THE INFLUENCE OF THE PHYSICAL STATE AND HABITUAL MASTICATION ON THE GLYCAEMIC RESPONSE AND SATIETY

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Abstract

The escalating levels of obesity highlight the need to better understand the mechanisms underlying energy intake and energy regulation. The blood glucose response (GR) has been shown to significantly influence short term food intake and therefore energy balance. Regulating the GR is also important in diabetes and impaired glucose tolerance; conditions which are also closely linked with obesity. Poor glycaemic control has moreover been shown to increase risks of other chronic diseases such as cardiovascular disease (CVD). Factors affecting the GR will therefore impact both on energy regulation and chronic diseases. A large number of factors influence the GR. A complete understanding of all these variables is essential if successful regulation of the GR is to be achieved.

The studies presented in this thesis focused on two factors affecting the GR that have hitherto received little research attention. These are the physical state (liquid-sold nature) of food and habitual mastication (ingested particle size). The first study investigated the effects of the physical state and showed that it affected the shape and amplitude of the GR and insulin response (IR) curves but not the total metabolic response. The response pattern implied that liquids were satiating for a shorter length of time compared to solids. The subsequent study then investigated the effect of carbohydrate-based energy containing beverages on satiety and short-term food intake and found that they were detected by the physiological energy regulatory systems and suitably compensated for. However, there was a notable gender-wise variation in compensation efficiency. Whilst consuming a carbohydrate beverage does not

appear to affect short-term energy balance of males it could induce a positive energy balance in females.

Using both *in vitro* and *in vivo* models, other studies forming this thesis showed that the degree of particle size breakdown during habitual mastication influenced the magnitude and pattern of the GR. Therefore, habitual mastication appears to be a significant contributor to between-individual variations in the GR. It was noted, however, that these effects were only observed with rice but not spaghetti. The thesis also showed that salivary α-amylase could potentially be a significant contributor to the GR, at least in those who spend a longer time masticating. The final study in the thesis showed further that the particle size of ingested food correlated inversely with the GR, IR and rate of gastric emptying. Differences in between-individual variations in the GR, IR, gastric emptying and post-gastric digestive aspects when ingesting food with varying particle sizes are also discussed.

Publications arising from the thesis

Ranawana V & Henry CJ (2011). Liquid and solid carbohydrate foods: comparative effects on glycemic and insulin responses, and satiety. *Int J Food Sci Nutr* **62(1)**, 71-81. (Chapter 3)

Ranawana DV & Henry CJ (2010). Are caloric beverages compensated for in the short-term by young adults? An investigation with particular focus on gender differences. *Appetite* **55**, 137-146. (Chapter 4)

Ranawana V, Monro JA, Mishra S & Henry CJ (2010). Degree of particle size breakdown during mastication may be a possible cause of interindividual glycemic variability. *Nutr Res* **30**, 246-254. (Chapter 5)

Ranawana V, Henry CJ & Pratt M (2010). Degree of habitual mastication seems to contribute to interindividual variations in the glycemic response to rice but not to spaghetti. *Nutr Res* **30**, 382-391. (Chapter 6)

Ranawana DV & Henry CJK (2010). Degree of habitual mastication may be a possible cause of inter-individual variation in *in vivo* glycaemic response to whole cereals. *Proc Nutr Soc* **69(OCE6)**, E407. (Chapter 6)

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Abbreviations

ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
CCK	Cholecystokinin
CFB	Change from baseline
CI	Confidence interval
cm	Centimeters
CNS	Central nervous system
CO_2	Carbon dioxide
CV	Coefficient of variation
DNS	Dinitro salicylic
EE	Energy expenditure
EI	Energy intake
EMG	Electromyography
FAO	Food and Agriculture Organisation
g	Grams
GE	Gastric Emptying
GI	Glycaemic Index
GIP	Gastric inhibitory peptide
GLP-1	Glucagon-like peptide-1
GR	Glycaemic response
HCL	Hydrochloric acid
IAUC	Incremental area under the curve
IR	Insulin response
kcal	Kilocalories
kg	Kilograms
L	Litres
LH	Lateral hypothalamus
m	Meters
M	Moles
mL	Millilitres

mm	Millimeters
MRI	Magnetic resonance imaging
MRP	Meal replacement products
n	Number
Na	Sodium
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NHS	National Health Service
nm	Nanometres
OJ	Orange juice
PYY	Peptide-YY
r	Correlation coefficient
R^2	Regression coefficient
RCS	Randomised control studies
RDS	Rapidly digestible starch
rpm	Revolutions per minute
RTD	Ready to drink
SAA	Salivary alpha amylase
SD	Standard deviation
SDS	Slowly digestible starch
SE	Standard error
SPSS	Statistical package for the social sciences
SSB	Sugar-sweetened beverage
SSS	Sensory specific satiety
T_{asc}	Ascension time
T_{half}	Half time
T_{lag}	Lag time
T_{lat}	Latency time
U	Units
UK	United Kingdom
UREC	University Research Ethics Committee
UV	Ultraviolet
VAS	Visual Analogue Scales
VH	Ventral hypothalamus
WHO	World Health Organisation

α	Alpha
µL	Microliters
µm	Micrometres
µmol	Micromoles
µU	Microunits

Introduction

The research work forming this thesis examined some factors influencing the glycaemic response to food in relation to satiety and energy regulation, namely the physical state (liquid-solid nature) and the effects of habitual mastication. The increasing prevalence of obesity and its unfavourable effects both at individual and population levels has prompted an increasing amount of research to focus on its causatives and control strategies. The regulation of the glycaemic response (GR) has been shown to be important both in the maintenance of energy balance and in the management of diabetes and related co-morbidities. Therefore, the findings in this thesis have practical significance in weight control, disease management and prevention.

The studies forming this thesis were carried out in the Functional Food Centre laboratories at Oxford Brookes University during the period January 2008-January 2011. The data is securely stored at the same location.

The thesis comprises of 8 chapters and begins with a literature review of the current state of the art (chapter 1). The materials, methods and protocols of all the experimental studies are described in the next chapter (2). This is followed by the five experimental study chapters (chapters 3-7) each consisting of an introduction and justification of the study, results and a detailed discussion of the results. The final chapter (8) summarises overall findings and conclusions, and lists recommendations for future work.

Chapter 1: Literature review

Following is a review of the available literature on the subject and begins by highlighting the increasing prevalence of obesity and its consequences on the health and well-being of the population. Since these are a result of a positive energy balance the review then deals with the intake side of the energy balance equation and the factors that influence short-term food intake. It then goes on to deal sequentially with satiety, factors affecting it, its measurement, the glycaemic response (GR), its impact on satiety and elements affecting it. The impact of the physical state of food (liquid-solid nature) and particle size on the GR and satiety are then reviewed followed by a description of the factors that affect particle size, namely, mastication and the oral processing phase. The review finally examines factors influencing gastric emptying time, its measurement and its relationship with the GR.

1.1 The obesity epidemic and its consequences

In nature food is a scarce resource for all animal species, and humans are no exception. Having evolved as omnivorous hunter gatherers, humans survived in an uncertain environment where access to food was limited and depended on seasonal and climatic factors. Food availability therefore, oscillated between periods of plenty and dearth, and nutrient intake correspondingly alternated. The presence of energy storage tissue in the human body was crucial for this lifestyle, as it enabled the build-up of compensatory reserves during times of plenty, for usage during shortages. The principle challenge to survival was under-nutrition and lack of adequate food. The human body therefore is physiologically programmed to over-consume when food is abundant. Indeed,

the human body is more sensitive to energy deficits as it is the more critical condition with regards to survival (Friedman, 2008). As agriculture developed through the years food production increased markedly, making energy no longer a limited resource. Populations in developed countries consequently reached their genetic potential for linear growth and began to gain weight in relation to height (Caballero, 2007). The consumption of energy dense (the amount of energy per gram of food) foods and a more sedentary lifestyle exacerbated weight gain. The year 2000 was a landmark which saw the number of overweight individuals in the world exceed those that were underweight (Caballero, 2007).

Obesity is defined as the excess accumulation of fat in the adipose tissue to an extent where health may be adversely affected (WHO, 2000). It is characterised by a body mass index (BMI) greater than 30 kg/m^2 and those between 25-30 kg/m^2 are considered overweight (DH, 2006). The incidence of obesity and associated non-communicable diseases is increasing both around the world (James *et al*., 2001), and in the UK (Rennie & Jebb, 2005). Although once an affliction limited to affluent countries, it is now rapidly rising in the urban sectors of low- and middle-income nations.

The global incidence of overweight and obese adults are estimated to be 1.6 billion and 400 million respectively (WHO, 2006), and these are projected to rise to 2.3 billion and 700 million by 2015. Similarly, approximately 20 million children under the age of five years are overweight or obese (WHO, 2006). Trends in the UK are no different, and prevalence has increased three fold since 1980 (Rennie & Jebb, 2005). In England, the percentage of obese adults

in 2007 was 24%, an overall increase of15% since 1993 (NHS, 2009). In the same year 17% of boys and 16% of girls (aged 2-15 years) were also obese (NHS, 2009). Men showed a greater proclivity towards being overweight than women (41% compared to 32%). Although regional differences within England are not significant, a greater prevalence is seen in Wales and Scotland (Jebb *et al.*, 2004).The high rates of obesity also incur considerable expenses to the state, and the direct cost of its treatment in the UK in 2002 was an estimated £47 million, and an additional £1000 million for dealing with its indirect consequences (NHS, 2009).

Obesity has far-reaching consequences on health as it is associated with other co-morbidities such as diabetes, hypertension, coronary heart disease (CHD), osteoarthritis and cancer (Must *et al.*, 1999; Marks & Raskin, 2000; James *et al.*, 2001). The risks of developing type 2 diabetes mellitus increase significantly with obesity. Whilst obese women are 13 times more likely to develop diabetes, obese men are five times as likely (NHS, 2006). The incidence of diabetes is rising rapidly and global numbers which stood at 197 million in 2007 are expected to increase by over 100% to 420 million by 2025 (Hossain *et al.*, 2007). Obesity accounts for 60-90% of diabetes prevalence and is therefore the largest predictor of the latter (Anderson *et al.*, 2003). The maintenance of a stable blood glucose response (GR) and avoiding wide fluctuations is the primary objective in diabetes control and prevention (Anon., 1998). This is also the basis of all nutrition based interventions, a cornerstone in diabetes management and prevention (Kelley, 2003). Considerable research has been therefore carried out to determine food factors influencing the GR and

how they can be manipulated to achieve beneficial outcomes (Bjorck *et al.*, 1994; Vosloo, 2005).

Weight gain occurs as a result of a positive energy balance and it is therefore reasonable that any interventions must stem from the management of the intake and expenditure components of the energy balance equation.

1.2 Energy balance

Energy balance is a term that encompasses the equilibrium between energy consumed and that expended. An adult is defined to be in energy balance if the difference between energy intake (EI) and expenditure (EE) is less than 150 kcal/day (Westerterp, 1994). Periods of negative and positive energy balance alternate in humans on a daily and weekly basis and these fluctuations are influenced by factors such as physical activity, number of meals, daily meal pattern, occupation, weather, hormonal cycles, food availability and seasonality (Westerterp, 1994). Humans characteristically demonstrate a sporadic eating pattern despite continuous energy expenditure, and the energy stores therefore play a crucial part in maintaining balance within this lifestyle (Strubbe, 1994).

1.2.1 Energy expenditure

Energy expenditure (EE) in adults is made up of three principle components; basal metabolism, thermogenesis and physical activity (Levine, 2005), of which only the last can be voluntarily controlled.

The majority of EE goes towards basal metabolism (60-80%), which is the energy required for the body's basic physiological functions (Hill *et al.*, 2006). The second largest component is physical activity and its proportion can vary between 10-40% based on the lifestyle. The increase in EE due to food ingestion (diet induced thermogenesis [DIT]) is approximately 7-10% of the energy content of the diet (Hill *et al.*, 2006). Although growth is also considered a component of energy expenditure, it accounts for a small proportion in humans (approximately 1-2%) (FAO, 2001). The amount of energy expended by humans depend on factors such as body size and composition, age, sex, diet, climate, genetics, hormonal state, psychological state, disease and medication (FAO, 2001; Hill *et al.*, 2006).

1.2.2 Energy Intake

Unlike EE which occurs through several channels EI is exclusively through food. The principal stimulus for eating is the need to maintain adequate energy pools in the body.

