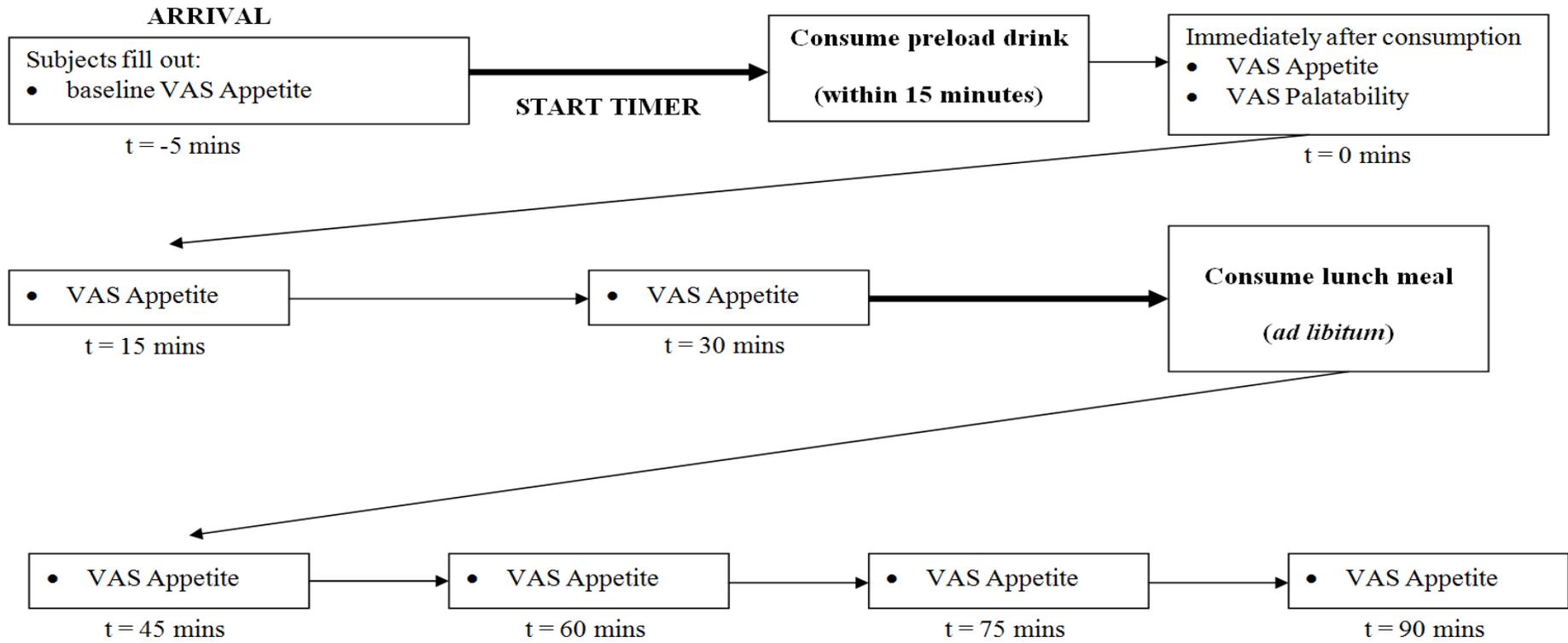
### **2.3.2. Design**

The study used a randomised, single-blind, within-subject crossover design. Each subject participated on four experimental occasions each 1 week apart. In each of the four sessions, subjects were provided with their usual breakfast, a preload and lunch. Subjects were randomised to the study using a repeated 4×4 Latin Square design.

Subjects were asked to maintain consistent levels of physical activity the night and morning before each experimental session. Subjects were also instructed to eat only the foods provided and to drink nothing else except water or the additional choice of hot chocolate, tea or coffee at breakfast. On the first test day, height and body weight were measured to calculate body mass index.

On the test day, each subject consumed his or her usual breakfast meal at home before 0900 h, followed only by water as desired until consumption of the test drink (Figure 2.1.). To ensure compliance with the consumption of

breakfast, the experimenters interviewed the volunteers upon arrival. Subjects came to the Nutrition Laboratory between 1130 and 1200 h. Each subject rated his or her feelings of appetite subjectively using a visual analogue scale (VAS). The VAS was a 10 cm line anchored at either end with opposing statements. Subjects marked the line at points indicating their feelings of hunger, desire to eat, fullness and prospective consumption (the amount of food they thought they could eat at that moment in time). Upon completion of the questionnaire, each subject consumed 300 ml of one of the four preloads and immediately following ingestion of the drink, used a VAS to rate palatability and his or her feelings of appetite. Subjects continued to assess their appetite using VAS every 15 min until 90 min after having been presented with the preload drink. No food or drink was available until 30 min after administration of the preload drink, whereby subjects were provided with a cafeteria style lunch meal *ad libitum*. Subjects were requested to eat until they felt full and did not wish to eat any more food. The amounts of food consumed (to the nearest 0.1 g) were determined by difference in the weight of food before and after the meal using an electronic scale.



t = time, based on the timer which was started immediately as the preload drink was consumed by the subject.

**Figure 2.1.** Experimental protocol.

VAS = visual analogue scales

### 2.3.3. Preload drink and lunch meal

Four preload drinks were formulated, the ingredient compositions of which are given in Table 2.1. The high carbohydrate control drink contained maltodextrin while the three proteinaceous drinks contained either a whey protein isolate (WPI) with 21% glycomacropeptide (GMP) (21% GMP WPI); a mixture of WPI with 21% GMP and added GMP (21% GMP WPI+GMP) or a WPI extracted from skim milk with no GMP (WPI no GMP). Drinks were made up to 300ml and were approximately iso-energetic. Beverages were made within 3 days of serving and stored in a refrigerator.

The macronutrient and energy contents of the preload drinks are also given in Table 2.1. The carbohydrate control was predominantly comprised of available carbohydrate but contained some protein from milk, which was common across the preloads. The amount of WPI in 300 ml of 21% GMP WPI, 21% GMP WPI+GMP and WPI no GMP was calculated to be 31.6, 15.8 and 40 g respectively. The 21% GMP WPI and 21% GMP WPI+GMP preloads contained 8.4 g and 24.2 g of GMP per 300 ml, respectively.

The lunch meal that subjects consumed *ad libitum* was an individual cafeteria style platter containing three hot meals (macaroni and cheese; fried rice; pasta spirals with tomato-based sauce), dairy milk chocolate bars, toffees and water. Subjects were presented with more of each food item than they were likely to consume and were instructed to eat as much or as little of any food item as they desired.

**Table 2.1.** Ingredient (g as-is basis) and nutrient (per 300ml) compositions of the preload drinks.

<b>Ingredient</b>	<b>Preload Drink</b>			
	<b>Carb</b>	<b>21% GMP WPI</b>	<b>21% GMP WPI + GMP</b>	<b>WPI no GMP</b>
Low-fat Milk <sup>1</sup> (g)	248	248	248	248
Sucrose <sup>2</sup> (g)	12	12	12	12
Maltodextrin <sup>3</sup> (g)	40	-	-	-
WPI ex cheese whey <sup>4</sup> (g)	-	40	20	-
Glycomacropeptide <sup>5</sup> (g)	-	-	20	-
WPI ex skim milk <sup>6</sup> (g)	-	-	-	40
Flavour <sup>7</sup>		0.9ml or 1.5ml (chocolate)		
Colour <sup>8</sup>		See index below		
<b>Energy or Nutrient</b>				
Gross energy <sup>9</sup> (kJ)	1379.2	1620.2	1573.8	1628.9
Metabolisable energy <sup>10</sup> (kJ)	1296.3	1273.2	1260.0	1259.4
Crude protein (g)	10.21	44.96	42.49	45.78
Available carbohydrate (g)	58.86	21.91	24.11	20.71
Crude fat (g)	3.79	4.15	3.92	3.95

<sup>1</sup> Lite milk (Pam's Products Ltd., Mt Roskill, Auckland, New Zealand)

<sup>2</sup> SIMPLY Pure Cane White Sugar (Kerry New Zealand Ltd., Auckland, New Zealand)

<sup>3</sup> Fieldose™ 10C (Penford New Zealand Ltd., Onehunga, Auckland, New Zealand)

<sup>4</sup> Whey protein isolate extracted from cheese whey containing 21% glycomacropeptide (Fonterra Ltd., Palmerston North, New Zealand)

<sup>5</sup> BioPURE-GMP™ (Davisco Foods International Inc., Eden Prairie, Minnesota, United States)

<sup>6</sup> Whey protein isolate extracted from skim milk not containing glycomacropeptide (Fonterra Ltd., Palmerston North, New Zealand)

<sup>7</sup> Alternative flavour:

Chocolate flavour (Sensient 61656, Sensient Technologies New Zealand, Otahuhu, Auckland, New Zealand)

Banana flavour (Sensient 419012, Sensient Technologies New Zealand, Otahuhu, Auckland, New Zealand)

Strawberry flavour (Sensient 1397, Sensient Technologies New Zealand, Otahuhu, Auckland, New Zealand)

Caramel flavour (Product Makers 000896, Belletech International Ltd. Takapuna, New Zealand)

Vanilla flavour (Product Makers 000708, Belletech International Ltd. Takapuna, New Zealand)

<sup>8</sup> Alternative colour:

Chocolate Brown colour (Sensient N1078, Sensient Technologies New Zealand, Otahuhu, Auckland, New Zealand): 0.2ml for caramel and 0.5ml for chocolate drink

Red colour (Sensient 962144/N928, Sensient Technologies New Zealand, Otahuhu, Auckland, New Zealand): 0.1ml for strawberry drink

Yellow colour (Hansells 102, Hansells Ltd. Greenlane, Auckland, New Zealand): 0.1ml for vanilla and 0.3ml for banana drink

<sup>9</sup> Gross energy determined using a bomb calorimeter

<sup>10</sup> Metabolisable energy calculated from the energy-producing food components using the energy conversion factors of 16.7 kJ/g for protein and available carbohydrate and 37.7 kJ/g for fat

#### 2.3.4. Chemical analysis

All food samples were freeze-dried at -20°C and finely ground. The samples were analysed for gross energy, moisture, ash, crude protein, crude fat and fibre.

The moisture and ash contents of the food samples were determined after drying the samples in a convection oven at 105°C for 16 h, followed by ashing in a furnace at 550°C for 16 h (AOAC, 2005).

The acid hydrolysis Mojonnier extraction method was used for determining crude fat in the dietary samples. Samples were moistened with 2 ml of alcohol to prevent lumping on addition of 10 ml of hydrochloric acid (mixed in a ratio of 25:11 v/v with water). The material was placed in a water bath held at 70–80 °C with stirring for 30–40 min for hydrolysis. After cooling and adding 10 ml of alcohol, the contents were extracted with a mixture of ethyl ether and petroleum ether in a Mojonnier flask. The solvent was evaporated and the extracted fat was weighed (AOAC, 2005; Nielsen, 2003).

Total nitrogen was determined using the total combustion method in a LECO analyser. Crude protein was calculated using the nitrogen conversion factor of 6.25 (AOAC, 2005).

The enzymatic–gravimetric method was used to determine the amount of total dietary fibre in the food samples (AOAC, 2005). Dry duplicate samples (fat extracted if containing > 10% fat) were digested sequentially with heat stable  $\alpha$ -amylase, protease, and amyloglucosidase to remove starch and protein. The digested material was treated with four volumes of ethanol to precipitate soluble dietary fibre before filtering, and the total dietary fibre residue was washed with ethanol and acetone, dried and weighed (Nielsen, 2003).

The carbohydrate content of the food samples was determined by difference. The level of available carbohydrate was calculated by subtraction of

the sums of the weights of crude protein, total fat, total dietary fibre, moisture, and ash from the total weight of the food (Nielsen, 2003).

The gross energy was determined by placing the samples in a bomb calorimeter (Gallenkamp Co. Ltd., London, Great Britain).

The metabolisable energy (ME) was calculated from the energy producing food components using the energy conversion factors of 16.7 kJ/g for protein and available carbohydrate and 37.7 kJ/g for fat (Athar *et al.*, 2003).

### **2.3.5. Statistical analysis**

Statistical analyses were facilitated by SAS version 9.1 (Statistical Analysis Systems, SAS Institute). Based on the results of Poppitt *et al.* (1998), a sample size of 51 subjects was required to detect a difference in *ad libitum* energy intake of 307 kJ to achieve power of 0.80 at a level of significance of 0.05. Amount of food, energy intake, nutrient intake at lunch and cumulative (food plus preload) energy intake were subjected to a two-way analysis of variance (ANOVA) with preload type and subject as factors. The test meal data from the female and male groups were analysed separately. Palatability scores (VAS) for the preload were also analysed using a two-way ANOVA. Scores for hunger, desire to eat, fullness and prospective consumption were subjected to a two-way repeated measures analysis of variance (ANOVA) with subjects and preload type as factors in the model. A p value of less than 0.05 was considered to indicate statistical significance. Where a main effect was found to be significant, Duncan's post hoc test was performed for mean comparisons. To analyse the relationship between food intake and changes in satiety ratings as well as body weight, Pearson's correlation analysis was applied. Data are presented as mean  $\pm$  standard error of the mean (sem) (Zar, 1999).

## **2.4. Results**

### **2.4.1. Subject characteristics**

Fifty-five subjects were recruited and of these, five did not complete the study due to compliance issues and their data were excluded. The age and BMI of the remaining 50 subjects were (mean  $\pm$  sem)  $24.6 \pm 0.8$  years and  $23.2 \pm 0.5$  kg/m<sup>2</sup>, respectively. The ages of the 19 men and 31 women were (mean  $\pm$  sem)  $25.4 \pm 1.3$  years and  $24.1 \pm 0.9$  years, respectively. The BMI (kg/m<sup>2</sup>) for the 19 men and 31 women were  $23.9 \pm 0.5$  and  $22.8 \pm 0.7$ , respectively. Cognitive dietary restraint scores were  $7.14 \pm 0.56$  overall and for the men and women,  $4.84 \pm 0.59$  and  $8.55 \pm 0.72$ , respectively.

### **2.4.2. Satiety ratings (VAS)**

The VAS results are shown in Figure 2.2. For hunger, desire to eat, fullness and prospective consumption, there was a change in response over time post ingestion of the preload drink ( $p < 0.001$ ) but there was no treatment by time interaction ( $p > 0.05$ ). There was no overall effect of preload treatment ( $p > 0.05$ ). Generally, subjects indicated that all the preloads suppressed hunger, desire to eat and prospective consumption within 30 min of preload consumption, and after the lunch test meal (30 min) these ratings decreased further. The feeling of fullness increased immediately upon preload ingestion (baseline -5 to 0) and increased further after lunch.

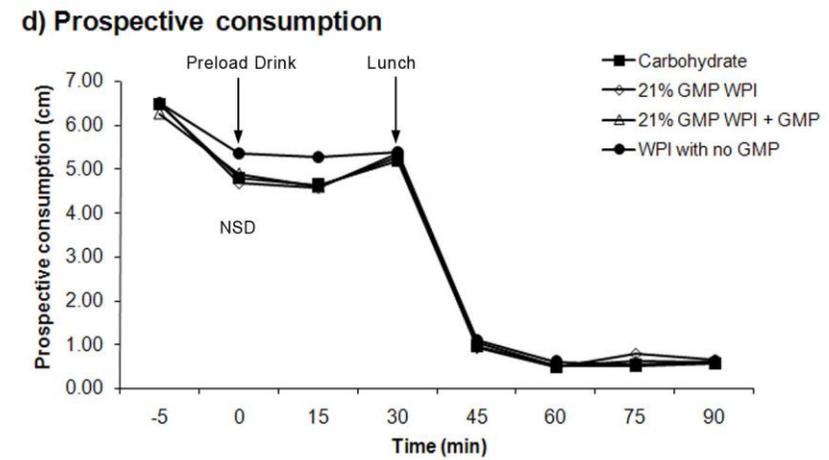
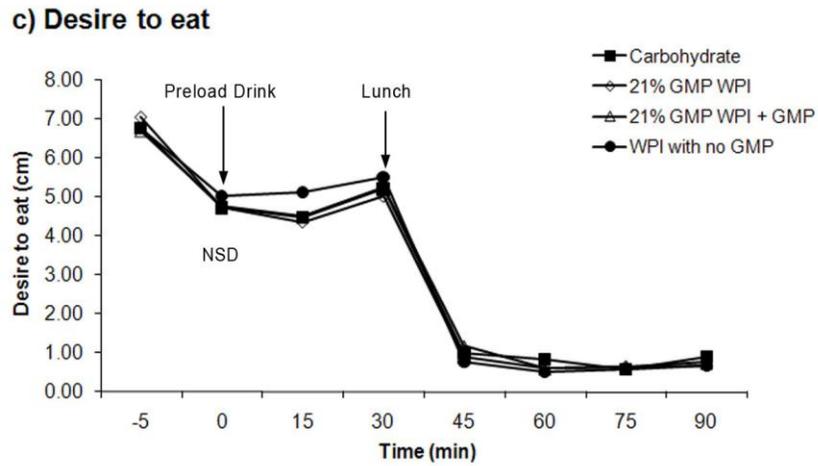
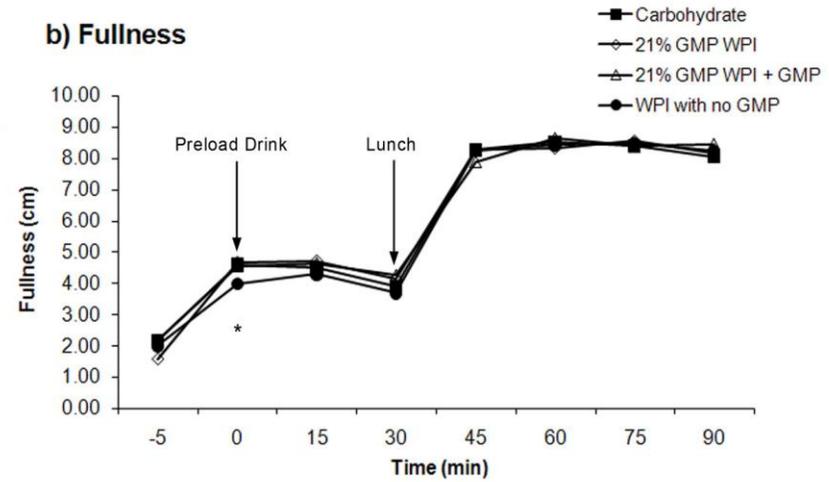
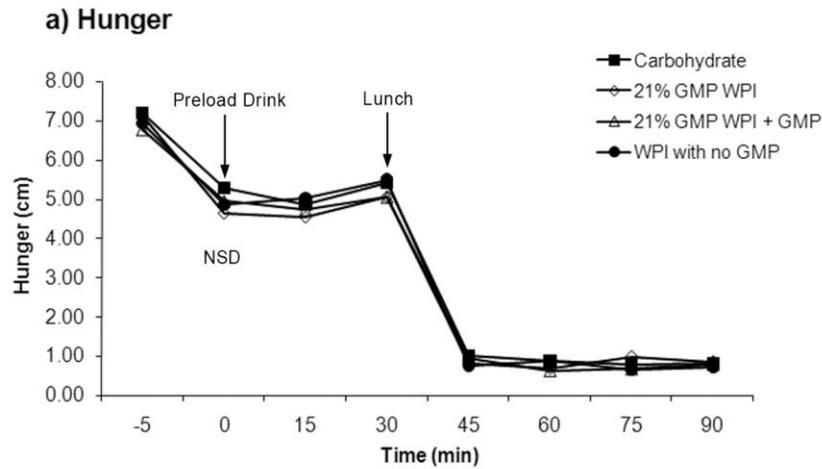
The VAS data were expressed as changes from baseline by subtracting the baseline scores (time -5) from the pre-meal data (time 0, 15, 30) allowing comparison of each subject's rating to his or her own pre-treatment score. For the hunger, desire to eat and prospective consumption questions, there was no effect of treatment ( $p > 0.05$ ). However, subjects rated themselves as feeling more full ( $p < 0.05$ ) with the lower GMP preload (21% GMP WPI) compared to the carbohydrate control and the other two proteinaceous preload drinks before

the lunch test meal. There was an effect of treatment on fullness at 0 min but none at 15 or 30 min after preload administration, though the results approached statistical significance ( $p=0.058$  and  $p=0.080$ , respectively).

### **2.4.3. Drink palatability**

The acceptability, likeability and textural acceptability of the preload drinks were determined using VAS (data not shown). There was an effect of treatment on likeability ( $p<0.001$ ), acceptability ( $p<0.02$ ) and textural acceptability ( $p<0.01$ ) of the preloads.

The carbohydrate drink was the most liked and the WPI with no GMP the least. Furthermore, the carbohydrate drink was more acceptable than that based on WPI with no GMP. The texture of the carbohydrate preload was more acceptable than that of the highest GMP level beverage and the WPI with no GMP drink. Overall, the carbohydrate preload was rated as the most palatable drink compared to the protein preloads.



**Figure 2.2.** a)-d). Subjective VAS ratings throughout the study for the different preload drinks: carbohydrate, WPI with naturally present GMP, WPI with added GMP and WPI with no GMP.

Values are expressed as means,  $n = 50$ . Significant differences in fullness scores to preloads after consumption is shown at 0 min time point (\*,  $p < 0.05$ ). NSD = not significantly different.

#### 2.4.4. Food and water intake

There were no differences ( $p>0.05$ ) among treatment groups for the total amount of food eaten or food energy and water ingestion at the *ad libitum* lunch. Cumulative (food plus preload) energy intake after the whey protein preloads was not significantly different from the control preload (Table 2.2.). There were no statistically significant differences between the preload drinks in lunch intakes of food dry matter or carbohydrate, protein, fat and fibre (Table 2.2.). The protein intakes, however, were numerically lower for the higher protein drinks and the treatment effect was close to significance ( $p=0.08$ ). When the carbohydrate, protein and fat energy intakes were expressed as proportions of the food energy intake for the lunchtime meal, energy from protein intake differed ( $p<0.05$ ) across the preloads with the higher protein preloads supporting lower protein energy as a percentage of total food energy. Subjects chose to eat more fried rice, which is higher in carbohydrate and lower in protein with the higher protein preloads and concomitantly less macaroni cheese.

The effect of the preloads on food, water and macronutrient intakes at the lunch test meal in men and women analysed separately was investigated (Table 2.3.). Within men, there was no effect of treatment on the amount of food or water consumed, nutrient or energy intake during the lunch meal ( $p>0.05$ ). When nutrient intake was analysed within women, however, it was found that protein, but not carbohydrate and fat, was significantly different across preloads ( $p<0.05$ ) with the least amount of protein being consumed after the highest GMP protein preload and the WPI with no GMP protein preload. Food intake was some 10% lower for the high GMP preload compared to the carbohydrate control, but the result did not reach statistical significance.

**Table 2.2.** Effect of preload drink on food, specific nutrient (weight or percentage of total food energy intake) and water intake at lunch 30 min after administration of preload (Mean;  $n = 50$ ).

	Preload Drink				Overall sem	<i>p</i>
	Carb	WPI no GMP	21% GMP WPI <sup>5</sup>	21% GMP WPI + GMP		
<b>Weight (g)</b>						
Food intake <sup>1</sup>	423.0	394.8	415.6	397.9	22.2	0.35
Dry matter	138.3	134.0	140.2	138.5	7.2	0.78
Carbohydrate	85.3	86.4	87.6	88.6	4.6	0.91
Fibre	3.8	3.6	3.7	3.5	0.2	0.51
Protein	17.8	15.7	17.2	16.2	1.0	0.08
Fat	26.3	23.2	26.3	24.9	1.8	0.15
Water intake	332.9	364.2	357.3	363.2	28.3	0.35
Food energy <sup>2</sup> (kJ)	2716.0	2583.8	2746.6	2693.5	145.0	0.58

<b>Percentage of total energy (%)<sup>3</sup></b>						
Carbohydrate	53.8	56.6	54.6	55.8	1.2	0.24
Protein	11.0 <sup>a</sup>	10.3 <sup>b</sup>	10.4 <sup>ab</sup>	10.1 <sup>b</sup>	0.3	<b>0.02</b>
Fat	35.2	33.1	34.9	34.0	1.1	0.34
<b>Food + Preload<sup>4</sup> (kJ)</b>	4012.3	3843.2	4019.8	3953.5	145.0	0.46

Means with different superscripts within a row were different,  $p < 0.05$  (Duncan's post hoc test).

<sup>1</sup> Weight of total foods consumed at lunch as-is

<sup>2</sup> Energy of total foods consumed at lunch

<sup>3</sup> The energy derived from carbohydrate, protein and fat was expressed as proportions of the food energy intake

<sup>4</sup> Cumulative energy intakes from lunch meal and the preload treatment

<sup>5</sup> This reading has one missing value ( $n = 49$ )

**Table 2.3.** Effect of preload drink on food, water and nutrient intakes at lunch in men and women (Mean)

	Preload Drink				Overall sem	<i>p</i>
	Carb	WPI no GMP	21% GMP WPI <sup>5</sup>	21% GMP WPI + GMP		
<b><i>Men (n = 19)</i></b>						
Food intake (g)	516.3	489.7	511.4	509.4	32.8	0.88
Carbohydrate (g)	99.8	100.3	101.8	98.8	7.1	0.98
Fibre (g)	4.9	4.7	4.7	4.7	0.4	0.90
Protein (g)	19.9	18.6	20.0	19.8	1.6	0.81
Fat (g)	27.2	25.8	30.2	28.0	2.9	0.53
Food energy (kJ)	3028.8	2958.6	3176.4	3038.4	223.0	0.82
Water intake (g)	391.7	407.4	460.4	415.7	49.0	0.30
<b><i>Women (n = 31)</i></b>						
Food intake (g)	365.7	336.8	356.8	327.3 <sup>1</sup>	23.8	0.25
Carbohydrate (g)	76.5	77.9	78.9	82.2 <sup>1</sup>	5.5	0.78
Fibre (g)	3.1	3.0	3.1	2.8 <sup>1</sup>	0.2	0.42
Protein (g)	16.5 <sup>a</sup>	14.0 <sup>b</sup>	15.5 <sup>ab</sup>	13.9 <sup>1b</sup>	1.2	<b>0.03</b>
Fat (g)	25.7	21.7	24.0	23.0 <sup>1</sup>	2.2	0.12

Food energy (kJ)	2524.3	2354.1	2483.2	2475.0 <sup>1</sup>	179.3	0.66
Water intake (g)	296.9	337.7	294.2	329.9 <sup>1</sup>	33.2	0.09

<sup>1</sup> This reading has one missing value ( $n = 30$ )

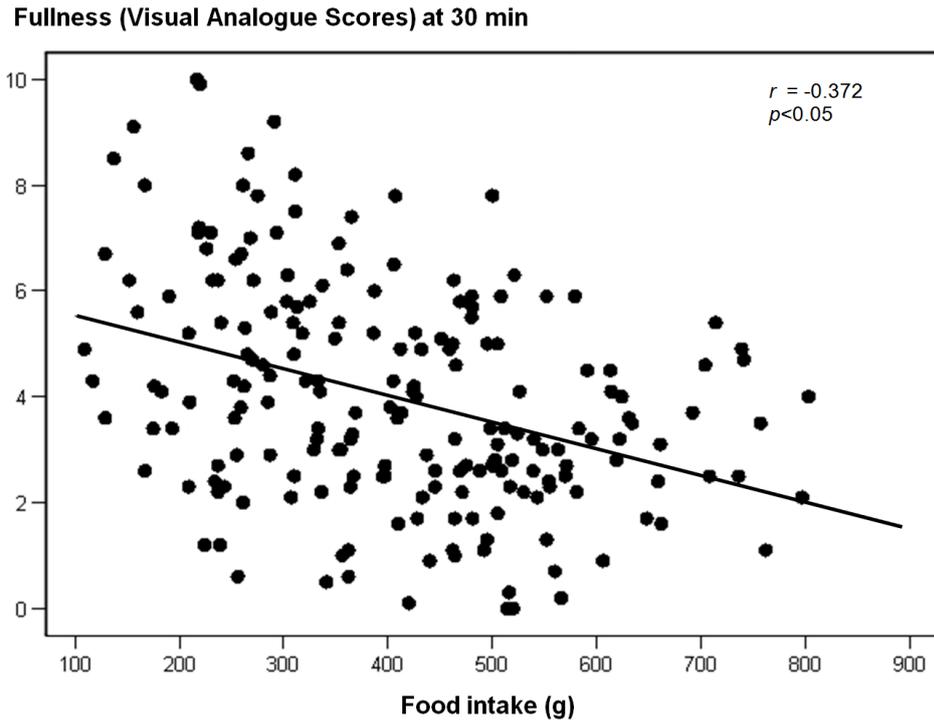
<sup>a</sup> Means with different superscripts within a row were different,  $p < 0.05$  (Duncan's post hoc test)

#### 2.4.5. Correlation analyses

There was a positive relationship between food intake and body weight of subjects ( $r = 0.58, p < 0.05$ ).

There was a positive correlation across individuals between amount of food eaten and hunger score at 30 min ( $r = 0.329, p < 0.05$ ), desire to eat score at 30 min ( $r = 0.372, p < 0.05$ ), and prospective consumption score at 30 min ( $r = 0.399, p < 0.05$ ). There was a negative correlation with fullness score at 30 min ( $r = -0.372, p < 0.05$ ) as shown in Figure 2.3. Overall hunger, desire to eat and prospective consumption ratings at 30 min correlated positively with total ingested dry matter, food energy as well as carbohydrate, fat and protein intake while fullness ratings correlated negatively (Table 2.4).

A key question was whether the lunch test meal intake was affected by the sensory qualities of the preloads. To test this, test meal intake was correlated with average sensory ratings. There was no relationship between food intake and drink likeability ( $r = 0.013, p > 0.05$ ), acceptability ( $r = 0.026, p > 0.05$ ) or textural acceptability ( $r = 0.027, p > 0.05$ ).



**Figure 2.3.** Scatter plot and linear regression line (dashed line) for fullness VAS scores at 30 minutes (cm) plotted as a function of food intake (g) ( $n = 50$ ).

**Table 2.4.** Pearson's correlations between food intake and satiety VAS ratings at 30 minutes in men and women

	<b>Food Energy (kJ)</b>	<b>Dry matter (g)</b>	<b>Carbohydrate (g)</b>	<b>Fat (g)</b>	<b>Protein (g)</b>
<b>Hunger (cm)</b>	0.250**	0.266***	0.260***	0.174*	0.283****
<b>Desire to eat (cm)</b>	0.331****	0.341****	0.319****	0.261***	0.356****
<b>Prospective consumption (cm)</b>	0.366****	0.371****	0.334****	0.312****	0.394****
<b>Fullness (cm)</b>	-0.280****	-0.294****	-0.278****	-0.209**	-0.310****

Marked Pearson's correlation coefficients ( $r$ ) are significant at: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$

## 2.5. Discussion

The present investigation followed an established methodology for the study of the effect of nutrients on satiety and food intake in human subjects (*Rolls & Hammer, 1995*). Our data showed an effect of a preload drink on subjective ratings of fullness. Subjects rated themselves as feeling more full with the lower GMP preload (21% GMP WPI) compared to the whey protein treatment with no GMP or with added GMP (21% GMP WPI+GMP) and the carbohydrate control. Food intake at lunch and cumulative (preload + lunch) food intake, however, were not affected by preload type but subjects ingesting the protein preloads consumed foods at lunch that were higher in carbohydrate.

Differences between the fullness ratings determined using the visual analogue scales (VAS) emerged immediately after the consumption of the preload (time 0), with the lower GMP preload being rated as more filling than the other preloads. VAS ratings have been shown to be reliable and reproducible for discrimination of different satiety conditions (*Porrini et al., 1995; Raben et al., 1995*) and our findings concur to some degree with others (*Hill & Blundell, 1986, 1990; Latner & Schwartz, 1999; Porrini et al., 1997; Rolls et al., 1988; Vandewater & Vickers, 1996*) that protein preloads are relatively more satiating than carbohydrate. The validity of the VAS may be determined by correlating the subjective satiety ratings with subsequent food intake. In this human feeding setting, the ratings of hunger, desire to eat, fullness and prospective food consumption correlated strongly with food intake as has been observed previously (*De Graaf et al., 1992; Hill et al., 1987; Porrini et al., 1995; Rolls et al., 1988*). The test meals resulted in large decreases in the mean scores for hunger, desire to eat and prospective food consumption and increased fullness scores independent of the test treatments. This suggested that subjects understood the VAS scales and adjusted their food intake at the lunch meal to their subjective satiety feelings.

The lunch food intake correlated with the body weight of the subjects, supporting the notion that an increase in body weight may be associated with increased food intake. The sensory properties of the preload drinks were found to be different with the carbohydrate drink being most liked, acceptable and texturally acceptable. Drink palatability was not associated with food intake so that the sensory properties of the preload did not appear to affect subsequent food intake.

The preloads had no influence on food or energy intakes at the lunch meal. Although the literature generally supports the view that protein ingestion suppresses food intake more than carbohydrate does (*Eisenstein et al., 2002*), some other studies have also failed to demonstrate this (*De Graaf et al., 1992; Geliebter, 1979; Stubbs et al., 1996*). The results of the present study support the observation that a protein preload does not affect food intake or the proportions of macronutrients consumed in a subsequent meal in comparison with carbohydrate. No statistically significant difference was observed for macronutrient intakes in men. However, high protein preloads led to significantly lower amounts of protein consumed at the lunch meal in women but this difference did not extend to differences in carbohydrate and fat ingestion.

There were some limitations to the present human feeding study. Subjects were instructed not to eat or drink anything (other than water) after consumption of their usual breakfast until the preload but subjects were not strictly monitored during this period. At the lunch meal, while an element of choice is clearly desirable, the range of foods might have been too great, leading to an overriding of physiological satiety signals (*Rolls et al., 1981*). As an alternative to the buffet style meal, a menu from which subjects pre-select in advance from a range of foods, may have merit.

Another issue was the lack of a control preload containing no energy (i.e. water control). We elected to incorporate a mainly carbohydrate preload control condition into the study design because our aim was not to examine the effect of a preload *per se* but rather the effect of a protein versus carbohydrate

preload. Similar to Portman *et al.* (2000) and Bertenshaw *et al.* (2008), we used a maltodextrin carbohydrate control because it is less sweet than sucrose or glucose and more of it could be used to manipulate carbohydrate content. Maltodextrin is derived from starch and is absorbed as rapidly as glucose. One preload containing only GMP would have been useful as the effects of added GMP in the present study might have been confounded with the WPI in the preload. Nonetheless, GMP is a peptide that empties rapidly from the stomach similarly to whey (Luhovyy *et al.*, 2007). Burton-Freeman (2008) recently reported that a preload containing GMP alone did not affect test meal intake compared to a preload containing whey protein isolate with a low amount of GMP or whey protein without GMP.

Another potential limitation of our study was the delay between the preload and ingestion of the test meal. Studies that have reported that protein exerts a greater satiating effect than carbohydrate vary in the time interval between meals (Latner & Schwartz, 1999; Marmonier *et al.*, 2000; Poppitt *et al.*, 1998; Porrini *et al.*, 1995; Stubbs *et al.*, 1996). The suppressive effects of protein and carbohydrate, consumed in the fasted state, on subsequent food intake are similar when assessed by voluntary food consumption within 60 min after their ingestion (Reid & Hetherington, 1997), whereas after longer time intervals, protein appears more suppressive of appetite and food intake (Poppitt *et al.*, 1998; Porrini *et al.*, 1995; Stubbs *et al.*, 1996). This is because the shorter the interval between the preload and test meal, the better the accuracy in energy compensation, and as the time lapse increases (1–5 h), the efficiency of the compensation decreases (Rolls *et al.*, 1991). Rolls *et al.* (1991) showed that in the 30 min delay condition, subjects accurately compensated for the calories in the preloads compared with a no-preload condition, but as the interval increased, compensation was less precise. Gustafson *et al.* (2001) applied a time interval of 1 h but failed to find an effect of caseinomacropptide on food intake and subjective measures of satiety. In a recent study reported by Bellissimo *et al.* (2008), an effect of protein and carbohydrate on subsequent food intake was found to be time-dependent. From 30 to 60 min, the effect of glucose decreased whereas the effect of whey protein increased in normal weight boys due to carbohydrates and proteins

having different post-ingestional consequences. Glucose and whey protein have different rates of intestinal absorption and elicit different metabolic responses. Glucose is rapidly absorbed to stimulate the satiety hormones glucagon-like peptide-1 (GLP-1) and insulin. In contrast, whey protein requires digestion before absorption (Hall *et al.*, 2003) and results in the slower stimulation of satiety hormones cholecystokinin (CCK) (Burton-Freeman, 2008) and GLP-1 (Bowen *et al.*, 2006; Hall *et al.*, 2003). Furthermore, the volume of preload used in this study might have been ineffective for some subjects. Rolls *et al.* (2000) reported that energy intake at lunch was reduced after consumption of a 600-ml preload compared to a 300-ml preload in normal weight men (BMI between 20 and 25).

The results of this study suggest that whey with GMP had an effect on the feeling of fullness but this did not translate into a lower food intake at the lunch meal. Further work is required understanding dose of protein, delivery mode of preload and timing between preload and subsequent test meal. The pattern of release of cholecystokinin and other signals of satiety would also assist in understanding the satiety response of protein.

## 2.6. Literature cited

- Anderson, G. H., & Moore, S. E. (2004). Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr*, *134*(4), 974S–979S.
- Anderson, G. H., Tecimer, S. N., Shah, D., & Zafar, T. A. (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.
- AOAC. (2005). *Official methods of analysis*. (18th ed). Gaithersburg, MD, USA: Association of Official Analytical Chemists.
- Athar, N., McLaughlin, J., & Taylor, G. (2003). *The concise New Zealand food composition tables*. 6th ed. Palmerston North, NZ: New Zealand Institute for Crop & Food Research; Wellington, NZ: Ministry of Health.

- Barkeling, B., Rössner, S., & Björvell, 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.
- Bellissimo, N., Desantadina, M. V., Pencharz, P. B., Berall, G. B., Thomas, S. G., & Anderson, G. H. (2007). A comparison of short-term appetite and energy intakes in normal weight and obese boys following glucose and whey-protein drinks. *Int J Obes*, *32*(2), 362–371.
- Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiol Behav*, *93*(3), 427–436.
- Beucher, S., Levenez, F., Yvon, M., & Corring, T. (1994). Effects of gastric digestive products from casein on CCK release by intestinal cells in rat. *J Nutr Biochem*, *5*(12), 578–584.
- Blundell, J. E., Lawton, C. L., Cotton, J. R., & Macdiarmid, J. I. (1996). Control of human appetite: implications for the intake of dietary fat. *Annu Rev Nutr*, *16*, 285–319.
- Bowen, J., Noakes, M., & 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.
- Brody, E. P. (2000). Biological activities of bovine glycomacropeptides. *Br J Nutr*, *84*(Suppl 1), S39–S46.
- Burton-Freeman, B. M. (2008). Glycomacropeptide (GMP) is not critical to whey-induced satiety, but may have a unique role in energy intake regulation through cholecystokinin (CCK). *Physiol Behav*, *93*(1-2), 379–387.
- De Graaf, C., Hulshof, T., Weststrate, J. A., & Jas, P. (1992). Short-term effects of different amounts of protein, fats, and carbohydrates on satiety. *Am J Clin Nutr*, *55*(1), 33–38.
- Driver, C. J. (1988). The effect of meal composition on the degree of satiation following a test meal and possible mechanisms involved. *Br J Nutr*, *60*(3), 441–449.

- Eisenstein, J., Roberts, S. B., Dallal, G., & Saltzman, E. (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), 189–200.
- Geliebter, A. A. (1979). Effects of equicaloric loads of protein, fat, and carbohydrate on food intake in the rat and man. *Physiol Behav*, *22*(2), 267–273.
- Guilloteau, P., Le Huerou-Luron, I., Chayvialle, J. A., Toullec, R., Legeas, M., Bernard, C., Roger, L., & Mendy, F. (1994). Effect of caseinomacropptide (CMP) on gastric secretion and plasma gut regulatory peptides in preruminant calves. *Reprod Nutr Dev*, *34*(6), 612–613.
- Gustafson, D. R., McMahon, D. J., Morrey, J., & Nan, R. (2001). Appetite is not influenced by a unique milk peptide: caseinomacropptide (CMP). *Appetite*, *36*(2), 157–163.
- Hall, W. L., Millward, D. J., Long, S. J., & Morgan, L. M. (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., & 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.
- Hill, A. J., & Blundell, J. E. (1986). Macronutrients and satiety: the effects of a high-protein or high-carbohydrate meal on subjective motivation to eat and food preferences. *Nutr Behav*, *3*(2), 133–144.
- Hill, A. J., & Blundell, J. E. (1990). Comparison of the action of macronutrients on the expression of appetite in lean and obese human subjects. *Ann N Y Acad Sci*, *580*(1), 529–531.
- Hill, A. J., Leathwood, P. D., & Blundell, J. E. (1987). Some evidence for short-term caloric compensation in normal weight human subjects: the effects of high- and low energy meals on hunger, food preference and food intake. *Hum Nutr Appl Nutr*, *41*(4), 244–257.
- Kirkmeyer, S. V., & Mattes, R. D. (2000). Effects of food attributes on hunger and food intake. *Int J Obes Relat Metab Disord*, *24*(9), 1167–1175.

- Kissileff, H. R., & Van Itallie, T. B. (1982). Physiology of the control of food intake. *Annu Rev Nutr*, 2, 371–418.
- Latner, J. D., & Schwartz, M. (1999). The effects of a high-carbohydrate, high-protein or balanced lunch upon later food intake and hunger ratings. *Appetite*, 33(1), 119–128.
- Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr*, 26(6), S704–S712.
- Marmonier, C., Chapelot, D., & Louis-Sylvestre, J. (2000). Effects of macronutrient content and energy density of snacks consumed in a satiety state on the onset of the next meal. *Appetite*, 34(2), 161–168.
- Nielsen, S. S. (2003). *Food analysis*. (3rd ed). New York: Kluwer Academic/Plenum Publishers.
- Poppitt, S. D., McCormack, D., & Buffenstein, R. (1998). Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiol Behav*, 64(3), 279–285.
- Porrini, M., Crovetti, R., Testolin, G., & Silva, S. (1995). Evaluation of satiety sensations and food intake after different preloads. *Appetite*, 25(1), 17–30.
- Porrini, M., Santangelo, A., Crovetti, R., Riso, P., Testolin, G., & Blundell, J. E. (1997). Weight, protein, fat, and timing of preloads affect food intake. *Physiol Behav*, 62(3), 563–570.
- Portman, R., Bakal, A., & Peikin, S. (2000). Ingestion of a premeal beverage designed to release CCK reduces hunger and energy consumption in overweight females. *Obes Res*, 8(Suppl 1), S117.
- Raben, A., Tagliabue, A., & Astrup, A. (1995). The reproducibility of subjective appetite scores. *Br J Nutr*, 73(4), 517–530.
- Reid, M., & Hetherington, M. (1997). Relative effects of carbohydrates and protein on satiety – a review of methodology. *Neurosci Biobehav Rev*, 21(3), 295–308.
- Rolls, B. J., & Hammer, V. A. (1995). Fat, carbohydrate, and the regulation of energy intake. *Am J Clin Nutr*, 62(5 Suppl), 1086S–1095S.
- Rolls, B. J., Bell, E. A., & Waugh, B. A. (2000). Increasing the volume of a food by incorporating air affects satiety in men. *Am J Clin Nutr*, 72(2):361–368.

- Rolls, B. J., Hetherington, M., & 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, S., McNelis, A. L., Fischman, M. W., Foltin, R. W., & Moran, T. H. (1991). Time course of effects of preloads high in fat or carbohydrate on food intake and hunger ratings in humans. *Am J Physiol*, *260*(4 Pt 2), 756–763.
- Rolls, B. J., Rowe, E. A., Rolls, E. T., Kingston, B., Megson, A., & Gunary, R. (1981). Variety in a meal enhances food intake in man. *Physiol Behav*, *26*(2), 215–221.
- Stubbs, R. J., Van Wyk, M. C., Johnstone, A. M., & Harbron, C. G. (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., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*, *29*(1), 71–83.
- Vandewater, K., & Vickers, Z. (1996). Higher-protein foods produce greater sensory-specific satiety. *Physiol Behav*, *59*(3), 579–583.
- Westerterp-Plantenga, M. S., Lejeune, M. P., Nijs, I., van Ooijen, M., & Kovacs, E. M. (2004). High protein intake sustains weight maintenance after body weight loss in humans. *Int J Obes Relat Metab Disord*, *28*(1), 57–64.
- Zar, J. H. (1999). *Biostatistical analysis*. 4th ed. Upper Saddle River, New Jersey: Prentice Hall.

## Chapter 3

### **Effect of time of consumption of preloads on measures of satiety in healthy normal weight women**

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Following consumption of preloads containing whey protein (including naturally present glycomacropeptide) compared to maltodextrin carbohydrate, subjects reported feeling fuller but intake at a subsequent *ad libitum* test meal 30 min later was similar (*Chapter 2*). The time of administration of the test meal after the preloads may account for the inability to detect an effect of whey protein on subsequent food intake. The objective of the present study was to investigate whether the satiating effect of whey protein (including glycomacropeptide) in comparison with maltodextrin carbohydrate was influenced by time of consumption.