The precise mechanisms of long-term food intake regulation are yet to be confirmed. Several theories have been proposed and these include the energostat model, set-point hypothesis and the settling-zone theory (Polivy & Herman, 1987; Strubbe, 1994; Levitsky, 2002; Friedman, 2008). All these theories proposed mechanisms by which the body presumably regulates energy intake and safeguards against imbalances. However, the escalating levels of obesity suggest the failure or non-existence of these mechanisms.

Recent literature has placed considerable emphasis on the role of internal and external energy intake cues, also known as regulatory and non-regulatory controls of food intake respectively (Friedman, 2008). Whilst regulatory cues are associated with the internal physiological systems controlling food intake, non-regulatory cues relate to environmental factors such as food availability, palatability, and social aspects. It is speculated that non-regulatory factors take precedence over regulatory controls in the short term (Friedman, 2008). This is reasonable from an evolutionary perspective since humans evolved in an environment with scarce resources. It was essential for survival to 'feast' in times of plenty and build up stores for times of paucity. Humans therefore are programmed to overeat when food is aplenty. However, this may be a disadvantage in the present day (in high-income populations) where food is not a limited resource.

1.3 Short-term food intake regulation

Short-term food intake is regulated by psycho-physiological mechanisms controlling appetite, satiety and satiation. Appetite is defined as the psychological desire to eat and is associated with the oro-sensory acceptability of a specific food (Yeomans & Bertenshaw, 2008). Eating will therefore occur only if appetising foods are available. Conversely, hunger is the subjective feeling that occurs when an individual wants to ingest food. It is the sensation that signals food deprivation to a degree that the next eating episode should be initiated. An individual will seek and eat an adequate amount of food only if both these stimuli are present.

The primary mechanisms that determine meal frequency and the quantity eaten on a daily basis are satiety and satiation. Satiety is the inhibition of the feelings of hunger and appetite in the post-meal phase (Vermunt *et al.*, 2003). Satiation in contrast is related to the termination of a meal as a result of inhibition of feelings of hunger and appetite during the meal. Therefore, satiation during a meal leads to termination of food intake and the commencement of the satiety phase. Whereas hunger and satiety are considered to be intrinsic feelings, appetite is often a learned response (Vermunt *et al.*, 2003). Both satiety and satiation are equally important, as together they determine total daily energy intake. A thorough grasp of the factors influencing these sensations is therefore required when determining strategies for managing energy intake.

Satiety and satiation are regulated by a complex series of mechanisms which begin when the food is consumed and continues after digestion and absorption. The factors determining satiety have been sequentially presented as a cascade by Blundell and colleagues (1994) (Figure 1.1). Post-meal satiety can be separated into two phases; the early and late, where the early phase is a result of sensory and cognitive factors associated with the food, and the late phase due to digestion and related biochemical signals.

Figure 1.1: The satiety cascade showing satiety and satiation progression over time (Blundell *et al.*, 1994)

Early phase satiation occurs as a response to oro-sensory stimulations to the food, the individual's current physiological state and previous experience with food (Hetherington *et al.*, 2002). The amount eaten at a single sitting depends on many factors (Bellisle, 2003) and include the level of physical activity (King, 1998; Elder & Roberts, 2007), preference for the food that is available (Levitsky, 2008), portion size (Levitsky & Youn, 2004), size of and time from previous meal (Levitsky, 2008) and food variety (Rolls *et al.*, 1981; Norton *et al.*, 2006; Brondel *et al.*, 2009). A large variety of food also minimises sensory specific satiety (SSS) and may cause overeating (Norton *et al.*, 2006). This phenomenon (SSS) is defined as the gradual decrease in perceived pleasantness to a particular food when it is eaten continuously, which ultimately leads to the cessation of the eating episode (Rolls & McDermott, 1991). A single-dish meal therefore elicits a rapid SSS compared to courses or a buffet where the greater food variety suppresses SSS. The repeated presentation of the same food to individuals also results in a decrease in the quantity eaten at each progressive sitting, a phenomenon known as the monotony effect (Siegel & Pilgrim, 1958). Both monotony and SSS therefore reduce food intake at a meal independent of physiological hunger and satiety cues.

Psychological aspects play a significant part in food intake; emotions and moods (stress, depression, premenstrual dysphoria) have been shown to affect food intake of individuals in different ways (Gibson, 2006). Levitsky (2008) showed that social factors surrounding an eating episode such as the location and others present also affected meal size. The ambience, familiarity and comfort of the location, and the relationship with the others present determine individual consumption. Cultural acceptances also govern the quantity eaten at

meals; while some cultures encourage overeating, others dissuaded it. Similarly, some cultures regard being overweight an acceptable and sometimes desirable disposition and others frown on it. Such social ideologies will influence the quantity of food eaten by individuals.

The cognitive aspects of the satiety cascade occur predominantly due to previous experiences with food (Blundell & Tremblay, 1995). The quantity of a particular food eaten at a single sitting by an individual may be habitual i.e. as accustomed portion sizes. Brunstrom and Rogers (2009) proposed that individuals determine food portion sizes based on expected satiation, which is quantified by previous experiences. In agreement, other studies have shown that people judge the quantity they can eat based on preconceived units such as slices, bowls, glasses, unit numbers etc (Rolls *et al.*, 2004). Consuming a quantity corresponding to their 'ideal' portion size will therefore produce a feeling of complete satiation while smaller or larger portions will be psychologically perceived as under- and over-eating respectively.

Post-ingestive satiety occurs due to the presence of food in the stomach and is stimulated by gastric distension and food composition (de Graaf *et al.*, 2004). Feedback on these aspects is sent to the hypothalamus via afferent nerve pathways. The earliest work in this area was carried out by Geliebter and colleagues (1988). Using water-filled balloons inserted into the stomach, they demonstrated that gastric volume was strongly correlated to the quantity of food eaten at a meal. In agreement, recent work suggested that the volume of food consumed may be more important in inducing satiety than its energy density (Rolls & Bell, 1999; Rolls *et al.*, 1999; Flood & Rolls, 2007; Rolls, 2009).

These studies showed a proportional decrease in subsequent food intake when the volume of the iso-caloric portions increased. Santangelo *et al.* (1998) fed healthy young men iso-caloric portions of food differing in consistency and observed a greater satiety for the treatments that spent a relatively longer time in the stomach. These findings also suggest that a slower rate of gastric emptying will enhance satiety as it would increase gastric holding time. Indeed, one study found an inverse correlation between gastric emptying and satiety (Villar *et al*., 1981).

The post-absorptive phase of satiety is the physiologically important stage as it occurs during and after digestion. It determines the long-term satiety following a meal and is the combined result of hormones, blood glucose levels, gut motility and actions of the central nervous system (Figure 1.2). These individual aspects are further discussed below. Broadly, the physiological mechanisms controlling satiety can be categorised as episodic and tonic. Episodic mechanisms produce short-term signals that occur immediately following food consumption, and tonic mechanisms generate long-term signals corresponding to body energy stores (Benelam, 2009).

Figure 1.2: A schematic illustration of physiological signals regulating satiety

Source: Benelam *et al*, 2009

1.4 Food intake control in the brain

Feeding and satiety are primarily controlled by the hypothalamus (Benelam, 2009) in which the lateral hypothalamus (LH) is the feeding centre and the ventral hypothalamus (VH), the satiety centre (Strubbe, 1994). All food intake regulation pathways can be broadly grouped into either those that are orexigenic (induce food intake) or anorexigenic (inhibit food intake). Both pathways function through direct neuronal signals from the gastro-intestinal tract, pancreas and adipose tissue, relative concentrations of glucose, fatty acids and other metabolic fuels in blood and concentration of certain hormones (Friedman, 2008). Both tonic and episodic signals act directly though receptors in the brain, or indirectly through other appetite control areas in the brain via the nervous system (Benelam, 2009).

1.5 Endocrine control of satiety

Hormones are thought to play a significant role in satiety, notably with the tonic regulation of food intake (Strader & Woods, 2005). As food enters the digestive system numerous hormones are secreted into the blood from the adipose tissue, pancreas and intestine and these include cholecystokinin (CCK), bombesin, ghrelin, insulin, Incretins (glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and gastric inhibitory peptide (GIP)), somatostatin, enterostatin, leptin, orexin, obestatin and nesfatin-1 (de Graaf *et al.*, 2004; Stanley *et al.*, 2005; Zhang *et al.*, 2005; Oh *et al.*, 2006). The hormones relatively more important in satiety regulation are believed to be leptin, insulin, ghrelin and incretins (de Graaf *et al.*, 2004).

Leptin is secreted from the adipose tissue and its plasma concentrations have been shown to inversely correlate with food intake and energy expenditure. (Trayhurn & Bing, 2006). Blood leptin concentrations correlate positively with body fat stores and therefore provide information on the magnitude of body fat reserves to the hypothalamus (de Graaf *et al.*, 2004; Stanley *et al.*, 2005). Low leptin concentrations may therefore induce greater food intake and adiposity. The episodic effects of leptin on short-term appetite and satiety however remain equivocal. One study showed that blood leptin concentrations were not correlated with appetite before and after meals in individuals in energy balance (Joannic *et al.*, 1998). Yet a strong negative correlation was observed in individuals not in energy balance (Chin-Chance *et al.*, 2000). Although leptin may be an important regulator of long-term energy balance, its role as a short-term biomarker of satiety remains to be verified.

Ghrelin, which is secreted in the fundic region of the stomach is the only recorded episodic orexigenic agent (stimulates short-term food intake and a positive energy balance) (Gil-Campos *et al.*, 2006). Ghrelin concentrations increase before a meal and decrease afterwards, in a pattern inverse to that of other satiety hormones (Cummings *et al.*, 2001). It has also been implicated in decreasing energy expenditure and increasing adipogenesis (Cummings *et al.*, 2001). Circulating ghrelin levels are inversely correlated with adiposity and anorectic and obese individuals therefore have high and low plasma levels respectively (Stanley *et al.*, 2005). An inverse relationship between plasma glucose and ghrelin concentrations was observed by Shiiya *et al* (2002) when subjects were given glucose loads, but no change in ghrelin levels when an equal volume of water was given. This suggests that ghrelin secretion does not

occur as a result of stomach distension and is stimulated by blood glucose levels. A significant inverse correlation between ghrelin concentrations and subjective feelings of appetite has also been observed (Blom *et al.*, 2003) which suggests a notable influence of this hormone on short term energy intake.

Following observations that the insulin response to orally administered glucose was greater than to an intravenous challenge, a group of insulin secretagogues named incretins were discovered (Drucker, 2006). This group consists of GIP, GLP-1 and PYY. The first incretin to be identified was GIP and this is produced in duodenal and jejunal enteroendocrine K cells. Whilst the predominant stimulus for its secretion is food intake, lowest and highest concentrations of it are observed in the fasted and postprandial stages respectively (Drucker & Nauck, 2006). Both GLP-1 and PYY are secreted from the enteroendocrine L cells of the intestinal mucosa (Drucker & Nauck, 2006). Since the majority of the L cells are located in the distal end of the gut and GLP-1 and PYY responses are seen soon after a meal, it is speculated that the initial secretion of these hormones is neurally mediated, whilst the latter phase is stimulated by nutrients (Teff & Kapadia, 2008). Simple carbohydrates and fats are the most potent stimulators of GLP-1 and proteins appear to have little effect (Teff & Kapadia, 2008). Together, GLP-1 and GIP account for nearly half of the postprandial insulin response (De Leon *et al.*, 2006; Drucker, 2006). Additionally, GLP-1 inhibits glucagon secretion, decreases liver glucose synthesis, slows gastric emptying, reduces appetite and induces satiety via the vagal nervous system (Drucker & Nauck, 2006; Teff & Kapadia, 2008). The hormone PYY is co-secreted with GLP-1 and concentrations of it

proportionately increase with the caloric and lipid content in the meal (Teff & Kapadia, 2008). It inhibits the release of neuropeptide Y, a strong appetite stimulant from the central nervous system (CNS) (de Graaf *et al.*, 2004). Externally administered PYY significantly reduced 24-hour food intake and proportionately affected subjective feelings of satiety and hunger (Batterham *et al.*, 2002; Batterham *et al.*, 2003) suggesting a possible role in the longer term regulation of satiety. Fasting concentrations of PYY showed a significant negative correlation with body mass index indicating a relationship of this hormone with adiposity and therefore long-term energy balance (de Graaf *et al.*, 2004). Similar to GIP and GLP-1, the relative amounts of carbohydrate, protein and fat in a meal also influence PYY secretion (Adrian *et al.*, 1985).