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### 3.1. Abstract

Differences in the time interval between preload and test meal may account for the variation in the satiating effects of whey protein found in previous preload studies. The objective was to compare the satiating effects (*ad libitum* meal intake at a set time after ingestion of preload) of whey protein (including glycomacropeptide) and maltodextrin carbohydrate and to determine whether such effects were influenced by the timing of preloads. On nine separate days, 19 healthy normal weight women consumed mixed composition preloads containing either water, or maltodextrin, or whey protein, 30, 60 or 120 min before an *ad libitum* test meal. Whey protein reduced food intake ( $p < 0.05$ ) at the test meal more than maltodextrin and water (respective food energy intakes were 2343, 2584 and 3135 kJ). The time interval between preload and test meal did not affect the food intake response. Total energy intake (preload + test meal) differed, with intake for the maltodextrin-enriched preload being greater than that for the whey protein-enriched preload, which was greater than for the water control. Total respective energy intakes were 3955, 3676 and 3135 kJ. Subjective ratings of appetite did not support a greater satiating effect of whey protein versus maltodextrin on food intake but there was evidence for a satiating effect of maltodextrin and whey protein versus the water control.

### 3.2. Introduction

The prevalence of obesity has increased rapidly in recent years and an understanding of factors regulating food intake and energy balance is central to an understanding of obesity and body weight control (Bjorntorp, 2001). Foods provided shortly before a meal (preload) that increase the feeling of fullness and reduce the amount of food eaten at the next meal and the total (preload plus test meal) amount of food energy consumed have potential. Dietary manipulations that enhance satiety are promising as they improve compliance with energy restricted diets (Westerterp-Platenga et al., 2004).

Dietary protein appears to be the most satiating macronutrient. In many human studies, high protein meals have been shown to increase feelings of satiety and to lead to a decrease in subsequent *ad libitum* energy intake compared to high carbohydrate or high fat meals (Anderson & Moore, 2004; Eisenstein et al., 2002; Halton & Hu, 2004). Preload studies report that subjects had increased feelings of satiety and reduced energy intake following the ingestion of protein compared to carbohydrate (Latner & Schwartz, 1999; Poppitt et al., 1998; Porrini et al., 1995). A few preload studies have found suppression of food intake at a subsequent meal but no change in feelings of satiety after a high protein meal (Barkeling et al., 1990; Johnson & Vickers, 1993). However, some preload studies report that protein did not affect food intake or subjective responses to satiety at all relative to carbohydrate (De Graaf et al., 1992; Geliebter, 1979; Raben et al., 2003; Vozzo et al., 2003).

Some methodological issues that may explain the inconsistent findings on the satiating effects of protein are preload volume and composition, protein source and the time interval between preload and test meal (Almiron-Roig, Chen, & Drewnowski, 2003; Anderson et al., 2004; Blundell et al., 2010; Reid & Hetherington, 1997). Previous studies on satiety in humans have generally used only one time delay interval between preload and subsequent meal consumption. A wide variation in time intervals has been employed across experiments determined by differences in appetite or plasma amino acid profiles (Luhovyy et al., 2007) or plasma hormones (Veldhorst et al., 2009). The suppressive effect of carbohydrate on subsequent food intake is thought to be within 60 min after ingestion, whereas protein appears more suppressive of food intake after longer time intervals (Fischer et al., 2004). However, two recent studies found that subsequent food intake was lower after a whey protein preload relative to a carbohydrate preload regardless of time interval (Bellissimo et al., 2007; Bertenshaw et al., 2008).

In an earlier study, we applied a time interval of 30 min but failed to observe a consistent effect of whey protein versus maltodextrin on subsequent food intake and several subjective measures related to satiety, though there was

some evidence for a satiating effect of a whey protein containing glycomacropeptide (GMP) (*Chung Chun Lam et al., 2009, see Chapter 2*). In the same study, we found that women were more sensitive to the whey protein preload intervention than men. It is unclear whether there is a critical time delay interval at which whey protein is more satiating than other macronutrients.

The objective of this study was to investigate the effects of different time delay intervals between preload and test meal on measures of satiety in healthy normal weight women. A secondary aim was to evaluate whether whey protein (containing GMP) is more satiating than maltodextrin. GMP may be an important factor for the satiating effect of whey protein (*Luhovyy et al., 2007*). The satiety responses (subsequent *ad libitum* food intake and subjective ratings of appetite) to maltodextrin-enriched, whey protein-enriched and water preloads administered at 30, 60 and 120 min prior to an *ad libitum* test meal were compared.

### **3.3. Subjects and methods**

#### **3.3.1. Subjects**

Healthy normal weight women aged 18–40 years and having a body mass index ranging between 19 and 25 kg/m<sup>2</sup> were recruited by public advertisement. All individuals were required to complete a health checklist to assess their suitability for the study. Inclusion criteria were: stable body weight, non-smoker, no known food allergies, no known current medical conditions affecting gastrointestinal motility or appetite, not pregnant or lactating, everyday breakfast consumers. Potential subjects were invited to attend an information session. Their height and weight were measured to allow calculation of their body mass index. Subjects completed the Three Factor Eating Questionnaire to assess their dietary restraint, disinhibition and hunger (*Stunkard & Messick, 1985*) and potential participants who were restrained (a

score of >11 out of 21 questions) were excluded (*Lowe & Thomas, 2009*). In addition, participants tasted each preload drink and test meal prior to the experiment. Subjects that rated the preloads and test meal less than 50 for taste on a 100 linear scale were excluded. Subjects were asked to fill in a 2-day breakfast food diary which helped ascertain that the amount of breakfast given during the study was appropriate for them. Eligible volunteers provided written informed consent to participate. The study was approved by the Massey University Human Ethics Committee (Application No. 08/38), New Zealand.

### **3.3.2. Preload drinks and test meal**

A water control (350 ml, no energy) was used at each time delay interval. The carbohydrate and protein preload drinks (300 ml, 1.3 MJ) comprised a basal mixture of commercial milk protein powder (brand Alfalite®, Alfa Foods Distributors, Auckland, New Zealand) dissolved in water with added sucrose (Kerry New Zealand Ltd., Auckland, New Zealand), a strawberry flavour compound (Formula Foods Corporation Ltd., Christchurch, New Zealand), a red colourant (Sensient Technologies, Auckland, New Zealand) and were enriched with either maltodextrin carbohydrate (Fieldose™ 10GV, Penford New Zealand Ltd., Auckland, New Zealand) or whey protein isolate (WPI 894 containing 21% glycomacropeptide, Fonterra Ltd., Palmerston North, New Zealand). In addition, water (50 ml) was imbibed with the carbohydrate or protein preload drinks to minimise the aftertaste of the preloads and thus all preloads were isovolumetric (350 ml). All beverage stocks were made at least one day prior to the testing sessions and stored in a refrigerator. Beverages were stirred thoroughly prior to serving. The preload drinks and a test meal were analysed for crude protein, crude fat and available carbohydrate using methods described by Chung Chun Lam *et al.* (2009) (*see Chapter 2*). Total dietary fibre was analysed in the test meal only. The ingredient, macronutrient and energy contents of the carbohydrate and protein preload drinks are given in Table 3.1.

**Table 3.1.** Composition of the preload drinks.

	<b>Preload Drink</b>	
	<b>Carbohydrate</b>	<b>Protein</b>
<b>Ingredient (g per 300ml drink)</b>		
Milk powder	22.35	22.00
Sucrose	10.77	10.60
Flavour	0.54	0.53
Colour	0.08	0.08
Maltodextrin	44.88	0
Whey Protein Isolate	0	44.19
Water	242.38	238.60
Weight/per 300ml	321	316
<b>Nutrient (per 300 ml serve)</b>		
Energy <sup>1</sup> (kJ)	1370.4	1333.0
Fat		
(g)	3.2	3.4
(% of energy)	8.9	9.6
Carbohydrate		
(g)	71.7	26.4
(% of energy)	87.3	33.1
Protein		
(g)	3.1	45.7
(% of energy)	3.8	57.3

<sup>1</sup> Metabolisable energy calculated from the energy-producing food components using the energy conversion factors of 16.7 kJ/g for protein and available carbohydrate and 37.7 kJ/g for fat

The lunch test meal that subjects consumed *ad libitum* and served warm was a single item meal (fried rice) consisting of rice, chicken, eggs, peas, corn, carrots, spring onions, chicken stock, salt, sugar and vegetable oil. The test meal contained 28.4 g of carbohydrate, 6.6 g of protein, 6.4 g of fat and 1 g of fibre per 100 g (as served) and was calculated to have a metabolisable energy content of 825 kJ per 100 g (as served). The fried rice was prepared in one batch every week, refrigerated until required, and reheated according to a standard protocol. Bottled spring water (750 ml) was also provided along with the test meal.

### **3.3.3. Experimental procedure**

A single-blind completely randomized factorial design with subjects as “blocks” was used. Women participated during the menses and follicular phase of their menstrual cycle to avoid metabolic and appetite shifts that may occur during ovulation and the luteal phase (*Buffenstein et al., 1995; Dye & Blundell, 1997*). Each test day was separated from the next by a minimum of one full day within the given 2 weeks. Each participant attended nine test session days in total and received each of the three preload drinks (water, carbohydrate and protein) administered at one of the three time delays of 30, 60 and 120 min. Each combination of preload drink and time delay was regarded as a treatment and all nine treatments were randomly allocated to each subject using random numbers.

On the day before a test session, subjects were instructed to refrain from alcohol and to consume only water from 2200 h onwards. On each test day, a standardised breakfast of toasted wholemeal bread with margarine, strawberry jam or peanut butter or marmite and coffee or tea with sugar and milk was provided to be eaten at least 3 h before the session appointment. Each subject consumed her breakfast at home, followed only by water as desired until consumption of the preload drink. Breakfast intake was recorded at each test day.

Subjects arrived at Massey University during their customary lunchtime and attended at the same time on each test day. They remained seated in individual cubicles with minimal disturbance from the investigators throughout the experimental session. Each subject rated her feelings of appetite subjectively using a visual analogue scale (VAS). Upon completion of the questionnaire, each subject received her preload drink to be consumed within 5 min and the time it took to consume the preload drink was recorded. Timing began after complete ingestion of the preload drink. Sensory ratings of the preload drink on VAS and appetite ratings were completed at that time. Subjects continued to rate their appetite using VAS every 15 min until either 30 or 60 min after having consumed the preload drink. For the time delay of 120 min, subsequent appetite ratings were taken at 15, 30, 45, 60, 75, 90 and 120 min after the first rating. At the end of these respective periods, subjects were provided with the test meal which was available to be consumed *ad libitum* within 15 min. Subjects were told to eat as much or as little as they would like. Fifteen and 30 min after the lunch test meal was taken away, subjects filled in VAS questionnaires for appetite. For the remainder of the day, each subject was requested to record her food intake by means of a diary.

#### **3.3.4. Measurements**

The time spent drinking the preloads and consuming the lunch test meal was recorded using a digital timer. The amounts of food and water consumed were determined by the differences in the weights of food or water before and after the lunch meal using an electronic scale (to the nearest 0.01 g). Food intake during the remainder of the day was weighed using kitchen scales and standard household measures. Items recorded in the food diary records were entered into the FoodWorks software (Xyris, Brisbane, Australia) to determine the energy and nutrient contents of the foods. Subjects rated the sensory properties of the carbohydrate and protein preload drinks immediately after consumption, for likeability, pleasantness of taste and likeability of texture. Ratings were made on a 100 mm line (VAS) end-anchored with

“dislike extremely” or “not at all pleasant” and “like extremely” or “extremely pleasant”. Before and after each preload drink and the test meal, subjects completed 100 mm VAS lines for feelings of hunger, desire to eat, fullness, prospective consumption and nausea. Left and right anchors for the appetite ratings were “not at all” or “very weak” or “nothing at all” and “extremely”, “very strong”, “a large amount”, respectively.

### 3.3.5. Statistical analysis

The overall design of the study was a single-blind completely randomized factorial design with subjects as “blocks” (Ott, 1993). According to power analysis based on results from previous studies investigating the effect of time delay interval between preload and test meal on food intake (Bellissimo *et al.*, 2007; Rolls *et al.*, 1991), a sample size of fifteen subjects was estimated to be required to detect differences in *ad libitum* food intake with 80% power. Data were tested for homogeneity of variance and the factors fitted in the model were subject, preload drink (water, carbohydrate or protein), time delay (consumption at 30, 60 or 120 min before the test meal) and the interaction between the latter two factors. Sensory ratings, time to consume the preload drink and test meal, and food intake data were subjected to an analysis of variance (ANOVA). The effect of session days on test meal intake was assessed by using a one-way ANOVA. As the diary records for food intake post the *ad libitum* lunchtime test meal were collected only for the time delay of 120 min, the “remainder of the day” and “total day” intakes were subjected to an ANOVA with only preload drink as a factor.

The ratings for each self-reported measure (hunger, desire to eat, fullness, prospective consumption and nausea) were compared by doing separate analyses for each time delay. For the time delay of 30 min, there were six time points whereas for the time delay of 60 min, there were eight and for the time delay of 120 min, there were 11. Repeated measures ANOVA was employed with preload drink (water, carbohydrate or protein) and time of rating as factors. Further analysis using treatment contrasts was used to

examine the statistical significance of the differences associated with preload drink and time of rating. Total and net incremental area under the curves (AUCs) of changes from 0 to 120 min were calculated by the trapezoidal method for hunger, desire to eat, fullness and prospective consumption. The AUCs for each rating across the eight time points were tested for a preload drink effect using ANOVA.

A *p* value of less than 0.05 was considered to indicate statistical significance. Where a main effect was found to be statistically significant, a Duncan post hoc test was performed for mean comparisons. To analyse the relationship between food intake at the test meal and sensory properties of the drink, Pearson's correlation analysis was applied. The results have been reported as means and standard errors (mean  $\pm$  sem). All statistical analyses were facilitated by the SAS software package (SAS version 9.2, SAS Institute Inc., Cary, NC, USA, 2010).

### **3.4. Results**

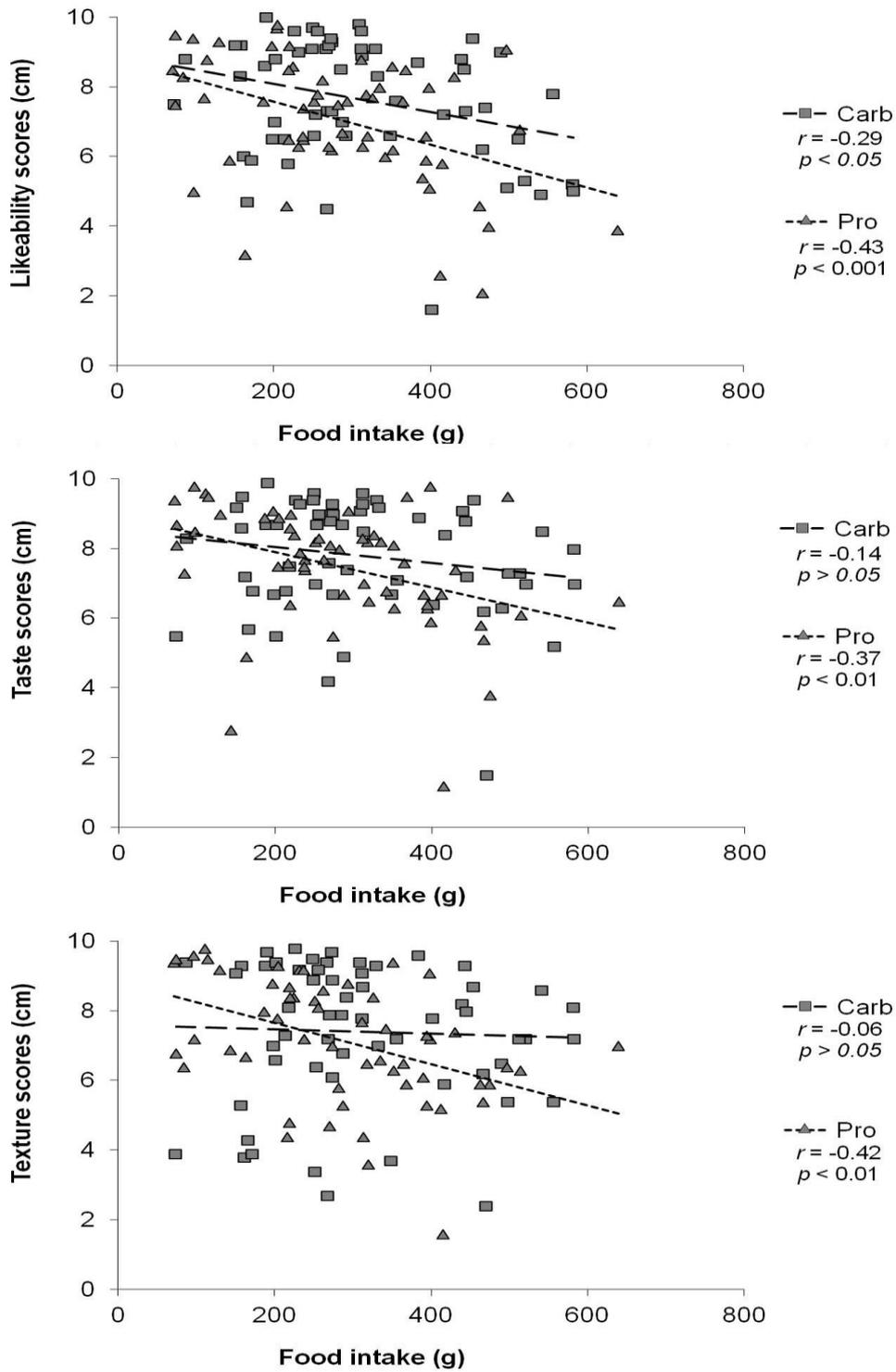
#### **3.4.1. Study subjects**

Twenty-two women were recruited for the study. Two women were excluded as they had BMI's above 25 kg/m<sup>2</sup> and one woman failed to complete the study due to compliance issues. The age, BMI and cognitive dietary restraint scores of the remaining 19 women were (mean  $\pm$  sem) 22.2  $\pm$  0.9 years, 22.9  $\pm$  0.4 kg/m<sup>2</sup> and 8  $\pm$  1, respectively. The *ad libitum* test meal intake for each treatment combination was not significantly different between the nine experimental session days ( $F(8,139) = 0.84$ ,  $p = 0.57$ )

### 3.4.2. Sensory ratings of the preload drinks

There was no significant preload drink x time delay interaction ( $p > 0.05$ ) for overall likeability ( $F(2,90) = 1.18, p = 0.31$ ), pleasantness of taste ( $F(2,90) = 0.34, p = 0.71$ ) and likeability of texture ( $F(2,90) = 2.01, p = 0.14$ ) of the preload drinks. Pleasantness of taste and likeability of texture were not affected by time delay ( $F(2,90) = 0.31, p = 0.73$  and  $F(2,90) = 0.11, p = 0.89$ , respectively) or preload drink ( $F(1,90) = 1.85, p = 0.17$  and  $F(1,90) = 0.94, p = 0.33$ , respectively). Overall likeability was not significantly different between the three time delays ( $F(2,90) = 0.36, p = 0.70$ ) but there was a significant effect of preload drink ( $F(1,90) = 8.17, p = 0.0053$ ) as subjects rated the carbohydrate preload ( $7.6 \pm 0.4$  cm) as being more liked (overall likeability) than the protein preload ( $7.0 \pm 0.4$  cm).

A key question was whether the amount of food consumed at the lunch test meal (g) was affected by the sensory ratings of the preload drinks. Pearson's correlation analyses of food intake with either the carbohydrate or protein preload drink palatability showed that food intake was negatively associated with the likeability of both the carbohydrate ( $r = -0.29, p = 0.0246$ ) and the protein ( $r = -0.43, p = 0.008$ ) preload drinks. Although significant, the Pearson correlation  $r$  values and scatter plots (Figure 3.1.) imply a weak association between the likeability of the preloads and food intake. The rated likeability of the preload drinks, therefore, did not appear to affect subsequent food intake.



**Figure 3.1.** Scatter plots and Pearson correlation between VAS-rated likeability of the carbohydrate (carb, ■) and protein (pro, ▲) preload drinks and amount of food consumed at the lunchtime test meal (g) in nineteen women.

### 3.4.3. Drinking and eating time

Drinking time was neither significantly different between the three time delays ( $F(2,144) = 0.30, p = 0.74$ ) nor between the three preload drinks ( $F(2,144) = 0.73, p = 0.48$ ) and there was also no significant preload drink x time delay interaction ( $F(4,144) = 0.28, p = 0.88$ ). Subjects consumed the test meal within the 15 min time period allowed. Eating time at the ad libitum test meal differed by preload drink ( $F(2,144) = 7.43, p = 0.0009$ ), but not by time delay ( $F(2,144) = 1.76, p = 0.17$ ) and there was no interaction between preload drink and time delay ( $F(4,144) = 0.68, p = 0.60$ ). Subjects spent longer eating the *ad libitum* test meal after consumption of water ( $9.91 \pm 0.69$  min) compared to carbohydrate ( $8.88 \pm 0.78$  min) and protein ( $8.30 \pm 0.81$  min).

### 3.4.4. Water intake

Water intake at the *ad libitum* test meal (Table 3.2.) was affected by time delay ( $F(2,144) = 16.29, p < 0.0001$ ) and preload drink ( $F(2,144) = 5.50, p = 0.005$ ) but there was no significant preload drink x time delay interaction ( $F(4,144) = 1.02, p = 0.40$ ). On the days that the time delay was 120 min, water intake was statistically significantly higher compared to the time delays of 30 and 60 min. Water intake at the test meal was statistically significantly lower when the water preload was consumed compared to the carbohydrate and protein preloads.

### 3.4.5. Ad libitum test meal intake

There was no interaction effect between preload drink and time delay ( $F(4,144) = 0.47, p = 0.75$ ) for the *ad libitum* test meal intake (Table 3.2.). Thus, there was no evidence that food intake following consumption of the preload drinks differed in response to the time interval between preload and test meal (time delay). However, there were significant main effects of both

time delay ( $F(2,144) = 6.95, p = 0.0013$ ) and preload drink ( $F(2,144) = 25.34, p < 0.0001$ ). When the time delay was 30 min, less food was consumed compared to 60 or 120 min. Subjects consumed less food energy at the test meal following the protein preload compared to the carbohydrate and water preloads. The carbohydrate preload led to a reduced food intake compared to water. Macronutrient intakes showed a similar result to that of food intake.

### **3.4.6. Total energy intake**

When the energy intake from the preload drink was added to the energy intake from the test meal, total energy intake (Table 3.2.) was affected by preload drink ( $F(2,144) = 26.72, p < 0.0001$ ) and time delay ( $F(2,144) = 6.95, p = 0.0013$ ), but there was no preload drink x time delay interaction ( $F(4,144) = 0.47, p = 0.75$ ). On the days that the time delay was 30 min, less total energy was consumed relative to 60 and 120 min. Total energy intake was higher for the carbohydrate preload drink compared to water and protein. Total energy consumption was greater following the protein preload compared to water.

**Table 3.2.** Effects of preload drink and time delay on water, test meal and total (preload + test meal) energy intakes in women (Mean;  $n = 19$ ).

	Preload Drink			Time Delay			Pooled SE	<i>p</i> value		
	Water	Carbohydrate	Protein	30 min	60 min	120 min		Preload Drink	Time Delay	Drink x Delay
<b>Water intake (g)</b>	255.0 <sup>b</sup>	292.1 <sup>a</sup>	303.6 <sup>a</sup>	250.2 <sup>b</sup>	260.8 <sup>b</sup>	339.7 <sup>a</sup>	17.1	<0.01	<0.0001	0.40
<b>Test meal energy (kJ)</b>	3134.8 <sup>a</sup>	2584.4 <sup>b</sup>	2342.6 <sup>c</sup>	2450.3 <sup>b</sup>	2750.2 <sup>a</sup>	2861.3 <sup>a</sup>	114.1	<0.0001	<0.01	0.75
<b>Total energy (kJ)</b>	3134.8 <sup>c</sup>	3954.7 <sup>a</sup>	3675.6 <sup>b</sup>	3351.4 <sup>b</sup>	3651.4 <sup>a</sup>	3762.4 <sup>a</sup>	114.1	<0.0001	<0.01	0.75

Overall means in a row with different superscript letters were significantly different ( $p < 0.05$ ).

### **3.4.7. Reported food intake for the remainder of the day and full 24h intake**

There were no differences among preload drinks for the reported energy ( $F(2,36) = 0.32, p = 0.72$ ), carbohydrate ( $F(2,36) = 0.02, p = 0.97$ ), protein ( $F(2,36) = 1.06, p = 0.35$ ), and fat ( $F(2,36) = 1.18, p = 0.32$ ) intakes for the remainder of the day (Table 3.3.). Although energy ( $F(2,36) = 0.78, p = 0.46$ ) and fat ( $F(2,36) = 0.52, p = 0.59$ ) intakes for the full 24 h measurement period did not differ by preload drink, carbohydrate and protein intakes varied ( $F(2,36) = 8.23, p = 0.0011$  and  $F(2,36) = 71.99, p < 0.0001$ , respectively). Carbohydrate intake over the whole test day was higher when the carbohydrate preload was given compared to water and protein. Similarly, for subjects receiving the protein preload, protein intake for the 24 h period was greater compared to water and carbohydrate.

**Table 3.3.** Effect of preload drink on food energy and nutrient intakes for the “remainder of the day” and “over the whole of the day” (breakfast + preload + test meal + remainder of the day; 24 hour) at the time delay of 120 minutes (Mean;  $n = 19$ ).

	Preload Drink			Pooled SE	<i>p</i> value
	Water	Carbohydrate	Protein		
<b>Remainder of the day (reported food intake)</b>					
Energy (kJ)	3650.7 <sup>1</sup>	3338.7 <sup>1</sup>	3274.1	289.3	0.73
Carbohydrate (g)	86.2	86.5	89.1	8.7	0.98
Protein (g)	31.7	37.3	33.5	2.3	0.36
Fat (g)	25.1	29.3	31.4	2.4	0.32
<b>Full 24 hour</b>					
Energy (kJ)	3650.7 <sup>1</sup>	3338.7 <sup>1</sup>	3274.1	306.1	0.47
Carbohydrate (g)	86.2	86.5	89.1	9.3	<0.01
Protein (g)	31.7	37.3	33.5	2.1	<0.0001
Fat (g)	25.1	29.3	31.4	2.3	0.60

Overall means in a row with different superscript letters were significantly different ( $p < 0.05$ ).

<sup>1</sup> On the days that subjects consumed the water and carbohydrate preload drinks, they reported alcohol consumption (average alcohol intake  $116.0 \pm 32.5$  g).

### 3.4.8. Subjective ratings of appetite

The VAS ratings for hunger, desire to eat, fullness and prospective consumption were analysed separately at each time delay. Results were similar for each time delay and therefore, only the data for the time delay of 120 min are shown (Figure 3.1.). As expected, hunger, desire to eat, fullness and prospective consumption were affected by time (hunger:  $F(10,530) = 100.04$ ,  $p < 0.0001$ ; desire to eat:  $F(10,530) = 107.62$ ,  $p < 0.0001$ ; fullness:  $F(10,520) = 153.70$ ,  $p < 0.0001$ ; prospective consumption:  $F(10,530) = 120.96$ ,  $p < 0.0001$ ). After consumption of the carbohydrate and protein preload drinks, hunger, desire to eat and prospective consumption decreased and then increased until the test meal, whereas fullness followed the inverse pattern.

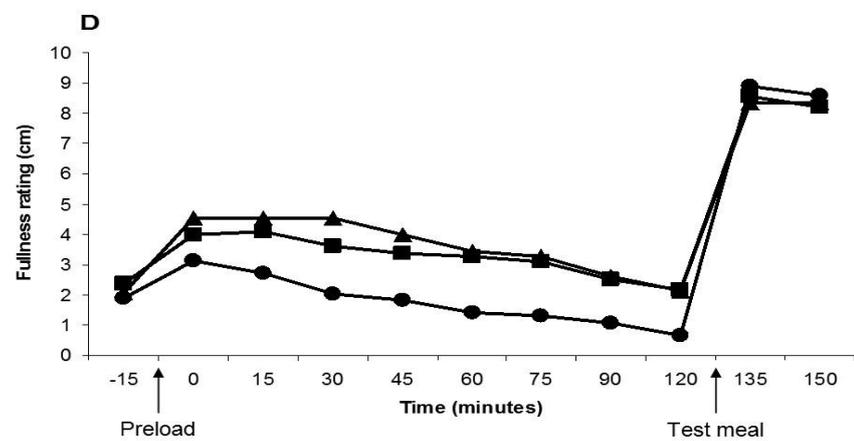
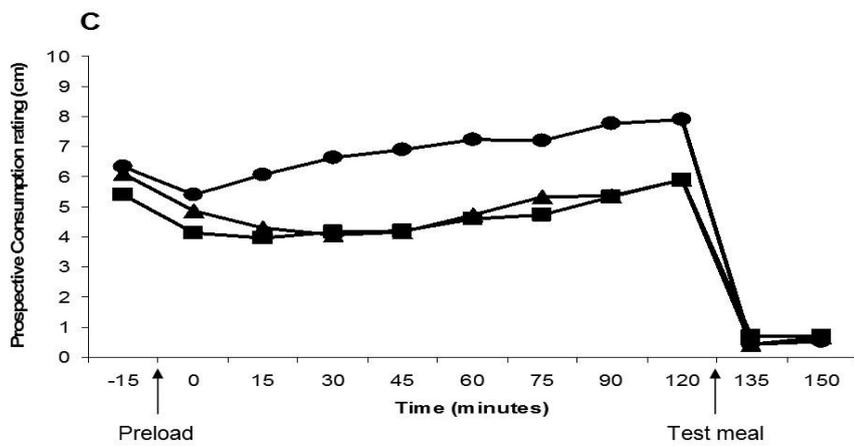
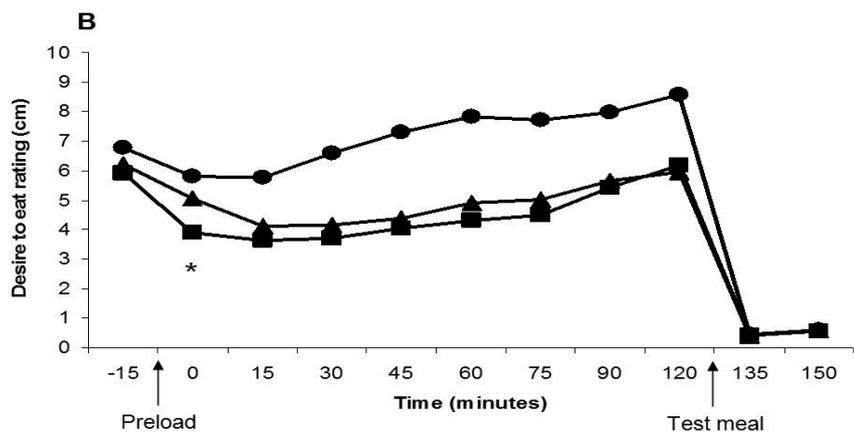
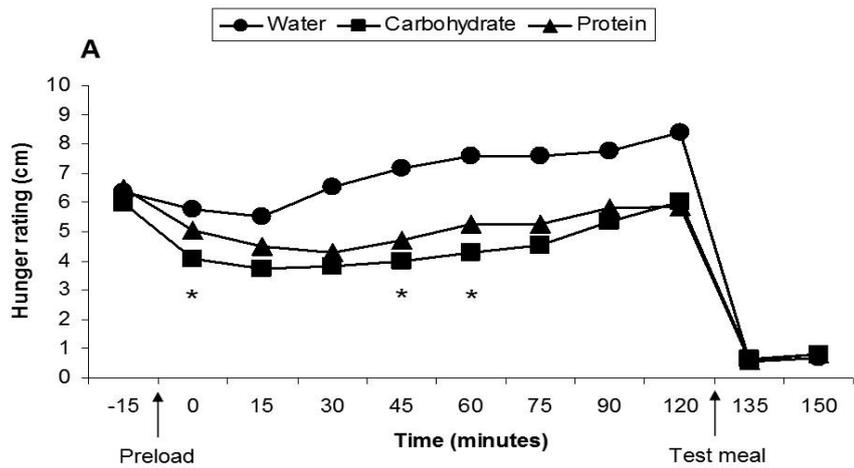
There was a significant interaction effect between preload drink and time of rating for all the measures (hunger:  $F(20,530) = 4.01$ ,  $p < 0.0001$ ; desire to eat:  $F(20,530) = 3.83$ ,  $p < 0.0001$ ; prospective consumption:  $F(20,530) = 4.13$ ,  $p < 0.0001$ ; fullness:  $F(20,520) = 2.33$ ,  $p = 0.001$ ). Hunger (Figure 3.1A), desire to eat (Figure 3.1B) and prospective consumption (Figure 3.1C) levels were higher after consumption of the water preload than after the carbohydrate and protein preloads ( $p < 0.05$ ), except for time point 0 min for all the measures and 15 min for hunger. Hunger, desire to eat and prospective consumption were higher immediately (0 min) after consumption of the water preload than the carbohydrate preload ( $p < 0.01$ ), but there was no difference between the water and protein preloads ( $p > 0.05$ ). At 15 min, carbohydrate led to decreased hunger more so than water ( $p < 0.01$ ). When subjects consumed the protein preload, they felt fuller (Figure 3.1D) initially (0 min) compared to when they had the water preload ( $p < 0.05$ ). Compared with water, fullness ratings were higher after the carbohydrate and protein preloads from 15 min until the test meal ( $p < 0.05$ ). The effect of preload drink on appetite ratings seems to occur within 15 min after consumption of the preload drinks. Differences between the carbohydrate and protein preloads emerged immediately after consumption of the preload drinks (0 min) for hunger ( $p =$

0.0422) and desire to eat ( $p = 0.0157$ ) ratings as well as at 45 ( $p = 0.0457$ ) and 60 min ( $p = 0.0335$ ) for hunger ratings.

There was a significant main effect of preload drink on area under the curves (AUCs) from 0 to 120 min for hunger ( $F(2,36) = 13.73, p < 0.0001$ ), desire to eat ( $F(2,36) = 14.69, p < 0.0001$ ), prospective consumption ( $F(2,36) = 16.70, p < 0.0001$ ) and fullness ( $F(2,36) = 8.84, p = 0.0008$ ). The AUC for hunger, desire to eat and prospective consumption were higher following consumption of water compared to both carbohydrate and protein, whereas the AUC for fullness was lower (Table 3.4.). The AUC responses of the carbohydrate and protein preloads for all the measures did not differ ( $p > 0.05$ ).

#### **3.4.9. Nausea**

A potential complication in the interpretation of effects of treatment on test meal intake would occur if preloads altered feelings of well-being post-ingestion. This was tested by analysing rated nausea separately within each time delay. Generally there were no differences in rated nausea at each time delay. The only exception was at the time delay of 60 min where nausea ratings were affected significantly by time ( $F(7,420) = 2.95, p = 0.005$ ).



**Figure 3.2.** Mean VAS ratings for subjects receiving the water (●), carbohydrate (■) and protein (▲) preloads at the time delay of 120 minutes.

There was a significant preload-by-time interaction (all  $p < 0.0001$ , except for fullness  $p < 0.001$ ). Hunger, desire to eat and prospective consumption scores were significantly higher ( $p < 0.05$ ) while fullness scores were significantly lower ( $p < 0.05$ ) following ingestion of the water preload compared with carbohydrate and protein at most time points. \* Mean value was significantly different between carbohydrate and protein preloads ( $p < 0.05$ ).

**Table 3.4.** Effect of preload drink on area under the curves (AUCs) for subjective ratings of appetite at the time delay of 120 minutes (Mean;  $n = 19$ ).

	Preload Drink			Pooled SE	<i>p</i> value
	Water	Carbohydrate	Protein		
<b>Total AUC (cm,120min)</b>					
Hunger	851.6 <sup>a</sup>	547.6 <sup>b</sup>	606.9 <sup>b</sup>	41.8	<0.0001
Desire to eat	872.5 <sup>a</sup>	543.6 <sup>b</sup>	587.8 <sup>b</sup>	45.1	<0.0001
Prospective consumption	839.3 <sup>a</sup>	548.5 <sup>b</sup>	573.8 <sup>b</sup>	38.0	<0.0001
Fullness	199.4 <sup>b</sup>	380.8 <sup>a</sup>	412.0 <sup>a</sup>	37.1	<0.0001
<b>Net incremental AUC (cm,120min)</b>					
Hunger	99.4 <sup>a</sup>	-192.6 <sup>b</sup>	-159.9 <sup>b</sup>	45.5	<0.0001
Desire to eat	77.4 <sup>a</sup>	-187.1 <sup>b</sup>	-141.8 <sup>b</sup>	42.3	0.0002
Prospective consumption	94.0 <sup>a</sup>	-171.2 <sup>b</sup>	-139.8 <sup>b</sup>	40.9	0.0004
Fullness	-30.5 <sup>b</sup>	97.0 <sup>a</sup>	162.4 <sup>a</sup>	36.0	<0.0001

Overall means in a row with different superscript letters were significantly different ( $p < 0.05$ ).

### 3.5. Discussion

Our primary aim was to determine whether the effects of different preload drinks on measures of satiety were affected by the time interval between the preload and an *ad libitum* test meal. We investigated the effects of three different preload drinks (water, carbohydrate-enriched and protein-enriched) administered at three different time delays (30, 60 and 120 min) on satiety. The results showed that there was no interaction between preload drink and time delay and thus, the time delay between preload and test meal had no influence on the effect of preload type on food intake at the subsequent test meal. The time interval between preload and test meal does not appear to be a major factor in determining the response of different preload drinks on subsequent food intake and this failed to explain inconsistencies in published findings of the effect of whey protein preloads on satiety.

#### 3.5.1. Energy intake

In the presently described study, it is of note that although there was no interaction between preload drink and time delay, there was a main effect of preload drink. The differences in *ad libitum* test meal intake between carbohydrate-enriched and protein-enriched preload drinks increased from 30 to 60 to 120 min (Table 3.5.), which would indicate that a time delay of 120 min would provide a more sensitive test for satiety than a time delay of 30 min. The time delay intervals of 60 and 120 min were chosen as the effect of carbohydrate on satiety and energy compensation has been reported to be short-lived (within 60 min and less than 120 min) (*Almiron-Roig et al., 2004; Fischer et al., 2004; Rolls et al., 1991*). By comparing the significant effect of preloads on subsequent food intake, we found that drinks enriched with maltodextrin carbohydrate and whey protein, when given in equal volumes and isocalorically (~1300 kJ) at various times before a subsequent test meal offered *ad libitum*, reduced food intake at the test meal more than water. The results

showed that the whey protein-enriched preload reduced energy intake at the lunchtime test meal by approximately 258 kJ more than carbohydrate and 826 kJ more than water overall. These findings are in line with previous studies that reported that protein is more satiating than carbohydrate (*Bellissimo et al., 2007; Bertenshaw et al., 2008; Latner & Schwartz, 1999; Porrini et al., 1995*). The effect of the whey protein on subsequent food intake may be attributed to the presence of GMP (20%) as GMP has been shown to stimulate the release of the satiety-related gut hormone cholecystokinin (CCK) (*Beucher et al., 1994; Pedersen et al., 2000*). The reduction in energy intake at the test meal, however, did not compensate for the energy consumed in the preload (1.3 MJ). Thus, total energy intake (energy in preload + test meal intake) was greater for subjects receiving the two caloric preloads than for subjects receiving the water preload, and greater following consumption of carbohydrate compared to protein. Although there was evidence for a satiating effect of the whey protein, the energy consumed in the preload to elicit this effect meant that total energy intake was higher compared to the water control. Furthermore, when energy intake for the full 24 h period (breakfast + preload + test meal + the remainder of the day) was compared, the cumulative intake of the total day did not differ between the preloads.

**Table 3.5.** Mean test meal energy intake (kJ) following consumption of three different preload drinks and for time delays of 30, 60, and 120 minutes in ninety women.

<b>Time Delay (min)</b>	<b>Preload Drink</b>			<i>p</i> value		
	Water	Carbohydrate	Protein	Preload Drink	Time Delay	Drink x Delay
<b>30</b>	2852.7	2289.0	2209.0	<0.0001	<0.01	0.75
<b>60</b>	3251.6	2611.4	2387.7			
<b>120</b>	3300.1	2852.6	2431.0			

### 3.5.2. Study design

There are some aspects of the present study that require discussion. In common with other satiety studies, as it is difficult to provide pure macronutrients due to palatability issues, we chose a sweet milky vehicle for our experimental preload drinks. Therefore, our carbohydrate preload drink contained predominantly maltodextrin but also some protein and our protein preload drink contained predominantly whey protein isolate but also carbohydrate. The results may potentially have been confounded by the mixed composition of the vehicle but our aim was to compare the effect of a whey protein-enriched preload versus a maltodextrin carbohydrate-enriched preload. Sugars and various sweeteners may affect satiety measures (*Anderson, 1995; Anderson & Woodend, 2003*). Therefore, we opted to use maltodextrin with a dextrose equivalent of 10. Maltodextrin is a simple carbohydrate derived from corn starch and has a low sweetness. Another methodological issue that may be important in studies of satiety but which is not considered to have greatly affected the outcome of the presently described study is the form (liquid versus solid) of the preload. There is some evidence that liquid beverages may elicit weaker satiating and compensatory responses than solid foods (*Almiron-Roig et al., 2003; Mattes & Campbell, 2009; Mourao et al., 2007*). However, a recent study found that the form of delivery of whey protein (sweet whey protein containing 15% GMP and acid whey protein devoid of GMP) did not influence food intake one hour later (*Akhavan et al., 2011*). Although a beverage was used here, there was still clear evidence for a satiating effect of whey protein.

In our earlier work (*Chung Chun Lam et al., 2009, see Chapter 2*), some limited evidence was obtained for an effect of whey protein on satiety in adult humans, but the result was not as definitive as in the present study. Several experimental factors that differed between the two studies may explain the different results. In our previous research, we found that women were better able to discriminate between different whey protein types than men and were

thus recruited in the current study. The quantity of crude protein in the carbohydrate preload of the present study was 3 g compared to 10 g in the previous experiment. Although the protein content of the protein preloads in both studies was around 45 g, the source was mostly from whey protein isolate in the current study. Women received their preloads and meals isolated in individual cubicles in the present study. Participants were not subjected to each other's eating behaviour, as was the case of the social setting of the previous study. In the present experiment, women were presented with a single homogeneous test meal, which may be a more sensitive method to study the effect of protein on satiety than an array of food items (*Long et al., 2000*). Normal-weight adult individuals were chosen to participate in the present study as they exhibit similar eating behaviour and circulating satiety-related hormonal responses compared to overweight and obese participants (*Hellstrom et al., 2004; Mela, 2001*).

### **3.5.3. Conclusion**

In conclusion, the feeding response to water and carbohydrate- and protein-enriched preloads was not influenced by the time interval between preload and test meal. The effects of a 45 g dose of carbohydrate or protein did not differ with the 30, 60 and 120 min employed as time delay intervals. The whey protein (containing GMP) preload was more satiating compared to carbohydrate, in that it led to the greatest suppression of food intake at a subsequent *ad libitum* test meal in normal weight women, independent of the time delay. However, carbohydrate and protein, although both being more satiating than water, failed to induce a lower overall consumption of energy over the total period of study (preload + test meal) and over the whole day. Subjects rated themselves as feeling more satiated following consumption of the energy-containing drinks relative to water but there were only minor differences in appetitive feelings between carbohydrate and protein. This work needs to be extended to a wider range of time delays and protein and carbohydrate sources.

### 3.6. Literature cited

- Akhavan, T., Luhovyy, B. L., & Anderson, G. H. (2011). Effect of drinking compared with eating sugars or whey protein on short-term appetite and food intake. *Int J Obes*, *35*(4), 562–569.
- Almiron-Roig, E., Chen, Y., & Drewnowski, A. (2003). Liquid calories and the failure of satiety: how good is the evidence? *Obes Rev*, *4*(4), 201–212.
- Almiron-Roig, E., Flores, S. Y., & Drewnowski, A. (2004). No difference in satiety or in subsequent energy intakes between a beverage and a solid food. *Physiol Behav*, *82*(4), 671–677.
- Anderson, G. H. (1995). Sugars, sweetness, and food intake. *Am J Clin Nutr*, *62*(1 Suppl), 195S–201S.
- Anderson, G. H., & Moore, S. E. (2004). Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr*, *134*(4), 974S–979S.
- Anderson, G. H., & Woodend, D. (2003). Consumption of sugars and the regulation of short-term satiety and food intake. *Am J Clin Nutr*, *78*(4), 843S–849S.
- Anderson, G. H., Tecimer, S. N., Shah, D., & Zafar, T. A. (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.
- Barkeling, B., Rössner, S., & Björvell, 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.
- Bellissimo, N., Desantadina, M. V., Pencharz, P. B., Berall, G. B., Thomas, S. G., & Anderson, G. H. (2007). A comparison of short-term appetite and energy intakes in normal weight and obese boys following glucose and whey-protein drinks. *Int J Obes*, *32*(2), 362–371.

- Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiol Behav*, *93*(3), 427–436.
- Beucher, S., Levenez, F., Yvon, M., & Corring, T. (1994). Effects of gastric digestive products from casein on CCK release by intestinal cells in rat. *J Nutr Biochem*, *5*(12), 578–584.
- Bjorntorp, P. (2001). *International textbook of obesity*. New York: Wiley.
- Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., van der Knaap, H., & Westerterp, M. (2010). Appetite control: methodological aspects of the evaluation of foods. *Obes Rev*, *11*(3), 251–270.
- Buffenstein, R., Poppitt, S. D., McDevitt, R. M., & Prentice, A. M. (1995). Food intake and the menstrual cycle: a retrospective analysis, with implications for appetite research. *Physiol Behav*, *58*(6), 1067–1077.
- Chung Chun Lam, S. M. S., Moughan, P. J., Awati, A., & Morton, H. R. (2009). The influence of whey protein and glycomacropeptide on satiety in adult humans. *Physiol Behav*, *96*(1), 162–168. (see Chapter 3)
- De Graaf, C., Hulshof, T., Weststrate, J. A., & Jas, P. (1992). Short-term effects of different amounts of protein, fats, and carbohydrates on satiety. *Am J Clin Nutr*, *55*(1), 33–38.
- Dye, L., & Blundell, J. E. (1997). Menstrual cycle and appetite control : implications for weight management. *Hum Reprod*, *12*(6), 1142–1151.
- Eisenstein, J., Roberts, S. B., Dallal, G., & Saltzman, E. (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), 189–200.
- Fischer, K., Colombani, P. C., & Wenk, C. (2004). Metabolic and cognitive coefficients in the development of hunger sensations after pure macronutrient ingestion in the morning. *Appetite*, *42*(1), 49–61.
- Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord*, *24*(1), 38–48.

- Geliebter, A. A. (1979). Effects of equicaloric loads of protein, fat, and carbohydrate on food intake in the rat and man. *Physiol Behav*, 22(2), 267–273.
- Halton, T. L., & 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.
- Hellström, P. M., Geliebter, A., Näslund, E., Schmidt, P. T., Yahav, E. K., Hashim, S. A., & Yeomans, M. R. (2004). Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. *Br J Nutr*, 92(Suppl 1), S47–S57.
- Johnson, J., & Vickers, Z. (1993). Effect of flavor and macronutrient composition of food servings on liking, hunger and subsequent intake. *Appetite*, 21(1), 25–39.
- Latner, J. D., & Schwartz, M. (1999). The effects of a high-carbohydrate, high-protein or balanced lunch upon later food intake and hunger ratings. *Appetite*, 33(1), 119–128.
- Long, S. J., Griffiths, E. M., Rogers, P. J., & Morgan, L. M. (2000). *Ad libitum* food intake as a measure of satiety: comparison of a single food test meal and a mixed food buffet. *Proc Nutr Soc*, 59, A7.
- Lowe, M. R., & Thomas, J. G. (2009). Measures of restrained eating. In : D. B. Allison, & M. L. Baskin (Eds.), *Handbook of assessment methods for eating behaviors and weight-related problems: measures, theory, and research* (pp. 137–185). CA, Sage: Thousand Oaks.
- Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr*, 26(6), S704–S712.
- Mattes, R. D., & Campbell, W. W. (2009). Effects of food form and timing of ingestion on appetite and energy intake in lean young adults and in young adults with obesity. *J Am Diet Assoc*, 109(3), 430–437.
- Matzinger, D., Degen, L., Fronterotta, D., & Beglinger, C. (2000). Effect of gastric distension on food intake and satiety in healthy human subjects. *Gastroenterology*, 118(4), A1071.
- Mela, D. J. (2001). Determinants of food choice: relationships with obesity and weight control. *Obes Res*, 9(Suppl 4), 249S–255S.

- Mourao, D. M., Bressan, J., Campbell, W. W., & Mattes, R. D. (2007). Effects of food form on appetite and energy intake in lean and obese young adults. *Int J Obes*, *31*(11), 1688–1695.
- Ott, L. (1993). *An introduction to statistical methods and data analysis*. 4th ed. Belmont, CA: Duxbury Press.
- Pedersen, N. L., Nagain-Domaine, C., Mahe, S., Chariot, J., Roze, C., & Tome, D. (2000). Caseinomacropptide specifically stimulates exocrine pancreatic secretion in the anesthetized rat. *Peptides*, *21*(10), 1527–1535.
- Poppitt, S. D., McCormack, D., & Buffenstein, R. (1998). Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiol Behav*, *64*(3), 279–285.
- Porrini, M., Crovetti, R., Testolin, G., & Silva, S. (1995). Evaluation of satiety sensations and food intake after different preloads. *Appetite*, *25*(1), 17–30.
- Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J. J., & Astrup, A. (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.
- Read, N., French, S., & Cunningham, K. (1994). The role of the gut in regulating food intake in man. *Nutr Rev*, *52*(1), 1–10.
- Reid, M., & Hetherington, M. (1997). Relative effects of carbohydrates and protein on satiety – a review of methodology. *Neurosci Biobehav Rev*, *21*(3), 295–308.
- Rolls, B. J., Kim, S., McNelis, A. L., Fischman, M. W., Foltin, R. W., & Moran, T. H. (1991). Time course of effects of preloads high in fat or carbohydrate on food intake and hunger ratings in humans. *Am J Physiol*, *260*(4 Pt 2), 756–763.
- Stubbs, R. J., Johnstone, A. M., O'Reilly, L. M., & Poppitt, S. D. (1998). Methodological issues relating to the measurement of food, energy and nutrient intake in human laboratory-based studies. *Proc Nutr Soc*, *57*(3), 357–372.

- Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*, 29(1), 71–83.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009b). Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety. *Appetite*, 52(2), 388–395.
- Vozzo, R., Wittert, G., Cocchiaro, C., Tan, W. C., Mudge, J., Fraser, R., & Chapman, I. (2003). Similar effects of foods high in protein, carbohydrate and fat on subsequent spontaneous food intake in healthy individuals. *Appetite*, 40(2), 101–107.
- Westerterp-Plantenga, M. S., Lejeune, M. P., Nijs, I., van Ooijen, M., & Kovacs, E. M. (2004). High protein intake sustains weight maintenance after body weight loss in humans. *Int J Obes Relat Metab Disord*, 28(1), 57–64.

## Chapter 4

### **Dietary whey protein influences plasma satiety-related hormones and plasma amino acids in adult women**

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A preload enriched with whey protein isolate (WPI) containing naturally present glycomacropeptide (GMP) was shown to suppress subsequent food intake compared with a maltodextrin carbohydrate-enriched preload in adult women (*Chapter 3*). The objective here was to compare changes in the response of blood parameters after consumption of the whey protein-enriched and maltodextrin carbohydrate-enriched preload drinks. A number of gastrointestinal hormones have been implicated in satiety, in particular cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide tyrosine-tyrosine (PYY). The response of plasma products of protein catabolism (amino acid, urea and ammonia) was also studied to establish a possible role in the satiating effect of whey protein.

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## 4.1. Abstract

Dietary protein has been shown to increase satiety compared to carbohydrate and fat in adult humans and a suppressive effect of whey protein on subsequent food intake in comparison with maltodextrin carbohydrate has been shown, but the mechanisms underlying this effect are unclear. The objective was to investigate how the ingestion of whey protein (including glycomacropeptide) affected postprandial plasma amino acid concentrations and several satiety-related gastrointestinal hormones. Eighteen normal weight women were studied in a single blind, randomised block design. Blood samples were taken at various time intervals for 120 min after consumption of either a carbohydrate (maltodextrin) enriched- or a protein (whey protein including glycomacropeptide) enriched-test drink matched for energy (approximately 1300 kJ) and volume (300 ml). Plasma active ghrelin concentrations decreased after both the carbohydrate- and protein-enriched test drinks ( $p<0.05$ ). The protein-enriched beverage led to increased plasma cholecystokinin (CCK) at 60 and 75 min ( $p<0.05$ ), glucagon-like peptide-1 (GLP-1) at 90 min ( $p<0.001$ ) and peptide tyrosine-tyrosine (PYY) at 90 and 120 min ( $p<0.01$ ) and pancreatic polypeptide (PP) from 15 to 120 min ( $p<0.05$ ) compared with maltodextrin. Total amino acid, urea and ammonia plasma concentrations were also higher after protein compared to maltodextrin ingestion ( $p<0.01$ ). Increased plasma concentrations of certain satiety-related gastrointestinal hormones, particularly the peptide hormone PP, as well as higher plasma concentrations of the amino acids and metabolites of amino acid catabolism are potential candidates, acting either singly or together, for mediating the observed satiety response to whey protein.

## 4.2. Introduction

The prevalence of obesity and associated health disorders has increased rapidly in recent years (*WHO, 2012*). Dietary manipulations that

enhance satiety are promising as they improve compliance with energy restricted diets and aid in maintaining a lower body weight after weight loss (*Westerberp-Plantenga et al., 2004*). It is widely believed that protein is the most satiating macronutrient (*Anderson et al., 2004; Eisenstein et al., 2002; Halton & Hu, 2004*) and different types of protein may exert different effects on satiety. There is some evidence that dairy whey protein elicits a stronger effect on satiety compared to carbohydrate and other protein sources (*Anderson et al., 2004; Hall et al., 2003; Luhovyy et al., 2007; Paddon-Jones et al., 2008; Veldhorst et al., 2008*). In a previously reported study with healthy adult women subjects conducted by our group (*Chungchunlam et al., 2012, see Chapter 3*), we found an effect of a whey protein (containing naturally present glycomacropeptide, GMP)-enriched preload drink on subsequent *ad libitum* food energy intake compared with a maltodextrin carbohydrate-enriched test drink. For a preload delay of 120 min, blood samples were collected sequentially and analysed for several hormones and metabolites. The plasma amino acid and hormone responses are the subject of this report.

Several systems appear to be involved in the regulation of food intake and satiety, including signals from the central nervous system, gastrointestinal tract, adipose tissue and a complex interacting system of both orexigenic (appetite stimulating) and anorexigenic (appetite suppressing) hormones (*Stanley et al., 2005; Strader & Woods, 2005*). Ghrelin is a peptide synthesised predominantly in the stomach (*Ariyasu et al., 2001*) and appears to have a role in the initiation of feeding (*Cummings et al., 2001; Cummings et al., 2004*). Cholecystokinin (CCK) is secreted from the endocrine I cells of the small intestine in response to dietary fat or protein and is involved in meal termination (*Degen et al., 2001; Rehfeld, 2004*). Other hormones that may be involved in the control of satiety are the incretin peptides that stimulate glucose-dependent insulin secretion. Following nutrient ingestion, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) are released from the intestinal L- and K-cells, respectively (*Baggio & Drucker, 2007; Elliott et al., 1993*). In contrast to GIP (*Meier et al., 2004*), GLP-1 has been found to promote satiety by interacting with its receptors in the brain and slowing gastric emptying (*Baggio & Drucker, 2007; Nauck et al.,*

1997; Stanley *et al.*, 2004). Members of the pancreatic polypeptide-fold family that are of interest in the regulation of satiety are pancreatic polypeptide (PP) and peptide tyrosine-tyrosine (PYY) (Stanley *et al.*, 2004). PP is released primarily from the endocrine cells of the pancreas (Adrian *et al.*, 1976) and PYY is mainly produced by the distal intestinal L-cells (Adrian *et al.*, 1985). The actions of PP and PYY on satiety appear to be mediated by the Y receptors in the central nervous system (Stanley *et al.*, 2004).

Observations on the effect of whey protein including GMP on satiety-related hormones are limited. The objective was to explore changes in plasma hormone and metabolite concentrations that may account for the observed (Chungchunlam *et al.*, 2012, *see Chapter 3*) satiety effect of whey protein containing GMP versus maltodextrin carbohydrate. Plasma glucose, insulin, leptin, ghrelin, CCK, GIP, GLP-1, PYY, PP, urea, ammonia, and amino acid concentrations were measured. This study builds upon previous meal response studies by comparing the effects of a preload enriched with either carbohydrate or whey protein on a wider range of gastrointestinal hormones, providing insight into potential mediators of the increased satiety response to whey protein.

### **4.3. Subjects and methods**

#### **4.3.1. Subjects**

Nineteen women, aged between 18 and 40 years with a BMI between 19 and 25 kg/m<sup>2</sup>, were recruited by public advertisement. Individuals completed questionnaires on lifestyle, medical history and dietary habits and the Three Factor Eating Questionnaire to assess dietary restraint (Stunkard & Messick, 1985) during an information session. Blood was collected following an overnight fast, for the analysis of glucose and insulin and subjects were excluded if the fasting levels of glucose and insulin were outside of the normal range. Subjects were selected on the basis of being non-smokers, body weight

stable, everyday breakfast consumers, not pregnant or lactating, and having regular menstrual cycles. Subjects were excluded if they reported a metabolic disease, or were on medication that may affect appetite or blood clotting. One woman did not tolerate the cannula and was excluded from the study. Eighteen women completed the study and their characteristics are given in Table 4.1. Written informed consent was obtained from the participants and the study was approved by the Massey University Human Ethics Committee (Application no. 08/38), New Zealand.

**Table 4.1.** Subject characteristics at baseline (Values are means  $\pm$  sem,  $n = 18$ ).

<b>Characteristic</b>	
Age (years)	21.9 $\pm$ 0.8
Height (cm)	165.9 $\pm$ 1.6
Body weight (kg)	62.8 $\pm$ 1.9
Body Mass Index (BMI, kg/m <sup>2</sup> )	22.7 $\pm$ 0.4
Three Factor Eating Questionnaire <sup>1</sup>	
Dietary restraint	8.1 $\pm$ 1.1
Disinhibition	6.2 $\pm$ 0.6
Hunger	5.9 $\pm$ 0.8
Fasting plasma glucose (mmol/l) <sup>2</sup>	4.48 $\pm$ 0.04
Fasting plasma insulin (pmol/l) <sup>3</sup>	45.2 $\pm$ 4.0

<sup>1</sup> The three factor eating questionnaire comprised of three different factors of human eating behaviour (dietary restraint: 21 questions, disinhibition: 16 questions, and hunger: 14 questions). Subjects who scored less than 10 for dietary restraint, 7 for disinhibition, and 7 for hunger were regarded as unrestrained eaters with less disinhibition of control of eating and were considered to be less susceptible to feelings of hunger (*Stunkard & Messick, 1985*).

<sup>2</sup> The normal range for fasting plasma glucose was accepted to be 3.5–5.5 mmol/l (Human plasma glucose using Roche hexokinase method, Roche Diagnostic Kit, Basel, Switzerland) (*ADA, 2004; Genuth et al., 2003*).

<sup>3</sup> The normal range for fasting plasma free insulin was accepted to be 10–80 pmol/l (Human insulin immunoassay using Roche Elecsys 2010, Roche Diagnostic Kit, Basel, Switzerland).

### 4.3.2. Test diets

The two test beverages, the test meal and preparation protocol were identical to those described earlier (*Chungchunlam et al., 2012, see Chapter 3*). The carbohydrate-enriched drink (300 ml, 1370 kJ) contained 87% of energy as carbohydrate, 4% of energy as protein and 9% of energy as fat. The amount of carbohydrate and protein in 300 ml of the carbohydrate-enriched drink was 72 g and 3 g, respectively. The primary source of carbohydrate in the carbohydrate beverage was maltodextrin (Fieldose™ 10GV, Penford New Zealand Ltd., Auckland, New Zealand). The protein-enriched drink (300 ml, 1333 kJ) contained 33% of energy as carbohydrate, 57% of energy as protein and 10% of energy as fat. The protein-enriched drink contributed 26 g carbohydrate and 46 g protein per 300 ml drink. The predominant source of protein in the protein beverage was whey protein isolate with 21% naturally present glycomacropeptide (WPI 894, Fonterra Co-operative Group Ltd., Palmerston North, New Zealand).

The *ad libitum* test meal (fried rice) consisted of a homogeneous combination of white rice, chicken meat, egg, oil and seasoning. The metabolisable energy (ME) and macronutrient contents (per 100 g) of the test meal were 835 kJ, 6.6 g protein, 28.4 g carbohydrate, 6.4 g fat and 1 g fibre, respectively. Subjects were initially screened to ensure that they liked this test meal. The *ad libitum* test meal was served in excess to allow subjects to eat until comfortably full. The test meal was accompanied by a bottle of spring water (750 ml) and subjects were allowed to request more water.

### 4.3.3. Experimental procedure

A single-blind randomised block design was used with the two test beverages randomly allocated to each subject using random numbers. The two

experimental days were separated by at least three days. Women participated during days 0–14 of their menstrual cycle, where day 0 is the first day of menstruation. Subjects were provided with a standardised breakfast which consisted of toasted wholemeal bread, margarine, strawberry jam, peanut butter, marmite and coffee or tea with sugar and milk to consume at home at least 3 h before receiving the preload drink on the study day. Other than the breakfast, they were asked to refrain from eating or drinking (apart from water) until they arrived at the laboratory at Massey University at their customary lunchtime. On arrival, baseline subjective measures of appetite were collected using 10 cm visual analogue scales (VAS) (Flint *et al.*, 2000). The questions asked were: “How hungry are you?”, “How strong is your desire to eat?”, “How full are you?” and “How much food would you like to eat?”. A catheter was inserted into a vein of the subject’s arm for arterialised venous blood sampling. After a blood sample was collected at baseline, the subject consumed the test drink within 5 min. Following complete ingestion of the test drink (time 0), a blood sample was drawn and subjects completed appetite ratings. Blood samples and appetite ratings were collected every 15 min for 90 min. A final blood sample and subjective appetite rating were taken at 120 min, before the cannula was removed. Subjects were then provided with the fried rice test meal (heated) to consume *ad libitum* within 15 min. Following the termination of the test meal, subjects completed appetite ratings at 15 and 30 min later, and they were then free to leave the laboratory.

#### **4.3.4. Blood sample collection and analysis**

Blood samples (10 ml) were obtained approximately 15 min prior to test drink ingestion and 0, 15, 30, 45, 60, 75, 90 and 120 min postprandially. Blood for plasma glucose analysis was collected in chilled fluoride oxalate tubes. Blood for plasma free amino acid, urea and ammonia analysis was collected in chilled tubes containing EDTA. Blood for plasma CCK was collected in chilled EDTA tubes containing aprotinin (500 KIU/ml blood; Phoenix Pharmaceuticals, Burlingame, CA, USA). Blood for plasma hormones (insulin, ghrelin, leptin, GIP, GLP-1, PP, PYY) was collected in

chilled EDTA tubes containing aprotinin (500 KIU/ml blood; Phoenix Pharmaceuticals, Burlingame, CA, USA) and dipeptidyl peptidase-IV (DPP-IV) inhibitor (10 µl/ml blood; Millipore, Missouri, USA). Plasma was isolated by centrifugation at 4°C for 10 min at 3000 g (Heraeus megafuge 1.0R, Hanau, Germany) within 10 min of collection and aliquots were stored at -80°C for future analysis.

Plasma glucose was determined by a hexokinase method (Roche Diagnostic Kit, Basel, Switzerland) and plasma urea was measured by an enzymatic method (Roche Diagnostic Kit, Basel, Switzerland) on a Flexor E analyzer (Vital Scientific NV, The Netherlands). Plasma ammonia was measured using an enzymatic method (Randox Laboratories Inc., Crumlin, United Kingdom). Plasma samples for free amino acid analysis were deproteinised by ultrafiltration and  $\gamma$ -amino-butyric acid was used as an internal standard. Amino acid concentrations were determined with the use of a RP-HPLC (Agilent Technologies, USA) after precolumn derivatization with o-phthalaldehyde (OPA). The amino acid system used for plasma free amino acid analysis gave poor separation for proline, therefore no data for this amino acid are shown.

Insulin, active ghrelin, leptin, GIP, active GLP-1, PP and total PYY were measured using a commercial Human Gut Hormone Milliplex® kit (Millipore, MA, USA). The detection limit for insulin was 137 pg/ml, ghrelin was 13.7 pg/ml, leptin 137 pg/ml, GIP 2.7 pg/ml, GLP-1 13.7 pg/ml, PP 13.7 pg/ml and PYY 13.7 pg/ml. The intra-assay coefficient of variation (CV) was less than 11% and the inter-assay CV was less than 19%. Ethanol extraction was performed on plasma for cholecystokinin analysis according to the manufacturer's instructions. Cholecystokinin-33 levels were determined using a commercial ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA, USA) with the intra-assay CV ranging between 5 and 10%.

### 4.3.5. Statistical analysis

A power calculation based on the results of Burton-Freeman (2008), where a difference in cholecystokinin area under the curve of 0.66 pmol/l was observed following consumption of preloads containing whey protein compared with a control preload in women, showed that a sample size of ten subjects was needed to achieve power of 0.80. The plasma hormonal, metabolite and amino acid concentrations were first tested for normality of distribution and the presence of statistical outliers (*Ott & Longnecker, 2010*). The response values for glucose, insulin, ghrelin, leptin, CCK, GIP, GLP-1, PYY, PP, urea, ammonia and amino acids were analysed using repeated measures two-way ANOVA with time and drink and their interaction (time x drink) as factors. Treatment contrasts were used to examine the statistical significance of the differences between drinks at each time point. Log transformations were conducted on PP and PYY concentrations to adjust for skewed distributions. Net incremental (area above and below baseline) areas under the curve (AUCs) (*Gannon et al., 1989*) of the response of each blood parameter after consumption of the test drink (from 0 to 120 min adjusted for baseline -15 min) were calculated by the trapezoidal method and tested for a drink effect by using a paired *t* test. Pearson's correlation analysis was used to examine potential relationships within blood parameters. A *p* value of less than 0.05 was regarded as being statistically significant. Data are presented as means and standard errors (Mean  $\pm$  sem). All data were analysed using the Statistical Analysis Systems statistical software package version 9.2 (SAS Institute, Cary, NC, USA).

## 4.4. Results

### 4.4.1. Glucose and Insulin

There was a significant drink x time interaction effect for plasma glucose ( $p < 0.0001$ ) and insulin ( $p = 0.0002$ ) concentrations (Figure 4.1.).

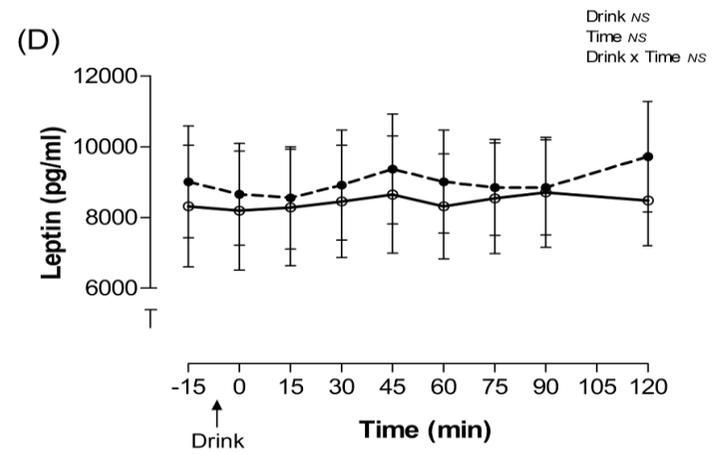
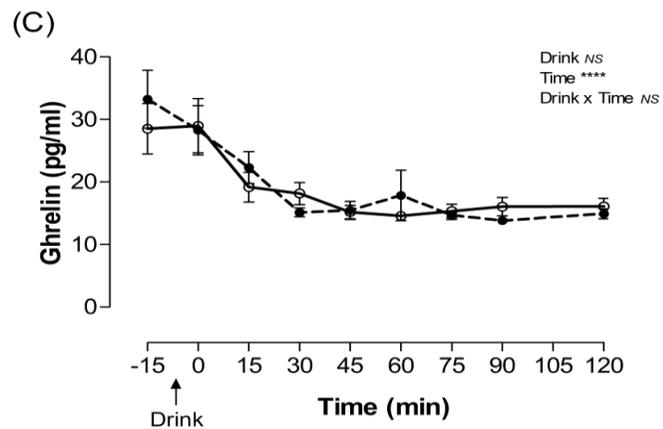
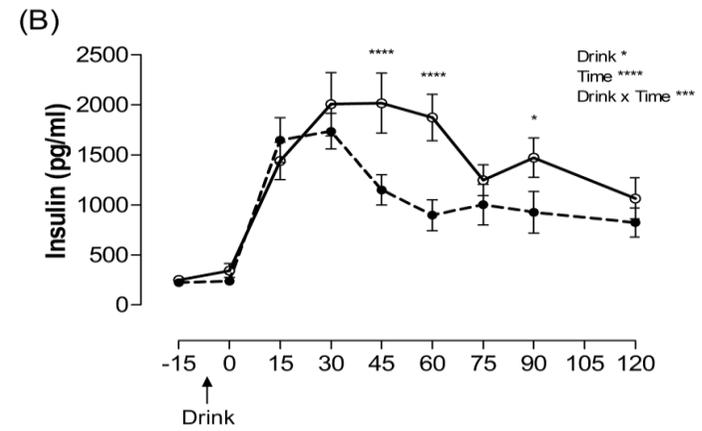
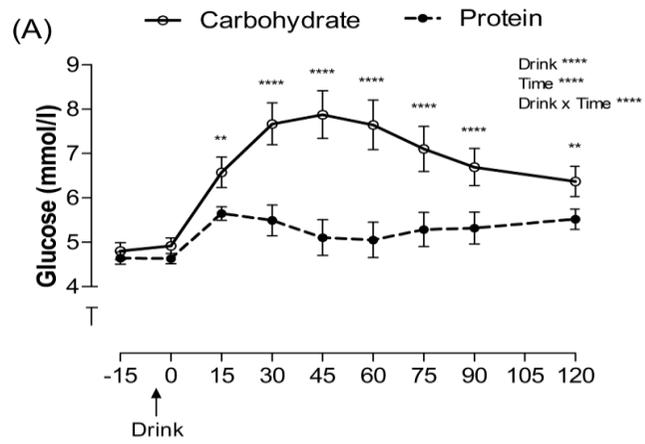
Glucose concentrations were significantly higher after consumption of the carbohydrate-enriched drink compared to the protein-enriched drink from 15 to 120 min (all  $p < 0.0001$ , except for  $p = 0.003$  for 15 min and  $p = 0.0065$  for 120 min). The carbohydrate-enriched drink elicited an insulin response that was significantly higher than that for protein at 45 ( $p < 0.0001$ ), 60 ( $p < 0.0001$ ) and 90 ( $p = 0.011$ ) min post-drink consumption. The net incremental AUC for glucose and insulin was greater after consumption of the carbohydrate-enriched drink than after the protein-enriched drink (Table 4.2.).

#### **4.4.2. Active Ghrelin**

There was no significant drink x time interaction effect ( $p = 0.81$ ) for the active ghrelin response (Figure 4.1.) and there was no significant effect of drink ( $p = 0.63$ ), but active plasma ghrelin concentrations were significantly influenced by time ( $p < 0.0001$ ). Postprandially, active ghrelin concentrations decreased rapidly after both test drinks and remained below the baseline level throughout the duration of the study. The net incremental AUC for the active ghrelin response was not different between the two beverages (Table 4.2.).

#### **4.4.3. Leptin**

Plasma concentrations of leptin (Figure 4.1.) were not significantly influenced by drink ( $p = 0.21$ ) or time ( $p = 0.28$ ) and there was no significant interaction between drink and time ( $p = 0.69$ ). The net incremental AUC for the leptin response was not different between the beverages over the 120 min period (Table 4.2.).



**Figure 4.1.** Plasma concentrations of (A) glucose, (B) insulin, (C) ghrelin, and (D) leptin in eighteen normal-weight women in response to ingestion of carbohydrate-enriched (○) and protein-enriched (●) beverages.

*P* values were derived by repeated-measures ANOVA and by contrast if the interaction between drink and time was significant: \*\*\*\*,  $p < 0.0001$ ; \*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ; *NS*, not significant. Significance indicators shown on the curve are for differences between the two drinks at each time point.

#### 4.4.4. Gastrointestinal hormones

There was no significant drink x time interaction effect ( $p=0.87$ ) but time ( $p<0.0001$ ) and drink ( $p=0.0222$ ) both had a significant effect on plasma GIP concentrations (Figure 4.2.). When the main effect of drink was examined, GIP concentrations were found to be more elevated after ingestion of the carbohydrate-enriched drink than after ingestion of the protein-enriched drink from 0 to 120 min ( $163.9 \pm 7.1$  vs.  $122.6 \pm 4.1$  pg/ml, respectively). The net incremental AUC for the GIP response was not different between test beverages (Table 4.2.).

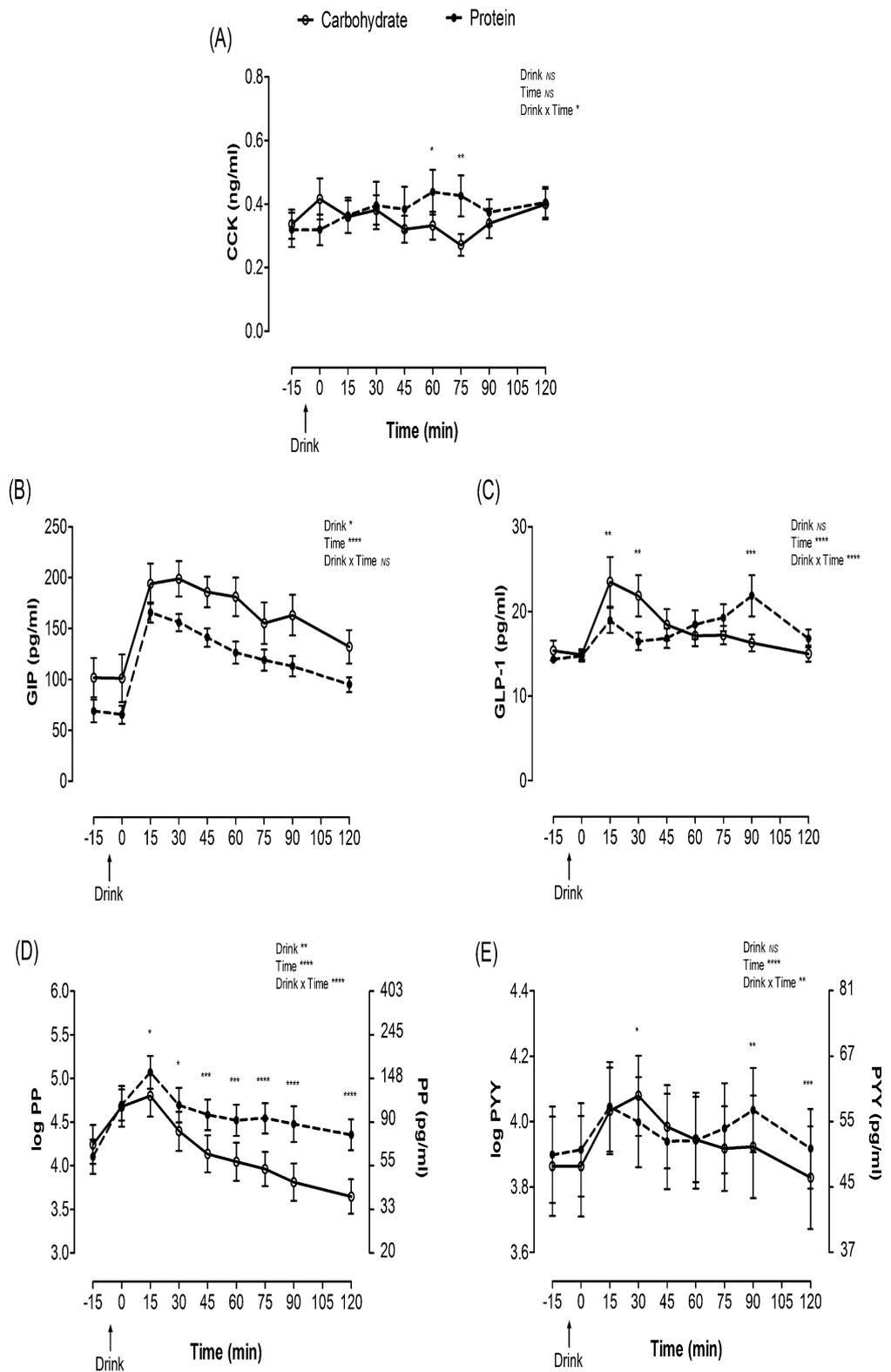
When all data were included in the analysis, there was no significant interaction between drink and time for the plasma CCK-33 response ( $p=0.43$ ) and there were no significant differences due to time ( $p=0.45$ ) or drink ( $p=0.58$ ). However, there were 7 statistical outliers from 322 total observations for the CCK-33 data. When the 7 statistical outliers for the CCK-33 data only were removed, there was a significant interaction effect between drink and time ( $p=0.0492$ ) (data shown in Figure 4.2.). The protein-enriched drink induced greater CCK-33 responses 60 ( $p=0.0406$ ) and 75 ( $p=0.0035$ ) min postprandially than the carbohydrate-enriched drink. The net incremental AUC for the CCK-33 response across the 120 min period did not differ between the two test beverages (Table 4.2.).

There was a significant drink x time interaction effect on the plasma concentrations of active GLP-1 ( $p<0.0001$ ), PP ( $p<0.0001$ ) and PYY ( $p=0.0042$ ) (Figure 4.2.). Following ingestion of the carbohydrate-enriched drink, active GLP-1 plasma concentrations at 15 ( $p=0.0054$ ) and 30 ( $p=0.0011$ ) min and PYY plasma concentrations at 30 min ( $p=0.0439$ ) were significantly higher than after protein ingestion. The protein-enriched drink elicited greater active GLP-1 plasma concentrations at 90 min ( $p=0.0005$ ) and PYY plasma concentrations at 90 ( $p=0.003$ ) and 120 ( $p=0.0006$ ) min compared with the carbohydrate-enriched drink. The mean plasma concentrations of PP peaked at 15 min and remained higher following ingestion of the protein-enriched drink

than following consumption of the carbohydrate-enriched drink until 120 min ( $p < 0.05$ ). The net incremental AUCs for the active GLP-1, PP and PYY responses across the 120 min period were not significantly different between beverages (Table 4.2.).

#### **4.4.5. Correlations between plasma hormones**

Pearson correlations showed a significant positive relationship between plasma insulin and plasma glucose ( $r = 0.58$ ,  $p < 0.0001$ ), GIP ( $r = 0.43$ ,  $p < 0.0001$ ) and GLP-1 ( $r = 0.12$ ,  $p = 0.0351$ ) across both test drinks and all time points, but a weak significant negative relationship with PP plasma concentrations ( $r = -0.21$ ,  $p = 0.0002$ ). In particular, plasma concentrations of insulin were negatively correlated with PP plasma concentrations at 120 min following consumption of both test drinks ( $r = -0.34$ ,  $p = 0.0414$ ). There was a weak but significant positive correlation between overall PYY plasma concentrations and overall plasma concentrations of GLP-1 ( $r = 0.12$ ,  $p = 0.0417$ ) and PP ( $r = 0.26$ ,  $p < 0.0001$ ). Plasma concentrations of GLP-1 and PP were positively correlated at 90 min following ingestion of the test drinks ( $r = 0.35$ ,  $p = 0.0341$ ) and the relationship at 120 min almost reached significance ( $r = 0.32$ ,  $p = 0.0549$ ).



**Figure 4.2.** Plasma concentrations of (A) CCK (cholecystokinin), (B) GIP (glucose-dependent insulinotropic polypeptide), (C) GLP-1 (glucagon-like peptide-1), (D) PP (pancreatic polypeptide), and (E) PYY (peptide tyrosine-tyrosine) in eighteen normal-weight women after ingestion of carbohydrate-enriched (○) and protein-enriched (●) beverages.

*P* values were derived by repeated-measures ANOVA and by contrast if the interaction between drink and time was significant: \*\*\*\*,  $p < 0.0001$ ; \*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ; *NS*, not significant. Significant probability levels shown on the curve are for differences between the two drinks at each time point.

**Table 4.2.** Net incremental area under the curve (iAUC) for postprandial responses following consumption of the “carbohydrate”-enriched and “protein”-enriched test drinks.

	Net iAUC <sup>1</sup>		Overall sem	<i>p</i> -value
	Carbohydrate	Protein		
Glucose (mmol/l,120min)	262.1	78.2	22.0	<0.0001
Insulin (pg/ml,120min)	149828.9	104301.9	12057.8	0.0162
Ghrelin (pg/ml,120min)	-1359.1	-1729.3	514.4	0.61
Leptin (pg/ml,120min)	15960.0	-6315.3	19511.0	0.43
CCK (ng/ml,120min)	1.84	9.47	5.08	0.30
GIP (pg/ml,120min)	7829.5	6807.5	1069.0	0.50
GLP-1 (pg/ml,120min)	326.3	489.0	102.1	0.27
PP (pg/ml,120min)	-34.2	5975.2	2494.1	0.10
PYY (pg/ml,120min)	357.1	435.6	106.7	0.60

<sup>1</sup> Net incremental area under the curve from 0 to 120 min, *n* = 18

#### 4.4.6. Urea and ammonia

There were differences in plasma urea and ammonia concentrations (Figure 4.3.) between drinks over time, characterised by a significant drink x time interaction effect ( $p < 0.0001$ ). The protein-enriched drink elicited a higher urea response from 45 min to the end of the study period (all  $p < 0.0001$ , except for  $p = 0.0138$  for 45 min) and led to significant increases in plasma ammonia concentrations from 15 to 120 min ( $P \leq 0.0001$ ) compared with carbohydrate. The net incremental AUC for the urea response was higher after ingestion of the protein-enriched beverage than that after the carbohydrate-enriched beverage ( $72.3 \pm 8.8$  vs.  $-15.2 \pm 11.4$  mmol/l,120min,  $p < 0.0001$ ). The net incremental AUC for the ammonia response was also greater after consumption of the protein-enriched beverage compared with carbohydrate ( $6.50 \pm 0.92$  vs.  $0.70 \pm 0.98$  mmol/l,120min,  $p = 0.0006$ ). Pearson correlation analyses showed a significant positive relationship between plasma urea and ammonia concentrations across both test drinks and all time points ( $r = 0.46$ ,  $p < 0.0001$ ).

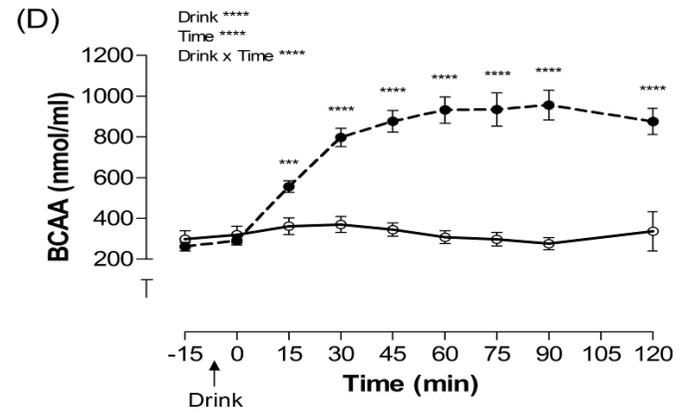
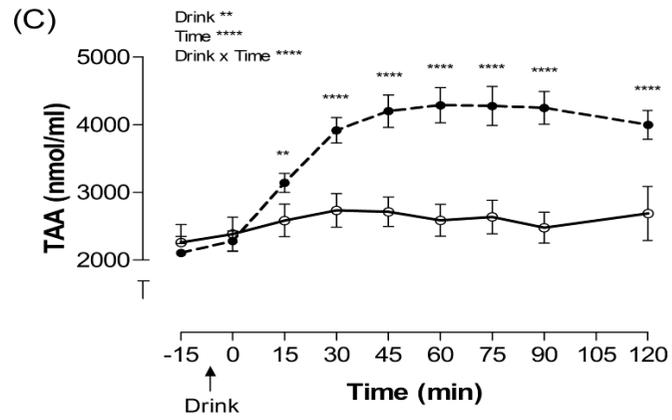
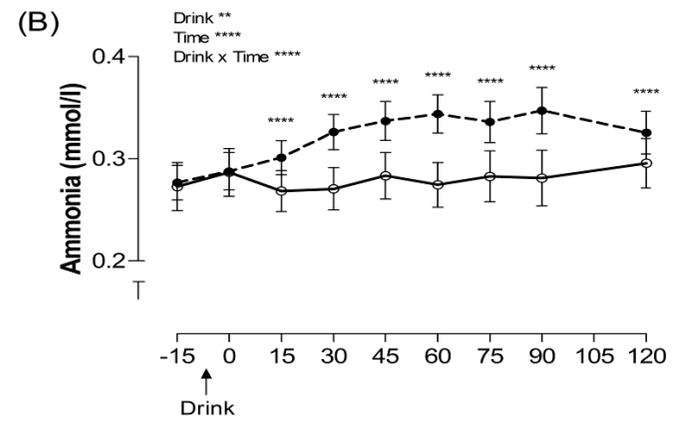
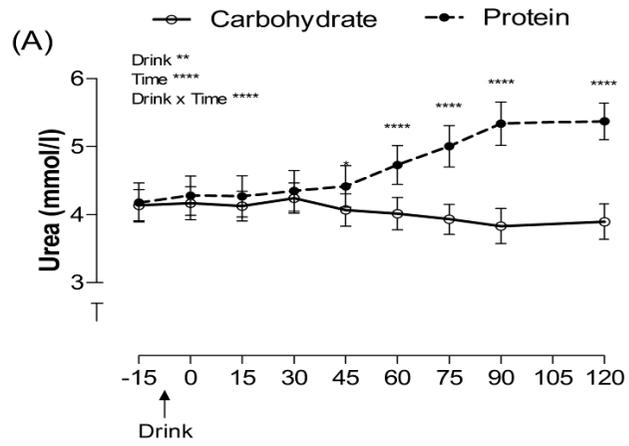
#### 4.4.7. Plasma amino acids

There was a significant drink x time interaction effect ( $p < 0.0001$ ) for total amino acid (TAA) and branched-chain amino acid (BCAA: isoleucine, leucine and valine) concentrations in plasma after drink ingestion (Figure 4.3.). Total amino acid and branched-chain amino acid levels were significantly more elevated from 15 to 120 min after protein drink ingestion than after carbohydrate drink ingestion (all  $p < 0.0001$ , except for  $p = 0.0048$  and  $p = 0.0002$  for 15 min for TAA and BCAA, respectively). The net incremental AUC for the total amino acids response was higher after consumption of the protein-enriched beverage compared with the carbohydrate-enriched beverage ( $218883.8 \pm 26528.5$  vs.  $42205.9 \pm 17644.3$  nmol/ml,120min,  $p = 0.0002$ ). The net incremental AUC for the branched-chain amino acid response was also greater after the protein-enriched beverage compared with the carbohydrate-

enriched beverage ( $66797.1 \pm 6431.9$  vs.  $3191.1 \pm 1766.5$  nmol/ml, 120min,  $p < 0.0001$ ).

The difference in individual plasma amino acid concentrations between the carbohydrate and protein drinks was determined by net incremental area under the curve (Table 4.3.). The net incremental AUC responses for glutamine, glycine, histidine, ornithine, taurine and tryptophan did not differ significantly after ingestion of the two test beverages ( $p > 0.05$ ). However, for  $\alpha$ -amino-butyric acid, alanine, arginine, asparagine, aspartic acid, citrulline, cysteine, glutamic acid, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine, and valine, the net incremental AUC responses were significantly higher after ingestion of the protein-enriched beverage compared with the carbohydrate-enriched beverage ( $p < 0.05$ ).

Pearson correlation analysis revealed weak significant negative relationships between overall plasma TAA and BCAA concentrations and overall plasma concentrations of glucose (TAA:  $r = -0.30$ ,  $p < 0.0001$ ; BCAA:  $r = -0.37$ ,  $p < 0.0001$ ) and GIP (TAA:  $r = -0.21$ ,  $p = 0.0002$ ; BCAA:  $r = -0.21$ ,  $p = 0.0003$ ) following consumption of both test drinks. Postprandial plasma TAA concentrations were weakly but significantly positively correlated with plasma CCK concentrations at 75 min ( $r = 0.34$ ,  $p = 0.0368$ ) and moderately correlated with plasma GLP-1 concentrations at 120 min ( $r = 0.58$ ,  $p = 0.0002$ ) after consumption of both test drinks. A positive correlation between plasma TAA and PP concentrations at 120 min ( $r = 0.30$ ,  $p = 0.0743$ ) approached significance. There was a significant positive correlation between plasma BCAA concentrations and plasma concentrations of GLP-1 at 75 ( $r = 0.36$ ,  $p = 0.0286$ ) and 120 ( $r = 0.67$ ,  $p < 0.0001$ ) min and PP at 120 min ( $r = 0.41$ ,  $p = 0.0125$ ). There was a weak but significant positive correlation between overall plasma concentrations of TAA and urea ( $r = 0.17$ ,  $p = 0.0025$ ).



**Figure 4.3.** Plasma concentrations of (A) urea, (B) ammonia, (C) TAA (Total amino acid), and (D) BCAA (Branched-chain amino acid) in normal-weight women in response to consumption of carbohydrate-enriched (○) and protein-enriched (●) beverages.

*P* values were derived by repeated-measures ANOVA and by contrast if the interaction between time and drink was significant: \*\*\*\*,  $p \leq 0.0001$ ; \*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ . Significance indicators shown on the curve are for differences between the two drinks at each time point.

**Table 4.3.** Net incremental area under the curve (iAUC) for plasma concentrations of individual amino acids following consumption of the carbohydrate-enriched and protein-enriched test drinks<sup>1</sup>.

<b>Amino acid net iAUC (nmol/ml,120min)</b>	<b>Carbohydrate-enriched Drink</b>	<b>Protein-enriched Drink</b>	<b>Overall sem</b>	<b><i>p</i>-value</b>
$\alpha$ -amino-butyric acid	528.2	1642.3	324.7	0.0267
Alanine	7503.5	21526.5	3378.1	0.0092
Arginine	1125.1	5041.1	870.6	0.0055
Asparagine	1632.7	5853.3	798.4	0.0016
Aspartic acid	46.8 <sup>2</sup>	522.6	109.7	0.0158
Citrulline	-225.5	1249.6	369.2	0.0117
Cysteine	-5044.9	14816.5	1920.2	<0.0001
Glutamic acid	1210.2	2849.8	389.6	0.0085
Glutamine	19486.2	29278.9	7926.0	0.39
Glycine	388.6	1509.2	884.0	0.38
Histidine	3012.6	5362.6	1573.8	0.30
Isoleucine	600.5	17884.5	1281.9	<0.0001

Leucine	-290.4	28586.7	2040.8	<0.0001
Lysine	261.2	20836.2	1952.4	<0.0001
Methionine	623.5	3720.3	434.6	0.0001
Ornithine	227.3	253.0	25.0	0.47
Phenylalanine	571.3	2939.5	484.8	0.003
Serine	2531.7	7850.6	1262.0	0.0084
Taurine	2214.3	2452.9	673.5	0.80
Threonine	3850.6	19146.9	2348.5	0.0003
Tryptophan	-75.6 <sup>3</sup>	85.6 <sup>4</sup>	82.2	0.20
Tyrosine	545.7	5082.6	739.0	0.0004
Valine	2881.0	20325.9	1918.3	<0.0001

<sup>1</sup> Net incremental area under the curve from 0 to 120 min,  $n = 18$

<sup>2</sup> Two missing data,  $n = 16$

<sup>3</sup> One missing data,  $n = 17$

<sup>4</sup> Three missing data,  $n = 15$

## 4.5. Discussion

Dietary protein has been shown to reduce *ad libitum* energy intake at a subsequent test meal and to reduce subjective feelings of appetite more than carbohydrate or fat. Moreover, there is some evidence that consumption of dairy whey protein is more satiating compared with other protein sources. The presently reported results relate to a larger study. In an earlier report (Chungchunlam *et al.*, 2012, *see Chapter 3*), results for the effect of time interval between a preload drink and *ad libitum* food intake at a subsequent test meal were reported for the same group of subjects discussed here. It was shown that, regardless of the time interval between preload drink and test meal, subjects consumed less food at an *ad libitum* test meal following a whey protein-enriched preload compared to a maltodextrin carbohydrate-enriched preload. Food intake at the *ad libitum* test meal for the preload delay of 120 min was greater following the carbohydrate-enriched drink compared with whey protein ( $2920 \pm 230$  vs.  $2442 \pm 290$  kJ, respectively,  $p=0.0345$ ). The latter finding provides a clear basis for the presently-reported investigation of the response of several plasma hormones and metabolites in the subjects.

Ingestion of the two test beverages enriched with maltodextrin carbohydrate or whey protein isolate (containing naturally present glycomacropptide) influenced several of the blood parameters differently, but plasma leptin and active ghrelin concentrations were not affected by the beverage type. Leptin concentrations in the circulation correlate positively with body fat stores (Considine *et al.*, 1996) and when subjects are in energy balance (or within normal BMI range), the acute postprandial leptin response shows only a weak relationship with appetite and satiety (Joannic *et al.*, 1998; Romon *et al.*, 1999). Plasma active ghrelin was found to decrease immediately after consumption of both the carbohydrate- and protein-enriched test drinks and the decrease was not statistically significantly different between the whey protein and maltodextrin carbohydrate. This is in line with several studies showing that high protein and high carbohydrate meals have no differential

effect on active plasma ghrelin levels (*Blom et al., 2006; Lejeune et al., 2006; Smeets et al., 2008*). The maltodextrin carbohydrate-enriched test drink was expected to have a greater effect on postprandial plasma glucose and insulin concentrations than the whey protein-enriched test drink, as was observed. After ingestion of the carbohydrate test drink, plasma glucose concentrations peaked at 45 min and increased to approximately 39% above baseline concentrations. This postprandial glucose response induced a rise in plasma insulin concentration, which in turn was associated with an increase in plasma concentrations of the incretin peptides, GIP and GLP-1. Plasma GIP concentrations over time and the incremental AUC GIP response were higher after the maltodextrin carbohydrate-insulin test drink relative to whey protein, and active GLP-1 plasma concentrations were higher 15 and 30 min after ingestion of the maltodextrin carbohydrate-enriched beverage.

Significantly, ingestion of the whey protein led to increased mean plasma concentrations of CCK, PYY and PP, and for GLP-1, an increase at 90 min. All or some of these hormones are potential candidates as mediators of the observed increased satiety response to whey protein. An effect of whey protein in comparison with maltodextrin carbohydrate on responses of plasma CCK, GLP-1 and PYY have been observed in other studies (*Batterham et al., 2006; Burton-Freeman, 2008; Calbet & Holst, 2004; Lejeune et al., 2006; Raben et al., 2003*). In the present study, the whey protein-enriched drink led to increased mean plasma concentrations of CCK-33 at 60 and 75 min, active GLP-1 at 90 min and total PYY from 90 to 120 min compared with the maltodextrin carbohydrate-enriched drink. Of particular interest was the significant elevation in mean plasma PP concentrations from 15 until 120 min after whey protein ingestion compared to maltodextrin carbohydrate. Although infusions of PP have been demonstrated to suppress food intake (*Batterham et al., 2003; Berntson et al., 1993; Jesudason et al., 2007*), little is known about the postprandial release of PP following the ingestion of a protein-rich meal (*Tomita et al., 1989; Zipf et al., 1983*). The presently reported data provide novel information about the response of plasma PP to whey protein when compared to maltodextrin carbohydrate, justifying further investigation. The finding that energy intake at the subsequent *ad libitum* test meal was lower

following consumption of the whey protein-enriched preload compared with that following ingestion of the carbohydrate-enriched preload (*Chungchunlam et al., 2012, see Chapter 3*) led to the hypothesis that the suppressive effect on subsequent food intake of whey protein may be associated with an increase in plasma gastrointestinal hormones (Pearson's correlation analysis). A significant inverse relationship between food energy intake at the *ad libitum* test meal and plasma concentrations of the hormone PP was found within individual subjects (overall:  $r = -0.21$ ,  $p=0.0003$ , and at 120 min:  $r = -0.33$ ,  $p=0.0464$ ). So, not only were mean plasma PP concentrations significantly higher at most times examined post-drink ingestion for the whey protein versus carbohydrate, but the effect was also seen among individual subjects, with subjects having higher plasma PP levels also showing lower food energy intake at the subsequent test meal. The hormone PP may have a role in mediating the satiating effect of whey protein, and this needs to be investigated further using PP administration and techniques to block the effect of released PP. The possibility that PP acts in concert with some of the other gastrointestinal hormones, the mean plasma concentrations of which were also elevated with whey protein, is also worthy of investigation.

A considerably elevated concentration of plasma amino acids within 15 min of consumption of the whey protein infers that plasma amino acids are possible candidate metabolites associated with the mechanism by which whey protein induces satiety. A sharp rise in mean circulating amino acid concentrations has been shown previously to bring about a reduction in appetite (*Mellinkoff et al., 1956*). The present observation that a rise in plasma amino acid concentrations was associated (Pearson's correlation analysis) with an increase in plasma concentrations of CCK, GLP-1, and PP is also in agreement with the hypothesis that plasma amino acids may be implicated in satiety. The products of postprandial protein metabolism, urea and ammonia, also increased after ingestion of the whey protein compared with carbohydrate, which has been reported by others (*Garlick et al., 1991*). The whey protein-enriched drink resulted in a higher initial (15 to 120 min) plasma ammonia response and a delayed (45 to 120 min) increase in plasma urea concentrations compared with maltodextrin carbohydrate. A secondary effect of plasma urea

and ammonia concentrations to the effect of plasma amino acids, on satiety should also not be excluded. The high energy costs associated with postprandial protein metabolism may contribute to diet-induced thermogenesis and increased satiety (*Acheson et al., 2011; Croveti et al., 1998; Westerterp-Plantenga et al., 1999*), though such an effect would not be specific to whey protein.

The literature on why whey protein is more satiating than other protein types and which component of whey protein induces satiety remains unclear. During the digestion of whey protein, peptides with biological activities are released and may influence gastrointestinal function to stimulate satiety (*Haque et al., 2008; Jahan-Mihan et al., 2011; Luhovyy et al., 2007; Madureira et al., 2010; Moughan et al., 2007; Rutherford-Markwick & Moughan, 2005*). The amino acid composition of whey protein in relation to other protein sources may play a role. Dairy whey protein is relatively high in branched-chain amino acids (BCAA: isoleucine, leucine and valine) (*Etzel, 2004; Moughan, 2008*) and this was reflected by the increased plasma BCAA concentrations for whey protein compared with carbohydrate. The BCAA leucine has been proposed as being the most important amino acid involved in the regulation of energy intake (*Garlick, 2005; Layman, 2003; Layman & Walker, 2006*). It is not clear whether these responses are specific to whey protein and further studies are required to examine how proteins from other sources influence satiety-related gastrointestinal hormones and metabolites of amino acid catabolism postprandially. The effect on satiety of the individual protein components present in the whey protein isolate (glycomacropeptide, beta-lactoglobulin, and alpha-lactalbumin) and the amino acid composition of the whey protein used here will be the focus of further investigation.

It appears, from the presently reported results, that the satiety effect of whey protein may be mediated by elevated plasma concentrations of the satiety-related gastrointestinal hormones; CCK, GLP-1, PYY and PP. Higher postprandial circulating levels of amino acids and products of amino acid catabolism after consumption of whey protein may also be involved in mediating the difference in satiety found between whey protein and

maltodextrin carbohydrate. The peptide hormone PP, in particular, may play a role in the satiating potential of whey protein. More studies examining the effect of whey protein and other protein sources on satiety-related gastrointestinal hormones and plasma amino acids are required. There is a need now for controlled intervention studies where potential regulatory hormones are administered or techniques for blocking the hormonal effects are applied. Further work is needed to identify the importance of these gastrointestinal hormone signalling responses, with underlying possible interactions, in relation to satiety to gain a better understanding of the control mechanisms of food intake and appetite.

#### 4.6. Literature cited

- Acheson, K. J., Blondel-Lubrano, A., Oquey-Araymon, S., Beaumont, M., Emady-Azar, S., Ammon-Zufferey, C., Monnard, I., Pinaud, S., Nielsen-Moennoz, C., Bovetto, L. (2011). Protein choices targeting thermogenesis and metabolism. *Am J Clin Nutr*, 93(3), 525–534.
- ADA (American Diabetes Association). (2004). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 27(Suppl 1), S5–S10.
- Adrian, T. E., Bloom, S. R., Bryant, M. G., Polak, J. M., Heitz, P. H., & Barnes, A. J. (1976). Distribution and release of human pancreatic polypeptide. *Gut*, 17(12), 940–944.
- Adrian, T. E., Ferri, G. L., Bacarese-Hamilton, A. J., Fuessl, H. S., Polak, J. M., & Bloom, S. R. (1985). Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology*, 89(5), 1070–1077.
- Anderson, G. H., & Moore, S. E. (2004). Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr*, 134(4), 974S–979S.
- Anderson, G. H., Tecimer, S. N., Shah, D., & Zafar, T. A. (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.

- Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K., & Nakao, K. (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.
- Baggio, L. L., & Drucker, D. J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology*, *132*(6), 2131–2157.
- Batterham, R. L., Heffron, H., Kapoor, S., Chivers, J. E., Chandarana, K., Herzog, H., Le Roux, C. W., Thomas, E. L., Bell, J. D., & Withers, D. J. (2006). Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab*, *4*(3), 223–233.
- Batterham, R. L., Le Roux, C. W., Cohen, M. A., Park, A. J., Ellis, S. M., Patterson, M., Frost, G. S., Ghatei, M. A., & Bloom, S. R. (2003). Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab*, *88*(8), 3989–3992.
- Berntson, G. G., Zipf, W. B., O’Dorisio, T. M., Hoffman, J. A., & Chance, R. E. (1993). Pancreatic polypeptide infusions reduce food intake in Prader-Willi syndrome. *Peptides*, *14*(3), 497–503.
- Blom, W. A. M., Lluch, A., Stafleu, A., Vinoy, S., Holst, J. J., Schaafsma, G., & Hendriks, H. F. (2006). Effect of a high-protein breakfast on the postprandial ghrelin response. *Am J Clin Nutr*, *83*(2), 211–220.
- Burton-Freeman, B. M. (2008). Glycomacropeptide (GMP) is not critical to whey-induced satiety, but may have a unique role in energy intake regulation through cholecystokinin (CCK). *Physiol Behav*, *93*(1-2), 379–387.
- Calbet, J. A., & Holst, J. J. (2004). Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. *Eur J Nutr*, *43*(3), 127–139.
- Chungchunlam, S. M. S., Moughan, P. J., Henare, S. J., & Ganesh, S. (2012). Effect of time of consumption of preloads on measures of satiety in healthy normal weight women. *Appetite*, *59*(2), 281–288. (see Chapter 3)
- Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., Ohannesian, J. P., Marco, C. C., McKee, L. J., Bauer,

- T. L., & Caro, J. F. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*, *334*(5), 292–295.
- Crovetti, R., Porrini, M., Santangelo, A., & Testolin, G. (1998). The influence of thermic effect of food on satiety. *Eur J Clin Nutr*, *52*(7), 482–488.
- Cummings, D. E, Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., & Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, *50*(8), 1714–1719.
- Cummings, D. E, Frayo, R. S., Marmonier, C., Aubert, R., & Chapelot, D. (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–E304.
- Degen, L., Matzinger, D., Drewe, J., & Beglinger, C. (2001). The effect of cholecystokinin in controlling appetite and food intake in humans. *Peptides*, *22*(8), 1265–1269.
- Eisenstein, J., Roberts, S. B., Dallal, G., & Saltzman, E. (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), 189–200.
- Elliott, R. M., Morgan, L. M., Tredger, J. A., Deacon, S., Wright, J., & Marks, V. (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.
- Etzel, M. R. (2004). Manufacture and use of dairy protein fractions. *J Nutr*, *134*(4), 996S–1002S.
- Flint, A., Raben, A, Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord*, *24*(1), 38–48.
- Foster-Schubert, K. E., Overduin, J., Prudom, C. E., Liu, J., Callahan, H. S., Gaylinn, B. D., Thorner, M. O., & Cummings, D. E. (2008). Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. *J Clin Endocrinol Metab*, *93*(5), 1971–1979.

- Gannon, M. C., Nuttall, F. Q., Westphal, S. A., Neil, B. J., & Seaquist, E. R. (1989). Effects of dose of ingested glucose on plasma metabolite and hormone responses in type II diabetic subjects. *Diabetes Care*, *12*(8), 544–552.
- Garlick, P. J. (2005). The role of leucine in the regulation of protein metabolism. *J Nutr*, *135*(6 Suppl), 1553S–1556S.
- Garlick, P. J., McNurlan, M. A., & Ballmer, P. E. (1991). Influence of dietary protein intake on whole-body protein turnover in humans. *Diabetes Care*, *14*(12), 1189–1198.
- Genuth, S., Alberti, K. G., Bennett, P., Buse, J., Defronzo, R., Kahn, R., Kitzmiller, J., Knowler, W. C., Lebovitz, H., Lernmark, A., Nathan, D., Palmer, J., Rizza, R., Saudek, C., Shaw, J., Steffes, M., Stern, M., Tuomilehto, J., Zimmet, P., & Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2003). Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care*, *26*(11), 3160–3167.
- Hall, W. L., Millward, D. J., Long, S. J., & Morgan, L. M. (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., & 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.
- Haque, E., Chand, R., Kapila, S. (2008). Biofunctional properties of bioactive peptides of milk origin. *Food Rev Int*, *25*(1), 28–43.
- Jahan-Mihan, A., Luhovyy, B. L., El Khoury, D., & Anderson, G. H. (2011). Dietary proteins as determinants of metabolic and physiological functions of the gastrointestinal tract. *Nutrients*, *3*(5), 574–603.
- Jesudason, D. R., Monteiro, M. P., McGowan, B. M., Neary, N. M., Park, A. J., Philippou, E., Small, C. J., Frost, G. S., Ghatei, M. A., & Bloom, S. R. (2007). Low-dose pancreatic polypeptide inhibits food intake in man. *Br J Nutr*, *97*(3), 426–429.
- Joannic, J. L., Oppert, J. M., Lahlou, N., Basdevant, A., Auboiron, S., Raison, J., Bornet, F., & Guy-Grand, B. (1998). Plasma leptin and hunger ratings in healthy humans. *Appetite*, *30*(2), 129–130.

- Layman, D. K. (2003). The role of leucine in weight loss diets and glucose homeostasis. *J Nutr*, *133*(1), 261S–267S.
- Layman, D. K., & Walker, D. A. (2006). Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr*, *136*(1 Suppl), 319S–323S.
- Lejeune, M. P., Westerterp, K. R., Adam, T. C., Luscombe-Marsh, N. D., Westerterp-Plantenga, M. S. (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. *Am J Clin Nutr*, *83*(1), 89–94.
- Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr*, *26*(6), 704S–712S.
- Madureira, A. R., Tavares, T., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2010). Invited review: physiological properties of bioactive peptides obtained from whey proteins. *J Dairy Sci*, *93*(2), 437–455.
- Meier, J. J., Goetze, O., Anstipp, J., Hafemann, D., Holst, J. J., Schmidt, W. E., Gallwitz, B., & Nauck, M. A. (2004). Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. *Am J Physiol Endocrinol Metab*, *286*(4), E621–E625.
- Mellinkoff, S. M., Frankland, M., Boyle, D., & Greipel, M. (1956). Relationship between serum amino acid concentration and fluctuations in appetite. *J Appl Physiol*, *8*(5), 535–538.
- Moughan, P. J. (2008). Milk proteins: a cornucopia for developing functional foods. In: A. Thompson, M. Boland, & H. Singh (Eds.), *Milk proteins: from expression to food* (pp. 483–499). London: Academic Press.
- Moughan, P. J., Fuller, M. F., Han, K. S., Kies, A. K., & Miner-Williams, W. (2007). Food-derived bioactive peptides influence gut function. *Int J Sport Nutr Exerc Metab*, *17* Suppl, S5–S22.
- Nauck, M. A., Niedereichholz, U., Ettlner, R., Holst, J. J., Orskov, C., Ritzel, R., & Schmiegel, W. H. (1997). Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol*, *273*(5 Pt 1), E981–E988.
- Ott, L., & Longnecker, M. (2010). *An introduction to statistical methods and data analysis*. 6th ed. Belmont, CA: Brooks/Cole Cengage Learning.

- Paddon-Jones, D., Westman, E., Mattes, R. D., Wolfe, R. R., Astrup, A., & Westerterp-Plantenga, M. (2008). Protein, weight management, and satiety. *Am J Clin Nutr*, 87(5), 1558S–1561S.
- Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J. J., & Astrup, A. (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.
- Rehfeld, J. F. (2004). Cholecystikinin. *Best Pract Res Clin Endocrinol Metab*, 18(4), 569–586.
- Romon, M., Lebel, P., Velly, C., Marecaux, N., Fruchart, J. C., & Dallongeville, J. (1999). Leptin response to carbohydrate or fat meal and association with subsequent satiety and energy intake. *Am J Physiol*, 277(5 Pt 1), E855–E861.
- Rutherford-Markwick, K. J., & Moughan, P. J. (2005). Bioactive peptides derived from food. *JAOAC Int*, 88(3), 955–966.
- Smeets, A. J., Soenen, S., Luscombe-Marsh, N. D., Ueland, O., & Westerterp-Plantenga, M. S. (2008). Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. *J Nutr*, 138(4), 698–702.
- Stanley, S., Wynne, K., & Bloom, S. (2004). Gastrointestinal satiety signals III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide. *Am J Physiol Gastrointest Liver Physiol*, 286(5), G693–G697.
- Stanley, S., Wynne, K., McGowan, B., & Bloom, S. (2005). Hormonal regulation of food intake. *Physiol Rev*, 85(4), 1131–1158.
- Strader, A. D., & Woods, S. C. (2005). Gastrointestinal hormones and food intake. *Gastroenterology*, 128(1), 175–191.
- Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*, 29(1), 71–83.
- Tomita, T., Greeley, G. Jr., Watt, L., Doull, V., & Chance, R. (1989). Protein meal-stimulated pancreatic polypeptide secretion in Prader-Willi syndrome of adults. *Pancreas*, 4(4), 395–400.

- Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N., & Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. *Physiol Behav*, *94*(2), 300–307.
- Westerterp-Plantenga, M. S., Lejeune, M. P., Nijs, I., van Ooijen, M., & Kovacs, E. M. (2004). High protein intake sustains weight maintenance after body weight loss in humans. *Int J Obes Relat Metab Disord*, *28*(1), 57–64.
- Westerterp-Plantenga, M. S., Rolland, V., Wilson, S. A., & Westerterp, K. R. (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.
- WHO. (2012). *Obesity and overweight (WHO fact sheet no. 311.)*: World Health Organization.
- Zipf, W. B., O'Dorisio, T. M., Cataland, S., & Dixon, K. (1983). Pancreatic polypeptide responses to protein meal challenges in obese but otherwise normal children and obese children with Prader-Willi syndrome. *J Clin Endocrinol Metab*, *57*(5), 1074–1080.

## Chapter 5

### **The underlying cause of the satiating effect of whey protein: effect of whey protein and glycomacropeptide isolate on measures of satiety in normal-weight adult women**

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Whey protein isolate (WPI) has been demonstrated to induce satiety compared with maltodextrin carbohydrate in adult humans (*Chapters 2 & 3*). This effect may be due to glycomacropeptide (GMP) present in the whey protein, but the previous studies reported in this dissertation have not investigated the effects of GMP alone. The aim of the present study was to compare the effects on satiety of WPI with a high level of naturally present GMP, WPI with a low level of GMP and GMP isolate alone. Maltodextrin carbohydrate was used as a baseline control.

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## 5.1. Abstract

Protein is the most satiating macronutrient and dairy whey protein is considered to be more satiating than other protein sources. The purported satiating effect of whey protein has been attributed by some investigators to the presence of glycomacropeptide (GMP), as GMP has been found to induce the secretion of the satiety-related hormone cholecystokinin. The objective was to investigate the satiating effects of isoenergetic (~1600 kJ) preload drinks enriched with maltodextrin carbohydrate, or enriched with whey protein isolate (WPI) with 2% naturally present GMP (“WPI-low GMP”), WPI with 21% naturally occurring GMP (“WPI-high GMP”) or GMP isolate (86% GMP, “GMP”). Satiety was assessed in twenty-two women (mean age 23.8 years, mean BMI 23.1 kg/m<sup>2</sup>) by determining the consumption of a test meal provided *ad libitum* 120 min following ingestion of a preload drink, and also by using visual analogue scales (VAS) for rating feelings of hunger, desire to eat, prospective food consumption and fullness (appetite). The *ad libitum* test meal intake was significantly different between the preload drinks ( $p=0.0003$ ), with food intake following ingestion of both of the WPI preload drinks being 18% lower compared with carbohydrate and GMP. There were no significant differences in the VAS-rated feelings of appetite between the preload drinks ( $p>0.05$ ). GMP alone did not reduce subsequent food intake consumption compared to maltodextrin carbohydrate but whey protein had a greater satiating effect than maltodextrin carbohydrate. It appears that the presence of GMP in whey is not the cause of the observed effect of whey protein on satiety.

## 5.2. Introduction

The rapid worldwide rise in the prevalence of obesity has led to growing interest in dietary modifications that can enhance satiety and reduce dietary energy intake (*Van Kleef et al., 2012*). Several scientific reviews have concluded that protein is the most satiating macronutrient (*Anderson & Moore,*

2004; Eisenstein *et al.*, 2002; Halton & Hu, 2004; Westerterp-Plantenga *et al.*, 2009). A previous study from our own group showed that dairy whey protein suppressed subsequent food energy intake in normal-weight adult women compared with maltodextrin carbohydrate, when consumed as a milkshake matched for energy content and volume (Chungchunlam *et al.*, 2012, *see Chapter 3*). The source of protein may influence the satiating effect of the dietary protein, and whey protein appears to be more satiating than other protein sources (Luhovyy *et al.*, 2007; Veldhorst *et al.*, 2008).

Cow's milk contains approximately 3.3% protein, 78% of which is casein and 19% whey proteins (Walstra *et al.*, 2006). During cheese making, whey is the soluble protein component of milk that is separated from the casein curd. This whey also contains glycomacropeptide (GMP), the soluble peptide cleaved from the action of rennet (chymosin) on  $\kappa$ -casein. GMP may play a role in satiety as GMP has been shown to be a potent stimulant of the release of the satiety-related hormone cholecystokinin (CCK) in rats (Beucher *et al.*, 1994; Yvon *et al.*, 1994). However, the potential of GMP to stimulate CCK release in humans remains to be conclusively demonstrated (Burton-Freeman, 2008; Keogh *et al.*, 2010). Studies investigating the effect of GMP on food intake and satiety have resulted in mixed findings. Veldhorst *et al.* (2009a) showed a decrease in food energy intake at a subsequent meal 180 min after consumption of a test breakfast containing whey protein with GMP compared with whey protein without GMP. However, in studies by Burton-Freeman (2008), Lam *et al.* (2009) (*see Chapter 2*), Gustafson *et al.* (2001), and Keogh *et al.* (2010), where GMP or caseinomacropeptide (CMP, the unglycosylated form of GMP) was tested, no difference in food energy intake at a subsequent test meal was found relative to a control preload (water, basal mixture, carbohydrate or whey protein). With respect to subjective measures of satiety, Gustafson *et al.* (2001), Keogh *et al.* (2010) and Veldhorst *et al.* (2009a) reported no change, whereas others (Burton-Freeman, 2008; Lam *et al.*, 2009, *see Chapter 2*) did find differences. In a study by Burton-Freeman (2008), ratings of satiety were greater after consumption of whey-containing milkshakes (whey protein with and without GMP) compared with a milk-based mixture and a GMP (0.8 g) milkshake in ten women, but this change in satiety

ratings was not seen in nine men. Consumption of a beverage containing beta-lactoglobulin (major component of whey protein) was found to increase feelings of fullness in comparison with beverages containing either whey protein concentrate or GMP (*S. Poppitt, pers. comm.*). In our previous study (*Lam et al., 2009, see Chapter 2*), using nineteen men and thirty-one women, ratings of fullness increased immediately after ingestion of a preload enriched with whey protein isolate (WPI) containing naturally occurring GMP compared with three other preload drinks (maltodextrin carbohydrate, WPI without GMP, and a mixture of WPI with GMP and added GMP isolate).

The presence of GMP in whey protein confounds investigation of the satiating effect of whey protein, and the effect of GMP alone on satiety and food intake remains to be addressed. The objective was to compare the effects of preload drinks enriched with maltodextrin carbohydrate, WPI with minimal (2%) naturally occurring GMP, WPI with 21% naturally present GMP and GMP isolate (86% GMP) on measures of satiety in normal-weight adult women.

### **5.3. Subjects and methods**

#### **5.3.1. Subjects**

Healthy women aged 18 to 40 years were recruited by public advertisement. Smokers, trained athletes, pregnant or lactating women and women not consuming breakfast regularly were excluded from the study. Subjects were also excluded if their body weight had changed over the past 6 months, if they had a history of menstrual irregularities, a gastrointestinal disorder or were taking any medication known to affect gastrointestinal motility or appetite (e.g. steroids or anti-psychotic drugs). All subjects attended an information session about the study procedure, signed a consent form, had their height and weight measured to ensure that they met the body mass index (BMI) criteria (BMI range: 19-26 kg/m<sup>2</sup>), completed the Three Factor Eating Questionnaire to assess their dietary restraint, disinhibition and hunger

(*Stunkard & Messick, 1985*) and tasted the preload drinks and test meal. The Massey University Human Ethics Committee (Application no. 11/47) approved the study. Twenty-two women completed the study and were compensated for their participation. The mean age was  $23.8 \pm 0.9$  years and the mean BMI was  $23.1 \pm 0.4$  kg/m<sup>2</sup>. According to the Three Factor Eating Questionnaire (*Stunkard & Messick, 1985*), subjects scored low in restraint ( $8.9 \pm 1.0$ ), disinhibition ( $5.9 \pm 0.4$ ) and perceived hunger ( $4.5 \pm 0.7$ ). Five out of the twenty-two subjects were considered as restrained eaters, scoring at least 12 on the restraint subscale of the Three Factor Eating Questionnaire (*Lowe & Thomas, 2009; Stunkard & Messick, 1985*), but were included in the study as results did not differ between restrained and unrestrained participants.

### **5.3.2. Preloads and test meal**

Preloads (Table 5.1) were approximately isovolumetric (~280 ml) and isoenergetic (~1600 kJ) drinks that comprised a basal mixture of skim milk powder, sucrose, natural sweetener, vanilla flavour, yellow colouring and water and either maltodextrin carbohydrate (“carbohydrate”), whey protein isolate (WPI) containing 21% naturally occurring glycomacropeptide (“WPI-high GMP”), WPI containing minimal 2% glycomacropeptide (“WPI-low GMP”), or glycomacropeptide isolate containing 86% GMP (“GMP”). The remaining 14% of the glycomacropeptide isolate comprised of 0.4% fat, 6.2% ash, 5.9% moisture and 1.5% undetermined material. The “carbohydrate” preload drink mainly comprised of maltodextrin carbohydrate (89.5% of metabolisable energy) but also contained some protein (7.2% of metabolisable energy), while the “protein” preload drinks were high in protein (66-76% of metabolisable energy) but also contained some carbohydrate (22-31% of metabolisable energy). The WPI-high GMP, WPI-low GMP and GMP preloads contained 12.8, 1.5 and 51.4 g of GMP per 300 g serve, respectively. The preload drinks were freshly prepared one to three days prior to the study day and stored in a refrigerator. Preloads were thoroughly mixed prior to serving.

**Table 5.1.** Composition of the four preload drinks.

	Carbohydrate <sup>1</sup>	WPI-high GMP <sup>2</sup>	WPI-low GMP <sup>3</sup>	GMP <sup>4</sup>
<b>Ingredient (g per 300 g serve)</b>				
Skim milk powder	15	15	15	15
Sucrose	9	9	9	9
Natural sweetener (Stevia)	0.06	0.06	0.06	0.06
Vanilla flavour	1.5	1.5	1.5	1.5
Yellow colour	0.09	0.09	0.09	0.09
Maltodextrin carbohydrate <sup>1</sup>	60	-	-	-
Whey Protein Isolate-high Glycomacropeptide <sup>2</sup>	-	60	-	-
Whey Protein Isolate-low Glycomacropeptide <sup>3</sup>	-	-	60	-
Glycomacropeptide isolate <sup>4</sup>	-	-	-	60
Water	214.35	214.35	214.35	214.35

<b>Nutrient (per 300 g serve)</b>				
Metabolisable energy (ME) <sup>5</sup> (kJ)	1650	1623	1558	1599
<b>Fat</b>				
(g)	1.4	1.2	1.0	1.3
(% of ME)	3.3	2.9	2.5	3.1
<b>Carbohydrate</b>				
(g)	88.4	22.8	20.4	29.3
(% of ME)	89.5	23.5	21.8	30.6
<b>Protein</b>				
(g)	7.2	71.6	70.6	63.5
(% of ME)	7.2	73.6	75.7	66.4

<sup>1</sup> Avondex 10, New Zealand Starch Ltd., Auckland, New Zealand

<sup>2</sup> Whey protein isolate containing 21% glycomacropeptide (WPI 894, Fonterra Co-operative Group Ltd., Palmerston North, New Zealand).

<sup>3</sup> Whey protein isolate containing 2% glycomacropeptide (WPI 895, Fonterra Co-operative Group Ltd., Palmerston North, New Zealand).

<sup>4</sup> Glycomacropeptide isolate containing 86% glycomacropeptide (BioPURE-GMP™, Davisco Foods International Inc., Minnesota, United States)

<sup>5</sup> Metabolisable energy (ME) calculated from the energy-producing food components using the energy conversion factors of 16.7 kJ/g for protein and available carbohydrate and 37.7 kJ/g for fat

Subjects received a hot lunchtime test meal of chicken fried rice provided in excess of the amount to be consumed (*ad libitum*) 120 min after ingestion of the preload drinks, as detailed previously (*Chungchunlam et al., 2012, see Chapter 3*). The food provided at the test meal contained 910 kJ metabolisable energy (ME), 29.5 g available carbohydrate, 9.7 g crude protein, 6.4 g crude fat and 2.1 g total dietary fibre per 100 g. Water was available with the meal.

### **5.3.3. Experimental procedure**

The study was a single-blind, completely randomised block design. Women participated during the menstrual period and follicular phase of their menstrual cycles and were tested in four sessions with a minimum of two days in between each session. On the day prior to each study session, subjects were requested to abstain from alcohol and to consume only water from 2200 h onwards. On the study day, subjects consumed a subject-specific breakfast that provided on average 1326 kJ of metabolisable energy (ME) with 58.4% of the ME derived from available carbohydrate, 11.3% from protein and 30.3% from fat. The breakfast meal consisted of toasted wholemeal bread with margarine and/or strawberry jam and coffee or tea with sugar and milk. Subjects were instructed to consume their breakfast at least 3 h before the test session appointment, followed only by water as desired.

On each study day, participants reported to the laboratory between 1200 and 1300 h and filled out a questionnaire to check for compliance with the eating and drinking restrictions on the evening before and the morning. Subjects who had not complied with the experimental protocol were scheduled for another test day. Each subject completed a questionnaire for her baseline assessment of appetite (hunger, desire to eat, prospective food consumption, fullness) and nausea using 10-cm visual analogue scales (VAS) (*Flint et al., 2000*). Subjects were provided with one of the four preload drinks (~280 ml)

immediately followed by 50 ml of water, all to be consumed within 5 min. After complete ingestion of the preload and water (0 min), subjects filled in a questionnaire assessing the palatability of the preloads, appetite and nausea. Subjects rated the palatability of the preloads on a 10-cm VAS for likeability, pleasantness of taste, likeability of texture and sweetness. Subsequent appetite and nausea ratings were completed at 15, 30, 45, 60, 75, 90 and 120 min after preload ingestion. After the 120 min VAS rating, subjects were seated in individual cubicles and offered the lunchtime test meal and water to be consumed within 15 min. Subjects were instructed to consume as much or as little as they desired and additional water was provided if requested. The lunchtime meal and water were weighed before and after the *ad libitum* meal using an electronic scale to determine the amounts consumed to the nearest 0.01 g. After the test meal and water were taken away, subjects rated the likeability of the test meal with the use of a 10-cm VAS. Appetite and nausea ratings were taken at 15 and 30 min after consumption of the lunch meal. This protocol has been described in full previously (*Chungchunlam et al., 2012, see Chapter 3*).

#### **5.3.4. Statistical analysis**

All statistical analyses were performed using the SAS software program for WINDOWS, version 9.2 (SAS Institute Inc., Cary, NC, USA). The design of the study was a single-blind randomised factorial design with subject as the blocking variable (*Ott & Longnecker, 2010*). According to a power analysis for a randomised block design (*Kastenbaum et al., 1970*) based on a previous study (*see Chapter 3*) using a similar experimental procedure with women subjects, a sample size of thirteen subjects is sufficient to detect a difference of 792 kJ in *ad libitum* test meal intake with a power of 0.80 at a significance level of 0.05. Primary analyses were undertaken to assess the normality of the distributions of data and to test for the presence of statistical outliers. VAS ratings for palatability of the preload and test meal (cm), test meal and water intake (g) and energy intake of test meal (kJ ME) were

analysed using an analysis of variance (ANOVA) with preload and subject as factors. The effect of study session days on test meal intake (kJ ME) was tested using a one-way ANOVA.

For the ratings of hunger, desire to eat, prospective consumption, fullness and nausea, a repeated-measures ANOVA was performed to examine the effect of preload and time of rating and their interaction (preload x time). If a significant interaction was found, this effect was further examined using the least squares means of the preload and time of rating combinations. When the interaction was not significant ( $p>0.05$ ), the main effects of preload and time of rating were examined using the least squares means of the preload and time of rating separately. Both procedures were conducted via Tukey's multiple comparison tests. The VAS ratings were further analysed by adjusting post-preload scores (0 to 120 min) for baseline measurements, by fitting them as covariates in the model. The nausea data were found to have a skewed distribution and were log transformed. The net incremental (area above and below the baseline rating) area under the curve (AUC) (*Gannon et al., 1989*) following consumption of the preload drinks (0 to 120 min) was calculated using the trapezoid rule and analysed for an effect of preload using a one-way ANOVA.

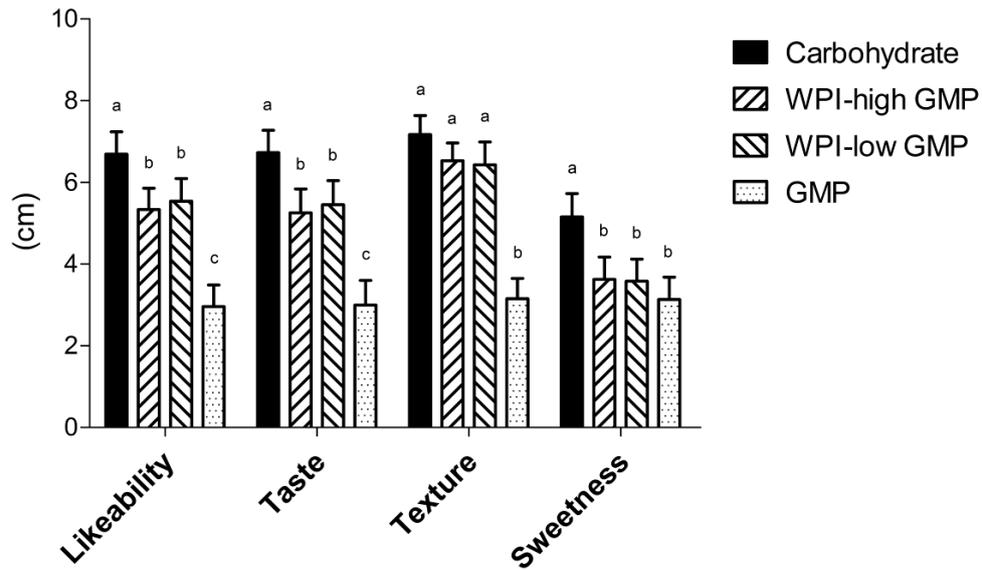
To examine the relationship between energy intake at the test meal (kJ ME) and palatability of the preload as well as ratings of appetite and nausea from 0 to 120 min, Pearson's correlation analysis was performed. Results were considered statistically significant at a  $p$ -value of less than 0.05. Duncan's multiple range test procedure was applied for multiple pairwise treatment comparisons. Results are reported as means and standard errors (mean  $\pm$  sem).

## 5.4. Results

### 5.4.1. Palatability of the preloads and test meal

When palatability was ascertained immediately after consumption of the preload drinks, there were significant differences in ratings of likeability ( $p < 0.0001$ ), pleasantness of taste ( $p < 0.0001$ ), likeability of texture ( $p < 0.0001$ ) and sweetness ( $p = 0.0003$ ) among the preload drinks (Figure 5.1.). The carbohydrate preload drink was rated as being more liked and pleasant in taste than the three other preload drinks. Moreover, overall likeability and pleasantness of taste of the two whey containing preload drinks were greater than that of the GMP preload drink. The texture of the GMP preload drink was the least liked compared with the perceived textures of the three other preload drinks. Subjects perceived the carbohydrate preload drink as being sweeter than the protein containing preload drinks.

The palatability of the lunchtime test meal was not significantly affected by preload type with subjects rating the test meal overall as being well liked ( $7.7 \pm 0.2$  cm,  $p > 0.05$ ).



**Figure 5.1.** VAS ratings of overall likeability, pleasantness of taste and likeability of texture and overall sweetness for the four preload drinks (Values are means  $\pm$  sem,  $n = 22$ ).

There was a significant effect of preload drink ( $p < 0.05$ ). Means in a column with different superscript letters were significantly different (Duncan's test,  $p < 0.05$ ).

#### 5.4.2. *Ad libitum* food intake at lunch

The intake of water at the lunchtime meal did not differ significantly by preload drink ( $p>0.05$ ) (Table 5.2.).

There was no effect of the study session day on test meal energy intake consumed at lunch ( $p>0.05$ ). Preload drink had a significant effect on the amount (g) and energy intake (kJ ME) of the test meal consumed *ad libitum* ( $p=0.0003$ ) (Table 5.2). *Ad libitum* energy intake at lunch was significantly higher when the carbohydrate and GMP preload drinks were consumed compared with the two whey protein-containing preloads. The mean food intakes ranked (numerically): GMP > Carbohydrate > WPI-high GMP > WPI-low GMP, consistent with WPI affecting satiety.

There were significant differences between preload drinks in total energy intake (the addition of energy intake from the preload drink and test meal) ( $p=0.0002$ ) (Table 5.2.). Following consumption of the carbohydrate and GMP preloads, total energy intake was significantly greater than following ingestion of the two whey protein preloads.

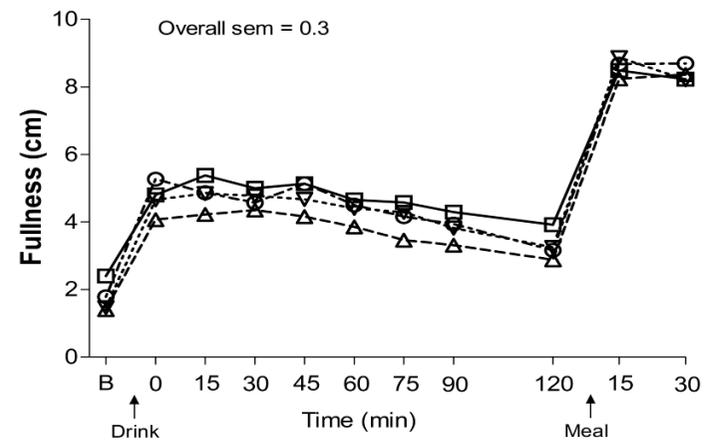
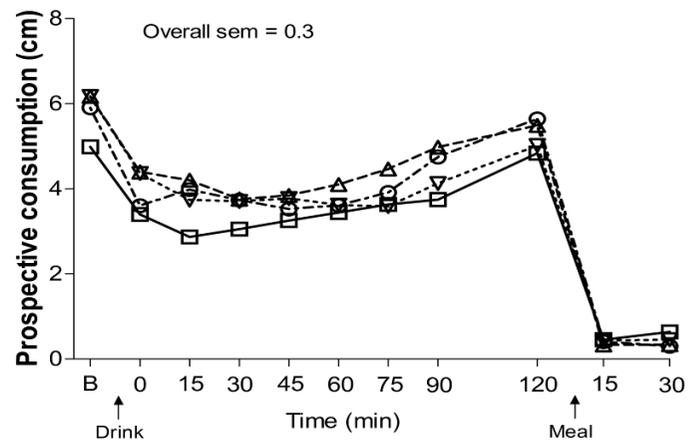
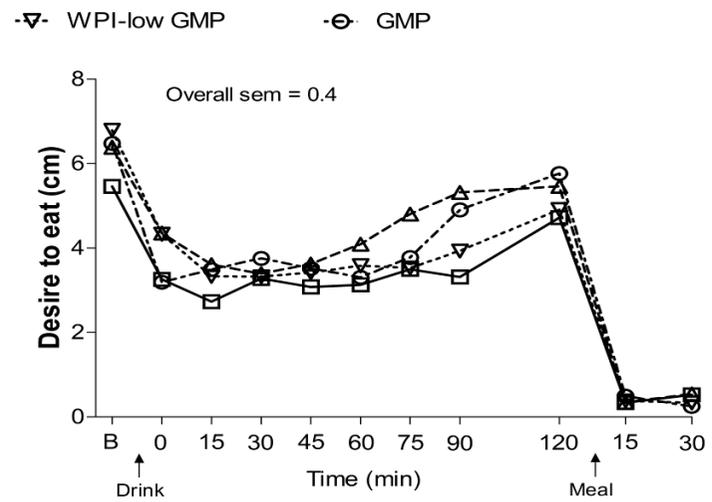
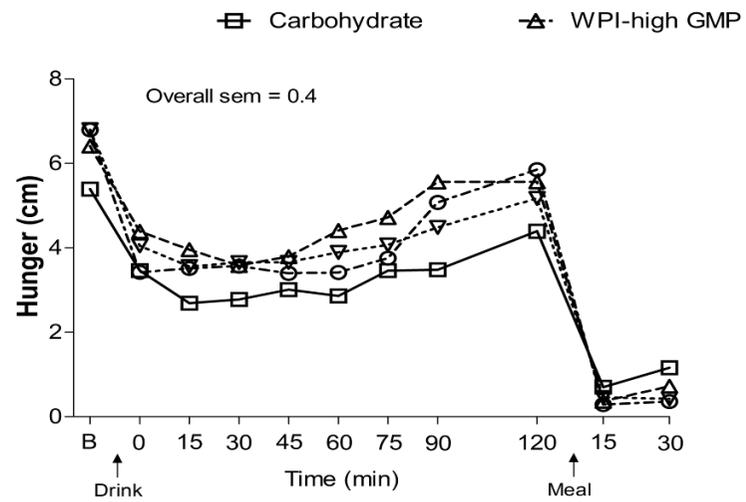
**Table 5.2.** Water intake, *ad libitum* test meal food (g) and energy (kJ ME) intakes and total (preload + test meal) energy intake after consumption of preloads containing carbohydrate, whey protein isolate with high glycomacropeptide (WPI-high GMP), whey protein isolate with low glycomacropeptide (WPI-low GMP) and glycomacropeptide isolate (GMP).