1.5.1 The physiology of insulin and its role in glucose homeostasis and satiety

Insulin is one of the most studied hormones in the context of food intake and is secreted from the β cells in the pancreatic islets of Langerhans. A small quantity is also produced in the brain (Gerozissis, 2008). The daily output of insulin by the pancreas is approximately 40-50 Units (15-20% of pancreatic insulin stores) (Keim *et al.*, 2006) and is secreted in response to blood glucose concentrations (Woods *et al.*, 2006) and incretin hormones (de Graaf *et al.*, 2004; Drucker, 2006) in a dose dependant manner. Carbohydrate is the most potent macronutrient secretagogue, protein and fats have mild and no influence respectively (Teff & Kapadia, 2008). Blood glucose directly stimulates the β cells to produce insulin in a dose dependant manner (Henquin, 2000). The glucose enters the cell by facilitated diffusion and undergoes oxidative glycolysis which increases the ATP content in the cell and therefore the

ATP:ADP ratio. A higher ratio subsequently causes the ATP-sensitive K^+ channels to close and the voltage-operated Ca^{2+} channels to open. The resulting increase in the intra-cellular free Ca^{2+} concentration stimulates insulin release by exocytosis (Henquin, 2000).

Insulin lowers blood glucose levels by facilitating its uptake into insulin-sensitive tissue (Keim *et al*., 2006). It also stops the production of glucose by the liver, stimulates glycogen production and inhibits glucagon secretion. Food factors appear to influence the magnitude of the insulin response (IR). Lee and Wolever (1998) observed that the IR to glucose, sucrose, fructose and bread were similar, although as the carbohydrate portion increased, a relatively greater increase in the IR was observed compared with the blood glucose response (GR). The starch structure also influences the IR, and one study showed that amylose produces a smaller IR than amylopectin, despite both foods producing a similar GR (Behall *et al*., 1988). Similarly Juntunen *et al.* (2002) found that the insulin responses to rye breads and pasta were lower than that observed for white wheat bread, despite all the foods eliciting a similar GR. The IR to a food therefore appears to depend more on its form and chemical structure than on fibre content or food type.

The sight and smell of food also stimulates insulin production (Sjostrom *et al*., 1980; Powley & Berthoud, 1985; Teff, 2000), indicating the psycho-sensory aspect to its expression (cephalic phase). The cephalic phase represents physiological responses by the body to the sight, thought and smell of food, and is characterized by a rise in hormone levels, notably insulin (Teff, 2000). The insulin released during this phase (which lasts for approximately 10

minutes) is important for glucose tolerance as it prepares the body against extreme glucose excursions prior to a meal (Ahren & Holst, 2001). A strong negative correlation between cephalic phase insulin release and the initial GR has been repeatedly observed (Del Prato, 2003) which demonstrates the importance of early-phase insulin secretion on glucose homeostasis.

Insulin has also been implicated in the regulation of circulating leptin levels (Trayhurn & Bing, 2006). Reciprocally, leptin stimulates insulin secretion (Houseknecht *et al.*, 1998) and the two hormones therefore function together to regulate energy stores and balance. Since insulin levels therefore are an indirect indicator of energy stores, the brain interprets low levels as evidence of a negative energy balance and causes the activation of orexigenic mechanisms as seen in diabetes (Schwartz *et al.*, 2000).

Elevated blood insulin concentrations increase satiety and suppress food intake in the short term (Holt & Miller, 1995; de Graaf *et al.*, 2004; Stanley *et al.*, 2005; Anderson *et al.*, 2006; Woods *et al.*, 2006; Flint *et al.*, 2007). An early prospective study conducted on Pima Indians determined the relationship between insulin and weight gain over three years and found that low insulin secretion was an independent biomarker of weight gain (Schwartz *et al.*, 1995). Circulating insulin levels therefore affect both short and long term energy balance. Animal studies have shown that short term food intake is affected when insulin is injected into the portal vein (VanderWeele, 1994), brain (Gerozissis, 2004; Woods, 2009) and when insulin receptors in the brain are disrupted (Obici *et al.*, 2002). Insulin deficient animals were also observed to be hyperphagic, and small doses of insulin injected to the brain reversed this effect

(Woods *et al.*, 2003). The satiating effects of insulin therefore appear to be through its effects on the brain.

Conversely, other studies utilizing euglycaemic and hyperglycaemic clamp methods found that food intake was not influenced by insulin when blood glucose was kept constant (Chapman *et al.*, 1998; de Graaf *et al.*, 2004) which suggests that the blood glucose response is the principle instigator of satiety. In agreement, Rodin *et al.* (1985) found that hyperinsulinaemia, independent of plasma glucose, paradoxically increased food intake. However these studies were conducted using exogenous insulin and require cautious interpretation. Studies investigating the effects of endogenous insulin reported an appetite-suppressing effect in lean (de Graaf *et al.*, 2004), but not obese (Speechly & Buffenstein, 2000; de Graaf *et al.*, 2004; Flint *et al.*, 2007) subjects. These findings may suggest that the satiogenic properties of insulin are dysfunctional in overweight subjects. Obese individuals however demonstrate more pronounced insulin secretion (Polonsky *et al.*, 1988b) and subdued clearance rates (Polonsky *et al.*, 1988a) and therefore have larger circulating levels compared to lean subjects. Obesity is also characterized by greater leptin and lower incretin levels compared to lean individuals (Schwartz *et al.*, 2000; Verdich *et al.*, 2001). These differences may be adversely influencing insulin induced satiety in the obese.

1.6 Measuring satiety and food intake

The methods used to measure satiety and food intake have been reviewed elsewhere (Hill *et al.*, 1995; Livingstone *et al.*, 2000; Benelam, 2009). Since satiety is influenced by physiological, psychological, cultural and environmental

aspects a series of factors need consideration during its measurement. A quandary when measuring satiety is the degree of control that must be exerted in the study design (Figure 1.3). Experimental conditions must be finely controlled within a laboratory setting if specific mechanisms and small differences are to be detected. However, results of such studies have little practical significance and application. To obtain practically useful satiety data, studies must be conducted in a free living state, where the degree of control is relaxed (Hill *et al.*, 1995). The outcomes of these studies however are less reliable due to the poor levels of control. Based on the hypothesis tested therefore, the study design is usually a compromise between these two ends. The sample size required for adequate statistical power also depends on where the study lies within this range.

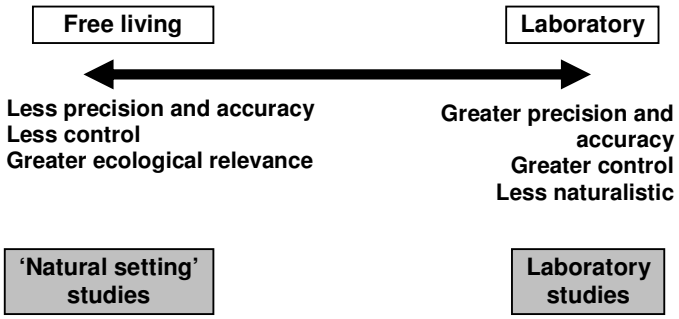


Figure 1.3: Schematic presentation of satiety and food intake measurement experimental designs

A within-subject crossover design is recommended for satiety studies, as the subjects then serve as their own controls, thus minimising both within-and between-individual variations (Livingstone *et al.*, 2000). In order to remove bias and preconceived expectations the subjects need to be blinded. In order to obtain a homogenous sample, the participant pool must be carefully vetted for physiological and behavioural confounders such as age, gender, body weight,

habitual diet, dietary restraint, eating disorders, psychopathology, eating attitudes, physical activity, menstrual cycle, smoking and weight control history (Livingstone *et al.*, 2000; Benelam, 2009). The design must also standardise the study setting to minimise social and environmental effects (Levitsky, 2008).

Satiety manifests both psychologically and physiologically and a good experimental design should therefore include techniques to collect data from both these aspects. This is accomplished by obtaining data on subjective feelings of satiety (psychological) and actual food intake (physiological) (Hill *et al.*, 1995). Additionally, biomarkers of satiety (hormones, plasma glucose) are measured (de Graaf *et al.*, 2004).

The standard method for measuring subjective feelings of hunger, appetite and satiety is visual analogue scales (VAS) (Raben *et al.*, 1995; Flint *et al.*, 2000; Stubbs *et al.*, 2000). This is a psychometric tool that takes the form of a straight line (100 mm) anchored at either end with opposing answers to a specific question. Corresponding to how the participants subjectively feel, they make a mark on the line. The specific questions asked include, 'how hungry do you feel?', 'how full do you feel?', 'how strong is your desire to eat?' and 'how much food do you think you can eat?' (Hill & Blundell, 1982). Advantages of VAS are that they are easy, quick and cheap to use, simple to analyse, and in a standardised format (Stubbs *et al.*, 2000). Their accuracy and reproducibility however depends on aspects such as sample size, participant training, study protocol and design, physiological and psychological variables, and external factors such as physical activity, weather, climate and previous meal characteristics (Flint *et al.*, 2000). Since subjective feelings are not an

inevitable result of underlying physiological mechanisms, but an individual's interpretation of their sensations (introspection), a precise correlation between psychometric data and actual feeding behaviour cannot always be expected (Stubbs *et al.*, 2000). Under controlled within-subject conditions however, and when used in conjunction with other methods VAS provide useful data regarding the psychological aspects of satiety.

Conversely, observing actual food intake gives a better measure of appetite and satiety reflective of physiological cues. Food intake in humans however, is a very complex process, and as discussed earlier, is influenced by environmental, psychological and social factors. In order to correct for all these variables studies measuring food intake are usually conducted in a laboratory setting (Benelam, 2009). Within controlled conditions the subjects are given an *ad libitum* test meal and the choice and quantity of food eaten is covertly measured. This is managed by adopting methods like unobtrusive observation, video recording, providing fixed portions, purpose-built instruments (Universal Eating Monitor), liquid food reservoirs, pumps and food dispensing machines (Hill *et al.*, 1995). The energy and macronutrient content in the quantity of food eaten and the percentage compensation (relative to the control) is then calculated for the different treatments (Cecil *et al.*, 2005). A cheaper alternative to providing a test meal is the use of food diaries/records which can be used to record past food intake by recall, consumption during a meal or following a treatment (Hill *et al.*, 1995). The obvious disadvantage of diaries and records is that the data is self-reported and therefore unreliable.

The standard study design used to measure short-term food intake regulation is based on the classical preload paradigm developed in the 1960s (Livingstone *et al.*, 2000). This involves the subject being given precisely prepared test and control foods that have been balanced in all respects (volume, texture sensory aspects, macronutrient composition) except for the attribute tested (Livingstone *et al.*, 2000; Benelam, 2009). Following the preload, data from VAS and biological samples for quantifying biomarkers are obtained at regular intervals. An *ad libitum* test meal is subsequently provided where food intake is unobtrusively recorded. The time interval between the preload and test meal, and the length of time during which measurements are obtained depend on the study objectives and previous research findings. A archetypal preload design is illustrated in Figure 1.4 and is typical of that used in the studies that make up the current thesis.

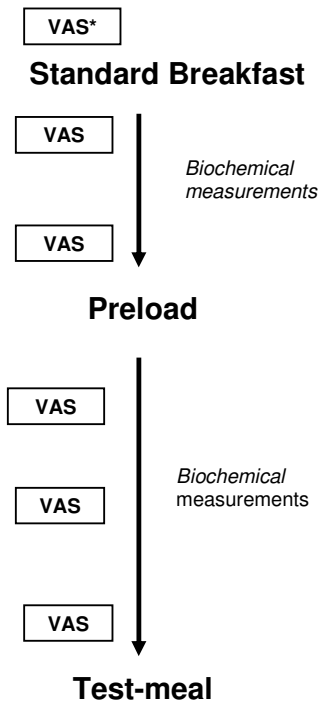


Figure 1.4: Schematic presentation of a typical preload paradigm.