	<b>Carbohydrate</b>	<b>WPI-high GMP</b>	<b>WPI-low GMP</b>	<b>GMP</b>	Overall sem	<i>p</i> -value
Water intake (g)	381.3	454.7	441.9	407.0	21.2	0.06
Test meal food intake (g)	338.9 <sup>a</sup>	290.2 <sup>b</sup>	273.6 <sup>b</sup>	348.3 <sup>a</sup>	13.5	0.0003
Test meal energy intake (kJ)	3084.2 <sup>a</sup>	2640.4 <sup>b</sup>	2490.0 <sup>b</sup>	3169.6 <sup>a</sup>	123.1	0.0003
Total energy intake (kJ)	4606.7 <sup>a</sup>	4121.9 <sup>b</sup>	3996.9 <sup>b</sup>	4689.2 <sup>a</sup>	123.2	0.0002

Means in a row with different superscript letters were significantly different (Duncan's test,  $p < 0.05$ )

### 5.4.3. Subjective ratings of appetite and nausea

There was no significant interaction between preload and time ( $p>0.05$ ) for the VAS ratings of hunger, desire to eat, prospective consumption and fullness (Figure 5.2.). There was a significant main effect of time ( $p<0.0001$ ) for each rating of appetite, but no main effect of preload was observed ( $p>0.05$ ). Analysis of the ratings following consumption of the preload drinks (from 0 to 120 min), adjusted for the baseline rating, revealed no significant interaction between preload and time ( $p>0.05$ ) for the ratings of hunger, prospective consumption and fullness. There was no main effect of preload ( $p>0.05$ ) but there was a main effect of time ( $p<0.0001$ ) for these ratings. A significant interaction between preload and time was found for desire to eat ( $p=0.0184$ ). Desire to eat ratings were significantly lower 90 min following consumption of the carbohydrate preload ( $3.3 \pm 0.6$  cm) compared with the GMP ( $4.9 \pm 0.6$  cm,  $p=0.0362$ ) and WPI-high GMP ( $5.3 \pm 0.6$  cm,  $p=0.0006$ ) preloads. There was no significant effect of preload on ratings of nausea ( $p>0.05$ ). When the subjective ratings of appetite and nausea were expressed as net incremental AUC over the 120 min period, no significant main effect of preload was found ( $p>0.05$ ) (Table 5.3.).



**Figure 5.2.** Mean subjective VAS ratings of hunger, desire to eat, prospective consumption, and fullness in response to ingestion of preload drinks containing carbohydrate, whey protein isolate with high glycomacropeptide (WPI-high GMP), whey protein isolate with low glycomacropeptide (WPI-low GMP) and glycomacropeptide isolate (GMP).

The preload drink ingestion (0 to 120 min) was preceded by a baseline (B) measurement. Ratings were also completed 15 and 30 min following consumption of the *ad libitum* lunchtime test meal. There was no significant preload by time interaction ( $p>0.05$ ) and no significant effect of preload drink ( $p>0.05$ ), but an effect of time was found ( $p<0.0001$ ) for each measure.

**Table 5.3.** Subjective ratings of appetite and nausea expressed as net incremental area under the curve (Net iAUC) following ingestion of preloads containing carbohydrate, whey protein isolate with high glycomacropeptide (WPI-high GMP), whey protein isolate with low glycomacropeptide (WPI-low GMP) and glycomacropeptide isolate (GMP).

<b>Net iAUC (cm,120min)</b>	<b>Carbohydrate</b>	<b>WPI-high GMP</b>	<b>WPI-low GMP</b>	<b>GMP</b>	<b>Overall sem</b>	<b><i>p</i>-value</b>
Hunger	-252.3	-221.2	-324.9	-323.1	44.9	0.27
Desire to eat	-247.5	-240.0	-362.6	-288.8	50.3	0.29
Prospective consumption	-170.3	-208.5	-263.5	-209.0	41.1	0.46
Fullness	272.8	279.6	338.6	309.1	47.6	0.75
Nausea	-31.8	49.8	64.9	-0.3	33.2	0.13

#### 5.4.4. Correlation analyses

Pearson's correlation analysis of the relationship between energy intake at the *ad libitum* test meal and subjective ratings of appetite following consumption of the preload drinks (from 0 to 120 min) showed a significant but weak positive correlation with overall hunger ( $r = 0.25$ ,  $p < 0.0001$ ), desire to eat ( $r = 0.28$ ,  $p < 0.0001$ ) and prospective food consumption ( $r = 0.38$ ,  $p < 0.0001$ ). This significant positive correlation was also observed 120 min following ingestion of the preload drinks and immediately preceding the consumption of the lunchtime test meal for hunger ( $r = 0.30$ ,  $p = 0.0047$ ), desire to eat ( $r = 0.36$ ,  $p = 0.0004$ ) and prospective food consumption ( $r = 0.46$ ,  $p < 0.0001$ ). A significant but weak negative correlation was found between test meal energy intake and overall ratings of fullness ( $r = -0.34$ ,  $p < 0.0001$ ) as well as fullness ratings at 120 min ( $r = -0.36$ ,  $p = 0.0005$ ). There was no significant relationship between test meal energy intake and ratings of nausea ( $p > 0.05$ ).

There was no statistically significant correlation found between test meal energy intake and the VAS-rated scores for likeability, pleasantness of taste, likeability of texture or sweetness of the preload drinks ( $p > 0.05$ ). The palatability scores of the preload drinks and post-drink nausea ratings (from 0 to 120 min) were not correlated ( $p > 0.05$ ). Although the palatability ratings differed among preload drinks, none of these differences were related to subsequent *ad libitum* food intake or ratings of nausea in the subjects.

### 5.5. Discussion

The study showed that consumption of a preload drink enriched with whey protein (WPI-high GMP and WPI-low GMP) suppressed energy intake at a subsequent *ad libitum* test meal, compared to either a preload drink containing GMP or a maltodextrin carbohydrate-enriched preload drink baseline control. There was no difference in food energy intake at the subsequent test meal between the GMP- and carbohydrate-enriched preload drinks. Consumption of the two whey protein-enriched preload drinks also

resulted in lower total energy intakes (preload + test meal energy intake) in comparison with the carbohydrate- and GMP-enriched preload drinks. The VAS ratings of appetite, however, did not differ significantly between preload drinks.

The finding that whey protein led to a suppressed food intake at an *ad libitum* test meal compared with a carbohydrate-enriched preload is in agreement with several other studies (*Bellissimo et al., 2007; Bertenshaw et al., 2008; Chungchunlam et al., 2012, see Chapter 3*), though not all studies have shown an effect of whey protein versus carbohydrate (*Burton-Freeman, 2008; Keogh et al., 2010*). The finding that whey protein resulted in a reduced subsequent food energy intake compared to GMP, rather than supporting similar food intakes, is novel. Results from a previous study (*Lam et al., 2009, see Chapter 2*) showed that *ad libitum* energy and macronutrient intakes 30 min after consumption of the preload drinks did not differ. The preload drinks (300 ml, 1200 kJ) contained either 40 g of maltodextrin carbohydrate, WPI without GMP, WPI with 21% naturally present GMP (8.4 g GMP) or a combination of 20 g of WPI with GMP and 20 g of GMP isolate (24.2 g GMP). *Burton-Freeman (2008)* found that energy intake at a meal 75 min later was similar following ingestion of a milkshake (300 ml, 1000 kJ) containing no added protein, 27 g of whey protein with GMP, 29 g of whey protein without GMP or 0.8 g of GMP in ten women and nine men (BMI range between 22 and 27 kg/m<sup>2</sup>). Similarly, *Gustafson et al. (2001)* also reported that CMP, at doses of 0.4 and 2.0 g in a beverage (100 ml, 0.034 kJ), had no effect on energy intake 60 min later in twenty men and thirty-two women. It has been suggested that the dosage level of CMP or GMP and glycosylated forms of CMP may have an effect on subsequent food intake. However, *Keogh et al. (2010)* observed no difference in energy intake at a test meal 180 min later in twenty overweight men (BMI > 25 kg/m<sup>2</sup>) who consumed preloads (895 kJ) containing either glucose, GMP-depleted whey protein concentrate (44.4 g protein), glycosylated GMP (42.3 g protein) or minimally glycosylated GMP (41.3 g protein). The finding that whey protein was more satiating compared to GMP, which is in contrast to the findings of others, may have been related to the higher dose of GMP (51.4 g) in the preload drink (~1600 kJ, 63.5 g protein)

and the time interval (120 min) used in the presently-reported study. Nonetheless, Veldhorst *et al.* (2009a) showed that GMP as a component of whey protein (amount of GMP present not reported) in a custard breakfast led to a lower *ad libitum* energy intake 180 min later compared with a breakfast containing whey protein without GMP fraction, regardless of the energy derived from protein (10 and 25%). It seems that GMP alone may not play an important role in satiety and the satiating effect of whey protein may not be influenced by the presence or absence of GMP at a high protein level (> 40% of energy).

There is some evidence of greater satiety and fullness ratings following consumption of whey protein, but not GMP alone (*Burton-Freeman, 2008; Lam et al., 2009, see Chapter 2*). However, the present observation that subjective VAS-rated direct measures of satiety did not differ between the preload drinks containing either whey protein or GMP has also been found in previous studies (*Gustafson et al., 2001; Keogh et al., 2010; Veldhorst et al., 2009a*). Nonetheless, and independent of the preload drink consumed, hunger, desire to eat and prospective food consumption scores were positively correlated with energy intake at the *ad libitum* test meal, while fullness was negatively related to test meal energy intake. The variation between subjects in response to the appetite scales may have been too high to allow any statistically significant differences among the preload drinks for the subjective measures of appetite to be detected, despite the differences observed in subsequent food intake.

The effect of perceived sensory differences between preload drinks may mask the potential effect of the carbohydrate or type of protein under investigation. Subjects perceived the preload drinks as being different in likeability, pleasantness of taste, likeability of texture and sweetness. As reviewed previously (*Bellisle et al., 2012; Rolls, 1991; Sorensen et al., 2012*), some studies suggest that sweeter preload beverages increase subsequent food intake and ratings of hunger more than water or less sweet preload drinks (*King et al., 1999; Rogers et al., 1988*), but others did not find an effect (*Black et al., 1991; Rodin, 1990; Rolls et al., 1990*). In the current study, the carbohydrate

beverage was rated as extremely sweet compared to the other three beverages. However, there was no statistically significant correlation between the palatability of the preload drinks and subsequent food intake, suggesting that the differences in the perception of the sensory properties of the preloads were not related to subsequent food intake.

A potential limitation of our study was the lack of a preload containing a whey protein completely devoid of GMP and a small difference in the composition of the whey products studied. Although the WPI-low GMP powder was anticipated to contain no GMP, protein analysis using reverse-phase high performance liquid chromatography showed that the GMP content in the WPI was 2%, most likely arising from contamination during processing. It is unlikely, that this small amount of GMP (1.5 vs. 12.8 g per 300 serve in the WPI-low GMP vs. WPI-high GMP preloads) would have contributed significantly to the suppressive effect of the whey protein-enriched preload drinks on subsequent food intake. The whey protein powders used here were also composed of beta-lactoglobulin (WPI-high GMP: 43% and WPI-low GMP: 64%) and alpha-lactalbumin (WPI-high GMP: 13% and WPI-low GMP: 16%). Studies investigating the effects of these components of whey protein on satiety are very sparse. An animal study showed that beta-lactoglobulin, the major whey protein component, induced a lower cumulative feed intake in rats compared with whey and whole milk proteins (*Pichon et al., 2008*). The satiating effects of isolated beta-lactoglobulin compared with whey protein in humans remains to be investigated. However, isolated alpha-lactalbumin was found to reduce subsequent food energy intake more than whey protein in human subjects (*Veldhorst et al., 2009b*). Further research will focus on the composition of whey protein and whether different characteristics of whey protein (beta-lactoglobulin, alpha-lactalbumin, free amino acid mixture simulating the amino acid composition of whey protein) influence satiety differently.

In summary, ingestion of a preload containing whey protein with high or low amounts of GMP suppressed food energy intake at an *ad libitum* test meal compared to GMP alone. Total energy intake (preload + test meal) was

also lower following consumption of the whey protein-enriched preloads than following ingestion of the maltodextrin carbohydrate- and GMP-enriched preloads. Subjective measures of satiety, however, did not differ between preloads. GMP alone does not reduce subsequent food intake in comparison to carbohydrate, and the presence of GMP does not appear to play an important role in the observed satiating effect of whey protein.

## 5.6. Literature cited

- Anderson, G. H., & Moore, S. E. (2004). Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr*, *134*(4), 974S–979S.
- Bellisle, F., Drewnowski, A., Anderson, G. H., Westerterp-Plantenga, M., & Martin, C. K. (2012). Sweetness, satiation, and satiety. *J Nutr*, *142*(6), 1149S–1154S.
- Bellissimo, N., Desantadina, M. V., Pencharz, P. B., Berall, G. B., Thomas, S. G., & Anderson, G. H. (2007). A comparison of short-term appetite and energy intakes in normal weight and obese boys following glucose and whey-protein drinks. *Int J Obes*, *32*(2), 362–371.
- Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiol Behav*, *93*(3), 427–436.
- Beucher, S., Levenez, F., Yvon, M., & Corring, T. (1994). Effects of gastric digestive products from casein on CCK release by intestinal cells in rat. *J Nutr Biochem*, *5*(12), 578–584.
- Black, R. M., Tanaka, P., Leiter, L. A., & Anderson, G. H. (1991). Soft drinks with aspartame: effect on subjective hunger, food selection, and food intake of young adult males. *Physiol Behav*, *49*(4), 803–810.
- Burton-Freeman, B. M. (2008). Glycomacropeptide (GMP) is not critical to whey-induced satiety, but may have a unique role in energy intake regulation through cholecystokinin (CCK). *Physiol Behav*, *93*(1-2), 379–387.

- Chungchunlam, S. M. S., Moughan, P. J., Henare, S. J., & Ganesh, S. (2012). Effect of time of consumption of preloads on measures of satiety in healthy normal weight women. *Appetite*, *59*(2), 281–288. (see Chapter 3)
- Eisenstein, J., Roberts, S. B., Dallal, G., & Saltzman, E. (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), 189–200.
- Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord*, *24*(1), 38–48.
- Gannon, M. C., Nuttall, F. Q., Westphal, S. A., Neil, B. J., & Seaquist, E. R. (1989). Effects of dose of ingested glucose on plasma metabolite and hormone responses in type II diabetic subjects. *Diabetes Care*, *12*(8), 544–552.
- Gustafson, D. R., McMahon, D. J., Morrey, J., & Nan, R. (2001). Appetite is not influenced by a unique milk peptide: caseinomacropeptide (CMP). *Appetite*, *36*(2), 157–163.
- Halton, T. L., & 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.
- Kastenbaum, M. A., Hoel, D. G., & Brown, K. O. (1970). Sample size requirements: randomized block designs. *Biometrika*, *57*(3), 573–577.
- Keogh, J. B., Woonton, B. W., Taylor, C. M., Janakievski, F., Desilva, K., & Clifton, P. M. (2010). Effect of glycomacropeptide fractions on cholecystokinin and food intake. *Br J Nutr*, *104*(2), 286–290.
- King, N. A., Appleton, K., Rogers, P. J., & Blundell, J. E. (1999). Effects of sweetness and energy in drinks on food intake following exercise. *Physiol Behav*, *66*(2), 375–379.
- Lam, S. M., Moughan, P. J., Awati, A., & Morton, H. R. (2009). The influence of whey protein and glycomacropeptide on satiety in adult humans. *Physiol Behav*, *96*(1), 162–168. (see Chapter 2)
- Lowe, M. R., & Thomas, J. G. (2009). Measures of restrained eating. In : D. B. Allison, & M. L. Baskin (Eds.), *Handbook of assessment methods for*

*eating behaviors and weight-related problems: measures, theory, and research* (pp. 137–185). CA, Sage: Thousand Oaks.

- Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr*, 26(6), 704S–712S.
- Mattes, R. D., & Popkin, B. M. (2009). Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. *Am J Clin Nutr*, 89(1), 1–14.
- Ott, L., & Longnecker, M. (2010). *An introduction to statistical methods and data analysis*. 6th ed. Belmont, CA: Brooks/Cole Cengage Learning.
- Pichon, L., Potier, M., Tome, D., Mikogami, T., Laplaize, B., Martin-Rouas, C., & Fromentin, G. High-protein diets containing different milk protein fractions differently influence energy intake and adiposity in the rat. *Br J Nutr*, 99(4), 739–748.
- Rodin, J. (1990). Comparative effects of fructose, aspartame, glucose, and water preloads on calorie and macronutrient intake. *Am J Clin Nutr*, 51(3), 428–435.
- Rogers, P. J., Carlyle, J. A., Hill, A. J., & Blundell, J. E. (1988). Uncoupling sweet taste and calories: comparison of the effects of glucose and three intense sweeteners on hunger and food intake. *Physiol Behav*, 43(5), 547–552.
- Rolls, B. J. (1991). Effects of intense sweeteners on hunger, food intake, and body weight: a review. *Am J Clin Nutr*, 53(4), 872–878.
- Rolls, B. J., Kim, S., & Fedoroff, I. C. (1990). Effects of drinks sweetened with sucrose or aspartame on hunger, thirst and food intake in men. *Physiol Behav*, 48(1), 19–26.
- Sorensen, L. B., Moller, P., Flint, A., Martens, M., & Raben, A. (2012). Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. *Int J Nutr*, 27(10), 1152–1166.
- Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*, 29(1), 71–83.
- Van Kleef, E., Van Trijp, J. C. M., Van Den Borne, J. J. G. C., & Zondervan, C. (2012). Successful development of satiety enhancing food products:

- towards a multidisciplinary agenda of research challenges. *Crit Rev Food Sci Nutr*, 52(7), 611–628.
- Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N., & Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. *Physiol Behav*, 94(2), 300–307.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009a). Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety. *Appetite*, 52(2), 388–395.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009b). A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. *Clin Nutr*, 28(2), 147–155.
- Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006) *Dairy science and technology*. 2nd ed. Boca Raton: CRC/Taylor & Francis.
- Westerterp-Plantenga, M. S., Nieuwenhuizen, A. Tome, D., Soenen, S., & Westerterp, K. R. (2009). Dietary protein, weight loss, and weight maintenance. *Annu Rev Nutr*, 29(7), 21–41.
- Yvon, M., Beucher, S., Guilloteau, P., Le Hueron-Luron, I., & Corring, T. (1994). Effects of caseinomacropptide (CMP) on digestion regulation. *Reprod Nutr Dev*, 34(6), 527–537.

## Chapter 6

### **Amino acid content of a whey protein isolate: determined using multiple hydrolysis times**

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Whey protein isolate with naturally present glycomacropeptide has been shown to induce satiety in adults in comparison with carbohydrate (*Chapters 2, 3 & 5*), and this effect may be mediated at least in part via an observed rapid increase in plasma amino acids after ingestion of whey (*Chapter 4*). In a further study (see *Chapter 7*), an intact whey protein was compared to its equivalent amino acid mixture given in the free form, in an attempt to assess if the amino acid composition *per se* is the basis for the enhanced satiety. Such a comparison requires that the amino acid composition of whey protein be known with accuracy. The objective of the study reported here was to determine the amino acid composition of a whey protein isolate with accuracy.

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## 6.1. Abstract

The aim was to determine with accuracy the amino acid composition of a whey protein isolate (WPI) with naturally present glycomacropeptide. This WPI was to be used in a subsequent study to determine if a reported satiating effect of the protein was due to the amino acid composition of whey protein *per se* or to other factors intrinsic to intact whey protein. The WPI powder was hydrolysed at multiple time intervals (0–144 h) and a least-squares nonlinear regression model applied to predict actual amino acid content as well as hydrolysis and loss rates for each amino acid. The predicted amino acid contents ( $A_0$ ) determined using this method were compared with mean standard 24-h (20-h for tryptophan) hydrolysis values. The 24-h hydrolysis method was shown to be suitable for the analysis of approximately one-half of the amino acids, as results similar to those obtained using the more accurate multiple hydrolysis model were observed. Aspartic acid, glutamic acid, histidine and methionine contents were overestimated by 3.5, 1.6, 2.1 and 2.5%, respectively whereas glycine, proline, serine and threonine were underestimated by 6.9, 4.8, 10.3 and 4.9%, respectively, by the 24-h hydrolysis method. The predicted (multiple hydrolysis times model) tryptophan content ( $A_0$ ) was found to be 23.1% greater than the equivalent 20-h hydrolysis value. The amino acid composition of whey protein isolate with naturally present glycomacropeptide, based on the more accurate multiple hydrolysis interval method, is reported.

## 6.2. Introduction

Dietary protein is considered to be the most satiating macronutrient (Anderson & Moore, 2004; Eisenstein *et al.*, 2002; Halton & Hu, 2004) and dairy whey protein has been shown to elicit a stronger effect on satiety compared to other protein sources (Anderson *et al.*, 2004; Hall *et al.*, 2003; Luhovyy *et al.*, 2007; Veldhorst *et al.*, 2008, 2009a). In our previous studies (Lam *et al.*, 2009, *see Chapter 2*; Chungchunlam *et al.*, 2012, *see Chapter 3*),

ingestion of whey protein with naturally present glycomacropeptide resulted in subjects consuming a lower energy intake at a subsequent meal compared with a maltodextrin carbohydrate control. The difference in satiety may be mediated by higher plasma levels of certain satiety-related hormones and amino acids (*data reported in Chapter 4*). The amino acid composition of whey protein *per se* may assist to explain the higher satiating property of whey protein with glycomacropeptide, and to test this it is important to know the amino acid composition of whey protein with accuracy.

The standard method used for the determination of amino acids (excluding tryptophan, cysteine, methionine, asparagine and glutamine) involves incubation of the protein in concentrated hydrochloric acid (6 M HCL) in an oxygen-free environment at 110°C for 24 h (*Moore & Stein, 1948, 1951*). During acid hydrolysis, the amide amino acids, asparagine and glutamine, are converted to their acid derivatives, aspartic and glutamic acids, respectively. As a result, the determined contents of aspartic acid and glutamic acid represent the sum of the respective acid and amide derivatives. Cysteine and methionine, referred to as the sulphur amino acids, are commonly oxidised to cysteic acid and methionine sulphone, respectively, prior to hydrochloric acid hydrolysis of the protein. Tryptophan is usually determined following alkaline hydrolysis of the protein in a nitrogen environment for 20 h at 110°C. After hydrolysis of the sample protein, the amino acids are separated and quantified using high-performance liquid chromatography (HPLC).

The chemistry and stability of amino acids during acid hydrolysis differ among amino acids, and the 24-h analysis time (20-h for tryptophan; *AOAC, 2008*) represents a compromise relative to the optimal hydrolysis time across amino acids leading to the maximal release of most amino acids from the protein while minimising amino acid degradation. The hydrolysis and degradation of amino acids occur simultaneously during hydrolysis and this needs to be recognised when determining the amino acid composition of proteins. By determining the amino acid content using multiple hydrolysis times, and using a least-squares nonlinear regression (*Robel & Crane, 1972*) to model the relationship between amino acid yield and hydrolysis time, the

actual protein-bound amino acid content of a protein source can be estimated more accurately. This latter method is more accurate than the standard 24-h hydrochloric acid hydrolysis-based amino acid analysis method (*Darragh & Moughan, 2005*).

It was necessary to determine with accuracy the amino acid composition of whey protein isolate to be used in the subsequent study because of variation in protein and amino acid ratios due to seasonal factors (changes in pasture availability and quality, physiological changes associated with the stage of lactation and pathological changes associated with the incidence of mastitis (*Auld et al., 1998*)), and extraction methods for amino acid analysis. The objective was to determine accurately the amino acid composition of a whey protein isolate with naturally present glycomacropeptide using multiple hydrolysis times combined with least-squares nonlinear regression.

### **6.3. Materials and methods**

#### **6.3.1. Sample**

Spray-dried whey protein isolate (WPI) from cheese whey, WPI 894, was donated by Fonterra Co-operative Group Ltd (Palmerston North, New Zealand) and contained (w/w) approximately 85.1% protein, 5.5% moisture, 4% ash, 1% fat and 2% lactose. The glycomacropeptide content of the WPI was 21.3%.

#### **6.3.2. Tryptophan analysis**

All amino acid analyses were conducted in triplicate at each hydrolysis time. Tryptophan was determined using the method described by Rutherford and Gilani (2009) with the following modification. The protein was hydrolysed with 8 ml of 4.5 M sodium hydroxide (NaOH) containing 5% (w/v)

maltodextrin carbohydrate as an oxygen scavenger. The solutions were hydrolysed at 110°C for 0, 2, 4, 6, 10, 14, 20, 24, 52, 92, and 144 h. Amino acid separation and quantification was performed with a reverse-phase (C<sub>18</sub> column) HPLC system (Agilent Technologies, USA) and detection was carried out by absorbance at 280 nm. The concentration of tryptophan was calculated by comparing the peak area of tryptophan in the sample to the area in the calibration tryptophan standards (AOAC, 2008). The free tryptophan molecular weight was used to calculate the tryptophan weight.

### **6.3.3. Sulphur amino acid analysis**

The performic acid oxidation procedure of Moore (1963) was used to convert cysteine and methionine to their more stable derivatives, cysteic acid and methionine sulphone, respectively. In brief, a 3 mg sample was oxidised with 1 ml of performic acid and after oxidation, 150 ml of 48% hydrogen bromide (HBr) was added to remove excess performic acid. Once dried down *in vacuo*, the material underwent acid hydrolysis as described below.

### **6.3.4. Analysis of the ‘acid-stable’ amino acids**

Acid hydrolysis was conducted as described in the basic protocol of Rutherford and Gilani (2009). Briefly, samples were hydrolysed in 1 ml of 6 M glass-distilled hydrochloric acid (HCL) containing 0.1% (v/v) phenol. The hydrolysates were degassed *in vacuo* with the use of a vacuum pump and sealed by melting the narrowed neck of the sample tube. These tubes were then hydrolysed at 110°C for 0, 2, 4, 6, 10, 14, 19, 24, 52, 92, and 144 h. Amino acid analysis was conducted in triplicate at each hydrolysis time. After hydrolysis, the tubes were cracked open before being dried in a centrifugal concentrator (Savant SpeedVac SC250EXP, Thermo Fisher Scientific Inc., New Zealand). The residues were reconstituted in 67 mM sodium citrate buffer (pH 2.2) containing 0.1% (w/v) phenol. Amino acids (except proline) were separated and quantified using derivatisation with o-phthalaldehyde (OPA)

followed by reverse-phase (C<sub>18</sub> column) chromatography using HPLC (Agilent 1200SL, Agilent Technologies, USA). Amino acids were detected by fluorescence at the excitation and emission wavelengths of 230 and 450 nm, respectively. Norvaline was added as an internal standard. Cysteine and methionine were measured in a separate run on the HPLC instrument as cysteic acid and methionine sulphone, respectively. Proline, which is a secondary amino acid, was quantified after derivatisation with 9-fluorenylmethyl chloroformate (FMOC) (*Einarsson et al., 1983*) followed by reverse-phase chromatography. The derivatised proline was detected using fluorescence with excitation and emission wavelengths of 266 and 315 nm, respectively. The weight of each amino acid was calculated using free amino acid molecular weights.

### 6.3.5. Prediction of amino acid concentrations

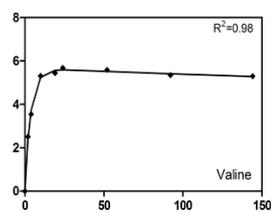
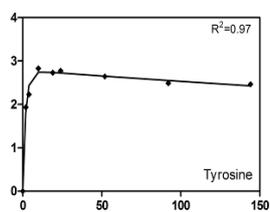
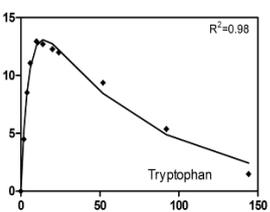
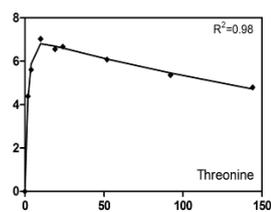
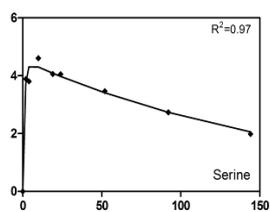
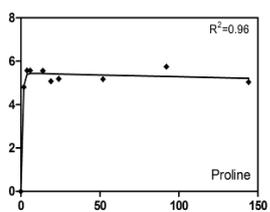
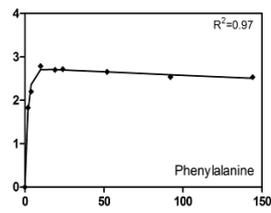
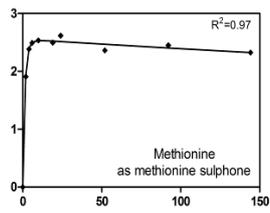
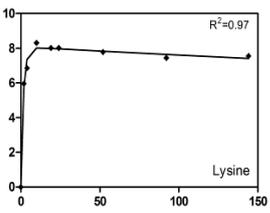
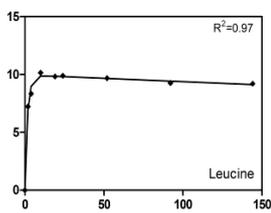
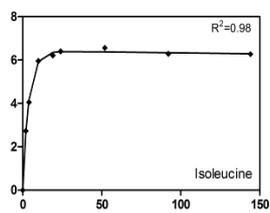
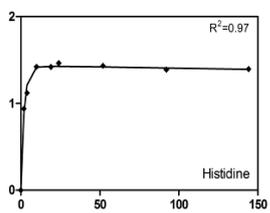
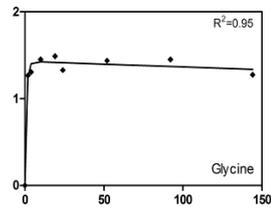
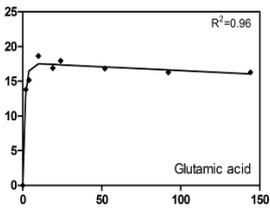
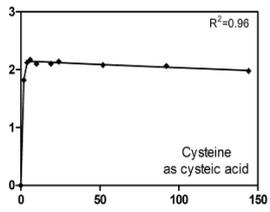
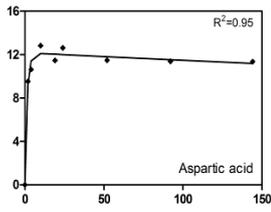
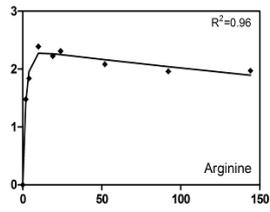
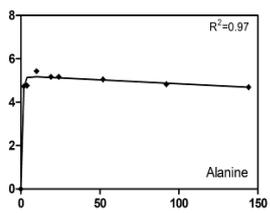
The determined amino acid concentration at each hydrolysis time was plotted against the hydrolysis time for each amino acid separately. A curve was fitted to each plot using the following equation (*Robel & Crane, 1972*):

$$B(t) = \frac{A_0 h (e^{-lt} - e^{-ht})}{h - l}$$

where  $B(t)$  is the amino acid concentration at hydrolysis time  $t$ ,  $h$  is the hydrolysis rate (the rate at which amino acids are released during hydrolysis),  $l$  is the loss rate (the rate at which amino acids are degraded after hydrolysis) and  $A_0$  is the amino acid content of the protein in the sample. A least-squares nonlinear regression (*Robel & Crane, 1972*) was used to predict  $A_0$ ,  $h$  and  $l$  for each amino acid with the constraints that  $A_0$  and  $h > 0$  (*Darragh et al., 1996*). Data were analysed using the R software package version 2.15.1 (<http://cran.r-project.org/>).

## 6.4. Results

The free amino acid content ( $B_0$ ) prior to hydrolysis (time 0 h) was negligible for each amino acid and therefore, the protein bound ( $A_0$ ) amino acid content was considered to be equal to the total (protein bound + free) amino acid content of the protein. The mean coefficient of variance between triplicates calculated over all hydrolysis times was 4.9% for the ‘acid-stable’ amino acids (excluding methionine), 6.1% for proline, 5.9% for cysteine (determined as cysteic acid) and methionine (determined as methionine sulphone), and 6.5% for tryptophan. The mean amino acid yield was plotted against hydrolysis time for each amino acid and these plots are shown in Figure 6.1. For all of the amino acids, the determined mean amino acid yield at each hydrolysis time correlated closely with the predicted amino acid yield based on the least-squares nonlinear regression model ( $R^2 = 0.95-0.98$ ), with an overall mean  $R^2$  of 0.97.



**Figure 6.1.** Effect of hydrolysis time (x-axis, h) on the concentration of amino acids (y-axis, g/100g) for whey protein isolate.

The line of best fit using least-squares nonlinear regression is plotted along with the determined mean amino acid contents (mean of triplicates) (◆).  $R^2$  values are given.

The estimated protein-bound amino acid content determined with the least-squares nonlinear regression model ( $A_0$ ) and the mean amino acid content after 24 h of hydrolysis for each amino acid are presented in Table 6.1. The values for alanine, arginine, cysteine (determined as cysteic acid), isoleucine, leucine, lysine, methionine (when determined without performic acid oxidation), phenylalanine, tyrosine and valine were within 1% of the equivalent 24-h hydrolysis values. The greatest difference was observed for tryptophan, which was underestimated by 23.1% after 20-h hydrolysis. Glycine, proline, serine and threonine were also underestimated by 6.9, 4.8, 10.3 and 4.9%, respectively. The standard 24-h hydrolysis method overestimated the amino acid content of aspartic acid (3.5%), glutamic acid (1.6%), histidine (2.1%) and methionine (determined as methionine sulphone) (2.5%). The hydrolysis rate ( $h$ ) and loss rate ( $l$ ) for each amino acid are given in Table 6.1. The hydrolysis rates ranged from 0.20 for tryptophan and 1.13 for alanine. When the loss rates were compared across all amino acids, the least stable amino acid was tryptophan (0.01361), followed by serine (0.00552) and threonine (0.00281). Interestingly, the loss rate for methionine (without performic acid oxidation) was similar to the loss rate for methionine sulphone (after performic acid oxidation prior to acid hydrolysis), where the determined loss rate for both methionine and methionine sulphone was 0.00065. The methionine content determined after performic acid oxidation was 15 and 17% higher than the equivalent value obtained using standard acid hydrolysis alone for the single 24-h acid hydrolysis and the multiple hydrolysis methods, respectively.

**Table 6.1.** The amino acid composition (g/100g powder on air-dry basis) of whey protein isolate estimated using a least-squares nonlinear regression model after multiple hydrolysis times ( $A_o$ ) (mean  $\pm$  sem), compared with the 24-h hydrolysis value (mean  $\pm$  sem) and estimated hydrolysis rate ( $h$ ) (amount of protein-bound amino acid released per hour) (mean  $\pm$  sem) and loss rate ( $l$ ) (amount of protein-bound amino acid destroyed per hour) for each amino acid (mean  $\pm$  sem)<sup>1</sup>.

	<b>24-h</b>	$A_o$	<b>Difference (%)<sup>2</sup></b>	$h$	$l$
Alanine	5.17 $\pm$ 0.05	5.21 $\pm$ 0.10	0.9	1.13 $\pm$ 0.19	0.00073 $\pm$ 0.00030
Arginine	2.31 $\pm$ 0.04	2.32 $\pm$ 0.05	0.5	0.47 $\pm$ 0.04	0.00143 $\pm$ 0.00035
Aspartic acid	12.62 $\pm$ 0.48	12.18 $\pm$ 0.31	-3.5	0.70 $\pm$ 0.09	0.00059 $\pm$ 0.00037
Cysteine <sup>3</sup>	2.14 $\pm$ 0.01	2.15 $\pm$ 0.04	0.6	0.95 $\pm$ 0.13	0.00056 $\pm$ 0.00028
Glutamic acid	17.94 $\pm$ 0.17	17.66 $\pm$ 0.39	-1.6	0.68 $\pm$ 0.08	0.00066 $\pm$ 0.00032
Glycine	1.33 $\pm$ 0.01	1.43 $\pm$ 0.04	6.9	1.02 $\pm$ 0.19	0.00046 $\pm$ 0.00036
Histidine	1.46 $\pm$ 0.01	1.43 $\pm$ 0.03	-2.1	0.47 $\pm$ 0.04	0.00020 $\pm$ 0.00029
Isoleucine	6.40 $\pm$ 0.06	6.41 $\pm$ 0.14	0.1	0.26 $\pm$ 0.02	0.00013 $\pm$ 0.00029
Leucine	9.89 $\pm$ 0.08	9.94 $\pm$ 0.20	0.5	0.58 $\pm$ 0.05	0.00060 $\pm$ 0.00030
Lysine	8.01 $\pm$ 0.20	8.07 $\pm$ 0.17	0.7	0.61 $\pm$ 0.06	0.00059 $\pm$ 0.00031
Methionine <sup>4</sup>	2.62 $\pm$ 0.02	2.55 $\pm$ 0.04	-2.6	0.69 $\pm$ 0.07	0.00065 $\pm$ 0.00027
Methionine <sup>5</sup>	2.16 $\pm$ 0.02	2.16 $\pm$ 0.04	-0.2	0.95 $\pm$ 0.12	0.00065 $\pm$ 0.00027

Phenylalanine	2.72 ± 0.01	2.74 ± 0.06	0.8	0.50 ± 0.04	0.00063 ± 0.00029
Proline	5.19 ± 0.32	5.45 ± 0.11	4.8	1.11 ± 0.20	0.00031 ± 0.00032
Serine	4.05 ± 0.09	4.51 ± 0.10	10.3	0.89 ± 0.12	0.00552 ± 0.00048
Threonine	6.68 ± 0.06	7.02 ± 0.12	4.9	0.46 ± 0.03	0.00281 ± 0.00029
Tryptophan <sup>6</sup>	1.23 ± 0.39 <sup>7</sup>	1.60 ± 0.04	23.1 <sup>8</sup>	0.20 ± 0.01	0.01361 ± 0.00082
Tyrosine	2.77 ± 0.01	2.78 ± 0.06	0.3	0.52 ± 0.05	0.00096 ± 0.00031
Valine	5.68 ± 0.03	5.65 ± 0.13	-0.5	0.27 ± 0.02	0.00047 ± 0.00030

<sup>1</sup> Samples were analysed in triplicate.

$$^2 \text{ Difference (\%)} = \frac{A_0 \text{ value} - 24\text{h value}}{A_0 \text{ value}} \times 100$$

<sup>3</sup> Detected as cysteic acid after performic acid oxidation followed by acid hydrolysis.

<sup>4</sup> Detected as methionine sulphone after performic acid oxidation followed by acid hydrolysis.

<sup>5</sup> Detected as methionine after acid hydrolysis.

<sup>6</sup> Tryptophan was uncorrected for the recovery of the internal standard

<sup>7</sup> 20-h hydrolysis value for tryptophan.

$$^8 \text{ Difference (\%)} = \frac{A_0 \text{ value} - 20\text{h value}}{A_0 \text{ value}} \times 100$$

## 6.5. Discussion

The aim was to accurately quantify the amino acid composition of a whey protein isolate containing naturally present glycomacropeptide. Estimates of the actual protein-bound amino acid content ( $A_0$ ), hydrolysis rate ( $h$ ) and loss rate ( $l$ ) were determined for each amino acid using a least-squares nonlinear regression model based on multiple hydrolysis time intervals. Overall, there was good agreement (<1% difference) between the amino acid content determined using multiple hydrolysis times and the 24-h hydrolysis values for approximately one-half of the amino acids. However, aspartic acid, glutamic acid, histidine and methionine (as methionine sulphone) were overestimated and glycine, proline, serine and threonine were underestimated by the standard 24-h hydrochloric acid hydrolysis method. Tryptophan was considerably underestimated (23.1%) by the 20-h alkaline hydrolysis method and this is in agreement with the findings of Rutherford *et al.* (2008), who determined the tryptophan content of several goat milk-based products using either the 20-h alkaline hydrolysis method or least-squares nonlinear regression method, where the tryptophan values were corrected for the recovery of an internal standard (5-methyl tryptophan) during hydrolysis (*S. Rutherford, pers. comm.*). These results highlight the inaccuracy of using a single hydrolysis time for labile amino acids, particularly tryptophan, serine and threonine. The amino acid contents of aspartic acid and glutamic acid are of particular interest because of the higher 24-h hydrochloric acid hydrolysis value relative to the predicted content ( $A_0$ ). The results are in agreement with data reported by Rutherford (2009) for five feedstuffs and Rutherford *et al.* (2008) for three goat milk infant formulas. The reason for this finding is not clear but may be related to the formation of the acid derivatives from the amide amino acids under the hydrochloric acid hydrolysis condition. It is important to note that the determined glutamic acid content (18.1%) reflects the amounts of glutamic acid and glutamine while the determined aspartic acid content (12.7%) represents the contents of aspartic acid and asparagine. The most abundant amino acid in the whey protein isolate analysed in the present study was

glutamic acid (18.1%), followed by aspartic acid (12.7%), leucine (10.0%) and lysine (8.1%).

The hydrolysis rates (*h*) for most amino acids were found to be higher in this study (39% on average) than those reported by Darragh & Moughan (2005) for whey protein isolate. In addition, the hydrolysis rates reported here were greater than those observed for whey protein concentrate (Darragh & Moughan, 2005), skim milk powder (Darragh & Moughan, 2005; Rutherford, 2009), human milk (Darragh & Moughan, 1998) and goat milk products (Rutherford *et al.*, 2008). The loss rates (*l*) were, in general, lower (48% on average) than those reported for whey protein isolate by Darragh & Moughan (2005). It is commonly known that serine and threonine are particularly labile during acid hydrolysis and as expected, losses of 13% for serine and 7% for threonine were found. A similar observation was reported by Darragh and Moughan (2005) for four milk protein products and Rutherford (2009) for five feedstuffs. The differences in the hydrolysis and loss rates for each amino acid across different protein sources may be due to differences in amino acid sequence and the relative proportions of amino acids in the protein.

In conclusion, this study reports accurate amino acid compositional data for a whey protein isolate determined using the least-squares nonlinear regression method with multiple hydrolysis time intervals. These results highlight that amino acid compositional data for some amino acids, particularly tryptophan, should be interpreted in relation to the errors inherent in their determination.

## 6.6. Literature cited

- Anderson, G. H., & Moore, S. E. (2004). Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr*, *134*(4), 974S–979S.
- Anderson, G. H., Tecimer, S. N., Shah, D., & Zafar, T. A. (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.

Association of Official Analytical Chemists (AOAC). (2008). *Official methods of analysis*. (18th ed). Gaithersburg, MD, USA.

Chungchunlam, S. M. S., Moughan, P. J., Henare, S. J., & Ganesh, S. (2012). Effect of time of consumption of preloads on measures of satiety in healthy normal weight women. *Appetite*, 59(2), 281–288. (see Chapter 3)

Darragh, A. J., Garrick, D. J., Moughan, P. J. & Hendriks, W. H. (1996). Correction for amino acid loss during acid hydrolysis of a purified protein. *Anal Biochem*, 236(2), 199–207.

Darragh, A. J., & Moughan, P. J. (1998). The amino acid composition of human milk corrected for amino acid digestibility. *Br J Nutr*, 80(1), 25–34.

Darragh, A. J., & Moughan, P. J. (2005). The effect of hydrolysis time on amino acid analysis. *JAOAC Int*, 88(3), 888–893.

Einarsson, S., Josefson, B., & Lagerkvist, S. (1983). Determination of amino acids with 9-fluorenylmethylchloroformate and reverse-phase high performance liquid chromatography. *J Chromatogr*, 282(C), 609–618.

Eisenstein, J., Roberts, S. B., Dallal, G., & Saltzman, E. (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), 189–200.

Hall, W. L., Millward, D. J., Long, S. J., & Morgan, L. M. (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., & 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.

Lam, S. M., Moughan, P. J., Awati, A., & Morton, H. R. (2009). The influence of whey protein and glycomacropeptide on satiety in adult humans. *Physiol Behav*, 96(1), 162–168. (see Chapter 2)

Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr*, 26(6), 704S–712S.

- Moore, S. (1963). On the determination of cystine as cysteic acid. *J Biol Chem*, 238(1), 235–237.
- Moore, S., & Stein, W. H. (1948). Photometric ninhydrin method for use in the chromatography of amino acids. *J Biol Chem*, 176(1), 367–388.
- Moore, S., & Stein, W. H. (1951). Photometric ninhydrin method for use in the chromatography of amino acids. *J Biol Chem*, 192(2), 663–681.
- Robel, E. J., & Crane, A. B. (1972). An accurate method for correcting unknown amino acid losses from protein hydrolysates. *Anal Biochem*, 48(1), 233–246.
- Rutherford, S. M. (2009). Accurate determination of the amino acid content of selected feedstuffs. *Int J Food Sci Nutri*, 60(Suppl 7), 53–62.
- Rutherford, S. M., & Gilani, G. S. (2009). Amino acid analysis. *Curr Protoc Protein Sci, Chapter11*(Unit 11.9), 1–37.
- Rutherford, S. M., Moughan, P. J., Lowry, D., & Prosser, C. G. (2008). Amino acid composition determined using multiple hydrolysis times for three goat milk formulations. *Int J Food Sci Nutri*, 59(7-8), 679–690.
- Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N., & Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. *Physiol Behav*, 94(2), 300–307.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., van Vught, A. J., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, M. S., & Westerterp-Plantenga, M. S. (2009). Dose-dependent satiating effect of whey relative to casein or soy. *Physiol Behav*, 96(4-5), 675–682.

## Chapter 7

### **The underlying cause of the satiating effect of whey protein: effect of whey protein components and a free amino acid mixture simulating whey protein on measures of satiety in adult women**

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Whey protein has been observed to have a higher satiating effect in adult women compared with maltodextrin carbohydrate (*Chapters 2, 3 & 5*). The purported satiating effect of whey protein may be due to the unique mixture of proteins in whey, to constituent individual proteins (beta-lactoglobulin, alpha-lactalbumin, and glycomacropeptide) and/or to its amino acid composition (amino acid analysis reported in *Chapter 6*). In a previous study (*Chapter 5*) where the effect of the component glycomacropeptide was investigated, the satiating effect of whey protein was found to be unique to whey, and glycomacropeptide *per se* did not appear to play a role. In the present study, the effects of dietary whey protein given as intact whey protein isolate (WPI), beta-lactoglobulin, alpha-lactalbumin, or a free amino acid mixture simulating the amino acid composition of WPI, on subsequent *ad libitum* food intake and subjective measures of satiety were investigated in healthy normal-weight adult women subjects.

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## 7.1. Abstract

Dairy whey protein has been shown to increase satiety relative to maltodextrin carbohydrate, but which characteristic of whey protein gives rise to its higher satiating effect remains to be elucidated. Satiety responses to preload meals enriched (52 g amino acid equivalent) with either intact whey protein isolate (WPI), beta-lactoglobulin (B-LG), alpha-lactalbumin (A-LA), or a free amino acid mixture simulating the amino acid composition of WPI (AA), were compared. A single-blind completely randomised block design included twenty healthy normal-weight women (mean age  $24.2 \pm 0.8$  years; mean BMI  $22.7 \pm 0.4$  kg/m<sup>2</sup>). Consumption of an *ad libitum* test meal 120 min following ingestion of the preload treatment and subjective feelings of appetite were measured. There were no significant differences in the *ad libitum* test meal intakes or total energy intakes (preload + test meal energy intakes) between the four preload conditions ( $p > 0.05$ ). Subjective feelings of appetite did not differ significantly in response to the four preload meals ( $p > 0.05$ ). Intact whey protein, isolated major whey protein fractions and a free amino acid mixture simulating whey protein showed similar effects on satiety. These results suggest that the amino acid composition of whey protein may play an important role, as the satiating effect of intact whey protein was mimicked by a free amino acid mixture simulating the amino acid composition of whey protein.

## 7.2. Introduction

Obesity is a global health concern (WHO, 2012) and there is growing interest in dietary strategies to promote body weight loss. Several scientific reviews conclude that the consumption of high-protein meals (>15% of energy from protein) results over time in a reduction of body weight (Eisenstein *et al.*, 2002; Halton & Hu, 2004; Te Morenga & Mann, 2012; Westerterp-Plantenga *et al.*, 2009). The effect of high-protein diets on body weight loss may be

related to the ability of protein to increase diet-induced thermogenesis and/or induce satiety and reduce food intake to a greater extent compared with other macronutrients (Anderson & Moore, 2004; Eisenstein et al., 2002; Halton & Hu, 2004; Westerterp-Plantenga et al., 2009). Several studies have reported a relationship between the source of protein and its satiety value, with dairy whey protein considered to be more satiating than other protein types (Luhovyy et al., 2007; Veldhorst et al., 2008). In previous studies with healthy normal-weight human subjects (Lam et al., 2009; Chungchunlam et al., 2012, see Chapters 2, 3 & 5), we found that whey protein suppressed subsequent food intake and increased subjective measures of satiety relative to maltodextrin carbohydrate. The satiating effect of whey protein may be attributed to an effect of the intact proteins themselves, bioactive peptides released during digestion (Haque et al., 2008; Jahan-Mihan et al., 2011; Madureira et al., 2010; Moughan et al., 2007; Rutherford-Markwick & Moughan, 2005), or to the amino acid composition of the whey protein (e.g. the high content of branched-chain amino acids) and the kinetics of digestion and amino acid uptake (reflected by postprandial plasma amino acids).

Amino acids may play an important role in the satiating effect of whey protein. Proteins release peptides and amino acids into the digestive lumen during digestion, and amino acids are transferred to the blood via amino acid transport systems present in the intestinal mucosa (Alpers, 1987; Ten Have et al., 2007). Human studies on the rate of digestion of milk protein and the pattern of circulating plasma amino acids revealed that whey protein empties from the stomach faster than casein to elicit a rapid initial increase in plasma amino acid concentrations (Boirie et al., 1997; Hall et al., 2003). We have previously reported that the consumption of whey protein results in a sharp increase in plasma amino acids compared with maltodextrin carbohydrate (see Chapter 4). There is some evidence that an increase in postprandial peripheral serum amino acid concentrations is associated with a reduction in subjective feelings of appetite (Mellinkoff et al., 1956). Moreover, whey protein is a rich source of branched-chain amino acids (isoleucine, leucine and valine) (Etzel, 2004; Moughan, 2008; see Chapter 6) and this is reflected in the postprandial plasma amino acid concentrations observed for whey protein (see Chapter 4).

Nilsson *et al.* (2004) also showed that plasma concentrations of the dietary essential amino acids (isoleucine, leucine, valine, lysine and threonine) were more pronounced after ingestion of whey protein than that after the ingestion of other protein sources (casein, skim milk, cod, and gluten). To our knowledge, the satiety effect of intact whey protein has not been compared against that of a free amino acid mixture simulating its amino acid content.

Whey protein is a term used to define a cluster of different soluble proteins. The predominant “whey protein” fraction occurring in the milk of cows is beta-lactoglobulin (50%), followed by alpha-lactalbumin (19%). In addition to minor proteins (e.g. lactoferrin and lactoperoxidase present in trace amounts), vitamins and minerals, whey protein also contains a mixture of immunoglobulins (12%), proteose peptones (12%), and bovine serum albumin (7%) (Walstra *et al.*, 2006). When whey protein is isolated after the manufacture of cheese, glycomacropeptide (GMP), a soluble protein originating from casein, becomes part of this whey (Walstra *et al.*, 2006). While the glycomacropeptide (GMP) fraction of whey protein has received much attention (Burton-Freeman, 2008; Gustafson *et al.*, 2001; Keogh *et al.*, 2010; Lam *et al.*, 2009, see Chapter 2; Veldhorst *et al.*, 2009a), our group and others have demonstrated that GMP does not seem to play a role in the satiating effect of whey, and the satiating effect of whey protein is unique to whey (see Chapter 5). In a rat study, Pichon *et al.* (2008) demonstrated that consumption of either whey protein concentrate (WPC) or beta-lactoglobulin-enriched whey protein isolate (WPI) reduced daily energy intake compared with whole milk protein. In a human study, eighteen lean men reported feeling fuller following consumption of beta-lactoglobulin than WPC but there was no difference in subsequent food intake 180 min later (S. Poppitt, *pers. comm.*). In another human study, Hursel *et al.* (2010) found that a breakfast containing alpha-lactalbumin-enriched whey protein decreased feelings of hunger and desire to eat to a greater extent than a whey protein-containing breakfast. Veldhorst *et al.* (2009b) also showed that isolated alpha-lactalbumin decreased energy intake at an *ad libitum* test meal 180 min later and increased feelings of satiety more than whey protein. Alpha-lactalbumin and beta-lactoglobulin would appear to be important fractions of whey protein with respect to its

satiating effect but the influence of the two major whey protein fractions (alpha-lactalbumin and beta-lactoglobulin) on satiety in humans remains unclear. We hypothesise that the satiating effect of whey protein may be due to the mixture of intact proteins in whey protein, to the effect of an individual intact protein in whey protein, to an effect of peptides arising from the digestion of these proteins, or to the plasma amino acid composition consequent upon the digestion of whey protein.

The objective of the present study was to assess, therefore, whether the higher satiating effect of whey protein is attributable to the whey protein *per se*, the major whey protein fractions or the amino acid composition of whey protein. The effect of whey protein, isolated beta-lactoglobulin, isolated alpha-lactalbumin and a mixture of free amino acids simulating the amino acid composition of whey protein, on subsequent *ad libitum* food intake and subjective feelings of appetite were studied in healthy normal-weight women.

### **7.3. Subjects and methods**

#### **7.3.1. Subjects**

Twenty women responded to public advertisement and were invited to participate in the present study. To be included in the study, subjects had to be aged between 18 and 40 years and with a body mass index (BMI) ranging from 19 to 26 kg/m<sup>2</sup>. Subjects were screened for exclusion criteria that included smoking, athletic training, a gastrointestinal disorder or eating disorder, dieting or taking medication known to affect appetite, not consuming breakfast every day, having a history of menstrual irregularities, pregnancy, lactation or trying to become pregnant. Participants who had an allergy or disliking regarding the test foods were not included in the study. All subjects had their height and weight measured in the laboratory to ensure that they met the BMI criteria, and completed the Three Factor Eating Questionnaire (*Stunkard & Messick, 1985*). Scores of the Three Factor Eating Questionnaire were not used as selection

criteria. The results were not affected when six restrained eaters, who scored more than 11 out of 21 on the restraint scale of the Three Factor Eating Questionnaire (Lowe & Thomas 2009; Stunkard & Messick, 1985), were excluded. So, the data of the six restrained eaters were included in the study (mean dietary restraint score  $9 \pm 1$ ). The study received ethical approval from the Massey University Human Ethics Committee (Application no. 11/47) and all volunteers provided written informed consent.

### **7.3.2. Test foods**

The breakfast meal consisted of toasted wholemeal bread and a selection of margarine, strawberry jam and either coffee or tea plus white sugar and full-fat milk. The same subject-specific breakfast was provided in packed containers before each study day and participants were instructed to consume all of the food items provided. The breakfast food consumption of each participant was recorded. The metabolisable energy (ME) content of the breakfast meal was 1503.1 kJ on average with 57, 11, and 32% of ME being derived from carbohydrate, protein, and fat, respectively.

The four test preloads consisted of an orange-flavoured marmalade spread (Jok'N'Al Orange marmalade, Joknal Products Ltd., Blenheim, New Zealand) to which was added either whey protein isolate (WPI), alpha-lactalbumin (A-LA), beta-lactoglobulin (B-LG) or a mixture of free amino acids simulating the amino acid composition of whey protein (AA). The preload spreads containing WPI, A-LA and B-LB were prepared in the laboratory one week prior to the study day and stored in a refrigerator. The preload spread containing the AA mixture was freshly prepared on each test day. Each preload spread (250 g) was accompanied with a slice (45 g) of toasted low-protein (1.2 g protein per 45 g) bread (Pavillion Original sliced gluten free bread, Pavillion Foods, Christchurch, New Zealand) and 100 ml of water. The WPI preload meal served as the control. The composition of the four preload meals (295 g) is given in Table 7.1. The preload meals were

isoenergetic (~1800 kJ) and contained a mixture of carbohydrate (52 g) and protein or amino acids (52 g amino acid equivalent).

A lunchtime test meal was served in private cubicles 120 min following consumption of the preload meals and comprised of a mixed homogeneous single item hot fried rice meal and a bottle of spring water. The hot fried rice test meal included white rice, minced chicken meat, eggs, peas, corn, carrots, chicken stock, salt, sugar, and vegetable oil. The fried rice test meal contained 36.8, 8.5, 5.8, and 1.0 g of available carbohydrate, crude protein, crude fat and total dietary fibre, respectively, per 100 g with a calculated ME content of 982.2 kJ per 100 g.

**Table 7.1.** Composition of the four preload meals enriched with whey protein isolate (WPI), alpha-lactalbumin (A-LA), beta-lactoglobulin (B-LG) and a free amino acid mixture simulating the amino acid composition of whey protein isolate (AA).

	Preload meal			
	WPI	A-LA	B-LG	AA
<b>Ingredient (g per 295 g serve)</b>				
Low-protein bread <sup>1</sup>	45	45	45	45
Orange-flavoured marmalade spread <sup>2</sup>	190	195	196	200
Whey protein isolate (WPI) <sup>3</sup>	60	-	-	-
Alpha-lactalbumin isolate (A-LA) <sup>4</sup>	-	55	-	-
Beta-lactoglobulin isolate (B-LG) <sup>5</sup>	-	-	54	-
Free amino acids (AA) <sup>6</sup>	-	-	-	50
<b>Calculated Nutrients (per 295 g serve)</b>				
Metabolisable energy (ME) <sup>7</sup> (kJ)	1849.2	1792.7	1793.3	1790.4
Fat				
(g)	1.8	1.3	1.3	1.2
(% of ME)	3.7	2.7	2.8	2.5

Carbohydrate				
(g)	53.9	51.9	52.1	52.7
(% of ME)	48.6	48.4	48.5	49.2
Protein (amino acids)				
(g)	52.8	52.5	52.3	51.8
(% of ME)	47.7	48.9	48.7	48.3

<sup>1</sup> Low-protein bread (Pavillion Original sliced gluten free bread, Pavillion Foods, Christchurch, New Zealand) (444.4 kJ ME, 1.2 g protein, 22.7 g carbohydrate, and 1.2 g fat per 45 g serve). The low-protein bread consisted of different starches (maize, modified maize, potato, tapioca), water, rice flour, egg, vegetable oil, yeast, iodised salt and sugar.

<sup>2</sup> Orange-flavoured marmalade spread (Jok’N’Al Orange marmalade, Joknal Products Ltd., Blenheim, New Zealand) (255.5 kJ ME, 0.3 g protein, 15.0 g carbohydrate, and 0 g fat per 100 g serve). The orange-flavoured marmalade spread was composed of water, 25% oranges (orange juice, orange peel), polydextrose, fructose, pectin, locust bean gum, citric acid, malic acid, potassium sorbate and sucralose.

<sup>3</sup> Whey protein isolate containing 21% glycomacropeptide (WPI 894, Fonterra Co-operative Group Ltd., Palmerston North, New Zealand).

<sup>4</sup> Alpha-lactalbumin isolate (Davisco Foods International Inc., Minnesota, United States)

<sup>5</sup> Beta-lactoglobulin isolate (Davisco Foods International Inc., Minnesota, United States)

<sup>6</sup>Free amino acids (Evonik Degussa GmbH, Hanau, Germany) were mixed in proportions corresponding to the amino acid composition of whey protein isolate containing 21% glycomacropeptide (*see Chapter 6* for amino acid composition of the whey protein)

<sup>7</sup>Metabolisable energy (ME) calculated from the energy-containing food components using the energy conversion factors of 16.7 kJ/g for protein and available carbohydrate and 37.7 kJ/g for fat

### 7.3.3. Experimental procedure

In a single-blind completely randomised block design, each woman subject participated in four session days, with a minimum of two days between test days. On the evening preceding each test day, subjects were instructed to abstain from strenuous physical activity and alcohol consumption, and to consume only water after 22:00 h. Women participated during the early phase of their menstrual cycle (menstruation and follicular phase) (*Buffenstein et al., 1995; Dye & Blundell, 1997*). On the morning of the test day, each subject consumed a subject-specific breakfast meal at home. The participants were instructed to consume all the food items provided at least 3 h before the test session appointment. Throughout the morning, participants were instructed to refrain from consuming anything except water.

Subjects arrived at the Human Nutrition Unit, Massey University around 1200 to 1300 h. Subjects who did not comply with restrictions on the evening before and the morning were scheduled for another test day. Upon arrival at the laboratory, a baseline questionnaire to assess feelings of appetite and nausea was completed. A test preload meal was served to be consumed within 15 min. Following complete ingestion of the preload meal (time 0 min), subjects completed a questionnaire assessing the palatability of the preload meals. Feelings of appetite and nausea were measured at 0, 15, 30, 45, 60, 75, 90, and 120 min. Following the 120 min measurement, subjects were seated in individual cubicles to consume the lunchtime test meal within 15 min. Subjects were instructed to consume the test meal at will (*ad libitum*) until such point as they felt comfortably full. The amount of the fried rice meal and water which were provided without restriction at lunch was measured before and after consumption, using an electronic scale (to the nearest 0.01 g). Subjects rated the likeability of the fried rice test meal on a questionnaire immediately after consumption. Subjects also completed questionnaires to assess subjective feelings of appetite and nausea 15 and 30 min after consumption of the lunchtime test meal.

#### **7.3.4. Questionnaires**

Each subject was instructed to fill in a booklet of questionnaires to assess subjective feelings of appetite (hunger, desire to eat, prospective food consumption and fullness) and nausea. Ratings were made on a 10-cm visual analogue scale (VAS) (*Flint et al., 2000*) labeled at each end with extremes: “not at all” and “extremely” for hunger, fullness, and nausea, “very weak” and “very strong” for desire to eat, and “nothing at all” and “a large amount” for prospective food consumption. VAS-rated appetite and nausea scores were collected immediately before consuming the test preload (baseline), during a 120 min period following consumption of the test preload (0, 15, 30, 45, 60, 75, 90, and 120 min), and 15 and 30 min following consumption of the lunchtime *ad libitum* test meal. Subjects rated the palatability of the preload meal (likeability, pleasantness of taste, likeability of texture and sweetness) and likeability of the fried rice test meal using a 10-cm VAS immediately after consumption of the preload meal and the lunchtime *ad libitum* test meal. The VAS was end-anchored with “dislike extremely” and “like extremely” for ratings of likeability and likeability of texture and with “not at all” and “extremely” for ratings of pleasantness of taste and sweetness. This booklet of questionnaires has been described previously (*Chungchunlam et al., 2012, see Chapter 3*).

#### **7.3.5. Statistical analyses**

Statistical analyses were performed with SAS software version 9.2 for WINDOWS (SAS Institute Inc., Cary, NC, USA). A single-blind randomised factorial design with subject as the blocking factor was used (*Ott & Longnecker, 2010*). Power analysis, based on the results from previous studies (*see Chapters 3 & 5*) using a randomised block design (*Kastenbaum et al., 1970*), indicated that a sample size of thirteen women subjects had sufficient

power of 0.80 at a level of significance of 0.05 to allow the detection of differences in *ad libitum* test meal intake. Each variable was examined for normal distribution and the presence of statistical outliers. Comparisons between the four preload meals were evaluated using an analysis of variance (ANOVA) with preload and subject as factors for VAS-rated palatability scores of preload and test meal (cm), water (g), test meal (g and kJ ME) and total energy (preload + test meal, kJ ME) intakes.

A repeated-measures ANOVA was used to evaluate the effect of preload, time of rating and their interaction (preload x time) on VAS-rated feelings of hunger, desire to eat, prospective consumption, fullness and nausea. In the case of a significant interaction between preload and time ( $p < 0.05$ ), Tukey-Kramer multiple comparison tests were used to examine the preload and time combinations. The pre-preload baseline measurement of each VAS rating showed some subject variation. To adjust for this, another repeated-measures ANOVA was performed using the baseline measurement as a covariate in the model. VAS-rated feelings of appetite and nausea were also reported as net incremental area under the curve (net iAUC) (Gannon *et al*, 1989) from 0 to 120 min using the trapezoidal rule and analysed using ANOVA for an effect of preload.

Pearson's correlation analysis was performed to test the relationship between energy intake (kJ ME) at the lunchtime *ad libitum* test meal and palatability of the preload as well as VAS-rated feelings of appetite and nausea. A  $p$ -value of less than 0.05 was considered statistically significant. Duncan's multiple range test was used for making multiple pairwise treatment comparisons. Results are expressed as means and their standard errors (mean  $\pm$  sem).

## **7.4. Results**

All twenty women, who were similar in age (mean age  $24.2 \pm 0.8$  years) and BMI (mean BMI  $22.7 \pm 0.4 \text{ kg/m}^2$ ), completed the four test session

days. The results for five of the subjects on the AA preload meal were excluded from the study due to a minor error in formulation of their preloads (proportions of amino acids). Therefore, for the AA test preload meal, the number of subjects is fifteen. When the data from the five subjects were included in the analysis, there was no difference in the overall findings of the study.

Subjects spent a similar amount of time consuming each of the four preload meals ( $8.8 \pm 0.5$  min on average). The time taken eating the *ad libitum* test meal and drinking water at the lunchtime meal was also similar across subjects ( $7.5 \pm 0.4$  min on average). The palatability of the lunchtime *ad libitum* test meal did not differ between preload groups (preload effect:  $p > 0.05$ ), with a mean overall likeability score of  $8.5 \pm 0.2$  cm.

No adverse effects of the preload meals were reported and VAS-rated feelings of nausea and net iAUC for nausea did not differ significantly between the four preload conditions ( $p > 0.05$ ). Concurrently, Pearson's correlation indicated that there was no relationship between postprandial feelings of nausea (0 to 120 min) or net iAUC for nausea and energy intake at the lunchtime *ad libitum* test meal ( $p > 0.05$ ).

#### **7.4.1. Palatability of the preload meals**

Ratings for the overall likeability, pleasantness of taste, likeability of texture and overall sweetness of the four preload meals are shown in Table 7.2. There were significant differences found in ratings for overall likeability ( $p = 0.011$ ), pleasantness of taste ( $p = 0.0003$ ) and overall sweetness ( $p = 0.0076$ ) between the preloads. The AA preload meal was rated as being the least liked (overall likeability), least pleasant in taste, and least sweet compared with the other three preload meals. There was no effect of preload on the ratings for likeability of texture ( $p > 0.05$ ).

**Table 7.2.** Palatability scores determined on a 10-cm visual analogue scale for the four preload meals containing either whey protein isolate (WPI), alpha-lactalbumin (A-LA), beta-lactoglobulin (B-LG), or a free amino acid mixture (AA)<sup>1</sup>.

	<b>WPI</b>	<b>A-LA</b>	<b>B-LG</b>	<b>AA</b>	Overall sem	<i>p</i> -value
Likeability	3.9 <sup>a</sup>	3.6 <sup>a</sup>	3.6 <sup>a</sup>	1.3 <sup>b</sup>	0.5	0.0011
Pleasantness of taste	4.3 <sup>a</sup>	3.5 <sup>a</sup>	3.1 <sup>a</sup>	1.1 <sup>b</sup>	0.5	0.0003
Likeability of texture	4.4	3.3	2.8	3.3	0.4	0.06
Sweetness	5.3 <sup>a</sup>	5.0 <sup>a</sup>	4.9 <sup>a</sup>	3.0 <sup>b</sup>	0.5	0.0076

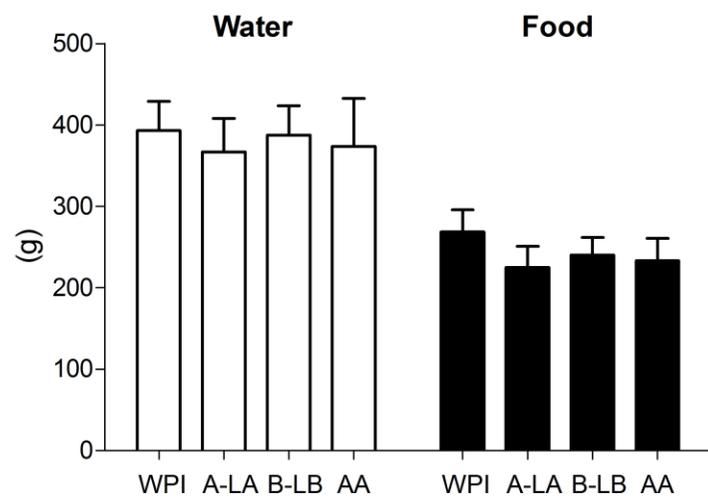
Means in a row with different superscript letters were significantly different (Duncan's test,  $p < 0.05$ )

<sup>1</sup> Values are means,  $n = 20$  for WPI, A-LA, and B-LG and  $n = 15$  for AA.

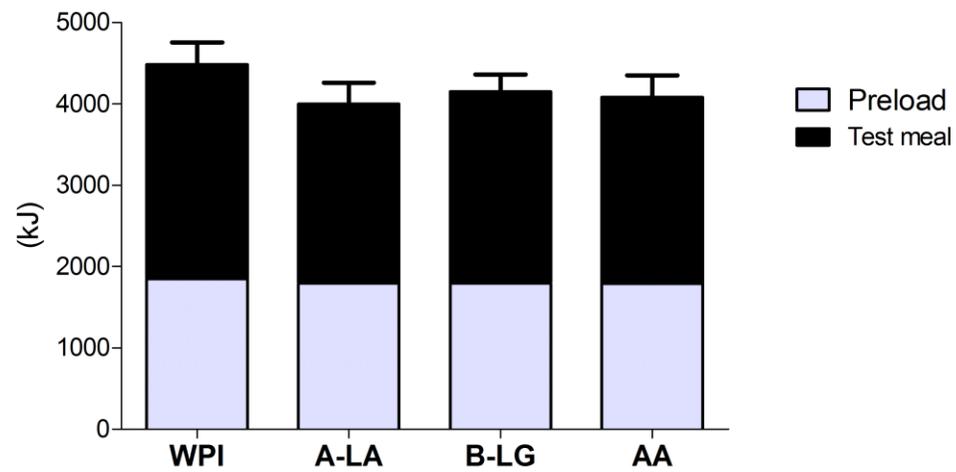
#### **7.4.2. *Ad libitum* test meal intake and total energy intake**

There were no significant differences in *ad libitum* water intake (Figure 7.1.) between the test preloads (preload effect:  $p>0.05$ ), with amount of water ingested at lunch averaging  $380.4 \pm 23.5$  g across all four preload conditions. There was no significant effect of preload ( $p>0.05$ ) on the amount (g) and energy intake (kJ ME) of the *ad libitum* food test meal consumed at lunch (Figure 7.1.). When the energy intake from the preload meal was added to the *ad libitum* food test meal energy intake at lunch, total energy intake did not differ significantly between the four preload meals ( $p>0.05$ ) (Figure 7.1.).

**(A) Amount**



**(B) Energy**



**Figure 7.1.** (A) Amount. (B) Energy.

**Figure 7.1(A). Amount.** *Ad libitum* test meal water and food intakes (g) at lunch following consumption of the four preload meals containing either whey protein isolate (WPI), alpha-lactalbumin (A-LA), beta-lactoglobulin (B-LG) or a mixture of free amino acids (AA). Values are means  $\pm$  sem,  $n = 20$  for WPI, A-LA, and B-LG and  $n = 15$  for AA. There was no significant effect of preload ( $p > 0.05$ ).

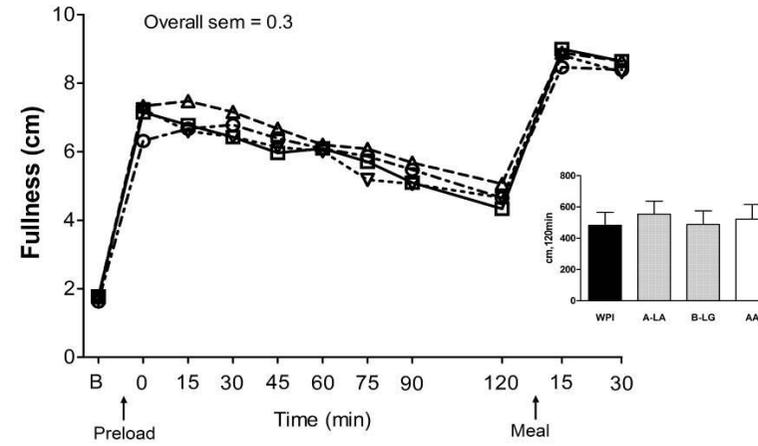
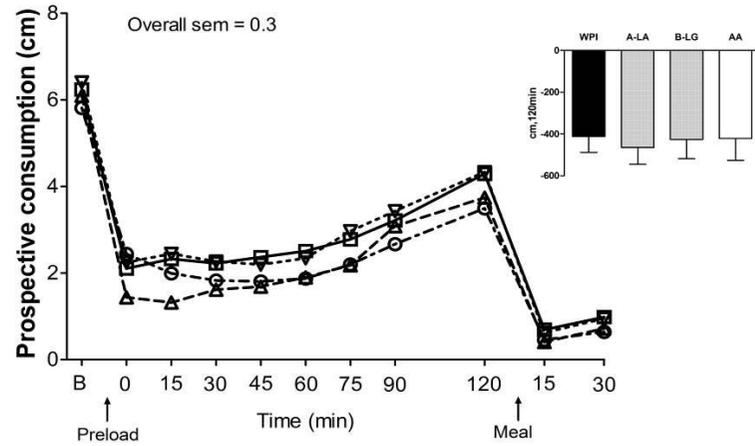
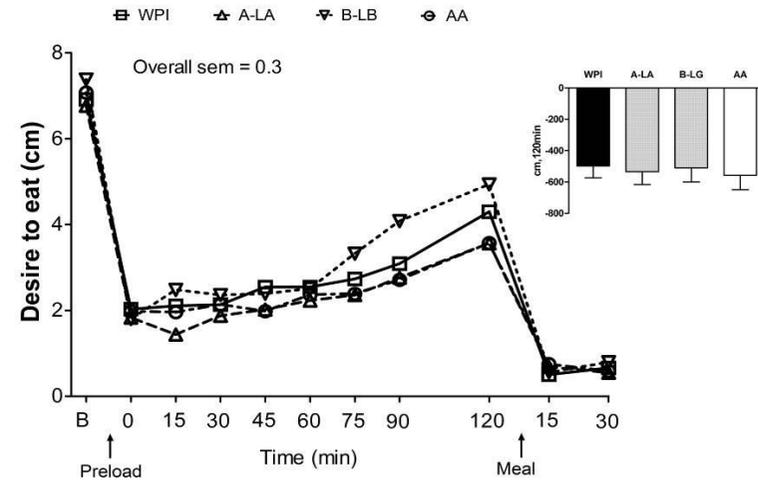
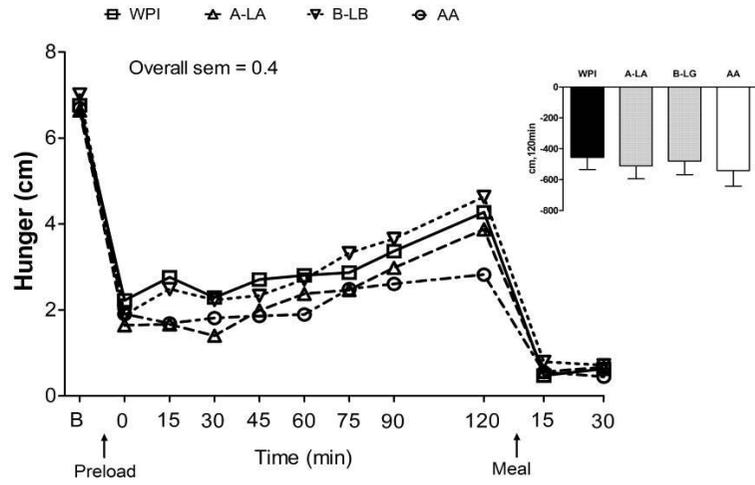
**Figure 7.1(B). Energy.** Total energy intake (preload + food test meal energy intakes, kJ ME) when the four preload meals enriched with either whey protein isolate (WPI), alpha-lactalbumin (A-LA), beta-lactoglobulin (B-LG) or a mixture of free amino acids (AA) were consumed. Values are means  $\pm$  sem,  $n = 20$  for WPI, A-LA, and B-LG and  $n = 15$  for AA. There was no significant effect of preload ( $p > 0.05$ ).

### 7.4.3. Subjective ratings of appetite

When the total testing period (~3 h) was examined, there was no significant interaction between preload and time ( $p>0.05$ ) for the VAS-rated feelings of hunger, desire to eat, prospective consumption, and fullness (Figure 7.2.). There was no significant main effect of preload ( $p>0.05$ ), but, as expected, there was a significant main effect of time ( $p<0.0001$ ) observed for each rating of appetite.

When the ratings determined following consumption of the preload meal (0 to 120 min) were adjusted for the baseline rating, there was no significant interaction between preload and time ( $p>0.05$ ) observed for all the VAS-rated feelings of appetite. The appetite ratings did not differ significantly between preloads ( $p>0.05$ ), but were influenced by time ( $p<0.0001$ ). When 4 statistical outliers from 592 total observations were removed for the desire to eat data, a significant interaction effect between preload and time was found ( $p=0.0224$ ). The preload meal containing B-LG increased the subjective feeling of desire to eat more than the preload meals containing either AA or A-LA at 90 and 120 min postprandially.

The feelings of hunger, desire to eat, prospective consumption, and fullness expressed as net iAUC following consumption of the preload meals (0 to 120 min) are shown in Figure 7.2. There were no significant differences found in the net iAUC responses for the appetite ratings between the four preload conditions (preload effect:  $p>0.05$ ).



**Figure 7.2.** VAS-rated subjective feelings of hunger, desire to eat, prospective consumption, and fullness before (baseline, B) and after consumption of the four preload meals, and 15 and 30 min following consumption of the *ad libitum* test meal. Inset: Net incremental areas under the curve (Net iAUC) from 0 to 120 min in response to the four preload meals.

The four preload meals were enriched with either whey protein isolate (WPI), alpha-lactalbumin (A-LA), beta-lactoglobulin (B-LG), or a free amino acid mixture (AA). Values are means,  $n = 20$  for WPI, A-LA, and B-LG and  $n = 15$  for AA. There was no significant interaction between preload and time ( $p > 0.05$ ) and there was no significant main effect of preload ( $p > 0.05$ ), but each VAS-rated feeling differed by time ( $p < 0.0001$ ). There was no significant main effect of preload on Net iAUCs for each subjective feeling ( $p > 0.05$ ).

#### 7.4.4. Correlation analyses

The relationship between palatability of the preload meals and subsequent energy intake at the *ad libitum* test meal was tested. Energy intake consumed at the subsequent test meal was not correlated to VAS-rated measures of overall likeability ( $r = -0.11, p > 0.05$ ), pleasantness of taste ( $r = -0.08, p > 0.05$ ), likeability of texture ( $r = 0.09, p > 0.05$ ), or overall sweetness ( $r = 0.05, p > 0.05$ ), for all of the four preload conditions.

Pearson's correlation analysis was performed to examine the relationship between energy intake at the subsequent test meal and subjective ratings of appetite following consumption of the preload (0 to 120 min). Overall, subsequent food energy intake was significantly weakly correlated with subjective feelings of hunger ( $r = 0.30, p < 0.0001$ ), desire to eat ( $r = 0.29, p < 0.0001$ ), prospective consumption ( $r = 0.31, p < 0.0001$ ), and fullness ( $r = -0.24, p < 0.0001$ ). Immediately before consumption of the *ad libitum* test meal (120 min), energy intake of the test meal was positively related to ratings of hunger ( $r = 0.39, p = 0.0005$ ), desire to eat ( $r = 0.29, p = 0.0106$ ), and prospective consumption ( $r = 0.32, p = 0.0041$ ), but negatively related to feelings of fullness ( $r = -0.30, p = 0.0087$ ).

### 7.5. Discussion

We have established in our own work that whey protein is more satiating in adult humans than carbohydrate (Chungchunlam *et al.*, 2012, see Chapters 3 & 5) and it has also been shown by others that whey protein is more satiating than other protein sources (Anderson *et al.*, 2004; Diepvens *et al.*, 2008; Hall *et al.*, 2003; Pal & Ellis, 2004; Veldhorst *et al.*, 2009c). In spite of this, little is known about the role of the major whey protein fractions (beta-lactoglobulin and alpha-lactalbumin) and the amino acid composition of whey protein in mediating this effect on satiety. The aim of the present study was to evaluate the effects of a preload meal containing intact whey protein isolate (WPI), beta-lactoglobulin (B-LG) alone, alpha-lactalbumin (A-LA) alone, or a

mixture of free amino acids simulating the amino acid composition of whey protein (AA), on subsequent *ad libitum* food intake and subjective feelings of appetite in twenty healthy normal-weight women.

The present work required the feeding of free amino acids. Dietary products based on free amino acids are generally low in palatability and may induce aversive responses due to their taste (*Kirimura et al., 1969; Nishimura & Kato, 1988*). The use of an orange-flavoured marmalade spread to mask the taste of free crystalline amino acids was effective, with subjects reporting no adverse effects or differences in feelings of nausea following consumption of the amino acid-based diet. Although the preload meal containing the free amino acids was perceived as being the least liked (overall likeability), least pleasant in taste, and least sweet in relation to the other three preload meals (WPI, B-LG, and A-LA), no relationship between the perceived sensory characteristics of the preload meal and subsequent food energy intake was observed. The intact whey protein powder used here was predominantly protein (85%) and protein analysis using high-performance liquid chromatography (HPLC) showed that the whey protein consisted (g/100g powder) of beta-lactoglobulin (43.705), glycomacropeptide (21.263), alpha-lactalbumin (13.984), proteose peptone-5 (3.184), immunoglobulins (1.706), bovine serum albumin (1.086), and lactoferrin (0.008). The effects of the minor protein components were not studied directly and it is possible that these minor proteins may have a role to play in promoting satiety. However, as the satiating effect of the intact whey protein did not differ from that of the free amino acid mixture, this suggests that the minor protein constituents of whey protein did not have an important satiating effect.

In healthy normal-weight adult women, ingestion of a preload meal, containing similar amounts of carbohydrate but enriched with WPI, B-LG, A-LA, or AA, resulted in similar *ad libitum* intakes of water (g) and food (g and kJ ME) at a test meal 120 min after ingestion of the preload. Total energy intake (preload + test meal energy intakes) also did not differ among preload meals. With respect to the subjective feelings of hunger, desire to eat,

prospective food consumption, and fullness (VAS scores and net iAUC), there were no significant differences between the four preload meals. The VAS scores for the subjective feelings of appetite were found to be related, as would be expected, with subsequent food energy intake. The satiating effect of intact whey protein was reproduced when whey protein was replaced by either beta-lactoglobulin, alpha-lactalbumin or free amino acids. These results suggest that the satiating effect of whey protein is mediated by virtue of its unique amino acid composition, while the whey protein fractions may only play a minor role.

It has been proposed that whey protein *per se* and whey protein fractions (beta-lactoglobulin and alpha-lactalbumin) differ in their ability to induce satiety. Consumption of isolated beta-lactoglobulin (*S. Poppitt, pers. comm.*) or alpha-lactalbumin (*Veldhorst et al., 2009b*) has previously been reported to have a stronger satiating effect in relation to intact whey protein. However, we failed to show any statistically significant differential effects of the four preload meals (WPI, B-LG, A-LA and AA) on subsequent *ad libitum* test meal intake or subjective feelings of appetite. Therefore, there is little evidence from the presently reported study, for a direct effect of the intact constituent protein structures or that the release of bioactive peptides during digestion of whey protein or whey protein fractions (*Haque et al., 2008; Jahan-Mihan et al., 2011; Moughan et al., 2007; Rutherford-Markwick & Moughan, 2005*) is the underlying reason for the satiating effect of whey protein. It is likely that the observed similar effects of the preloads on *ad libitum* food intake and subjective feelings of appetite reflect in part a similar rate of gastric emptying of the four preload meals. Whey protein is thought to be a “fast” protein that empties rapidly from the stomach and leads to relatively rapid intestinal absorption as peptides and amino acids (*Boirie et al., 1997; Hall et al., 2003; Ten Have et al., 2007*). Interestingly, several studies have shown that intact and hydrolysed whey protein (mostly in the form of peptides) elicit similar rates of gastric emptying (*Calbet & Holst, 2004; Power et al., 2009*). The rate of gastric emptying of free amino acids in relation to proteins or peptides is not clear, as free amino acids may empty from the stomach quickly, as the amino acids are readily available for intestinal absorption (*Ten Have et al., 2007*), or slowly via stimulation of osmoreceptors found in the

duodenum (*Burn-Murdoch et al., 1978*). Whey protein, beta-lactoglobulin, alpha-lactalbumin and free amino acids may all exhibit similar rates of appearance of amino acids in the peripheral blood. This is supported by the results (*S. Chungchunlam, unpublished data*) of an accompanying study with the growing rat where stomach emptying rates for the four experimental diets used here were determined using a magnetic resonance spectroscopy technique. Similar stomach emptying rates were found.

To the best of our knowledge, this is the first study comparing the effects of intact whey protein and a mixture of free amino acids simulating the amino acid composition of the whey protein on satiety. Whey protein, beta-lactoglobulin, and alpha-lactalbumin are rich in dietary essential amino acids and branched-chain amino acids (*Etzel, 2004; Moughan, 2008*). A number of studies have reported more rapid increases in plasma total amino acids (TAA), dietary essential amino acids (EAA), and branched-chain amino acids (BCAA: isoleucine, leucine, and valine) for whey in comparison with other protein sources (*Boirie et al., 1997; Calbet & Holst, 2004; Hall et al., 2003; Morifuji et al., 2010; Nilsson et al., 2004; Veldhorst et al., 2009a*). Moreover, the ability of beta-lactoglobulin (*Farnfield et al., 2009*) or alpha-lactalbumin (*Veldhorst et al., 2009b*) intake to increase circulating plasma amino acids to a similar extent as to whey protein has been shown in other human studies. With regards to free amino acid meals, two studies have demonstrated a similar profile of plasma amino acids when peptides and an equivalent mixture of free amino acids were consumed in human subjects (*Hegarty et al., 1982; Silk et al., 1979*). A relationship between the amino acid composition of the test meal and the postprandial changes in circulating plasma amino acids has also been reported by others (*Adibi & Mercer, 1973; Hegarty et al., 1982; Marrs et al., 1975; Silk et al., 1979*) and in an earlier study (*see Chapter 4*). In particular, whey protein elicited a greater increase in plasma branched-chain amino acids (BCAA) relative to maltodextrin carbohydrate, and a significant relationship ( $p < 0.05$ ) between plasma concentrations of BCAA and satiety-related hormones, glucagon-like peptide-1 and pancreatic polypeptide, was observed (*see Chapter 4*). The similar effect of preload meals enriched with whey protein or its equivalent free amino acids in inducing satiety, as observed here, provides

some evidence that amino acids may be involved in the suppression by whey protein of subsequent food energy intake and VAS-rated subjective feelings of appetite. One of the limitations of the present study is that we were unable to measure rate of gastric emptying in the subjects and the concentrations of amino acids in the peripheral blood, which may have helped to elucidate the postprandial kinetics of the four preload meals and extend the interpretation of the results.

Consumption of intact whey protein, isolated whey protein fractions, and a free amino acid mixture reflecting the amino acid composition of whey protein influenced satiety to a similar extent in adult women. Therefore, the underlying cause of the satiating effect of whey protein seems to be related to its unique amino acid composition. This warrants further investigation by assessing the rate of whey protein digestion, the dynamics of uptake of dietary amino acids and the appearance of circulating amino acids, and their potential association with satiety responses. Concomitantly, there is a need for further studies to compare the satiating effects of whey protein with other protein sources, in terms of amino acid composition, and plasma amino acid concentrations post-absorption.