*Visual analogue scales

1.7 Blood glucose homeostasis

The blood glucose concentration is a dynamic but finely regulated entity in the human body. In healthy individuals the post-absorptive/fasting concentration is maintained between 4-6 mmol/L (Van den Berghe *et al.*, 2009). Both hypo- and hyperglycaemia have been shown to adversely affect health and wellbeing (Davidson, 2004). Glucose is the fuel for the brain and red blood cells and a constant supply in the blood is therefore critically important. The blood glucose concentration is regulated by balancing the rate of food consumption and intestinal absorption of dietary carbohydrates, rate of removal and release of glucose by the liver, rate of uptake of glucose by peripheral tissue and rate of loss and synthesis by the kidney (Nordlie *et al.*, 1999). The apparent blood glucose concentration is therefore dependant upon all these factors (Figure 1.5).

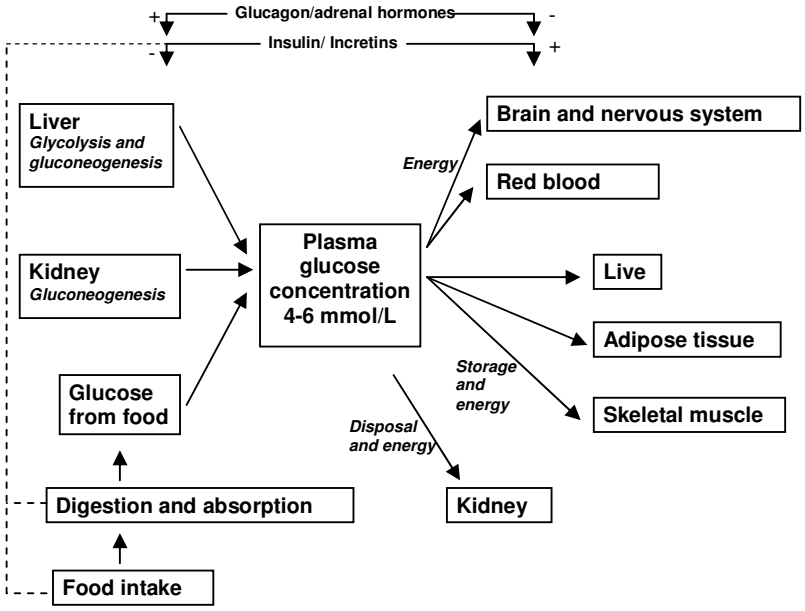


Figure 1.5. : Graphical representation of blood glucose homeostasis

In the fasted and post-absorptive states blood glucose homeostasis is maintained by the regulation of hepatic and renal glucose production and the amount of glucose taken up predominantly by non-insulin-dependant tissue (nervous system, red blood cells, skin, smooth muscles etc) (Cherrington, 1999). In the postprandial state glucose homeostasis is maintained by controlling the rate of glucose appearance from the digestive system and the uptake by non-insulin-dependant tissue, peripheral tissue (skeletal muscle and adipocytes), kidney and the liver. In the postprandial hyperglycaemic state hepatic production ceases and tissues exclusively utilise glucose derived from the food (Moore *et al.*, 2003).

The appearance of glucose in the blood is from either exogenous (digestion of food and absorption) or endogenous (liver and kidney) sources (Stumvoll *et al.*, 1995; Corssmit *et al.*, 2001). When a carbohydrate is ingested liver glucose synthesis is suppressed and splanchnic and tissue glucose uptake is stimulated. Glucose absorbed from the intestine first travels into the liver via the portal vein where it is redistributed into storage (glycogen), energy production and release into plasma glucose (Woerle *et al.*, 2003). The apparent glucose concentrations in the plasma will therefore depend on how much is held back by the liver for glycolysis and glycogenesis (synthesis of glycogen from glucose). The quantity retained by the liver would depend on the magnitude of prevailing ATP and glycogen stores in the body. It is estimated that approximately 33% of oral glucose is retained by the liver (Cherrington, 1999; Moore *et al.*, 2003). The principle mechanisms by which the liver regulates blood glucose homeostasis are glycogenesis (storage and uptake of glucose), glycogenolysis (synthesis of glucose from glycogen) and gluconeogenesis

(synthesis of glucose from non-glycogen sources) (Nordlie *et al.*, 1999). These processes are auto-regulated by enzymes. The activity of the two enzymes specific for gluconeogenesis, phosphoenolpyruvate carboxykinase and fructose-1.6-biphosphatase are controlled by the glycolytic enzymes pyruvate kinase and 6-phosphofructo-1-kinase. Similarly, the activity of the glycogenolytic enzyme glycogen phosphorylase is modulated by the glycogenic enzyme glycogen synthase. Additionally, the enzyme glucose-6-phosphatase catalyses the terminal steps in gluconeogenic and glycogenolytic pathways, and its activity is controlled by the glycolytic enzyme glucokinase. The cross-interactions of all these enzymes collectively produce an auto-regulatory system for blood glucose control.

Although the liver has been largely considered the primary organ for maintaining glucose homeostasis, recent work indicates that the kidney plays an equally important role. In the post-absorptive state the kidney has been shown to account for up to 25% of systemic glucose production (Stumvoll *et al.*, 1997). The kidney produces glucose primarily via gluconeogenesis and appears to be as important as the liver in this respect. Lactate, glycerol and glutamine are the most important gluconeogenic renal precursors (Stumvoll *et al.*, 1997). Conversely the kidney is also important in the uptake of glucose and has been shown to absorb 10-20% of the circulating glucose (Stumvoll *et al.*, 1995; Cherrington, 1999). Therefore, similar to the liver, the kidney plays a significant part in post-absorptive glucose homeostasis.

Glucose in the blood is taken up by nervous tissue for meeting energy needs. Although the metabolic rate of the brain has been shown to be relatively

constant during physiological alterations of cerebral activity, the rate of energy metabolism in discrete parts of the brain may differ depending on the state of functional activity (Sokoloff, 1977; Hawkins *et al.*, 1979). Red blood cells also utilise glucose as their primary energy source. Together, the nervous system and red blood cells account for approximately 64% of glucose utilisation in the post-absorptive state (Cherrington, 1999).

Excess glucose in the plasma is taken up by peripheral skeletal and adipose tissue, the specific mechanisms by which are not yet completely understood (Moore *et al.*, 2003). However, evidence strongly suggests that it is through the action of insulin. Insulin stimulates the translocation of the GLUT-4 glucose transporter to the muscle plasma membrane and facilitates the diffusion of glucose into the muscle. The absorbed glucose is either converted to glycogen following phosphorylation into glucose-6-phosphate or undergoes glycolysis for energy production. The majority of glucose absorption in the post-absorptive state occurs in non-insulin-dependant tissue whilst the bulk of the glucose in the post-prandial state occurs in the insulin-dependant muscles (Kelley *et al.*, 1988). Therefore insulin plays an important role in maintaining post-prandial glucose homeostasis. Exercise induces greater glucose uptake by stimulating the translocation of GLUT-4 (Moore *et al.*, 2003). In addition, increased Ca^{2+} and Nitric oxide have also been proposed as mediators of exercise induced glucose uptake. The degree of physical activity will therefore influence glucose homeostasis.

Hormones play a significant role in orchestrating glucose homeostasis. As described above, insulin stimulates glucose uptake by peripheral tissues. It also

suppresses endogenous glucose production (Moore *et al.*, 2003). Conversely, glucagon which is produced in the α-cells of the pancreas stimulates the conversion of glycogen to glucose during hypoglycaemic conditions. It further safeguards against hypoglycaemia by regulating gluconeogenesis (Cherrington *et al.*, 1978). Gucagon like peptide-1 has been suggested to significantly influence glucose homeostasis as it stimulates insulin secretion during hyperglycaemia, suppresses glucagon production and slows gastric emptying (Perfetti & Merkel, 2000). It has further been implicated in stimulating skeletal muscle glucose uptake (Moore *et al.*, 2003). Adrenal hormones (cortisol and adrenaline) have also been shown to increase plasma glucose levels and affect glucose metabolism (Hakanson *et al.*, 1986; Khani & Tayek, 2001). Therefore, stress and related states will influence blood glucose homeostasis.

Thus, it is evident that the regulation of the blood glucose concentration is a complex process that involves many organs, enzymes, hormones and their inter-relationships. A good understanding of the mechanisms by which the blood glucose concentration is controlled is needed when conducting GR research. Controlling for participant characteristics and adopting a within-subject controlled design could minimise the influence of these variables when conducting GR research. However, their confounding effects on treatment outcomes cannot be discounted entirely.

1.8 The blood glucose response

1.8.1 Impact on satiety

The GR refers to the changes that can be observed in the blood glucose concentration following the consumption of a carbohydrate food. The earliest recorded classification of carbohydrates was as simple (mono- and disaccharides) and complex (polysaccharides), and work in the early 1920s showed that the former induced hyperglycaemia at a faster rate than polysaccharides in dogs (Allen, 1920). Later studies confirmed that equal portions of different types of carbohydrates influenced the GR in different ways (Crapo *et al.*, 1977; Schauberger *et al.*, 1977). Although it was earlier thought that these differential effects were due to fibre (Wolever, 2006) subsequent studies showed that other factors (independent of the fibre content) were also responsible for these differences (Jenkins *et al.*, 1981b). Since glucose is the primary form of energy for the brain it is one of the most precisely regulated substrates in the human body. A steady concentration of it is maintained in the blood throughout the day by managing exogenous (food) and endogenous (liver) sources of carbohydrate (Keim *et al.*, 2006). Blood glucose concentrations rise above normal levels following a carbohydrate-containing meal and are rapidly cleared by the action of insulin. The rise in glucose following a meal also causes endogenous glucose production to cease (Keim *et al*, 2006).

According to the energostatic theory, meal initiation occurs when the brain perceives an energy deficit (Friedman, 2008). This was first observed as a decline in blood glucose levels, leading Mayer (1953) to propose the glucostatic theory. It stated that hunger resulted when critical areas in the brain reduced

their glucose utilisation, and that, increased glucose utilisation by these same critical brain areas led to satiation and cessation of eating. Mayer suggested that decreased glucose utilisation (metabolic hypoglycaemia), or "the point at which peripheral arteriovenous differences in blood glucose becomes negligible and glucose is no longer entering metabolisable cells," was the cue for food intake. Since then considerable research has been carried out to examine the validity of the hypothesis (Stunkard & Wolff, 1956; Van Itallie, 1990). More recent work on the effect of blood glucose on food intake has been carried out by Campfield and colleagues (Smith & Campfield, 1993; Campfield *et al.*, 1996; Campfield & Smith, 2002). Using a cholinergic agonist to induce declines in blood glucose in rats, Smith and Campfield (1993) continuously recorded blood glucose and insulin levels, and spontaneous food intake. The authors observed a pattern where spontaneous food intake consistently occurred following a brief dip and subsequent rise in blood glucose. This was then evaluated in humans (Campfield *et al*, 1996). Using healthy subjects blinded from food and time cues the authors observed spontaneous food intake following brief transient declines in blood glucose similar to that observed in rats. These trends were confirmed in healthy subjects in other studies which observed blood glucose response patterns and spontaneous eating following carbohydrate and fat preloads (Melanson *et al.*, 1999c) and fat, sugar and aspartame preloads (Melanson *et al.*, 1999b).

The above authors went on to investigate these patterns under exercise conditions and found that meal initiation occurred with transient declines in blood glucose only if subjects were not glycogen depleted (Melanson *et al.*, 1999a). Similar outcomes were observed in another study where no patterns

between blood glucose and time-blinded food intake were observed in overweight men in negative energy balance (Kovacs *et al.*, 2002). This suggests that glucostatic mechanisms function only in a normal physiological state.

However, these findings do not definitively confirm a causal relationship between the GR and food intake. Indeed it may be other factors stimulated by the GR that eventually impact on food intake. In a meta-analysis of seven studies Flint *et al.* (2007) found that insulin but not glucose was associated with short-term appetite regulation in normal subjects. However, since the blood glucose response is the single largest stimulator of insulin secretion it is also the primary instigator for these subsequent metabolic reactions.