## **7.6. Literature cited**

- Adibi, S. A., & Mercer, D. W. Protein digestion in human intestine as reflected in luminal, mucosal, and plasma amino acid concentrations after meals. *J Clin Invest*, 5297), 1586–1594.
- Alpers, D. H. (1987). Digestion and absorption of carbohydrate and proteins. In: L. R. Johnson (Ed), *Physiology of the gastrointestinal tract* (pp. 1469–1487). New York: Raven Press.
- Anderson, G. H., & Moore, S. E. (2004). Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr*, 134(4), 974S–979S.
- Anderson, G. H., Tecimer, S. N., Shah, D., & Zafar, T. A. (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.
- Boirie, Y., Dangin, M., Gachon, P., Vasson, M. P., Maubois, J. L., & Beaufrère, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci U S A*, 94(26), 14930–14935.
- Buffenstein, R., Poppitt, S. D., McDevitt, R. M., & Prentice, A. M. (1995). Food intake and the menstrual cycle: a retrospective analysis, with implications for appetite research. *Physiol Behav*, 58(6), 1067–1077.
- Burn-Murdoch, R. A., Fisher, M. A., & Hunt, J. N. (1978). The slowing of gastric emptying by proteins in test meals. *J Physiol*, 274, 477–485.
- Burton-Freeman, B. M. (2008). Glycomacropeptide (GMP) is not critical to whey-induced satiety, but may have a unique role in energy intake regulation through cholecystokinin (CCK). *Physiol Behav*, 93(1-2), 379–387.
- Calbet, J. A., & Holst, J. J. (2004). Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. *Eur J Nutr*, 43(3), 127–139.
- Chungchunlam, S. M. S., Moughan, P. J., Henare, S. J., & Ganesh, S. (2012). Effect of time of consumption of preloads on measures of satiety in healthy normal weight women. *Appetite*, 59(2), 281–288. (see Chapter 4)
- Diepvens, K., Häberer, D., & Westerterp-Plantenga, M. (2008). Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *Int J Obes*, 32(3), 510–518.
- Dye, L., & Blundell, J. E. (1997). Menstrual cycle and appetite control : implications for weight management. *Hum Reprod*, 12(6), 1142–1151.
- Eisenstein, J., Roberts, S. B., Dallal, G., & Saltzman, E. (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), 189–200.
- Etzel, M. R. (2004). Manufacture and use of dairy protein fractions. *J Nutr*, 134(4), 996S–1002S.

- Farnfield, M. M., Trenerry, C., Carey, K. A., & Cameron-Smith, D. (2009). Plasma amino acid response after ingestion of different whey protein fractions. *Int J Food Sci Nutr*, *60*(6), 476–486.
- Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord*, *24*(1), 38–48.
- Gannon, M. C., Nuttall, F. Q., Westphal, S. A., Neil, B. J., & Seaquist, E. R. (1989). Effects of dose of ingested glucose on plasma metabolite and hormone responses in type II diabetic subjects. *Diabetes Care*, *12*(8), 544–552.
- Gustafson, D. R., McMahon, D. J., Morrey, J., & Nan, R. (2001). Appetite is not influenced by a unique milk peptide: caseinomacropeptide (CMP). *Appetite*, *36*(2), 157–163.
- Hall, W. L., Millward, D. J., Long, S. J., & Morgan, L. M. (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., & 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.
- Haque, E., Chand, R., Kapila, S. (2008). Biofunctional properties of bioactive peptides of milk origin. *Food Rev Int*, *25*(1), 28–43.
- Hegarty, J. E., Fairclough, P. D., Moriarty, K. J., Clark, M. J., & Dawson, A. M. (1982). Comparison of plasma and intraluminal amino acid profiles in man after meals containing a protein hydrolysate and equivalent amino acid mixture. *Gut*, *23*(8), 670–674.
- Hursel, R., van der Zee, L., & Westerterp-Plantenga, M. S. (2010). Effects of a breakfast yoghurt, with additional total whey protein or caseinomacropeptide-depleted alpha-lactalbumin-enriched whey protein, on diet-induced thermogenesis and appetite suppression. *Br J Nutr*, *103*(5), 775–780.

- Jahan-Mihan, A., Luhovyy, B. L., El Khoury, D., & Anderson, G. H. (2011). Dietary proteins as determinants of metabolic and physiological functions of the gastrointestinal tract. *Nutrients*, *3*(5), 574–603.
- Kastenbaum, M. A., Hoel, D. G., & Brown, K. O. (1970). Sample size requirements: randomized block designs. *Biometrika*, *57*(3), 573–577.
- Keogh, J. B., Woonton, B. W., Taylor, C. M., Janakievski, F., Desilva, K., & Clifton, P. M. (2010). Effect of glycomacropeptide fractions on cholecystokinin and food intake. *Br J Nutr*, *104*(2), 286–290.
- Kirimura, J., Shimizu, A., Kimizuka, A., Ninomiya, T., & Katsuya, N. (1969). Contribution of peptides and amino acids to the taste of foods. *J Agric Food Chem*, *17*(4), 689–695.
- Lam, S. M., Moughan, P. J., Awati, A., & Morton, H. R. (2009). The influence of whey protein and glycomacropeptide on satiety in adult humans. *Physiol Behav*, *96*(1), 162–168. (see Chapter 3)
- Lowe, M. R., & Thomas, J. G. (2009). Measures of restrained eating. In : D. B. Allison, & M. L. Baskin (Eds.), *Handbook of assessment methods for eating behaviors and weight-related problems: measures, theory, and research* (pp. 137–185). CA, Sage: Thousand Oaks.
- Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr*, *26*(6), 704S–712S.
- Marrs, T. C., Addison, J. M., Burston, D., & Matthews, D. M. (1975). Changes in plasma amino acid concentrations in man after ingestion of an amino acid mixture simulating casein, and a tryptic hydrolysate of casein. *Br J Nutr*, *34*(2), 259–265.
- Mellinkoff, S. M., Frankland, M., Boyle, D., & Greipel, M. (1956). Relationship between serum amino acid concentration and fluctuations in appetite. *J Appl Physiol*, *8*(5), 535–538.
- Morifuji, M., Ishizaka, M., Baba, S., Fukuda, K., Matsumoto, H., Koga, J., Kanegae, M., & Higuchi, M. (2012). Comparison of different sources and degrees of hydrolysis of dietary protein: effect on plasma amino acids, dipeptides, and insulin responses in human subjects. *J Agri Food Chem*, *58*(15), 8788–8797.

- Moughan, P. J. (2008). Milk proteins: a cornucopia for developing functional foods. In: A. Thompson, M. Boland, & H. Singh (Eds.), *Milk proteins: from expression to food* (pp. 483–499). London: Academic Press.
- Moughan, P. J., Fuller, M. F., Han, K. S., Kies, A. K., & Miner-Williams, W. (2007). Food-derived bioactive peptides influence gut function. *Int J Sport Nutr Exerc Metab*, *17 Suppl*, S5–S22.
- Nilsson, M., Holst, J. J., & Björck, I. M. (2007). Metabolic effects of amino acid mixtures and whey protein in healthy subjects: studies using glucose-equivalent drinks. *Am J Clin Nutr*, *85*(4), 996–1004.
- Nishimura, T., & Kato, H. (1988). Taste of free amino acids and peptides. *Food Rev Int*, *4*(2), 175–194.
- Ott, L., & Longnecker, M. (2010). *An introduction to statistical methods and data analysis*. 6th ed. Belmont, CA: Brooks/Cole Cengage Learning.
- Pal, S., & 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.
- Pichon, L., Potier, M., Tome, D., Mikogami, T., Laplaize, B., Martin-Rouas, C., & Fromentin, G. High-protein diets containing different milk protein fractions differently influence energy intake and adiposity in the rat. *Br J Nutr*, *99*(4), 739–748.
- Potier, M., Fromentin, G., Lesdema, A., Benamouzig, R., Tomé, D., & Marsset-Baglieri, A. (2010). The satiety effect of disguised liquid preloads administered acutely and differing only in their nutrient content tended to be weaker for lipids but did not differ between proteins and carbohydrates in human subjects. *Br J Nutr*, *104*(9), 1406–1414.
- Power, O., Hallihan, A., & Jakeman, P. (2009). Human insulinotropic response to oral ingestion of native and hydrolysed whey protein. *Amino Acids*, *37*(2), 333–339.
- Rutherford-Markwick, K. J., & Moughan, P. J. (2005). Bioactive peptides derived from food. *JAOAC Int*, *88*(3), 955–966.
- Silk, D. B., Chung, Y. C., Berger, K. L., Conley, K., Beigler, M., Slesinger, M. H., Spiller, G. A., Kim, Y. S. (1979). Comparison of oral feeding of peptide and amino acid meals to normal human subjects. *Gut*, *20*(4), 291–299.

- Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*, 29(1), 71–83.
- Te Morenga, L., & Mann, J. (2012). The role of high-protein diets in body weight management and health. *Br J Nutr*, 108(Suppl 2), S130–S138.
- Ten Have, G. A., Engelen, M. P., Luiking, Y. C., & Deutz, N. E. (2007). Absorption kinetics of amino acids, peptides, and intact proteins. *Int J Sport Nutr Exerc Metab*, 17 Suppl, S23–S36.
- Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N., & Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. *Physiol Behav*, 94(2), 300–307.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., van Vught, A. J., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009c). Dose-dependent satiating effect of whey relative to casein or soy. *Physiol Behav*, 96(4-5), 675–682.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009a). Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety. *Appetite*, 52(2), 388–395.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009b). A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. *Clin Nutr*, 28(2), 147–155.
- Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006) *Dairy science and technology*. 2nd ed. Boca Raton: CRC/Taylor & Francis.
- WHO. (2012). *Obesity and overweight (WHO fact sheet no. 311)*. World Health Organization.
- Westerterp-Plantenga, M. S., Nieuwenhuizen, A. Tomé, D., Soenen, S., & Westerterp, K. R. (2009). Dietary protein, weight loss, and weight maintenance. *Annu Rev Nutr*, 29(7), 21–41.

# **Chapter 8**

## **General Discussion and Conclusion**

Protein is the most satiating macronutrient (*Crovetti et al., 1998; Geliebter, 1979; Johnson & Vickers, 1993; Marmonier et al., 2000; Poppitt et al., 1998; Stubbs et al., 1996*). There is also an effect of dietary protein type on satiety, with dairy whey protein having been shown to elicit a higher satiating effect compared with other protein sources in adult humans (*Anderson et al., 2004; Diepvens et al., 2008; Hall et al., 2003; Pal & Ellis, 2010; Veldhorst et al., 2009a*). This dissertation reports work from several studies on the effect of whey protein on satiety in adult humans. The first study (*Chapter 2*) aimed to confirm that dietary whey protein induces satiety compared with maltodextrin carbohydrate. The objective here, was to provide a baseline for the following studies. However, the results obtained showed equivocal evidence for this and several methodological variables of the first study were considered to be important. In particular, the time delay interval between preload and test meal was proposed as being critical and was investigated in a second study (*Chapter 3*). In addition, the second study was designed to measure circulating hormones and metabolites as potential mediators by which whey protein may induce satiety (*Chapter 4*). Human preload studies identifying which characteristic of whey protein may cause its satiating effect are scarce and the results remain inconclusive. Two studies were thus undertaken (*Chapters 5 & 7*) to compare the effects on satiety of major individual constituent proteins of whey protein and a free amino acid mixture simulating the amino acid composition of whey protein, with the effect of intact whey protein *per se*. For the latter purpose, the amino acid composition of whey protein needed to be determined accurately and this was the aim of the study reported in Chapter 6.

In the studies reported in this dissertation (*Chapters 2, 3, 4, 5 & 7*), maltodextrin was the carbohydrate source tested and this carbohydrate was chosen as the control for several reasons. Maltodextrin is an oligosaccharide, made up of glucose monomers present in corn starch, which are absorbed as rapidly as free glucose (*Southgate, 1995*). As maltodextrin is less sweet than either glucose or sucrose, a large amount can be added to a meal to increase the carbohydrate content without affecting overall sweetness. The aim was to

compare the effect of protein-enriched preloads versus carbohydrate-enriched preloads, and as such the “carbohydrate” preload treatments were predominantly comprised of maltodextrin but also contained some protein, while the “protein” preload treatments contained predominantly protein but also carbohydrate. It is possible that the non-protein composition (mainly carbohydrate providing 20-50% of metabolisable energy) of the preloads may have lessened the effect of the protein preloads. On the other hand, the enriched preloads, containing both protein and carbohydrate, provided the subjects with a more balanced and possibly acceptable preload meal. Moreover, it should be noted that although the dose of protein provided has been shown to be effective in promoting satiety in acute short-term studies (*see Chapter 1 & Appendix 1*), the amount, in practice is large, representing the required protein intake of an adult human per day (50 g of protein required for a 60 kg adult human per day) (*WHO, 2002*).

The objective of the first study (*Chapter 2*) was to confirm that dietary whey protein induces satiety compared with maltodextrin carbohydrate in normal-weight adult humans. Moreover, a combination of whey protein and glycomacropeptide (GMP), where GMP is known to stimulate the release of the satiety-related hormone cholecystokinin, was hypothesised to have a stronger effect on satiety than whey protein without GMP. Nineteen men and thirty-one women consumed a preload beverage enriched with either maltodextrin carbohydrate (40 g), acid whey protein devoid of GMP (40 g), cheese whey protein containing 21% naturally occurring GMP (40 g), or a mixture of cheese whey protein (20 g) and GMP isolate (20 g). Subjects consumed a “cafeteria” test meal 30 min following administration of the preload drink and rated their subjective feelings of appetite using visual analogue scales (VAS). Ingestion of the preload beverages enriched with protein (40 g) did not reduce subsequent *ad libitum* food intake relative to maltodextrin carbohydrate. Subjects rated themselves as feeling fuller immediately following consumption of the preload drink containing cheese whey protein including GMP compared to the other three preload drinks. Given that the effects of whey protein and GMP on satiety were not clear in the first

study, it was elected to evaluate several potential methodological limitations of the first study that may have masked the effect of whey protein. In particular the time delay interval between preload and test meal was a concern, and this was investigated directly in the second study (*Chapter 3*).

The experimental protocol used in the first study (*Chapter 2*) followed an established methodology for the study of the effect of macronutrients on food intake and satiety in human subjects (*Rolls & Hammer, 1995*). In the first study, women compensated for the high amount of protein in the protein preloads by choosing to eat a subsequent meal that was lower in protein. The finding that women may be more sensitive to dietary manipulations and parameters of satiety than men is in agreement with the results of other studies (*Burley et al., 1993; Burton-Freeman et al., 2004; Drapeau et al., 2007; Schneeman et al., 2003*). This needs to be investigated in more detail. Participants were studied in a “cafeteria” setting and were provided with a buffet style test meal after administration of a preload. As such, environmental factors (*De Castro, 1988*) and the variety of foods at the test meal (*Rolls et al., 1981*) may have overridden the physiological satiety signals. Consequently, in the subsequent studies undertaken (*Chapters 3, 4, 5 & 7*), only women were recruited. The women consumed their test meals in individual private cubicles, and a single-item test meal consisting of a mixed homogeneous fried rice meal was used. Women also participated only during the menstrual period and follicular phase of their menstrual cycle to avoid metabolic and appetite shifts that may occur during ovulation and the luteal phase (*Buffenstein et al., 1995; Davidsen et al., 2007; Dye & Blundell, 1997*). Moreover, the dose of whey protein (40 g contributing ~32 g of absolute protein) used in the first study (*Chapter 2*) may have been too low to enable an effect to be seen. Previous preload studies have found that 40-50 g of whey protein was effective in suppressing food intake and feelings of appetite (*see Appendix 1* for review; *Akhavan et al., 2010; Astbury et al., 2010*) and such higher doses of whey protein were used in the subsequent studies (45-60 g contributing ~42-54 g of absolute protein).

The second human study had two main objectives. The first aim was to assess whether the time interval between ingestion of a preload and consumption of an *ad libitum* test meal was an important factor in determining the satiating response to whey protein (*Chapter 3*). The second aim was to determine the effect of ingesting a whey protein-enriched preload beverage on the postprandial plasma hormones related to the regulation of food intake and satiety, and plasma metabolites associated with protein catabolism, in comparison with maltodextrin carbohydrate (*Chapter 4*). There was no effect of time delay interval between preload and test meal (30, 60 or 120 min) on satiety (*Chapter 3*). This is in line with results from similar studies (*Bellissimo et al., 2007; Bertenshaw et al., 2008; Potier et al., 2010*). However, the results showed that the ingestion of whey protein containing GMP consistently led to a reduced subsequent food energy intake compared with that of maltodextrin carbohydrate and water, but no difference was observed in VAS-rated subjective feelings of appetite (*Chapter 3*). At the time delay of 120 min, when adult women consumed either the carbohydrate- or whey protein-enriched preload drink, blood samples were collected at regular time intervals to determine possible biochemical and metabolic mechanisms by which whey protein may induce satiety versus maltodextrin carbohydrate (*Chapter 4*). It was found that increased postprandial plasma concentrations of pancreatic polypeptide (PP) may be a likely biochemical indicator of the satiating effect of whey protein, as a lower subsequent food energy intake was related to elevated plasma concentrations of PP. The extent to which protein promotes satiety via PP release in humans requires further investigation. In addition, consumption of whey protein resulted in an increased concentration of plasma amino acids related to its amino acid composition (specifically branched-chain amino acids). An increase in serum amino acid concentrations acts as a satiety signal upon protein ingestion (*Mellinkoff et al., 1956*). It seems that elevated concentrations of plasma amino acids coincided with increased plasma PP concentrations, implying a correlated effect with PP to contribute to the induction of satiety. While satiety and metabolic responses to the consumption of particular free amino acids have been studied (*Butler et al., 1981; Nilsson et al., 2007; Veldhorst et al., 2009c*), the contribution of the ratios of amino acids is unknown and needs further study. Moreover, it has been suggested that the

timing of administration of a subsequent test meal relative to a preload may coincide with the pattern of change of circulating amino acids or hormones (Luhovyy *et al.*, 2007; Veldhorst *et al.*, 2009b). A decrease in plasma concentrations of the orexigenic hormone ghrelin (but no difference between whey protein and carbohydrate), and an increase in plasma concentrations of amino acids and the anorexigenic hormone PP was shown to occur 15 min following ingestion of the whey protein preload and was sustained over the duration of the test period (120 min) (Chapter 4). Looking at the other anorexigenic hormones, consumption of whey protein induced an increase in plasma concentrations of cholecystokinin (CCK) at 60 and 75 min, glucagon-like peptide-1 (GLP-1) at 90 min, peptide tyrosine-tyrosine (PYY) at 90 and 120 min. Therefore, it is not appropriate to use blood parameters to determine the optimal time delay interval between preload and test meal and it is important to emphasise that the hormonal and metabolic factors present in the peripheral blood are only indicators of the promotion of satiety and may not have a causal effect.

The remainder of the studies (Chapters 5, 6 & 7) aimed at investigating the underlying cause for the higher satiating effect of whey protein. It may be attributable to the predominant constituent protein fractions (beta-lactoglobulin, glycomacropeptide, or alpha-lactalbumin), the amino acid profile of whey protein *per se*, or a combination of the factors. The third human study (Chapter 5) showed that pure glycomacropeptide does not affect subsequent food intake and subjective feelings of appetite, as previously reported (Burton-Freeman, 2008; Gustafson *et al.*, 2001; Keogh *et al.*, 2010).

Whey-protein induced satiety may be mediated by plasma amino acids related to its amino acid composition, the intact proteins themselves, or to bioactive peptides released during gastrointestinal digestion (Haque *et al.*, 2008; Jahan-Mihan *et al.*, 2011; Madureira *et al.*, 2010; Moughan *et al.*, 2007; Rutherford-Markwick & Moughan, 2005). A free amino acid mixture simulating the amino acid composition of whey protein was formulated to be used in the fourth human study (Chapter 7). The amino acid composition of the

they protein had been determined with a high level of accuracy (*Chapter 6*) using multiple hydrolysis times and by fitting a nonlinear model which takes into account the simultaneous hydrolysis of bound amino acids and degradation of free amino acids. The fourth study (*Chapter 7*) was then undertaken to compare the effect, on food intake and satiety, of intact whey protein, isolated alpha-lactalbumin, isolated beta-lactoglobulin, and a free amino acid mixture simulating the amino acid composition of whey protein. This is the first study comparing the satiating response to consumption of whey protein with that following a free amino acid mixture simulating the amino acid composition of whey protein in adult humans. A novel finding of this dissertation is that whey protein constituents and the free amino acid mixture were satiating to a similar extent to intact whey protein *per se* (*Chapter 7*). Therefore, this provides preliminary evidence that the satiating effect of whey protein may be attributed to its amino acid composition and possibly elevated plasma concentrations of amino acids, relative to carbohydrate, but this requires further investigation. The four preload treatments exhibited similar rates of emptying from the stomach (*S. Chungchunlam, unpublished data*) and likely similar dynamics of uptake of the amino acids. This should be considered more fully in future studies.

It is known that proteins differ in their amino acid composition (*Gilbert et al., 2011*) but it is not clear whether such differences are the underlying cause for reported differences in the promotion of satiety. Whey protein, egg albumen, and soya protein are all considered to be “complete” proteins as they provide all of the dietary essential amino acids, but differ in their satiating efficiency. Other protein sources are completely devoid of specific amino acids in the pure form, such as the lack of phenylalanine, tryptophan, tyrosine, arginine, cysteine, and histidine in glycomacropeptide (GMP), no tryptophan present in gelatin, and no lysine in zein. Therefore, future research should investigate the extent to which dietary manipulation of amino acid intakes (possibly at levels to meet adult amino acid requirements) affects the induction of satiety and the regulation of food intake. More information on the mechanisms involved is needed.

The dose at which protein was provided in the studies (50 g amino acid equivalent) was high, meeting the daily protein requirements of a 60 kg adult human (WHO, 2002). In the first study (Chapter 2), when given a choice of test meals, women compensated for the high amount of protein in the preload by choosing to eat a test meal lower in protein. However, in the second study (Chapter 3), this compensation effect was not seen in the reported protein intake (food diary) for the remainder of the day, and the total protein intake for the day (breakfast + preload + test meal + reported food diary intakes) was higher when the whey protein preload was consumed compared with carbohydrate or water. Interestingly, total food energy intake for the day when a preload was ingested, independent of the energy and macronutrient contents, was similar (Chapter 3). Another study has also found that daily food energy intake, when a preload containing either protein or carbohydrate was consumed, was similar to that when no preload was ingested (Potier *et al.*, 2010). This implies that normal-weight adult humans are able to maintain a constant daily food energy intake. This raises important questions concerning the short-term measures of satiety and the limitations of their results. It should be considered that the maximum impact on the suppression of further food intake induced by consumption of whey protein as determined in acute studies does not necessarily endure over longer time periods.

The most common method for measurement of feelings of appetite in satiety studies is the visual analogue scale (VAS). In the studies reported in this dissertation, this validated scale was used to assess appetite feelings, including hunger, desire to eat, prospective food consumption, and fullness. As expected, VAS-rated feelings of appetite differed over time and were associated with the consumption of a meal. However, it is not clear why VAS measurements of appetite feelings were not effective in detecting differences across preloads postprandially in most studies, where differences in *ad libitum* food intake were found. Possible reasons for this include a small sample size, variation between subjects, inability to detect small differences, and the time point of ratings. It may be useful to test for more appropriate times of measurement of

VAS-rated feelings of appetite in future studies. In addition, VAS have been used extensively to assess ‘non-appetite’ sensations due to their ease of design and interpretation. For instance, in the reported studies (*Chapters 3, 5 & 7*), VAS was used to measure the perception of sensory properties of preloads immediately following consumption of the preloads. The results obtained showed that this tool was sensitive enough to detect differences, but the reproducibility and validity of the VAS scales to describe ‘non-appetite’ perceptions is not clear, suggesting caution in their use in this respect.

It should be recognised that although the preload-test meal design has been preferentially used in satiety studies, a limitation of this method is that it does not adequately reflect free-living conditions. Subjects are constrained within a laboratory setting and their eating behaviour and physical activity are restricted. Another disadvantage of running a preload study is that it is expensive and time-consuming. Its relevance to real life conditions is questionable and a large sample size may be required to enable differences in the measures of satiety to be detected. It has been queried as to whether a better approach would be to have subjects placed in a laboratory deprived of time cues and knowledge of the nutritional composition of a preload, to assess the time of request for a subsequent test meal. Moreover, most satiety studies have been conducted using normal-weight subjects as they are in a relatively stable energy balance and exhibit similar feeding behaviour. There is little information regarding the promotion of satiety in overweight and obese individuals (*Bowen et al., 2006a, 2006b, 2007, 2008; Hill & Blundell, 1990; Leidy et al., 2010; Poppitt et al., 2011*). Nonetheless, the findings of satiety studies in normal-weight subjects have been extrapolated to dietary interventions aimed at body weight loss in overweight and obese participants.

The main results of the acute short-term studies in this dissertation suggest that whey protein is more effective in reducing subsequent food intake compared to maltodextrin carbohydrate. The studies provide an insight into the underlying mechanisms associated with long-term effects. Long-term studies suggest that consumption of a high protein diet (providing > 15% of energy)

results in body weight loss. The question remains as to whether short-term acute studies are consistent with the body composition changes observed in long-term feeding studies, in particular the contribution of whey protein. A recent study by Frestedt *et al.* (2008) showed that when obese participants supplemented their energy-restricted diet (500 kcal per day) with a specialised whey protein fraction (intact whey protein, peptides and minerals, e.g. calcium, 20 g protein per day), they lost more body fat and had a lower lean body mass at 12 weeks compared with receiving an isoenergetic supplement containing maltodextrin. Both groups lost body weight, but there was no statistically significant difference between the supplements. This highlights the importance of body composition measurements in addition to body weight measurements and demonstrates that a relationship between short-term and long-term studies does exist. Further studies investigating the longer-term (at least 6 months) effects of dietary whey protein supplements in overweight and obese subjects are required. There is also an important opportunity for research to explore whether the consumption of whey protein over the long term results in a decrease in body fat and an increase in the retention of lean tissue mass in normal-weight subjects, and thus reduces the risk of developing obesity.

## 8.1. Conclusions

With respect to the relatively greater satiety induced by whey protein in comparison with carbohydrate, the following conclusions can be made:

- (1) Consumption of 45 g whey protein (which included glycomacropeptide) promotes satiety to a greater extent than maltodextrin carbohydrate.
- (2) The time of administration of an *ad libitum* test meal following ingestion of a preload does not appear to be a factor in determining the satiating response to a preload containing whey protein.

- (3) Increased postprandial plasma concentrations of anorexigenic hormones (CCK, GLP-1, PYY, PP) and metabolites of protein catabolism (amino acids, urea and ammonia) are potential mediators by which whey protein induces satiety.
- (4) Little evidence was found for the hypothesis that the satiating effect of whey protein is attributable to its major individual protein constituents (glycomacropeptide, beta-lactoglobulin, and alpha-lactalbumin).
- (5) It can be concluded that the satiating effect of whey protein is mediated by virtue of its amino acid composition, as the satiety response to ingestion of a free crystalline amino acid mixture simulating the amino acid composition of whey protein was similar to that of intact whey protein.

## **8.2. Recommendations for future research**

The studies in this dissertation were based on the assumption that protein is more satiating than carbohydrate. The question remains as to whether other types of protein affect satiety differently from whey protein.

Regarding subject characteristics contributing to the effect of protein on satiety, several aspects remain to be elucidated. Gender and/or body size and body composition need to be further investigated as factors influencing the outcomes of satiety studies.

A finding presented in this dissertation is that the reduction in food intake following ingestion of whey protein was related to a postprandial increase in plasma concentrations of the anorexigenic hormone pancreatic polypeptide (PP). The role of PP in protein-mediated satiety needs to be further

explored and a possible synergistic effect with other satiety-related hormones or metabolites of protein catabolism should also be assessed.

With respect to the characteristics of whey protein contributing to whey protein-induced satiety, the individual major protein fractions (glycomacropeptide, alpha-lactalbumin, and beta-lactoglobulin) did not seem to play a role. It would be of interest to study the effects of the minor protein constituents of whey protein on the regulation of food intake and satiety.

The studies in this dissertation showed that the satiating effect of whey protein may be attributed to its amino acid composition and postprandial plasma amino acid (specifically branched-chain amino acid) response. The effects of different types of protein, with particular amino acid compositions, on satiety and the appearance of plasma amino acids postprandially remain to be investigated.

Studies investigating the long-term effects of high protein intake on changes in body composition in overweight or obese human subjects have shown successful body weight loss and maintenance of body weight. It remains to be established whether intake of a high-protein diet, using whey protein as the protein source in comparison with other proteins, contributes to favourable body weight loss and weight maintenance. Measurements of the short-term effects of protein consumption will provide additional insight into the underlying mechanisms associated with the beneficial effects of high-protein diets over the long term.

### **8.3. Literature cited**

Akhavan, T., Luhovyy, B. L., Brown, P. H., Cho, C. E., & Anderson, G. H. (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.
- Anderson, G. H., Tecimer, S. N., Shah, D., & Zafar, T. A. (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.
- Astbury, N. M., Stevenson, E. J., Morris, P., Taylor, M. A., & Macdonald, I. A. (2010). Dose-response effect of a whey protein preload on within-day energy intake in lean subjects. *Br J Nutr*, 104(12), 1858–1867.
- Bellissimo, N., Desantadina, M. V., Pencharz, P. B., Berall, G. B., Thomas, S. G., & Anderson, G. H. (2007). A comparison of short-term appetite and energy intakes in normal weight and obese boys following glucose and whey-protein drinks. *Int J Obes*, 32(2), 362–371.
- Bertenshaw, E. J., Lluch, A., & Yeomans, M. R. (2008). Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiol Behav*, 93(3), 427–436.
- Bowen, J., Noakes, M., & Clifton, P. M. (2006a). 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., & Clifton, P. M. (2007). Appetite hormones and energy intake in obese men after consumption of fructose, glucose and whey protein beverages. *Int J Obes*, 31(11), 1696–1703.
- Bowen, J., Noakes, M., & Clifton, P. M. (2008). Role of protein and carbohydrate sources on acute appetite responses in lean and overweight men. *Nutr Diet*, 65(S3), S71–S78.
- Bowen, J., Noakes, M., Trenerry, C., & Clifton, P. M. (2006b). Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *J Clin Endocrinol Metab*, 91(4), 1477–1483.
- Buffenstein, R., Poppitt, S. D., McDevitt, R. M., & Prentice, A. M. (1995). Food intake and the menstrual cycle: a retrospective analysis, with implications for appetite research. *Physiol Behav*, 58(6), 1067–1077.

- Burley, V. J., Paul, A. W., & Blundell, J. E. (1993). Influence of a high-fibre food (myco-protein) on appetite: effects on satiation (within meals) and satiety (following meals). *Eur J Clin Nutr*, 47(6), 409–418.
- Burton-Freeman, B. M. (2008). Glycomacropeptide (GMP) is not critical to whey-induced satiety, but may have a unique role in energy intake regulation through cholecystokinin (CCK). *Physiol Behav*, 93(1-2), 379–387.
- Burton-Freeman, B., Davis, P. A., & Schneeman, B. O. (2004). Interaction of fat availability and sex on postprandial satiety and cholecystokinin after mixed-food meals. *Am J Clin Nutr*, 80(5), 1207–1214.
- Butler, R. N., Davies, M., Gehling, N. J., & Grant, A. K. (1981). The effect of preloads of amino acid on short-term satiety. *Am J Clin Nutr*, 34(10), 2045–2047.
- Crovetti, R., Porrini, M., Santangelo, A., & Testolin, G. (1998). The influence of thermic effect of food on satiety. *Eur J Clin Nutr*, 52(7), 482–488.
- Davidson, L., Vistisen, B., & Astrup, A. (2007). Impact of the menstrual cycle on determinants of energy balance: a putative role in weight loss attempts. *Int J Obes*, 31(12), 1777–1785.
- De Castro, J. M. (1988). Physiological, environmental, and subjective determinants of food intake in humans: a meal pattern analysis. *Physiol Behav*, 44(4-5), 651–659.
- Diepvens, K., Häberer, D., & Westerterp-Plantenga, M. (2008). Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *Int J Obes*, 32(3), 510–518.
- Drapeau, V., King, N., Hetherington, M., Doucet, E., Blundell, J., & Tremblay, A. (2007). Appetite sensations and satiety quotient: predictors of energy intake and weight loss. *Appetite*, 48(2), 159–166.
- Dye, L., & Blundell, J. E. (1997). Menstrual cycle and appetite control: implications for weight management. *Hum Reprod*, 12(6), 1142–1151.
- Frestedt, J. L., Zenk, J. L., Kuskowski, M. A., Ward, L. S., & Bastian, E. D. (2008). A whey-protein supplement increases fat loss and spares lean muscle in obese subjects: a randomized human clinical study. *Nutr Metab (Lond)*, 5, 8.

- Geliebter, A. A. (1979). Effects of equicaloric loads of protein, fat, and carbohydrate on food intake in the rat and man. *Physiol Behav*, 22(2), 267–273.
- Gilbert, J. A., Bendsen, N. T., Tremblay, A., & Astrup, A. (2011). Effect of proteins from different sources on body composition. *Nutr Metab Cardiovasc Dis*, 21 (Suppl 2), B16–B31.
- Gustafson, D. R., McMahon, D. J., Morrey, J., & Nan, R. (2001). Appetite is not influenced by a unique milk peptide: caseinomacropeptide (CMP). *Appetite*, 36(2), 157–163.
- Hall, W. L., Millward, D. J., Long, S. J., & Morgan, L. M. (2003). Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br J Nutr*, 89(2), 239–248.
- Haque, E., Chand, R., Kapila, S. (2008). Biofunctional properties of bioactive peptides of milk origin. *Food Rev Int*, 25(1), 28–43.
- Hill, A. J., & Blundell, J. E. (1990). Comparison of the action of macronutrients on the expression of appetite in lean and obese human subjects. *Ann N Y Acad Sci*, 580(1), 529–531.
- Jahan-Mihan, A., Luhovyy, B. L., El Khoury, D., & Anderson, G. H. (2011). Dietary proteins as determinants of metabolic and physiological functions of the gastrointestinal tract. *Nutrients*, 3(5), 574–603.
- Johnson, J., & Vickers, Z. (1993). Effect of flavor and macronutrient composition of food servings on liking, hunger and subsequent intake. *Appetite*, 21(1), 25–39.
- Keogh, J. B., Woonton, B. W., Taylor, C. M., Janakievski, F., Desilva, K., & Clifton, P. M. (2010). Effect of glycomacropeptide fractions on cholecystokinin and food intake. *Br J Nutr*, 104(2), 286–290.
- Leidy, H. J., Armstrong, C. L., Tang, M., Mattes, R. D., & Campbell, W. W. (2010). (2010). The influence of higher protein intake and greater eating frequency on appetite control in overweight and obese men. *Obesity*, 18(9), 1725–1732.
- Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr*, 26(6), 704S–712S.

- Madureira, A. R., Tavares, T., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2010). Invited review: physiological properties of bioactive peptides obtained from whey proteins. *J Dairy Sci*, *93*(2), 437–455.
- Marmonier, C., Chapelot, D., & Louis-Sylvestre, J. (2000). Effects of macronutrient content and energy density of snacks consumed in a satiety state on the onset of the next meal. *Appetite*, *34*(2), 161–168.
- Mellinkoff, S. M., Frankland, M., Boyle, D., & Greipel, M. (1956). Relationship between serum amino acid concentration and fluctuations in appetite. *J Appl Physiol*, *8*(5), 535–538.
- Moughan, P. J., Fuller, M. F., Han, K. S., Kies, A. K., & Miner-Williams, W. (2007). Food-derived bioactive peptides influence gut function. *Int J Sport Nutr Exerc Metab*, *17* Suppl, S5–S22.
- Nilsson, M., Holst, J. J., & Björck, I. M. (2007). Metabolic effects of amino acid mixtures and whey protein in healthy subjects: studies using glucose-equivalent drinks. *Am J Clin Nutr*, *85*(4), 996–1004.
- Pal, S., & 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.
- Poppitt, S. D., McCormack, D., & 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., Wiessing, K. R., Falk, S., Xin, L., Budgett, S. C., Darragh, A., & Hall, R. S. (2011). Low-dose whey protein-enriched water beverages alter satiety in a study of overweight women. *Appetite*, *56*(2), 456–464.
- Potier, M., Fromentin, G., Lesdema, A., Benamouzig, R., Tomé, D., & Marsset-Baglieri, A. (2010). The satiety effect of disguised liquid preloads administered acutely and differing only in their nutrient content tended to be weaker for lipids but did not differ between proteins and carbohydrates in human subjects. *Br J Nutr*, *104*(9), 1406–1414.
- Rolls, B. J., & Hammer, V. A. (1995). Fat, carbohydrate, and the regulation of energy intake. *Am J Clin Nutr*, *62*(5 Suppl), 1086S–1095S.

- Rolls, B. J., Rowe, E. A., Rolls, E. T., Kingston, B., Megson, A., & Gunary, R. (1981). Variety in a meal enhances food intake in man. *Physiol Behav*, 26(2), 215–221.
- Rutherford-Markwick, K. J., & Moughan, P. J. (2005). Bioactive peptides derived from food. *JAOAC Int*, 88(3), 955–966.
- Schneeman, B. O., Burton-Freeman, B., & Davis, P. (2003). Incorporating dairy foods into low and high fat diets increases the postprandial cholecystokinin response in men and women. *J Nutr*, 133(12), 4124–4128.
- Southgate, D. A. (1995). Digestion and metabolism of sugars. *Am J Clin Nutr*, 62(1 Suppl), 203S–210S, discussion 211S.
- Stubbs, R. J., Van Wyk, M. C., Johnstone, A. M., & Harbron, C. G. (1996). Breakfasts high in protein, fat or carbohydrate: effect on within-day appetite and energy balance. *Eur J Clin Nutr*, 50(7), 409–417.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., van Vught, A. J., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009a). Dose-dependent satiating effect of whey relative to casein or soy. *Physiol Behav*, 96(4-5), 675–682.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009b). Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety. *Appetite*, 52(2), 388–395.
- Veldhorst, M. A., Nieuwenhuizen, A. G., Hochstenbach-Waelen, A., Westerterp, K. R., Engelen, M. P., Brummer, R. J., Deutz, N. E., & Westerterp-Plantenga, M. S. (2009c). A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. *Clin Nutr*, 28(2), 147–155.
- WHO (World Health Organisation). (2002). *Protein and amino acid requirements in human nutrition (WHO technical report series 935.)*. Report of a Joint WHO/FAO/UNU Expert Consultation. Geneva: World Health Organization.

# Appendices

# Appendix 1

## Whey protein and satiety: implications for diet and behavior

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The objective of this chapter, presented in the form of a published review, was to extend the review of literature (*Chapter 1*) and to review the effects of whey protein on the regulation of food intake and satiety. The evidence associating protein intake with satiety and body weight is reviewed first, followed by a brief review of the potential mechanisms of action of protein on satiety. The effect of dairy whey protein, particularly glycomacropeptide, on food intake and satiety are discussed.

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## Chapter 72

# Whey Protein and Satiety: Implications for Diet and Behavior

Sylvia M.S. Chung Chun Lam and Paul J. Moughan

### Abbreviations

VAS	Visual analogue scales
GMP	Glycomacropeptide
BCAAs	Branched chain amino acids
$\beta$ -Ig	$\beta$ -Lactoglobulin
$\alpha$ -Ia	$\alpha$ -Lactalbumin
BSA	Bovine serum albumin
Ig	Immunoglobulins
CCK	Cholecystokinin
GLP-1	Glucagon-like peptide-1
PYY	Peptide YY
DIT	Diet-induced thermogenesis

### 72.1 Introduction

In recent years, the prevalence of obesity and overweight has increased rapidly in various populations and across all age groups, but particularly among the young. Consequently, the risk of diseases associated with obesity such as type-2 diabetes, cardiovascular disease, hypertension, and stroke has also risen dramatically and this is a major health concern (Bjorntorp 2001).

The regulation of food intake has been the subject of intensive study driven by the need to understand the problem of overweight and obesity. It is well established that the satiating effect of foods is an important part of overall food intake regulation. Satiety is defined as the feeling of fullness and suppression of hunger feelings after a meal resulting from the ingestion of food. Foods that maximize satiety are promising as they help to reduce the amount of food consumed at the next eating episode and increase the time interval between two meals (Van Itallie and Vanderweele 1981; Kissileff and Van Itallie 1982; Blundell et al. 1996).

Various satiety-inducing food ingredients have been investigated (Kissileff et al. 1984; Rolls et al. 1990; Holt et al. 1995). It would appear that energy density (Stubbs et al. 2000) and fiber content

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(Burton-Freeman 2000; Howarth et al. 2001) of a food are important factors modulating food intake and satiety. Consumption of low energy-dense foods and/or high-fiber foods is directly related to lower energy intake and increased satiety. Although there is some evidence that simple carbohydrate and fat may have an effect on satiety, there is increasing data indicating that protein is the most satiating macronutrient independent of its calorific value (Eisenstein et al. 2002; Anderson and Moore 2004; Halton and Hu 2004).

## 72.2 Dietary Protein, Food Intake, and Satiety

In many human studies protein appears to be more satiating than the other macronutrients (available carbohydrate, fat, or alcohol). Marmonier et al. (2000) convincingly demonstrated that a high-protein meal delayed the request for food for 60 min compared with 34 min for carbohydrate and 25 min for fat. Eisenstein et al. (2002) reviewed ten preload studies that included measurement of food intake as well as satiety ratings, and found that eight out of the ten studies showed a lower energy intake at the subsequent meal with a high protein preload than with a low protein preload. More direct evidence regarding the effect of high-protein meals in reducing food energy intake and increasing feelings of satiety comes from studies by Booth et al. (1970), Porrini et al. (1995), Poppitt et al. (1998), and Latner and Schwartz (1999) (see Table 72.1). In the study of Poppitt et al. (1998) where the satiating effect of the four dietary components (fat, carbohydrate, protein, and alcohol) was investigated, subjects reported feeling less hungry and more satiated throughout the study period and ate less food at lunch following a protein preload relative to the other macronutrients. In the Latner and Schwartz (1999) study, subjects consumed 31% more energy at dinner after a high-carbohydrate lunch and 20% more energy after a mixed carbohydrate and protein lunch compared to a high-protein lunch. The latter condition suppressed hunger more relative to the other two diets. Porrini et al. (1995) found that when twelve normal-weight men consumed a carbohydrate-rich meal, their subsequent food intake was higher versus consuming a protein-rich meal. Ratings of fullness and satiety were also increased after the high-protein preload compared to the high-carbohydrate preload. In a study of nine subjects who ate a protein-rich or a protein-poor lunch, Booth et al. (1970) found that energy intake at a meal 2–3 h later was lower after consumption of the protein-rich lunch compared to the low-protein lunch.

A few studies found suppression of food intake at a subsequent meal but no change in feelings of satiety after a high-protein meal (Table 72.1). Johnson and Vickers (1993) showed that a smaller amount of food and fewer calories were consumed after a high-protein meal compared with a high-carbohydrate or high-fat meal in 14 normal-weight subjects. However, there was no difference between carbohydrate and protein on feelings of satiety as both carbohydrate and protein were rated as more satiating than fat. Although Barking et al. (1990) did not find any change in subjective ratings of satiety between a high-protein meat casserole and a high-carbohydrate vegetarian casserole, they showed a decrease in food intake at the subsequent meal when subjects ate the high-protein meat casserole compared to when they ate the high-carbohydrate vegetarian casserole.

With respect to subjective ratings of satiety, studies by Stubbs et al. (1996), Vandewater and Vickers (1996), Crovetto et al. (1998), and Smeets et al. (2008) have shown that protein is more satiating than carbohydrate or fat (Table 72.1). Stubbs et al. (1996) reported that energy intake at a lunch test meal was similar for all the breakfast treatments but there was a greater suppression of the feeling of hunger after a high-protein meal compared to a meal high in carbohydrate or fat. Similar findings were observed by Crovetto et al. (1998) who studied intake at a test meal and satiety feelings by means of a satiety rating questionnaire in ten normal-weight women. There was no difference between the

**Table 72.1** Compilation of recorded effects of dietary protein on food intake and subjective ratings of satiety in humans

References	Subjects	Diets	Measure	Results: food intake	Results: satiety
Barkeeling et al. (1990)	Twenty normal-weight women	High-protein meat casserole (64.5 g P, 43% of E from P, 21% F, 36% C) High carbohydrate vegetarian casserole (15.5 g P, 10% of E from P, 21% F, 69% C)	Food intake 4 h after casserole lunch was measured using a universal eating monitor and subjective feelings of satiety were also rated.	Subjects consumed 12% fewer calories after the high-protein meal compared to the high-carbohydrate meal.	No change in subjective ratings between treatments.
Booth et al. (1970)	Nine normal-weight subjects (no further specification)	Protein-rich lunch Protein-poor lunch	Food intake 2–3 h after lunch	Energy intake was lower after consumption of the protein-rich lunch compared to the protein-poor lunch.	Not measured
Crovetti et al. (1998)	Ten normal-weight women	High-protein meal (68.1% of E from P, 19.2% F, 12.6% C) High carbohydrate meal (69.1% of E from C, 20.7% F, 10.1% P) High fat meal (70.1% of E from F, 21.3% C, 8.6% P)	Food intake 7 h later and satiety feelings were rated using a satiety rating questionnaire.	No difference on total test meal intake.	Subjects felt fuller after the high-protein meal over the 7 h test period than after the high carbohydrate or high fat meals.
De Graaf et al. (1992)	Twenty-nine normal-weight women	Ten liquid breakfasts: A zero condition (0.03 MJ) and the nine other preloads varied in energy levels (0.42, 1.05, and 1.67 MJ) and macronutrient content.	Food intake after breakfast was recorded in a diary. Satiety ratings were also assessed after consumption of breakfast.	No difference in energy intake for the remainder of the day.	The rated responses to satiety for the macronutrients did not differ.
Geliebter (1979)	Twelve normal-weight men	Protein load (egg albumin) Fat (corn oil) Carbohydrate (corn starch)	Test meal was presented 70 min after the preload and satiety ratings were measured.	No difference in energy intake during test meal.	Ratings of satiety did not differ.