Food intake occurs due to a transient decrease in the GR, and therefore maintaining consistent and elevated blood glucose levels following a meal delays the time until the next meal and extends satiety (Chaput & Tremblay, 2009). This was demonstrated by Anderson *et al.* (2002) who observed food intake 60 minutes after the ingestion of iso-carbohydrate (75 g) portions of glucose, polycose™, sucrose, amylopectin, fructose-glucose and amylose. They reported a lower subsequent food intake following the drinks eliciting a greater GR (glucose, polycose and sucrose). Recently, Bornet *et al.* (2007) reviewed 32 studies investigating the impact of the GR on satiety and concluded that foods with a low GR were more satiating than those with a high GR. Although this observation appears to disagree with that of Anderson *et al.* (2002), both reports showed that an elevated GR did induce satiety. The differences were based on the pattern of the GR curve (Figure 1.5). A highly digestible

carbohydrate food causes a large initial blood glucose peak and an accompanying insulin surge which subsequently causes a rapid reversion of the blood glucose level back to baseline. A slowly digestible carbohydrate conversely produces a lower GR and IR but also one that sustains above baseline for a longer time period. Based on the glucostatic hypothesis therefore, these low GR foods maintain satiety for a longer time (Figure 1.5). A high GR food, due to its greater initial glucose response will in comparison suppress food intake at first, but lose its satiating properties rapidly. Whilst a high GR food therefore is more satiating in the short-term (60 minutes or less) (Anderson *et al.*, 2002), a low GR food remains satiating for longer (Bornet *et al.*, 2007).

Figure 1.6: The application of the glucostatic theory to high and low GR foods (Bornet *et al.*, 2007)

The satiating effects of carbohydrates have been consistently demonstrated in previous studies (Feinle *et al.*, 2002). Compared to a carbohydrate-free control Blundell *et al.* (1994) observed a decrease in food intake following a soup containing maltodextrin. Similarly, Rogers and Blundell (1989) found that food intake one hour after glucose or starch supplemented yogurts was lesser than that following unsweetened and artificially-sweetened controls. Intra-gastric infusion of dextrose also reduced food intake 30 minutes later compared to a saline control which had no effect on food intake (Shide *et al.*, 1995). The same study also observed that food intake was not affected when the same treatments were administered intravenously and this was later confirmed in another study (Lavin *et al.*, 1996). Therefore the satiating effects of carbohydrates appear to be mediated through the gastro-intestinal tract. Cecil and colleagues (1998) showed that appetite suppression after soup preloads was greatest when consumed orally, followed by when infused into the stomach and small intestine respectively. This confirms that satiety sensations are generated throughout the digestive system as food passes through. A protracted digestion time will therefore consequently also lengthen satiety duration.

A large volume of recent work on the GR was carried out in the context of the glycaemic index (GI). The GI concept, first proposed in 1981 (Jenkins *et al.*, 1981a) is a physiological measurement of the blood glucose raising potential of a carbohydrate food. It is defined as the blood glucose response elicited by a 50 g available carbohydrate portion of a food, expressed as a percentage of the response following 50 g of a reference (anhydrous glucose or white bread) when taken by the same subject (Wolever, 2006). Since the GI is a ratio

between two blood glucose responses, the physiological principles pertaining to it are similar to those of the GR. A high GI food causes the GR to rise rapidly and subsequently fall below baseline as a result of rapid counter-regulatory hormonal responses. Low GI foods in comparison produce a low, stable and positive blood glucose level for a longer time period. Low GI foods therefore also prolong satiety and increase the interval between meals (Wolever, 2006). In a review, Roberts (2003) summarised studies comparing food GI and satiety and concluded that energy intake after a high GI food was 81% greater than after a low GI food.

1.8.2 Food-associated determinants of the blood glucose response, and its variations

Glucose appearance rate in the blood following a meal depends on the digestion and absorption rate. Factors influencing the digestibility of carbohydrates will therefore also impact on the GR. Food factors affect the GR by either slowing transit time or limiting starch accessibility to digestive enzymes (Fardet *et al.*, 2006). Different carbohydrates impact on the GR in different manners; glucose elicits the greatest response compared to disaccharides and polysaccharides as it does not require digestion, and is therefore rapidly absorbed (Lee & Wolever, 1998). In comparison fructose produces a relatively lower GR as it is poorly absorbed in the intestine (Lee & Wolever, 1998). In the case of disaccharides, sucrose and maltose elicit similar responses whilst lactose produces a relatively smaller GR (Vosloo, 2005).

The GR of polysaccharides depend on their digestibility which is in turn dictated by their chemical and physical characteristics. The principle digestible polysaccharide in the human diet is starch and this is made up of amylose and

amylopectin. The relative amounts of these two components determine the GR of starch. Amylose has been consistently shown to elicit a smaller GR than amylopectin (Behall *et al.*, 1988; Behall *et al.*, 1989; Ranawana *et al.*, 2009), possibly because the long chains of amylose are more resistant to digestion (Benmoussa *et al.*, 2007). Amylose also reacts with other food components such as lipids and forms complexes that are impervious to digestion (Holm *et al.*, 1983). Different varieties/strains within a single crop type therefore elicit different GRs due to differences in the amylose:amylopectin ratio (eg: wheat, rice) (Vosloo, 2005; Ranawana *et al.*, 2009).

The presence of other macronutrients reduces the GR of a carbohydrate food (Thorne *et al.*, 1983; Spiller *et al.*, 1987; Gatti *et al.*, 1992; Vosloo, 2005; Henry *et al.*, 2006), through changing chyme viscosity and creating an enzyme resistant barrier around the carbohydrate molecules (Henry *et al.*, 2006). Fibre influences the GR through similar mechanisms (Bjorck *et al.*, 1994). However, the effects of fibre depends on its type (soluble and insoluble) and origin (natural or added) (Wolever & Miller, 1995). Whilst soluble fibre has a significant effect when externally added, insoluble fibre influences the GR only when naturally present. This suggests that insoluble fibre influences the GR through its effects on food structure. Soluble fibre affects digestion by slowing gastric emptying and sugar absorption through increasing chyme viscosity (Vosloo, 2005). The presence of other compounds in the food such as polyphenols in legumes (Thompson *et al.*, 1984), phytates in wheat (Yoon *et al.*, 1983) and organic acids such as propionates in bread (Todesco *et al.*, 1991) also reduces the GR. Similarly, the addition of salt has also been shown

to significantly increase plasma glucose and insulin responses (Thorburn *et al.*, 1986).

The digestibility of a carbohydrate food is directly affected by the degree of cooking and gelatinisation. Hydration due to exposure to wet heat makes the starch structure more accessible to amylase (Bjorck *et al.*, 1994). Studies with rice showed that a longer cooking time elicited a higher GR due to a greater degree of starch gelatinisation (Panlasigui *et al.*, 1991; Ranawana *et al.*, 2009). However, subsequent cooling and prolonged storage of gelatinised starch causes it to re-crystallise and retrograde, leading to the formation of resistant starch (RS) (Mitra *et al.*, 2007) which is conversely poorly digested by amylase (YoungHee & SeungHo, 2004). Retrograded starch therefore lowers GR as evident in parboiled rice (Ranawana *et al.*, 2009).

The degree of milling and particle size of the carbohydrate food has also been repeatedly shown to have an inverse correlation with digestibility, and therefore the GR (Thorne *et al.*, 1983; Bjorck *et al.*, 1994; Vosloo, 2005). Using rice as the model O'dea *et al.* (1980) demonstrated that the ground form elicited a greater GR and IR compared to the whole grains. The authors asserted that particle size is more important towards the GR than fibre content. Similar results were also observed with other cereals (Heaton *et al.*, 1988). The increased surface area of small particles provides greater access for enzyme activity and therefore faster digestion.

The degree of processing also affects digestibility, and one study showed that instant rice, rice bubbles, corn chips, cornflakes and instant potato elicited

greater glycaemic responses compared to whole rice, corn and potato (Brand *et al.*, 1985). A greater degree of processing makes the starch more digestible through gelatinisation, weakening the structure and reducing particle size. In the case of liquids the volume affects the GR. One study investigated the effects of 25 g of glucose, fructose and sucrose on the GR when dissolved in volumes of water ranging between 200-600 ml, and found a volume dependant increase in the GR (Sievenpiper *et al.*, 1998). A higher dilution produces a more hypotonic solution that is easily absorbed compared to a low-volume hypertonic solution.

The above discussed factors are all associated with the food and are therefore external variables that influence the GR. Even when all these factors are controlled for, a notable degree of variation can be observed in the GR even when the same food is given to a cohort of healthy individuals matched for age, BMI, health status, physical activity, lifestyle etc. Between-individual variations can be considerably large and account for 20-80% of the total variation observed during GR testing and have a co-efficient of variation (CV) as high as 55% (Vega-Lopez *et al.*, 2007; Wolever *et al.*, 2008). A study that repeatedly measured the GR of rice, spaghetti and bread found that between-individual variations accounted for 62% of the total variation and was thus the greatest contributor to it (Wolever *et al.*, 1990). Likewise, the within-subject variability, i.e. the variations observed when the same food is given repeatedly to the same person under standardised conditions can also be considerable. In the study by Wolever *et al.* (1990) this component accounted for 16% while in another study, normal subjects showed inter- and intra-individual variations of 26 and 25% respectively in response to repeated challenges of 50 g of glucose

(Wolever & Jenkins, 1985). A similar study compared the within-individual variations to a liquid (75 g glucose) and solid (mixed test meal bar) challenge and found them to be 39 and 31% respectively (Wolever, 2006). Within-individual variations in the insulin response were also measured in this study, and these amounted to 21 and 20% for the liquid and solid respectively.

Explanations for these variations are limited, although time of day, nature of the previous meal consumed, between-meal time gap, physical activity and length of fasting have been suggested (Wolever, 2006). However, Campbell *et al.* (2003) comparatively observed the effects of the previous meal, physical activity and the fasting period on the GR and found that these factors do not significantly contribute to within-individual variations. Armario *et al.* (1996) showed that blood glucose concentrations were positively correlated with biomarkers of stress which suggests that the psychological state also affects plasma glucose levels. Differing psychological states in individuals could therefore contribute to GR variations. Using an insulin clamp Chiu *et al.* (2000) demonstrated that insulin sensitivity differed between ethnic groups (Asian, African, Caucasian, Mexican), which suggest possible ethnicity-based differences in glucose homeostasis. Using an ethnically mixed cohort of subjects could therefore also contribute to GR variations. Gastro-intestinal motility and absorption rates have also been suggested to differ between individuals and thereby contribute to between-individual variations in the GR (Wolever, 2006).

Methodological aspects associated with GR testing also contribute to data variations. The time taken to consume the test food has been shown to affect

the GR; a study by Heine *et al.* (1983) demonstrated a 50% reduction in the GR when glucose or hydrolysed starch was consumed in one minute compared to 10 minutes. It is therefore advocated that the eating time be standardised during GR testing (Wolever, 2006). The source of blood sampling could also contribute to differences between individuals. Measuring glucose concentrations in venous blood was associated with a greater random error and intra-individual variation compared to capillary blood (Wolever & Bolognesi, 1996; Wolever *et al.*, 2003). Also, smaller changes in plasma glucose concentrations were more rapidly detected in capillary blood (Granfeldt *et al.*, 1995). Capillary blood is therefore recommended for GR determinations (Wolever, 2006). In conclusion, the above discussion illustrates the complexities surrounding the GR and the number of internal, external and methodological factors influencing it. Therefore, it is imperative that all these factors are considered as practically possible when conducting GR studies.

1.9 The effect of the physical state of food and its impact on satiety and the glycaemic response

It has been speculated that energy delivered in liquid form may not trigger satiety mechanisms (DiMeglio & Mattes, 2000; St-Onge *et al.*, 2004) and that the body's regulatory systems are stimulated mainly by the physical form (liquid or solid) of the substrate in which it is provided (Mattes, 1996; Mattes, 2006a; Mattes, 2006b). Energy consumed in a liquid medium is believed to be less efficiently detected by the physiological compensatory systems in the body and thus lead to a passive overconsumption of energy (Gibson & Neate, 2007). Zijlstra *et al.* (2008) observed differences in eating rates between liquids, semi-

solids and solids. They demonstrated that the consumption rate of a liquid was 14 and 30% higher compared to a nutrient-matched semi-solid and solid respectively. More liquid can therefore be consumed within a defined time period compared to a solid or viscous food due to the absence of an oral processing phase.

Several studies have investigated the comparative effects of liquid and solid iso-caloric foods on satiety, and found that the solids elicited a greater degree of fullness. Using liquid and semi-solid dairy products, one study showed that participants consumed significantly more of the liquid compared to the semi-solid to achieve the same degree of satiation (de Wijk *et al.*, 2008). Therefore, the higher consumption rate of liquids will result in a greater energy intake compared to solids independent of satiety cues.