(continued)

Table 72.1 (continued)

References	Subjects	Diets	Measure	Results: food intake	Results: satiety
Johnson and Vickers (1993)	Nine normal-weight women and six men	High-protein meal High-carbohydrate meal High-fat meal	A mini buffet lunch was served 90 min following the preloaf and subjects rated their hunger, stomach fullness, and prospective consumption 2 and 90 min after the preloaf.	A smaller amount of food and fewer calories were consumed after the high-protein meal compared to the high-carbohydrate or high-fat meals.	The high-carbohydrate and high-protein preloads decreased hunger and prospective consumption and increased stomach fullness more than the high-fat preloaf.
Latner and Schwartz (1999)	Twelve normal-weight women	High-Protein Lunch (80 g of whey protein, 71.5% of E from P) High Carbohydrate Lunch (113 g of polyose supplement 99% of E from C) Mixed Lunch (62 g C & 40 g P, 55.1% of E from C & 35.7% P)	Buffet-style dinner served 4.5 h after lunch consumption. Pre- and postmeal satiety ratings were assessed.	Thirty-one percent more energy was consumed at dinner following the high-carbohydrate lunch and 20% more following the mixed lunch than following the high-protein lunch.	Hunger was less in the high-protein condition than for the high-carbohydrate and mixed treatments. The mixed lunch also reduced hunger more than the high-carbohydrate lunch.
Poppitt et al. (1998)	Twelve normal-weight women	1 MJ baseline meal and drink to which 1MJ of carbohydrate, protein, fat and alcohol was added	Food intake 90 min after treatment and prior to, and at 30, 90, and 120 min. Subjective feelings of hunger and satiety were measured.	Energy intake was statistically significantly lowest after the protein treatment relative to the other macronutrients.	Subjects reported feeling less hungry and more satiated throughout the test period on the protein meal compared to the other macronutrients.
Porrini et al. (1995)	Twelve normal-weight men	High-Protein meal (56% of E from P, 25% F, 19% C) High Carbohydrate meal (17% of E from P, 27% F, 56% C)	Food intake at an ad libitum meal was measured and subjects rated their feelings of satiety.	Subsequent food intake was lower for the high-protein meal compared with the high-carbohydrate meal.	Ratings of fullness and satiety were higher following ingestion of high-protein meal compared with the high-carbohydrate meal.
Raben et al. (2003)	Nineteen normal-weight women and 10 men	Breakfasts differed by percentage E contributed by each macronutrient: 1. P (32%) 2. C (65%) 3. F (65%) 4. Alcohol (23%)	Food intake 5 h after breakfast meals and subjective satiety ratings were measured before, after and every 30 min until 5 h after the test meals.	No difference in subsequent energy intake.	No difference in satiety sensations after the four meals.

Smeets et al. (2008)	Nineteen normal-weight women and 11 men	High-Protein lunch (25% of E from P, 45% C, 30% F) Adequate-Protein lunch (10% of E from P, 60% C, 30% F)	Satiety ratings were measured immediately, at 30, 60, 120, 180, and 240 min following the meal.	Not measured	The ratings for hunger and satiety were higher 30 and 120 min after the high-protein lunch than after the adequate-protein lunch.
Stubbs et al. (1996)	Six men	High-Protein breakfast (59% of E from P) High-Carbohydrate breakfast (18% of E from P) High-Fat breakfast (22% of E from P)	Energy intake at a test meal lunch and throughout the rest of the day was assessed and subjective hunger, fullness, and appetite was measured hourly.	Lunch and after lunch energy intakes were similar following all the breakfast treatments.	The high-protein breakfast produced greater suppression of hunger between breakfast and lunch and over 24 h than the High-Carbohydrate or High-Fat treatments.
Vozzo et al. (2003)	Sixteen normal-weight men	High-Protein yoghurt (29% of E from P, 44% C, 24% F) High-Carbohydrate yoghurt (60% of E from C, 24% F, 14% P) High-Fat yoghurt (40% of E from F, 14% P, 42% C)	Food intake for the remainder of the day (7 h) was measured. Subjective rating scales assessing hunger and fullness were used at 15 min intervals for the duration of the study.	Subsequent food intake least but statistically non-significantly after the High-Protein yoghurt than High-Carbohydrate and high-fat yoghurts.	No difference in ratings of satiety.

The weight of the evidence suggests that dietary protein induces satiety more strongly than available carbohydrate, fat, or alcohol in humans as indicated by objective and subjective measures. Meals high in protein decrease food intake at a subsequent meal and subjective ratings of satiety also showed that protein is more satiating than carbohydrate, fat, or alcohol

h hour, MJ megajoules, min minutes; E energy, P protein, C carbohydrate, F fat

diets on test meal intake but subjects felt fuller after the high-protein meal over the 7-h test period than after the high-carbohydrate or high-fat meals. In the Smeets et al. (2008) study, the visual analogue scales (VAS) for hunger and satiety were higher after the high-protein lunch than after the adequate-protein lunch. In addition, results from a study by Vandewater and Vickers (1996) showed that protein-rich meals produced a greater feeling of satiety. The high-protein versions of yoghurt or sandwich induced a greater decrease in hunger and increase in stomach fullness ratings than the low-protein versions.

Contrary to the above discussed results, however, Geliebter (1979), de Graaf et al. (1992), Raben et al. (2003), and Vozzo et al. (2003) found that protein did not affect food intake or subjective responses to satiety (Table 72.1). There was no difference in ratings of satiety and energy intake at a test meal presented 70 min after equicaloric loads of protein (egg albumin), fat (corn oil), and carbohydrate (corn starch) (Geliebter 1979). In the de Graaf et al. (1992) study, energy intake at a test meal, energy intake for the remainder of the day, and satiety feelings did not differ with preloads that varied in macronutrient content. Raben et al. (2003) showed no change in subsequent energy intake or satiety feelings after meals rich in one of the four macronutrients; protein, carbohydrate, fat, or alcohol. In the Vozzo et al. (2003) study, spontaneous food intake and ratings of satiety did not differ significantly after preloads high in protein, carbohydrate, or fat. Subsequent food intake tended to be suppressed 29% by the high-protein preload, 20% by the high-fat preload, and 17% by the high-carbohydrate preload.

Several trials have investigated the relationship between high dietary protein intake and reduced body weight as well as long-term weight maintenance as summarized in Table 72.2. In a 6-month study, Skov et al. (1999) compared the effects of diets varying in percentage energy from protein (25% vs. 12%) on body weight and body composition in 65 otherwise healthy overweight and obese subjects. Weight loss after 6 months was 5.1 kg in the low-protein group vs. 8.9 kg in the high-protein group and fat loss was 4.3 and 7.6 kg, respectively. Subjects spontaneously limited their food intake on the high-protein diet which may have been associated with an enhanced satiety of the high-protein diet. In another study, when 19 subjects increased the proportion of dietary energy supplied by protein from 15% to 30% for 12 weeks, they reported greater feelings of satiety, a larger decrease in spontaneous energy intake, and lost more body weight and body fat on the high-protein diet (Weigle et al. 2005). Layman et al. (2003) monitored the weight management progress of 24 overweight women, who were either on a carbohydrate to protein ratio of 3.5 (68 g protein/day) or a ratio of 1.4 (125 g protein/day). Participants in both groups lost 7–7.5 kg after 10 weeks but women on the low carbohydrate to protein ratio diet reported feeling more satiated compared to the women that were on the high carbohydrate to protein ratio diet. A 4-week study (Baba et al. 1999) of 13 obese hyperinsulinemic normoglycemic men on a hypoenergetic diet that provided 80% of their resting energy expenditure found that those who consumed a high-protein diet (45% protein, 25% carbohydrate, and 30% fat as a percentage of dietary energy) lost more weight (8.3 vs. 6.0 kg) compared to those who consumed a high carbohydrate diet (12% protein, 58% carbohydrate, and 30% fat).

The weight of the evidence suggests that protein is more satiating than the other macronutrients. Preload studies looking at differences in food intake at the subsequent meal and/or feelings of satiety report an increased satiety value of protein. However, some studies did not find any change in subsequent intake or subjective ratings of satiety. Discrepancies in observations from preload studies may be affected by preload volume and composition and the time interval between preload and test meal (Rolls and Hammer 1995). In a recent study by Bellissimo et al. (2007), when the time interval between preload and test meal was increased from 30 to 60 min, the effect of carbohydrate preload decreased whereas the effect of whey protein increased. Carbohydrates and proteins have different post-ingestional consequences. The satiating effect of protein may improve over time

**Table 72.2** Summary of results of studies that investigated the long-term effects of high-protein diets in overweight and obese humans

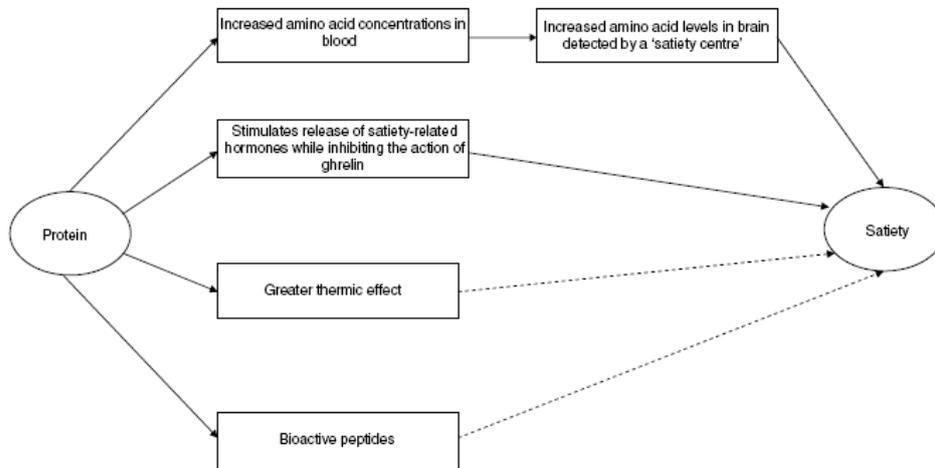
References	Subjects	Diets	Duration	Effects
Baba et al. (1999)	Thirteen men	High-Protein diet (45% of E from P, 25% C, 30% F) Low-Protein diet (12% of E from P, 58% C, 30% F)	Four weeks	Greater weight loss with the High-Protein diet than with the Low-Protein, High-Carbohydrate diet.
Layman et al. (2003)	Twenty-four women	High-Protein diet, carbohydrate to protein ratio of 1.4 (125 g protein/day; 30% of E from P, 41% C, 29% F) Low-protein diet, carbohydrate to protein ratio of 3.5 (68 g protein/day; 16% of E from P, 58% C, 26% F)	Ten weeks	Decrease in body weight in both diets but the ratio of fat/lean loss was found to be greater in the High-Protein diet compared with the Low-Protein, High-Carbohydrate diet. Greater satiety with the High-Protein diet was demonstrated than with the Low-Protein diet.
Skov et al. (1999)	Sixty-five subjects; 19 women and 6 men on each treatment diet	High-protein diet (25% of E from P, 45% C, 30% F) Low-protein diet (12% of E from P, 58% C, 30% F)	Six months	Greater body weight loss and body fat loss with the High-Protein diet compared to the Low-Protein diet. Subjects also spontaneously reduced their total food intake on the High-Protein diet.
Weigle et al. (2005)	Sixteen women and 3 men	High-protein diet (30% of E from P, 50% C, 20% F) Low-protein diet (15% of E from P, 50% C, 35% F)	Twelve weeks	Decreased spontaneous energy intake, body weight, and body fat on the High-Protein diet. Subjects on the High-Protein diet also reduced their spontaneous energy intake and reported greater feelings of satiety.

The effects of high-protein intake on body weight and body composition in overweight and obese humans  
*E* energy, *P* protein, *C* carbohydrate, *F* fat

(after 30 min) but the effect may become less precise as the duration to the next meal increases up to 3 h. The effect of the time interval between the ingestion of protein and subsequent food intake on the measurement of satiety appears to be critical and deserves more investigative attention. Nonetheless, consumption of high-protein meals in the long term may reduce body weight and body fat; effects that may be useful in the treatment of obesity. The short-term effects of protein on satiety may be an important factor.

### 72.3 Mechanism of Action of Protein on Satiety

Dietary protein may influence food intake and satiety via a number of mechanisms. Firstly, higher dietary protein intakes influence the plasma concentrations of amino acids which in turn may influence satiety-related hormones. Proteins have a high thermic effect and bioactive peptides released during digestion in the gut may also play a role (Rutherford-Markwick and Moughan 2005; refer to Fig. 72.1).



**Fig. 72.1** Mechanisms of action of dietary protein. This figure illustrates the mechanisms whereby dietary protein may exert its effect on satiety. Protein ingestion results in the release of amino acids and small peptides. The circulating levels of amino acids rapidly increase and this may contribute to satiety through increased amino acid concentrations in the brain. Amino acids may also stimulate the release of satiety-related hormones including cholecystokinin, glucagon-like peptide-1, and peptide YY while inhibiting the action of ghrelin, a hormone known to stimulate feeding. Part of the satiety effect of dietary protein may be due to the higher thermic effect of protein and the bioactive peptides released during protein digestion. → proven mechanism, --> probable mechanism

### 72.3.1 Amino Acids

The aminostatic hypothesis of feeding put forward by Mellinkoff et al. (1956) suggests that plasma amino acid concentration is inversely related to fluctuations in appetite. Studies in rats fed amino-acid-imbalanced diets indicate that low plasma concentrations of certain amino acids may lead to increases or decreases in food intake. The changes in plasma amino acid concentrations result in changes in amino acid concentrations in the brain, which are sensed by a “satiety centre” in the brain. The rise in plasma amino acids elicited by protein ingestion may assist in the suppression of food intake and the onset of satiety (Mellinkoff et al. 1956). Specific amino acids may have different effects on the satiety centre, which could explain differences among protein sources in inducing satiety.

### 72.3.2 Satiety Hormones

A number of studies show that dietary protein intake is correlated to the release of a number of satiety-related hormones such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and to a decrease in ghrelin, a hormone known to stimulate feeding. In the study by Hall et al. (2003), the suppression of food intake by whey was shown to be associated with increases in CCK and GLP-1. Compared with whey or casein, carbohydrate consumption decreased ghrelin and increased CCK to a lesser extent (Bowen et al. 2006b). However, no difference in ghrelin, GLP-1, and PYY was found between a high-protein (25% of energy from protein) and an adequate-protein diet (10% of energy from protein) (Smeets et al. 2008). Further, in a metabolic trial in which the energy

from protein was increased from 10% to 30%, plasma ghrelin remained unchanged, but after dinner GLP-1 concentrations were higher on the high-protein diet compared with the adequate-protein diet (Lejeune et al. 2006).

### **72.3.3 Thermic Effect of Protein**

The higher thermic effect of dietary protein may be partly responsible for its satiating effect. Consuming high-protein diets results in greater levels of heat production, partly related to increased energy expenditure, compared to carbohydrate and fat, which is referred to as the thermic effect of protein or diet-induced thermogenesis (DIT). Karst et al. (1984) studied the thermic effect of three protein sources (egg white, gelatine, and casein), two carbohydrates (starch and hydrolyzed starch), and two fats (sunflower oil and butter) in 12 healthy men for 6 h. While fat produced no observed thermic effect, the thermic effect of protein was three times larger than that of carbohydrate. A review of data from 19 trials revealed that for each 1% change in energy from protein, the thermic effect of the meal increased by 0.22% (Westerterp 2004). Crovetti et al. (1998) argued that the greater thermic effect of protein relative to carbohydrate or fat might be related to satiety. Similar conclusions were made by Westerterp-Plantenga et al. (1999), Lejeune et al. (2006), and Smeets et al. (2008). In the Westerterp-Plantenga et al. (1999) study, a high-protein meal (29% of energy from protein, 61% from carbohydrate and 10% from fat) increased DIT more than that for a high fat meal (9% of energy from protein, 30% from carbohydrate, and 61% from fat). They concluded that satiety assessed by VAS was associated with the thermic effect of the diets.

The higher thermic effect of protein is believed to be related to satiety and might explain at least in part why high-protein meals promote body weight loss. The role of dietary protein in body weight regulation and underlying mechanisms has been the subject of recent reviews (Halton and Hu 2004; Moughan 2008; Westerterp-Plantenga et al. 2009). High-protein diets seem to be effective in sustaining or increasing fat-free body mass. The preservation of a higher lean mass has an added energy cost and appears to have a significant effect on resting energy expenditure. Consumption of dietary protein above the required level will increase protein turnover by increasing protein synthesis and protein breakdown in the body. All of the processes associated with high-protein diets result in a lower metabolic efficiency of energy utilization that may contribute to weight loss.

## **72.4 Protein Source and Food Intake and Satiety**

The source of dietary protein appears to be a factor in the suppression of food intake. Some evidence indicates that consumption of milk proteins, particularly whey protein may increase satiety in animals and humans.

### **72.4.1 Animal Studies**

Studies in animals indicate that whey protein consumption may reduce food intake more than other protein sources. Yu et al. (2009) investigated the effects of whey, soy, and gluten proteins on inter-meal interval, meal number, meal size, and meal duration in male mice. Mice fed the whey protein

diet showed the greatest reduction in energy intake over seven days and gained less weight than those fed the other protein diets. The decrease in energy intake in mice fed the whey protein diet was attributed to a low meal number and a long intermeal interval rather than a decrease in meal size. Using obese minipigs, Ferrari et al. (2005) and Dunshea et al. (2007) compared the effects of whey or soy proteins on food intake and body weight. Both studies found that obese pigs fed the high whey protein diet reduced their food intake to a greater extent than pigs fed the high soy protein diet. Furthermore, pigs fed the high-protein diets experienced less weight and fat gain than pigs fed the low-protein diets, with the greater effect demonstrated by whey protein than soy protein.

#### 72.4.2 Human Studies

The evidence regarding a stronger effect of whey protein compared to other protein sources on satiety in humans is limited (Table 72.3). In one study, food intake at a test meal 60 min later was reduced to a greater extent after consumption of whey protein than after soy protein or egg albumen (Anderson et al. 2004). In another study, although food intake at lunch 180 min after consumption of the treatments did not differ, whey protein had a greater effect in suppressing hunger than either casein or soy (Veldhorst et al. 2009a). Within milk protein types, Hall et al. (2003) reported that whey suppressed food intake at a buffet meal 90 min later more than casein. Subjective ratings of satiety were also greater following consumption of the whey preload.

However, not all studies have found whey to have an effect on food intake and satiety. In one study, Bowen et al. (2006b) investigated the role of whey and casein proteins, relative to lactose or glucose in energy intake and satiety and in a second study, Bowen et al. (2006a) compared the effects of whey, soy, gluten, and glucose. In both studies, energy intake at a buffet lunch 3 h later was 10% higher after the glucose treatment compared with all the other preloads. There was no difference in subjective ratings for hunger, satiety, emptiness, or desire to eat between the treatments. Although the researchers observed a greater reduction in food intake after consumption of the high-protein meals than the high carbohydrate meals, they did not find any significant effect of protein source (whey, soy, gluten, and casein). The time interval of 180 min between preload and test meal may have been too long for the effect of the different protein sources on food intake to be detected.

Several studies have compared the effect of different dietary proteins on satiety but it remains unclear whether whey protein has a stronger effect on satiety than other protein sources. There is evidence for an effect of whey protein, but the situation is not definitive.

#### 72.5 Composition of Whey Protein

It is of interest to enquire as to what properties of whey protein could lead to the purported satiety effect. Is it related to the amino acid composition of whey, peptides released upon digestion or some other attribute?

Cow's milk contains approximately 3.3% protein, and milk proteins fall into two main categories: caseins and whey proteins, as illustrated in Fig. 72.2. Caseins represent ~80% while whey proteins represent the rest (~20%) of the total milk proteins (Walstra et al. 2006). During cheese making, whey is the soluble component of milk that is separated from the casein curd. For years, whey was considered as a waste product of the cheese making process but recently, whey has become a valuable ingredient in numerous food products, particularly in protein fortified products (Walzem et al. 2002).

**Table 72.3** Compilation of research studies that investigated the effects of protein source on satiety in humans

References	Subjects	Treatments	Measure	Results: food intake	Results: satiety
Anderson et al. (2004)	Thirteen normal-weight men	Fifty grams of egg albumen, whey and soy protein were placed in sweet and flavored beverages in addition to water control.	Food intake 1 h after the treatments	Whey and soy protein decreased food intake relative to the control, whereas egg protein did not.	Not measured
Bowen et al. (2006a)	Seventy-two overweight men	Liquid preloads (1.1 MJ) containing 50 g whey, soy, gluten or glucose	An ad libitum buffet lunch was served 3 h after consuming the liquid breakfast and ratings of satiety were measured after preload consumption.	Energy intake after glucose was statistically significantly higher compared to the other preloads.	No effect of treatment on subjective ratings
Bowen et al. (2006b)	Nineteen overweight men	Liquid breakfasts (1 MJ) consisting of 50 g of whey, 55 g of casein, 56 g of lactose or 50 g of glucose	An ad libitum buffet lunch was served 180 min after consuming the liquid breakfast and ratings of satiety were measured after preload consumption.	Energy intake after glucose was statistically significantly higher compared to the other preloads and there was no difference between protein sources.	Satiety ratings did not differ significantly between the four treatments.
Hall et al. (2003)	Eight lean women and 8 men	Liquid preloads (1,700 kJ) containing 48 g of whey or casein protein	A buffet meal was served 90 min after consumption of the preloads	The whey preload reduced energy intake at buffet meal by 829 kJ or 18% compared with the casein preload.	No difference in subjective satiety ratings.
Hall et al. (2003)	Eight lean women and 1 man	Liquid preloads (1,700 kJ) containing 48 g of whey or casein protein	A fixed lunch was offered at 42 g/kg 90 minutes after preload and ratings of hunger, satiety and desire to eat were measured.	Not measured	Greater fullness and reduced desire to eat followed the whey test meal over 180 minutes.

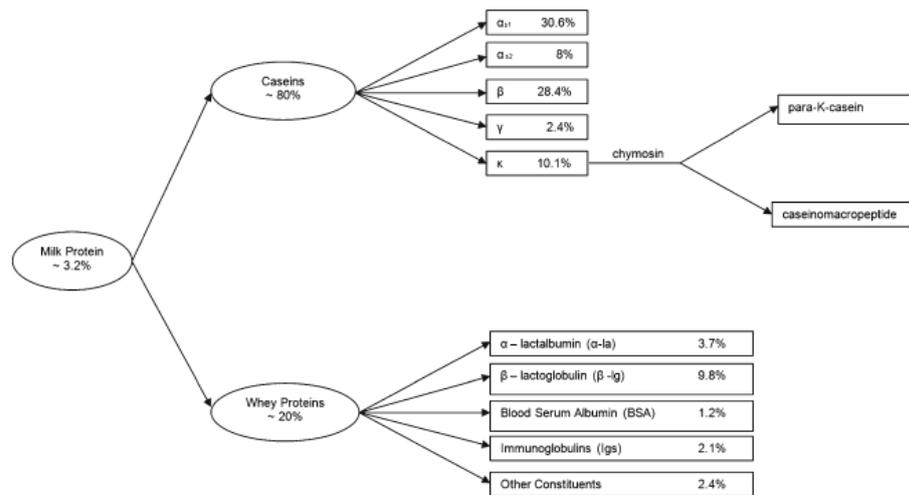
(continued)

**Table 72.3** (continued)

References	Subjects	Treatments	Measure	Results: food intake	Results: satiety
Veldhorst et al. (2009a)	Fourteen normal-weight women and 11 men	Custard breakfasts with either casein, soy or whey protein at two different energy levels (Normal Protein, 10% of E from P; 55% C, 35% F or High Protein, 25% of E from P; 55% C, 20% F)	Satiety ratings were recorded for 4 h and an ad libitum lunch was offered 180 min after the preload.	No difference between protein source and protein concentration for food intake at lunch.	There was no difference in satiety ratings between protein sources when the protein level was high (25% P). At 10% P, whey decreased hunger compared to casein or soy.

Protein source may determine the satiating effect of protein, but the evidence from studies in humans is limited. Whey protein derived from milk has been suggested as a potential protein that may contribute more strongly than other protein sources to short-term satiety in humans

h hour, min minutes, MJ megajoules, kJ kilojoules, g grams, kg kilograms; E energy, P protein, C carbohydrate, F fat



**Fig. 72.2** Protein composition of bovine milk. Bovine milk contains about 3.3% protein. The two major fractions of milk proteins are casein and whey protein, which comprise about 80% and 20% of the total milk proteins. Casein and whey protein are divided into other components. During cheese making, the enzyme chymosin breaks down  $\kappa$ -casein into para- $\kappa$ -casein and caseinomacropeptide. The latter is water-soluble and becomes part of the whey fraction (Adapted from Walstra et al. 2006, with permission. ~ = approximately;  $\alpha$  = alpha;  $\beta$  = beta;  $\gamma$  = gamma;  $\kappa$  = kappa)

The nutritive value of whey protein is related to its amino acid composition as well as the high bioavailability of these amino acids. Whey protein has a very high concentration of branched chain amino acids (BCAAs). These BCAAs are thought to be beneficial to athletes and individuals looking to achieve optimal lean muscle mass as they help to increase the bioavailability of carbohydrate in the muscles and prevent muscle protein breakdown during exercise (Walzem et al. 2002). The most important components of whey proteins are  $\beta$ -lactoglobulin ( $\beta$ -lg),  $\alpha$ -lactalbumin ( $\alpha$ -la), bovine serum albumin (BSA), and immunoglobulins (Ig). Other nitrogenous constituents include lactoferrin, lactoperoxidase, and many other bioactive factors and enzymes. They also contain caseinomacropeptide, a polypeptide of 64 amino acid residues, formed from the action of the enzyme chymosin on  $\kappa$ -casein. Glycomacropeptide (GMP), the glycosylated fragment of caseinomacropeptide has been shown to stimulate the secretion of the gut hormone cholecystokinin (CCK) (Beucher et al. 1994), which may induce satiety. Glycomacropeptide would appear to be a prime candidate for explaining a satiating effect of whey. Having said this, the empirical evidence for an effect of glycomacropeptide on satiety is not overwhelming (refer to Table 72.4).

## 72.6 Effect of Glycomacropeptide on Food Intake and Satiety in Humans

Caseinomacropeptide, the unglycosylated form of GMP, at doses of 0.4 and 20 g did not show any effect on food intake or subjective ratings of satiety over 1 h in a clinical trial (Gustafson et al. 2001). In our own work and with a carefully controlled study, we did not find any difference in energy intake at a cafeteria meal offered 30 min after administration of whey preloads without GMP, or with naturally present GMP or with added GMP (Chung Chun Lam et al. 2009). However, Veldhorst et al. (2009b)

**Table 72.4** Compilation of human studies assessing the effects of whey protein and glycomacropeptide on food intake and subjective ratings of satiety

References	Subjects	Diets	Measure	Results: food intake	Results: satiety
Burton-Freeman (2008)	Ten normal-weight men and 10 women	Preload milkshakes (1 MJ): 1. Whey; Whey Protein Isolate (44% of E from P) 2. Whey-GMP; whey protein without GMP (44% P) 3. Control; low protein (2% P) 4. GMP; GMP isolate (3% P)	Food intake 75 min after the preloads and satiety ratings recorded before and after preload consumption	No difference between preloads for food intake at the lunch test meal.	Greater satiety with Whey and Whey-GMP preloads compared to the Control preload in women. However, no difference in satiety ratings observed in men. No difference between the GMP and control preloads on subjective satiety in participants.
Chung Chun Lam et al. (2009)	Thirty-one normal-weight women and 19 men	Preload milkshakes (1.3 MJ): 1. Whey; Whey Protein isolate (8.4 g GMP) 2. Whey + GMP; Whey with added GMP (24.2 g GMP) 3. Whey-GMP; Whey without any GMP 4. Carbohydrate (maltodextrin)	A buffet lunch was served 30 min after preload administration and ratings of satiety were measured before and after preload consumption.	Equal suppression of food intake by protein preloads compared with carbohydrate.	Whey induced a greater feeling of fullness immediately after consumption of the preload compared to the other treatments.
Gustafson et al. (2001)	Thirty-two normal-weight women and 20 men	Preloads (34 J) were prepared by dissolving 0.4 or 2.0 g of CMP in a noncaloric fruit-flavored beverage vehicle.	Food intake 1 h after consuming the beverage. Participants assessed their feelings of satiety pre- and post preload and test meal and every hour until bedtime on 100 mm VAS.	No difference in food intake during test meal.	No effect of treatment on subjective ratings of satiety.
Veldhorst et al. (2009b)	Fourteen normal-weight women and 11 men	Custard breakfasts (2.5 MJ) with either whey or whey without GMP at two different energy levels (Normal Protein, 10% of E from P, 55% C, 35% F or High Protein, 25% of E from P, 55% C, 20% F).	Satiety ratings were recorded for 4 h and an ad libitum lunch was offered 180 min after the preload.	Protein concentration did not have an effect on food intake at lunch. However, there was a greater reduction in energy intake after the Whey breakfast than after the breakfast with Whey without GMP.	There was no difference in satiety ratings between protein sources but satiety ratings were higher after the normal protein breakfasts than after the high-protein breakfasts.

The presence of glycomacropeptide may be a determinant of the satiating effect of whey protein but the limited evidence from studies in humans is not strong

MJ megajoules, min minutes, J joules, E energy, P protein, GMP glycomacropeptide, WPI whey protein isolate, SMP skim milk powder

found that the presence of GMP as part of whey protein in a breakfast meal reduced energy intake at lunch, 180 min later. To test the effects of a preload containing GMP alone on food intake, feelings of satiety, and CCK release, Burton-Freeman (2008) fed 20 adults either a low-protein preload, or a whey protein preload with GMP, or a whey protein preload without GMP, or a GMP preload. Test meal intake 75 min later, subjective satiety or CCK release was not affected by whey protein or GMP. Contradictory findings on the effect of GMP on satiety may be related to differences in the dose of whey protein used and the time interval between preload and test meal. Human trials looking at the long term effect of GMP and whey protein are limited. In a 12-month randomized trial, Keogh and Clifton (2008) compared the effects of GMP-enriched whey powder versus skim milk powder meal-replacements in 127 overweight and obese subjects. Subjects who consumed the GMP-enriched whey protein meal replacement had greater weight loss after 12 months than those who consumed skim milk meal-replacements (9.9 vs. 10.8 kg), but the difference was not statistically significant. The role of GMP on CCK release and CCK-mediated satiety requires further work.

## 72.7 Applications to Other Areas of Health and Disease

The need to understand the regulation of food intake and the termination of eating is essential to the understanding and effective treatment of obesity and eating disorders. Obese people or individuals with eating disorders such as anorexia nervosa, bulimia nervosa, or binge eating disorder may exhibit different hunger and satiety mechanisms compared to normal-weight people or people not suffering from eating disorders. Binge eating is a central characteristic of bulimia nervosa and binge eating disorder and is defined as the consumption of a very large amount of food until the person feels uncomfortably full (American Psychiatric Association 1994).

People with bulimia nervosa and binge-eating disorder exhibit distorted satiety responses and tend to overeat throughout the day (Rossiter et al. 1992) and during binge episodes (Yanovski et al. 1992). Individuals who binge-eat were found to consume a higher proportion of energy from fat and a lower percentage of intake from protein. Hetherington et al. (1994) showed that the percentage of dietary energy from protein intake during the day was lower for women with bulimia nervosa compared with controls. Similarly, women with binge-eating disorders ate a lower proportion of energy from protein than controls when asked to binge-eat (Yanovski et al. 1992). Consumption during binge episodes in subjects suffering from binge-eating disorders was shown to be high in carbohydrates but during non-binge days, a greater amount of protein was consumed (Rossiter et al. 1992). Protein intake may assist eating-disordered and obese individuals from overeating or binge-eating.

For individuals who binge-eat, the satiating effect of different macronutrients may be a factor in the loss of control over eating. There is strong evidence that protein, in comparison with other macronutrients, is more effective in promoting satiety and suppressing food intake (Table 72.5). Latner and Wilson (2004) investigated the effect of protein on binge eating, food intake at a test meal and rated satiety in subjects with bulimia nervosa or binge-eating disorder. Over the course of 2 weeks, eighteen women with bulimia nervosa or binge eating disorder ingested a high-protein or a high-carbohydrate supplement three times daily. Food intake at test meals was reduced and subjects felt less hungry and fuller after protein supplementation compared with carbohydrate supplementation. Subjects experienced less binge eating episodes during protein supplementation than during carbohydrate supplementation. Dietary protein may in part correct the satiety impairment in people with bulimia nervosa and binge eating disorders. The role of protein in inducing satiety and reducing binge eating episodes may have some implications for the long-term treatment of eating disorders.

**Table 72.5** Key features of satiety

1. Satiety is defined as the feeling of fullness that determines the time interval between two meals (the intermeal interval) and the amount of food consumed at the next eating event.
2. Foods that increase satiety offer promise to encourage an individual to prolong the intermeal interval and reduce food intake. This is useful for the overweight and people on energy-restricted diets that have difficulty achieving satiety while complying with the energy restriction.
3. Many dietary factors affect satiety including the energy density, fiber content and macronutrient composition of a food. It is widely believed that protein is the most satiating macronutrient independent of its caloric value. Whey protein derived from milk may be more effective than other protein sources in inducing satiety.
4. Three main approaches used to measure satiety:
  - The time taken to request food after a test meal.
  - Rating scales are used to measure subjective feelings of satiety. The most common is the visual analogue scales which are usually 100 mm long lines labeled at each end with extremes of the subjective feeling.
  - Food intake at a subsequent meal.

This table lists the key features underlying the concept of satiety including the definition of satiety, why satiety is important, the dietary factors affecting satiety and the measurement of satiety

### Summary

- Satiety is related to the time interval between meals and the amount of food eaten at the next eating occasion.
- Dietary protein appears to be more satiating and suppresses food intake more than carbohydrate or fat.
- The effect of protein on food intake and satiety may be source-dependent with whey protein derived from milk, potentially having an effect on food intake suppression and increased satiety.
- Glycomacropeptide, found in whey protein, is a peptide claimed to induce satiety by stimulating the release of the hormone cholecystokinin involved in the control of food intake.
- The mechanisms of action of protein related to food intake suppression and satiety include increased secretion of satiety-related hormones, a greater thermic effect of protein, the plasma concentration of amino acids, and possibly bioactive peptides released during digestion.
- Dietary protein may play a role in the long-term treatment of eating disorders by promoting satiety and reducing binge-eating episodes.

### Key Terms

**Amino acids:** The building blocks of proteins.

**Binge eating:** The uncontrollable consumption of an excessive amount of food.

**Bioactive peptides:** Peptides which are active either locally in the gut or systemically to impact on a range of physiological functions, including modifying nutrient absorption and excretion, immunoregulatory effects, and antihypertensive activity. Some are released from foods during digestion.

**Branched chain amino acids:** Three out of the nine essential amino acids that cannot be produced by the body. They are leucine, isoleucine, and valine.

**Casein protein:** The major fraction of milk protein comprising of 80% of the total protein.

**Energy density:** The energy content in kilojoules or Calories per unit of weight or volume.

**Fiber:** Plant materials which are resistant to digestion by alimentary enzymes.

**Glycomacropeptide:** The glycosylated fragment of caseinomacropeptide, a polypeptide of 64 amino acid residues. When milk is treated with the enzyme chymosin during the formation of

cheese curd, caseinomacropeptide is released from bovine kappa-casein and moves out of the curd with the whey fraction.

**Peptide:** Two or more amino acids chained together by a bond. Peptides can serve as messengers within the body such as enzymes, hormones, antibodies, and structural components.

**Satiety:** The feeling of fullness that occurs after eating a meal.

**Visual analogue scales:** A subjective measure of satiety. The scale is usually 100 or 150 mm long labeled at each end with extremes of the subjective feeling to be quantified.

**Thermic effect of food or diet-induced thermogenesis:** Levels of heat produced during the absorption and metabolism of ingested nutrients and partly related to energy expenditure.

**Whey protein:** The soluble component of milk that is separated from the casein curd during cheese making and represents 20% of the total milk protein.

## References

- American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Washington: American Psychiatric Association; 1994.
- Anderson GH, Moore SE. *J Nutr.* 2004;134:974S–9S.
- Anderson GH, Tecimer SN, Shah D, Zafar TA. *J Nutr.* 2004;134:3011–5.
- Baba NH, Sawaya S, Torbay N, Habbal Z, Azar S, Hashim SA. *Int J Obes.* 1999;23:1202–6.
- Barkeling B, Rössner S, Björvell H. *Int J Obes.* 1990;14:743–51.
- Bellissimo N, Desantadina MV, Pencharz PB, Berall GB, Thomas SG, Anderson GH. *Int J Obes.* 2007;32:362–71.
- Beucher S, Levenez F, Yvon M, Corring T. *J Nutr Biochem.* 1994;5:578–84.
- Bjorntorp P. *International textbook of obesity.* New York: Wiley; 2001.
- Blundell JE, Lawton CL, Cotton JR, Macdiarmid JJ. *Annu Rev Nutr.* 1996;16:285–319.
- Booth DA, Chase A, Campbell AT. *Physiol Behav.* 1970;5:1299–1302.
- Bowen J, Noakes M, Clifton PM. *J Clin Endocrinol Metab.* 2006a;91:2913–9.
- Bowen J, Noakes M, Trenerry C, Clifton PM. *J Clin Endocrinol Metab.* 2006b;91:1477–83.
- Burton-Freeman B. *J Nutr.* 2000;130:272S–5S.
- Burton-Freeman B. *Physiol Behav.* 2008;93:379–87.
- Chung Chun Lam SMS, Moughan PJ, Awati A, Morton HR. *Physiol Behav.* 2009;96:162–8.
- Crovetti R, Porrini M, Santangelo A, Testolin G. *Eur J Nutr.* 1998;52:482–8.
- de Graaf C, Hulshof T, Weststrate JA, Jas P. *Am J Clin Nutr.* 1992;55:33–8.
- Dunshea FR, Ostrowska E, Ferrari JM, Gill HS. *Aust J Exp Agric.* 2007;47:1051–9.
- Eisenstein J, Roberts SB, Dallal G, Saltzman E. *Nutr Rev.* 2002;60:189–200.
- Ferrari JM, Ostrowska E, Muralitharan M, Tatham BG, McCauley I, Cox ML, Eason PJ, Kerton DJ, Dunshea FR. *FASEB J.* 2005;19:A1478.
- Geliebter AA. *Physiol Behav.* 1979;22:267–73.
- Gustafson DR, McMahon DJ, Morrey J, Nan R. *Appetite.* 2001;36:157–63.
- Hall WL, Millward DJ, Long SJ, Morgan LM. *Br J Nutr.* 2003;89:239–48.
- Halton TL, Hu FB. *J Am Coll Nutr.* 2004;23:373–85.
- Hetherington MM, Altemus M, Nelson ML, Bernat AS, Gold PW. *Am J Clin Nutr.* 1994;60:864–73.
- Holt SH, Miller JC, Petocz P, Farmakalidis E. *Eur J Nutr.* 1995;49:675–90.
- Howarth NC, Saltzman E, Roberts SB. *Nutr Rev.* 2001;59:129–39.
- Johnson J, Vickers Z. *Appetite.* 1993;21:25–39.
- Karst H, Steiniger J, Noack R, Steglich HD. *Ann Nutr Metab.* 1984;28:245–52.
- Keogh JB, Clifton P. *Am J Clin Nutr.* 2008;87:1602–5.
- Kissileff HR, Gruss LP, Thornton J, Jordan HA. *Physiol Behav.* 1984;32:319–32.
- Kissileff HR, Van Itallie TB. *Ann Rev Nutr.* 1982;2:371–418.
- Latner JD, Schwartz M. *Appetite.* 1999;33:119–28.
- Latner JD, Wilson GT. *Int J Eat Disord.* 2004;36:402–15.
- Layman DK, Boileau RA, Erickson DJ, Painter JE, Shiue H, Sather C, Christou DD. *J Nutr.* 2003;133:411–7.
- Lejeune MPM, Westerterp KR, Adam TCM, Luscombe-Marsh ND, Westerterp-Plantenga MS. *Am J Clin Nutr.* 2006;83:89–94.

- Marmonier C, Chapelot D, Louis-Sylvestre J. *Appetite*. 2000;34:161–8.
- Mellinkoff SM, Frankland M, Boyle D, Greipel M. *J Appl Physiol*. 1956;8:535–8.
- Moughan PJ. In: Thompson A, Boland M, Singh H, editors. *Milk proteins: from expression to food*. London: Academic; 2008. pp. 483–99.
- Poppitt SD, McCormack D, Buffenstein R. *Physiol Behav*. 1998;64:279–85.
- Porrini M, Crovetti R, Testolin G, Silva S. *Appetite*. 1995;25:17–30.
- Raben A, Agerholm-Larsen L, Flint A, Holst JJ, Astrup A. *Am J Clin Nutr*. 2003;77:91–100.
- Rolls BJ, Fedoroff IC, Guthrie JF, Laster LJ. *Appetite*. 1990;15:115–26.
- Rolls BJ, Hammer VA. *Am J Clin Nutr*. 1995;62:1086S–95S.
- Rossiter EM, Agras WS, Telch CF, Bruce B. *Int J Eat Disord*. 1992;11:111–20.
- Rutherford-Markwick KJ, Moughan PJ. *J AOAC Int*. 2005;88:955–66.
- Skov AR, Toubro S, Rønn B, Holm L, Astrup A. *Int J Obes*. 1999;23:528–36.
- Smeets AJ, Soenen S, Luscombe-Marsh ND, Ueland O, Westerterp-Plantenga MS. *J Nutr*. 2008;138:698–702.
- Stubbs J, Ferres S, Horgan G. *Crit Rev Food Sci Nutr*. 2000;40:481–515.
- Stubbs RJ, Van Wyk MC, Johnstone AM, Harbron CG. *Eur J Clin Nutr*. 1996;50:409–17.
- Van Itallie TB, Vanderweele DA. In: Howard AN, editor. *Recent advances in obesity research*. London: Newman; 1981. pp. 278–88.
- Vandewater K, Vickers Z. *Physiol Behav*. 1996;59:579–83.
- Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, van Vught AJAH, Westerterp KR, Engelen MPKJ, Brummer R-JM, Deutz NEP, Westerterp-Plantenga MS. *Physiol Behav*. 2009a;96:675–82.
- Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, Westerterp KR, Engelen MPKJ, Brummer R-JM, Deutz NEP, Westerterp-Plantenga MS. *Appetite*. 2009b;52:388–95.
- Vozzo R, Wittert G, Cocchiaro C, Tan WC, Mudge J, Fraser R, Chapman I. *Appetite*. 2003;40:101–7.
- Walstra P, Wouters JTM, Geurts TJ. *Dairy science and technology*. Boca Raton: CRC; 2006.
- Walzem RL, Dillard CJ, German JB. *Crit Rev Food Sci Nutr*. 2002;42:353–75.
- Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, Purnell JQ. *Am J Clin Nutr*. 2005;82:41–8.
- Westerterp K. *Nutr Metab*. 2004;1:1–5.
- Westerterp-Plantenga MS, Rolland V, Wilson SA, Westerterp KR. *Eur J Clin Nutr*. 1999;53:495–502.
- Westerterp-Plantenga MS, Nieuwenhuizen A, Tome D, Soenen S, Westerterp KR. *Annu Rev Nutr*. 2009;29:21–41.
- Yanovski SZ, Leet M, Yanovski JA, Flood M, Gold PW, Kissileff HR, Walsh BT. *Am J Clin Nutr*. 1992;56:975–80.
- Yu Y, South T, Huang X-F. *Appetite*. 2009;52:372–9.

## **Appendix 2**



**MASSEY UNIVERSITY**  
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**STATEMENT OF CONTRIBUTION  
TO DOCTORAL THESIS CONTAINING PUBLICATIONS**

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

**Name of Candidate:** Sylvia Chung Chun Lam

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**Name of Published Research Output and full reference:**

Chung Chun Lam, S. M. S., Moughan, P. J., Awati, A., & Morton, H. R. (2009). The influence of whey protein and glycomacropeptide on satiety in adult humans. *Physiology & Behavior*, 96(1), 162–168.

**In which Chapter is the Published Work:** Chapter 2

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:  
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Sylvia Chung designed and conducted the experimental study, and contributed to the interpretation of the data, and drafted the manuscript. The manuscript was edited by the supervisors.

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**In which Chapter is the Published Work:** Chapter 3

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Sylvia Chung designed and conducted the experimental study, and contributed to the interpretation of the data, and drafted the manuscript. The manuscript was edited by the supervisors.

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Chung Chun Lam, S. M. S., & Moughan, P. J. (2011). Whey protein and satiety: implications for diet and behaviour. In: V. R. Preedy, R. R. Watson, & C. R. Martin (Eds), Handbook of Behavior, Food and Nutrition (pp. 1107–24). New York, USA: Springer.

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Sylvia Chung drafted the original published work and largely contributed the ideas, and theories, and discussion in the report. The supervisor discussed ideas and edited the manuscript.

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