Preload studies have also shown that solids are better compensated for than liquids. This has been demonstrated using isocaloric portions of jellybeans and liquids (DiMeglio and Mattes, 2000), solid, gel and liquid forms of sucrose (Lavin *et al.*, 2002), solid and liquid forms of nutrient matched meal replacement products (MRP) (Tieken *et al.*, 2007; Stull *et al.*, 2008), pureed and whole forms of carrot (Moorehead *et al.*, 2006) and the pureed, whole and juiced forms of fruits (Haber *et al.*, 1977; Bolton *et al.*, 1981; Flood-Obbagy & Rolls, 2009). These findings were confirmed in a series of experiments conducted by Tournier and Louis-Sylvestre (1991) which collectively showed that subsequent food intake was greater following a liquid lunch preload compared to a solid of similar composition. Another study comparatively observed hunger ratings for 3.5 hours following a liquid preload, and the same

after solidifying with gelatine and locust-bean gum, and found that the solid forms suppressed hunger more than the liquid (Hulshof *et al.*, 1993). Therefore, there is convincing evidence to suggest that solids are more satiating than liquids and are better compensated for in the short term.

However, there is also evidence to indicate that liquids and solids do not impact differentially on satiety (Almiron-Roig *et al.*, 2003). The same group in one study compared hunger ratings and food intake (either 120 or 20 minutes after the preload) following the consumption of iso-caloric (300 kcal) portions of either a cola drink or cookies and found that the physical form had no effect on hunger and food intake (Almiron-Roig *et al.*, 2004). Energy intakes were however relatively less when lunch was consumed 20 minutes after the preload, suggesting that the time between preload and next meal may be more important than the physical form.

The time lapse till onset of hunger following a meal has on the other hand, been shown to correlate with the viscosity of food (Kissileff, 1985; Mattes & Rothacker, 2001). A more viscous/solid food appears to elicit a greater degree of fullness, and this may be due to feedback signals arising from oral processing. Indeed, Zijlstra and colleagues (2008) observed longer oral processing times with more viscous foods and this resulted in greater satiety and lesser food intake. The absence of chewing also results in decreased pancreatic exocrine and endocrine responses associated with hunger and satiety (Almiron-Roig *et al.*, 2003). Mars and others (2009) showed that greater viscosities led to longer oro-sensory stimulation and resultant learned satiation

which suggests that semi-solid and solid foods are also psychologically perceived as more satiating.

Other studies have however reasoned that food volume may play a greater role in satiety than viscosity. Russell and Delahunty (2004) used rice milk beverages adjusted to two volumes and viscosities and showed a significant reduction in hunger and a longer time to lunch following the higher volume treatment, but no effect of viscosity. This is in agreement with work done by Rolls and colleagues who showed that volume has a larger influence on satiety than most other factors (Rolls *et al.*, 1998; Rolls *et al.*, 1999; Flood & Rolls, 2007; Rolls, 2009). However, although a higher volume will elicit a greater post-ingestive satiety, the long-term satiety effects mediated by post-absorptive factors will depend on other characteristics of the food such as composition, texture and digestibility.

In summary, results from past studies suggest that liquids are less satiating than solids and this may be due to psychological preconceptions, poorer physiological responses and differences in viscosity. However, the presence of contradicting findings highlights the need for more research.

1.9.1 The effect of liquid calories on satiety and food intake

The debate on sugar-sweetened beverages (SSB) and their implication as instigators of passive over consumption of energy (Gibson & Neate, 2007) is largely fuelled by the outcomes of prospective and cross-sectional studies, and bolstered by population statistics. A five fold increase in soft drink consumption (from 180ml per week in 1975 to 976ml per week in 2000) was reported in the

UK (UKSA, 2008) and a similar trend was observed in the US where the contribution from soft drinks to total daily calorie intake increased by 135% between 1977 and 2001 (Nielsen & Popkin, 2004). Since the rise in soft drink consumption and obesity rates occurred in tandem, caloric beverage consumption has been implicated as a cause of the obesity epidemic (Malik *et al.*, 2006).

The literature on SSBs and their effects on energy balance have been previously reviewed (Anderson & Woodend, 2003a; Vermunt *et al.*, 2003; Bawa, 2005; Anderson, 2006; Bachman *et al.*, 2006; Malik *et al.*, 2006; Pereira, 2006). The majority of cross-sectional and longitudinal studies suggest a positive relationship between SSB consumption and both weight gain (Ludwig *et al.*, 2001; Giammattei *et al.*, 2003; Mrdjenovic & Levitsky, 2003; Nicklas *et al.*, 2003; Berkey *et al.*, 2004; Schulze *et al.*, 2004; Bes-Rastrollo *et al.*, 2006; Striegel-Moore *et al.*, 2006; Harrington, 2008), and incidence of chronic diseases (WHO, 2003; Apovian, 2004). However, outcomes of cross-sectional and longitudinal studies do not confirm direct causal effects. These can be determined only through randomised-control studies. Indeed, SSB consumption has been associated with lifestyle factors such as high fat and energy consumption and low physical activity, (Almiron-Roig *et al.*, 2003; Gibson, 2008) and these aspects could have substantially contributed to the relationships reported in the observational studies.

Short-term randomised control studies investigating the acute effects of SSBs on food intake and satiety have shown mixed results. There is some evidence to show that energy-containing drinks are compensated for at the following

meal. Woodend and Anderson (2001) found a dose dependant reduction in food intake at a pizza lunch 60 minutes following liquid sucrose preloads (25-75 g) by young males. Similarly, Holt and colleagues (2000) observed non-significant differences in total energy intakes following sucrose, sugar-free and mineral water preloads, suggesting that sugar-rich drinks elicited a higher level of compensation compared to the sugar-free alternatives. Another study reported that sucrose containing drinks significantly reduced the desire to eat compared to water and artificially sweetened drinks (Canty & Chan, 1991). Pure glucose and fructose preloads were also found to decrease food intake at the subsequent meal, suggesting a calorie compensatory effect for liquid calories irrespective of the chemical form of the carbohydrate (Spitzer & Rodin, 1987; Rodin *et al.*, 1988; Rogers *et al.*, 1988; Rodin, 1990; Anderson, 2002). Therefore, there is convincing evidence to show that carbohydrate-based caloric liquids are detected by the physiological regulatory systems and compensated for in the short term.

However, other studies have shown no compensation for liquid calories at the subsequent *ad libitum* meal provided 30-240 minutes later (Anderson *et al.*, 1989; Rolls *et al.*, 1990; Almiron-Roig & Drewnowski, 2003; DellaValle *et al.*, 2005). Rolls *et al.* (1990) compared the effects of sucrose (20 and 40 g) and aspartame sweetened lemonade on food intake when taken with the meal, and 30 or 60 minutes before, and found food intake to be equal after all the treatments. Similarly, Almiron-Roig and Drewnowski (2003) observed no difference in food intake 135 minutes after the consumption of iso-caloric (248 kcal) portions of orange juice, cola, low-fat milk and water.

Studies with children have also shown a good level of compensation for caloric drinks in the short term. Anderson (1995) observed a 68% and 63% reduction in food intake by 9-10 year old children, 30 minutes following the consumption of a 300ml drink containing 45 and 90g of sucrose respectively. A similar study observed a significant reduction in food intake by children (pre-school age) 30 and 60 minutes following caloric drinks (Birch *et al.*, 1989). Children may be better able at compensating for calories due to their limited experience with external stimuli and greater dependence on internal signals. Adults may be less responsive to internal physiological cues due to stronger environmental stimulants.

The above findings provide evidence both supporting and disproving the hypothesis that energy provided in a liquid media is detected and compensated for. However, closer examination of the studies shows that the observation of a compensatory effect appears to depend on the study design. The time interval between preload and test meal, and the caloric content of the preload have significant influences on the observation of compensatory effects. Those with preloads having a relatively large energy content (>150 kcal) and time gaps less than 60 minutes between preload and test meal consistently showed good levels of compensation compared to studies with smaller energy preloads and time gaps greater than 60 minutes. This suggests that energy in liquids are detected and compensated for within a specific time window. The length of the time window and magnitude of compensation is dictated by the energy content in the preload and physiological aspects (Almiron-Roig *et al.*, 2003). The results of short-term randomised experiments therefore do not support the conclusions reached by the majority of the observational studies that SSBs are a significant

cause of passive overeating and obesity. More data from well designed studies are however required before firm conclusions can be reached.

1.9.2 Effect of the physical state on the glycaemic response

Limited research has comparatively observed the GR of liquids and solids. Doyle *et al.* (1997) observed the GR and insulin response (IR) to liquid, solid and gel forms of a carbohydrate and found no significant differences. However, they obtained measurements for a brief time period (90 minutes); GR measurements should be obtained for a minimum period of 120 minutes if the entire response is to be observed (Wolever, 2006). Several studies observed the GR to the solid and juiced forms of fruits. Haber *et al.* (1977) compared the GR and IR to whole apple and its juiced and pureed forms and found that the latter two elicited greater glycaemic and insulin responses than the former. They also observed a striking rebound fall after the GR peak for the juice which was absent with the whole apple and concluded that the liquid form decreased satiety, disturbed glucose homeostasis and favoured over-nutrition. Another study observed similar patterns when whole oranges and orange juice were compared (Bolton *et al.*, 1981).

While there is some evidence therefore to suggest that the physical state affects the GR there is inadequate data to establish firm conclusions. Although the above studies have observed the GR after changing the phase of the same food, no studies have within a single design comparatively investigated the GR of foods naturally differing in their physical state. This aspect requires investigation.

1.10 Food particle size and effects on the glycaemic response and satiety

The particle size of food demonstrates an inverse relationship with digestibility. Small particles have a larger surface area: starch ratio which results in greater accessibility of the digestive enzymes to the starch (O'Dea *et al*., 1980). Particle size therefore is a rate-limiting factor in the digestion of solid carbohydrates. Through its effects on digestion rate food particle size also influences the GR (Bjorck *et al*., 1994; Vosloo, 2005). While large particles produce a lower GR small particles show a relatively larger response. O'Dea *et al.* (1980) observed the effect of whole and ground forms of polished and brown rice on the GR and IR. The authors found that the peak GR and IR for ground rice were significantly higher than that for the whole rice, as was the total area under the curve (AUC) for the initial 60 minutes. However, the AUC for the total 240 minutes was not significantly different for the two treatments, suggesting that the particle size had a significant influence only during the initial response phase. A significant and sustained increase in the IR was observed with ground rice compared to whole rice, which indicated that particle size also affected insulin secretion. Similar results were found in a study which compared the GR and IR to whole and ground rice (Collier & O'Dea, 1982). The authors reported that both parameters demonstrated significantly lower responses for whole compared to ground rice. Heaton *et al.* (1988) evaluated the GR and IR of whole and cracked grains, and course and fine flour of wheat maize and oats. The IR for the test foods increased in the order; whole grains, cracked grains, course flour, fine flour. The peak to nadir rebound was also more striking with the flour than the whole and cracked grains, which implied that the

pattern and rate of change in magnitude of the response were both affected by particle size.

Behall and colleagues (1999) fed participants breads made using conventionally milled white and whole-grain flour, and ultra-fine whole-grain wheat flour, and measured the GR and IR for the subsequent 180 minutes. Contrary to the findings in the above studies the GR and IR were not significantly different between the treatments. However, the particle size in the treatments was considerably small (ranged between 37-150 μm) and may have not been sufficiently large enough to affect digestion rate. This view is supported by another report that showed no significant differences in the GR between ground rice and pure glucose (Collier & O'Dea, 1982) which suggests that the digestibility of cereals ground to a flour is maximal and particle size is not a rate limiting factor for them.

The relationship between particle size and digestibility may be more important in foods where the starch resides within cells. In support of this Tovar *et al.* (1992) found that the GR of milled and cooked bean flour (rich in free starch) was greater than that of boiled and milled flour (rich in cell enclosed starch). Studies with fruit also showed that the disintegration of the structural and cellular integrity increased its digestibility and therefore the GR (Haber *et al.*, 1977; Bolton *et al.*, 1981). The particle size may not be as important in foods produced with highly processed grains and flour, where the majority of the starch is in its free form. Brand and colleagues (1985) demonstrated that the digestibility of instant rice, rice bubbles, cornflakes, instant potato and potato crisps was greater than that of boiled rice, sweet corn and potato. Whilst

milling, beating, shearing, refining and other processing methods affect cell and granule integrity, they also promote water absorption and gelatinisation which subsequently increases starch digestibility (Vosloo, 2005). Highly processed and milled carbohydrate foods therefore produce greater GRs than the whole forms.

A limited number of studies have attempted to discern food particle size effects on satiety. The significant effects of particle size on the GR suggest that it has the potential to influence satiety through glucostatic mechanisms (Bornet *et al.*, 2007). Additionally, Holt and Miller (1994) evaluated appetite following the ingestion of whole, cracked, coarse and finely milled wheat and found that satiety increased with particle size. Similarly, French and Booth (1991) fed subjects meat chunks of different sizes and showed that satiety increased with particle size. No other studies have addressed this aspect and data from more experiments are required before firm deductions can be made.

1.11 Mastication of foods, its measurement and effects on physiology and satiety

Mastication is the process of chewing food, and involves taking food into the mouth and crushing it to form a bolus (Hanawa *et al.*, 2007). The primary objective of mastication is the breakdown of food into small particles that will form a smooth cohesive bolus suitable for deglutition (van der Bilt *et al.*, 2006). Mastication occurs as a sequence which begins with the introduction of the food into the mouth and finishes with swallowing (Woda *et al.*, 2006a). The sequence consists of a succession of individual chewing cycles, each of which

comprises one jaw opening movement and closing movement. During chewing the food is mixed with saliva to moisten it and salivary mucins bind the food particles together to form a cohesive bolus. The formation of a smooth bolus acts as the cue for the initiation of voluntary swallowing (van der Bilt *et al.*, 2006).

Since mastication consists of a regular succession of chewing cycles rhythm is a principle characteristic and this is governed by a brain stem controlled central pattern generator and regulated by feedback systems (Woda *et al.*, 2006a). The masticatory system is a functional unit and consists of the teeth, their supporting structures, the jaws, tempero-mandibular joints, masticatory muscles (masseter and temporal), and the vascular and nervous systems supplying to these tissues (Soboleva *et al.*, 2005). Mastication is also the first step in the digestion process. Whilst the food is crushed and broken down, α-amylase and lipase in saliva begin breaking down carbohydrates and fats respectively (Humphrey & Williamson, 2001).

When given equal quantities of the same food, individuals chew it to different degrees before swallowing (Jiffry, 1981; Peyron *et al.*, 2004). Engelen *et al* (2005) observed habitual chewing rates of 266 dentate individuals when they were given a variety of foods (peanuts, toast, cheese, carrot and cake). They found a significant inter-individual variation in the number of chews taken before swallowing. Another study observed masticatory rates for gram and soy beans, and reported that the number of chewing cycles individuals' used varied between 9-15 and 10-18 respectively per unit weight of food (Jiffry, 1981). The number of chewing cycles per mouthful is however relatively

constant within a person for a particular food (van der Bilt *et al.*, 2006; Woda *et al.*, 2006a). The study by Engelen and colleagues (2005) also found that participants utilising a small number of chews for one type of food consistently also used small numbers for other foods. This suggests that all individuals can be broadly categorised as either high or low masticators.

One objective of chewing is the physical breakdown of food. Mastication efficiency can therefore be quantified by the particle size distribution in the chewed food (van der Bilt *et al.*, 2006; Gambareli *et al.*, 2007). The final degree of breakdown has been reported to depend on chewing parameters such as the number of cycles, chewing strength and chewing time, all of which vary between individuals (Lassauzay *et al.*, 2000).

When eating the same food van der Bilt *et al.* (2006) found that the particle size distribution in the chewed bolus varied between individuals. Whilst some broke down the food to a greater degree others swallowed relatively larger particles. This observation was confirmed in other studies (Jiffry, 1981; Jiffry, 1983). The particle size distribution in food ingested following habitual mastication therefore varies between subjects.

A large between-individual variation in particle size distribution was also seen when the same food is masticated for a standard number of chewing cycles by different individuals. Fontijn-Tekamp *et al.* (2004) gave 6 mm cubes of a Silicon based test material to a group of 87 dentate individuals and found that the median particle size ranged between 1.60-5.27 mm after 15 chewing cycles. The number of chewing cycles therefore does not appear to predict the degree

of breakdown during mastication. Instead, it suggests that the intensity of chewing varies significantly between individuals.

Mastication efficiency depends on several intrinsic (body) and extrinsic (food) factors. Intrinsic factors include the intensity of mastication which has been shown to differ between males and females (Woda *et al.*, 2006b) although the total number of cycles used to prepare a food for swallowing are comparable (Lassauzay *et al.*, 2000). Males demonstrate a greater intensity of mastication due to their relatively larger jaws and associated muscles. Mastication efficiency also decreases with age due to loss of masticatory muscle mass. The consequent decrease in bite force results in the total number of chewing cycles increasing with age (Woda *et al.*, 2006a). The number of teeth and the quality of the contact surfaces significantly influence mastication efficiency and a diminished performance has been observed in individuals with dental malocclusions, tempero-mandibular disorders and missing teeth (Woda *et al.*, 2006a).

The eating rate has been shown to also differ between lean and obese individuals (Bellisle & Le Magnen, 1981). Compared to lean subjects those obese demonstrated shorter chewing times and intra-meal pauses when presented with palatable foods. Therefore, overweight individuals appear to eat faster and chew less.

The degree of mastication is also affected by the level of saliva secretion. Saliva production rate varies between individuals and Engelen *et al.* (2005) showed that those secreting more saliva used a lesser number of chewing

cycles before swallowing. Saliva production is influenced by many factors which include hydration status, smoking, medications, cephalic phase responses, size of salivary glands, body weight, physical activity, alcohol and age (de Almeida *et al.*, 2008). Other studies have however shown that saliva flow rate correlates weakly with the swallowing threshold and explains only 2% of the variance in the swallowing threshold (van der Bilt *et al.*, 2006). Therefore variations in saliva secretion may not be significantly influencing between-individual variations in mastication.

The extrinsic factors influencing masticatory performance are those associated with the food such as texture, flavour, size, shape, hardness, water content and macronutrient composition (van der Bilt *et al.*, 2006; Woda *et al.*, 2006a). The principal food attributes affecting mastication however are hardness and dryness, both of which are positively correlated with chewing time. A study comparing the number of mastication cycles taken to swallow a variety of different foods reported a greater degree of chewing for hard (carrot and peanuts) and dry (melba toast and toast) compared to soft and moist (cake and cheese) foods (Engelen *et al.*, 2005). Similar observations were made elsewhere using nuts and vegetables (Peyron *et al.*, 2004). Food hardness also affects mastication force, jaw muscle activity and mandibular jaw movements (van der Bilt *et al.*, 2006).

The amount of food taken in a mouthful has been shown to influence the degree of mastication (van der Bilt *et al.*, 2006; Woda *et al.*, 2006a; Zijlstra *et al.*, 2009). Fontijn-Tekamp *et al.* (2004) fed 87 dentate subjects peanuts, cheese and carrots and found that the number of mastication cycles increased

linearly with food volume per mouthful. These findings were confirmed in another study using peanuts (Lucas & Luke, 1984), and the authors also showed that the particle size of swallowed food increased as food volume increased.

Mastication has also been shown to affect the digestive process through several modes. Hoebler and colleagues (1998) found significant physical and chemical transformations in food as a result of mastication and therefore concluded that oral digestion influenced the subsequent digestion process to a considerable extent. The cephalic phase (physiological reactions occurring in the body before consuming a meal as a response to sensory stimulations) can also significantly affect digestive outcomes by stimulating early phase hormonal responses especially insulin (Teff, 2000). Since the length of the cephalic phase is influenced by the time spent masticating, the degree of oral processing may have indirect effects on food intake and satiety by altering hormonal responses (Powley & Berthoud, 1985). This concurs with the concept of 'Fletcherism' coined by Horace Fletcher (1849-1919) (Christen & Christen, 1997), who advocated deliberate and extended mastication until the 'food swallowed itself'. He believed that prolonged chewing resulted in less overeating and reduced overall food intake. In agreement with this Zijlstra and colleagues (2009) observed an inverse relationship between food intake and oral processing time.

Read *et al.* (1986) showed that the GR was greater when subjects masticated food thoroughly compared to when they swallowed them without chewing. However, no work has been carried out to observe how the habitual degree of

chewing affects the GR. Since individuals habitually masticate to different degrees, differences in the degree of breakdown may be contributing to the between-individual variations observed in the GR. On the other hand, the GR also affects satiety through glucostatic mechanisms. The degree of habitual mastication may therefore also impact on satiety through particle size effects and the GR. The influence of the habitual degree of mastication on the GR and satiety requires investigation in future work.

1.11.1 Measurement of mastication

The most widely used method for measuring mastication is electromyography (EMG) (Mioche & Martin, 1998; Kemsley *et al.*, 2002; van der Bilt *et al.*, 2006; Woda *et al.*, 2006a). The use of EMG to characterise chewing behaviour was first proposed by Brown (1994). Using chewing gum as the model Brown showed that EMG measurements can be effectively used to observe variations in chewing between individuals. The method records bioelectrical activities taking place in the principle mastication related muscles (left and right side masseter and temporalis muscles) during an eating episode (Woda *et al.*, 2006a) (Figure 1.6). Since data is collected via electrodes mounted on the skin surface it is a non-invasive method that does not interfere with the eating process and can therefore be used to measure habitual eating patterns. A series of parameters pertaining to mastication can be measured with EMG and include the number of chewing cycles, muscular work, masticatory frequency, mastication time, opening and closing durations, vertical and lateral amplitudes and opening and closing velocities (Lassauzay *et al.*, 2000).

Figure 1.7: Muscles involved in mastication (Source: www. srgholisticdentist.com)

Mastication efficiency can be quantified by measuring the particle size distribution of the masticated food bolus. This is accomplished by instructing subjects to chew a standard amount of a test food to the point where they feel the need to swallow and then getting them to expectorate into a container. The particle size distribution in the resulting sample is determined by washing through a series of sieves (Jiffry, 1981; Jiffry, 1983; Fontijn-Tekamp *et al.*, 2004; Peyron *et al.*, 2004; Gambareli *et al.*, 2007; McKiernan & Mattes, 2010). The dry weight of particles collected on each sieve is expressed as a percentage of the entire sample. The use of sieves is simple, inexpensive and requires little skill. The particle size distribution can also be determined using optical scanning (laser diffraction) methods (Hoebler *et al.*, 2000). Although this is a method that provides accurate data it is expensive and requires skill. Gambarelli *et al.* (2007) assert that optical methods are better when particles are large, and sieves when the particles are small.

1.11.2 Secretion and functions of saliva and its role in carbohydrate digestion

Saliva is predominantly water (99%) and contains in addition to enzymes, inorganic and organic constituents such as bicarbonates, sodium, calcium,

potassium and proteins (Dodds *et al*., 2005). A healthy individual produces 1-1.5 L of saliva per day (de Almeida *et al*., 2008). In response to neurotransmitter stimulations, saliva is secreted into the oral cavity from acinar cells located in three salivary glands; the parotid, submandibular and sublingual glands (Baum, 1993; de Almeida *et al*., 2008). Whilst the latter two make up the bulk of un-stimulated saliva, the parotid gland contributes more than 50% of the stimulated form (Rohleder & Nater, 2009). The main functions of saliva are lubrication and food bolus formation, buffering of oral cavity pH, maintenance of tooth integrity, antibacterial activity, taste and digestion (Humphrey & Williamson, 2001).

Alpha-amylase is the principle starch digestion enzyme in saliva and this cleaves starch into maltoses (Pedersen *et al*., 2002). More than 80% of the α-amylase is produced in the parotid gland and factors such as age, smoking, alcohol, prescription drugs, caffeine, physical activity and somatic and psychiatric diseases affect its concentrations in saliva (Rohleder & Nater, 2009). Gender does not influence salivary α-amylase concentrations (Rohleder & Nater, 2009). The most widely used unit for measuring α-amylase activity is enzyme units per millilitre (U/ml). An enzyme unit is defined as the quantity that catalyses the conversion of 1 µmol of substrate per minute (Rohleder & Nater, 2009).

Chewing time may affect the degree of digestion in the mouth where those masticating for longer will expose the food to salivary α-amylase for a greater period of time. The digestibility of the swallowed bolus may therefore be greater in those masticating for longer and this could influence the pattern and

magnitude of the GR. However, no research has been carried out in this respect. Even so, Humphrey and Williamson (2001) argue that the contribution of salivary α-amylase to starch digestion is limited because mastication involves a relatively short time and salivary α-amylase is rapidly deactivated in the stomach by the acidic conditions. Firm conclusions cannot be reached until further research is carried out.

1.12 Gastric emptying and its effects on satiety, glycaemic response and insulin response

The stomach functions as a reservoir for food, and prepares it for intestinal digestion by chemically and physically breaking it down (Hellstrom *et al.*, 2006) and eliminating microbes through reductions in the pH (Low, 1990). The two functional compartments in the stomach are the proximal fundus (serves as the food reservoir), and the distal antrum (responsible for mixing and breakdown of food) (Low, 1990).

Gastric emptying (GE) refers to the rate at which food is emptied from the stomach (Clegg & Shafat, 2010) and the physical characteristics of the food determines its dynamics. The emptying pattern of liquids and solids differ markedly and it is well documented that liquids empty faster than solids (Read & Houghton, 1989; Collins *et al.*, 1991). Ingested liquids are rapidly distributed throughout the stomach and emptying begins almost immediately in an exponential manner at a rate proportional to the volume present (Hellstrom *et al.*, 2006). The emptying of liquids is speculated to occur as a result of the pressure gradient between the stomach and duodenum (Low, 1990). The GE of

solids in comparison shows a biphasic pattern (Siegel *et al.*, 1988). In the first phase (lag phase) food is held in the stomach and chemically and physically broken down to form a chyme, and then redistributed from the fundus into the antrum. This stage is then followed by the second phase (linear emptying phase) where the chyme is passed through the pylorus into the duodenum. The type of data arising from GE studies therefore depend on the physical form of the test food (Hellstrom *et al.*, 2006).

Gastric emptying, especially of solids, depends on the characteristics of the ingested food. Factors such as meal volume, particle size, viscosity, osmolarity, pH, protein, fat and energy content influence GE by altering the lag phase (Low, 1990). Using large and small portions of ground beef, Doran *et al.* (1998) showed a positive correlation between meal size and GE. The particle size of food correlates inversely with GE; foods with larger particles are held in the stomach for longer and broken down by grinding before being released into the duodenum (Torsdottir *et al.*, 1984; Vincent *et al.*, 1995). Meyer (1980) observed that the stomach does not release food into the duodenum until it is fragmented into particles smaller than 1000 μm. The shape of the particles however, does not appear to affect GE (Meyer *et al.*, 1989).

Since digestion and absorption occurs after the food has passed through the stomach GE influences the appearance rate of metabolites in the blood. Food factors delaying GE therefore affect the GR and satiety. Indeed, Fardet *et al.* (2006) have suggested that GE is one of only two principle mechanisms that influence carbohydrate digestion rate and thereby the GR. Mourot *et al.* (1988) used rice, spaghetti, French bread and mashed potato and showed that the GR

and IR to the four test foods were closely correlated with the GE. Similar relationships were shown by Torsdottir *et al.* (1984) using potato and rice meals, by Liljeberg and Bjorck (1998) using breads fortified with organic acids and by Horowitz *et al.* (1993) using 75g boluses of glucose. The last authors also found that GE accounts for up to 34% of the variance observed in the peak GR. These studies clearly demonstrate that the GR and related metabolic mechanisms are significantly influenced by GE.

The post-ingestive satiety phase in the satiety cascade occurs as a result of feedback signals from the stomach regarding the type and quantity of food consumed (Blundell & Tremblay, 1995). Gastric distention induces satiety (Geliebter *et al.*, 1988; de Graaf *et al.*, 2004) and a greater holding time therefore lengthens post-ingestive satiety. In agreement, animal studies showed that food intake does not stop when food was continuously drained from the stomach through a cannula, but did so when the cannula was closed (Davis & Smith, 1990). This effectively demonstrated the impact of gastric distention on food intake. Other studies have shown that the stomach is also able to detect meal characteristics and differentially stimulate the secretion of satiety hormones such as incretins and insulin (Santangelo *et al.*, 1998; Peracchi *et al.*, 2000). Similarly, Berry *et al.* (2003) showed that the early phase of GE is a key influencer of total insulin release and glycaemia, which further substantiates the importance of GE in post-meal physiology.

The measurement of GE is carried out using indirect methods as direct techniques involve invasive procedures. Indirect methods broadly use serial dilution, radiological, radioisotopic and stable isotopic techniques (Low, 1990;

Ghoos *et al.*, 1993). The serial test is one of the earliest procedures employed and uses a non-absorbable marker added to the test beverage (Low, 1990). The entire contents of the test-drink are then aspirated through an oro-gastral tube at periodic intervals and the volume and dilution of the beverage and marker are used as indicators of GE and gastric secretion respectively. However, this method can only be used with liquids. Radiological methods generally use Barium meals as qualitative indicators of GE (Low, 1990). This too however, can be used only with liquids. The use of magnetic resonance imaging (MRI) has been evaluated as a method of measuring GE but has again shown reliable results only with liquids (Feinle *et al.*, 1999).

The current gold standard method used to measure GE is scintigraphy (Urbain & Charkes, 1995). The technique involves the impregnation of the test food with a radioisotope (usually ^{99m}technetium and ^{111}indium for solids and liquids respectively), and following its movements after ingestion using gamma cameras (Low, 1990). Although the method provides accurate and reproducible data the technique requires expensive instrumentation, trained personnel and is time-consuming. Since the procedure employs radioactive markers it cannot be safely used with vulnerable groups, and for repeated applications in the same individual.

To overcome the disadvantages in scintigraphy an alternative method of measuring GE using non-radioactive stable isotope markers was later developed (Ghoos *et al.*, 1993). The accuracy of the method has been evaluated in comparative studies with scintigraphy which have shown that the data obtained from the two methods have a correlation co-efficient (r) greater

than 0.87 for liquids, solids and semi-solids (Ghoos *et al.*, 1993; Braden *et al.*, 1995; Choi *et al.*, 1998; Braden *et al.*, 2007). The procedure is non-invasive, accurate, relatively cheap and is quicker than scintigraphy. The absence of radioactive compounds also makes it suitable for repeated use in individuals and in vulnerable groups such as children and pregnant women (Braden *et al.*, 2007). The method was developed using octanoic acid as the isotope carrier compound (Ghoos *et al.*, 1993). Since this is however a fatty acid and is only fat soluble studies using water-based test foods alternatively use sodium acetate which produces equally reliable data (Braden *et al.*, 1995; Clegg & Shafat, 2010).

The technique uses stable isotopes of carbon (^{13}C and ^{14}C) which are incorporated into the food. The test principle is based on the stable isotope being oxidized after absorption in the duodenum. The resulting concentration of $^{13/14}CO_2$ in the exhaled breath is measured using mass spectrometry. The appearance rate of $^{13/14}CO_2$ in the breath is therefore used as an indirect indicator of GE.

The data derived from mass spectrometry analysis of breath samples is used to model a temporal curve representing the GE dynamics of the food (see section 2.2.6 in materials and methods for details). Several parameters quantifying GE are determined from this curve and include lag time (T_{lag}), half time (T_{half}), latency phase (T_{lat}) and ascension time (T_{asc}) (Figure 1.7). The T_{lag} is the time taken to maximal rate of $^{13}CO_2$ excretion (Jackson *et al.*, 2004) and is equivalent to the time of the inflection point (highest point in the GE curve) (Ghoos *et al.*, 1993). It gives an indication of the time it takes for the maximum

gastric emptying rate to occur. The T_{half} is the time it takes 50% of the ^{13}C dose to be excreted and is an indicator of the speed of gastric emptying (Ghoos *et al.*, 1993; Jackson *et al.*, 2004). Schommartz *et al.* (1998) later proposed T_{lat} and T_{asc} and reported that these parameters better described the different phases of the gastric emptying curve. The T_{lat} is defined by the point of intersection of the tangent at the inflection point of the $^{13}CO_2$ excretion curve and represents the initial delay in the excretion curve (Schommartz *et al.*, 1998) which is an indication of the holding time of food in the stomach prior to the initiation of emptying. The ascension time is the time period between the T_{lat} and T_{half} and represents the phase during which the highest $^{13}CO_2$ excretion rate can be observed (Schommartz *et al.*, 1998) and is representative of the period where GE occurs at the fastest rate.

Figure 1.8: Gastric emptying time points (source: Clegg and Shafat, 2010)

Aims and Objectives

The overarching objective of the work carried out in this thesis was to further elucidate factors influencing the glycaemic response. The literature review highlighted some gaps in the current state of the art in this area and the thesis attempts to address these, namely the physical state and the particle size of ingested food, and their effects on the GR. Food particle size effects on the GR were studied in the context of habitual mastication- the resulting particle size breakdown and its effects on individuals' GR.

The escalating levels of obesity highlight the urgency for a better understanding of energy intake control mechanisms,a and how they can be manipulated to prevent a positive energy balance. The literature review described the role of the blood glucose response (GR) in energy intake and balance. Regulating the GR is also important for the avoidance and control of diabetes and impaired glucose tolerance, conditions which are again closely linked with obesity. Factors affecting the GR will therefore impact both on energy regulation and chronic diseases. Outcomes of the studies forming this thesis are discussed in relation to both these aspects. A thorough understanding of all the factors influencing the GR is essential if its successful regulation is to be achieved. A major obstacle to establishing unequivocal conclusions is the consistent observation of large GR variations even under standardised testing conditions. Therefore, it is imperative that factors influencing the GR are studied also in the context of elucidating reasons for these variations and how they may be reduced.

A limited number of studies have attempted to compare the GR of solid and liquid carbohydrate foods. Although the literature suggests that the physical state influences the GR the dearth of studies preclude the formation of firm conclusions. It is also speculated that liquids are less satiating than solids, and that the former result in a passive overconsumption of energy. The veracity of this hypothesis also remains unconfirmed due to the limited amount of research. No previous attempts have investigated speculated differences in satiety between liquids and solids from a glucostatic theory perspective. The thesis examines the GR and satiety to liquid and solid carbohydrate foods and also investigates the effects of energy provided in a liquid form on short term energy intake and satiety.

Secondly, the thesis explores the role of habitual mastication and the resulting degree of particle size breakdown on the GR. Food particle size affects digestibility and thereby the GR. It is therefore likely that the degree of habitual mastication will impact on individuals' GR to a carbohydrate food. Previous studies have shown that the degree of habitual mastication differs significantly between people. Individual differences in mastication may therefore contribute to between-subject variations in the GR. The thesis also examines the effect of ingested food particle size on gastric emptying and the insulin response.

Research into the effects of the physical state on the GR is both timely and critical as the consumption of carbohydrate based drinks have increased at a near exponential rate in the recent times. Although it is widely speculated that carbohydrate containing liquids lead to excess energy intake, obesity and chronic diseases, there is little evidence to support it. The findings in this thesis

will allow for better public health recommendations in this regard. The work carried out in this thesis is the first instance where the effects of mastication on the GR have been investigated. The findings will be useful when formulating practical advice and intervention strategies for blood glucose and weight control for those who require it.

The specific objectives of the thesis are:

- To observe the effects of liquid and solid carbohydrate foods on the GR, insulin response (IR) and subjective feelings of satiety (Chapter 3).

- To determine the short-term effects of carbohydrate-based energy containing beverages on subjective hunger and food intake in humans (Chapter 4).

- To investigate inter-individual differences in habitual mastication and resulting particle size breakdown, and their effects on *in vitro* and *in vivo* glycaemic potency (Chapter 5 and 6).

- To examine the effects of ingested food particle size on GR, IR, gastric emptying and observe between-individual differences in the GR and IR when external, food and mastication factors are standardised (Chapter 7).

Chapter 2: Materials and Methods

2.1 Introduction

This chapter comprises of two sections:

Section 1: Common methodologies

Describes standard methodologies used in the experiments

Section 2: Experiment-specific methods, participant characteristics and protocols

Describes experiment specific protocols, participant characteristics and statistical methods.

2.2 Section 1: Common methodologies

2.2.1 Participant recruitment and preparation

Participants for studies were recruited by means of advertisements, flyers and personal communications. After expressing interest individuals were sent details of the study via email. If further interested, they were given a printed information sheet and individually briefed. Volunteers completed a self reported health questionnaire (giving details of food allergies/intolerances, metabolic diseases, special dietary needs and smoking habits, eating habits and disorders, physical activity and medication) (section 2.2.1.1) and were subjected to an anthropometric assessment (section 2.2.1.2). The inclusion criteria for the studies were,

- BMI: 18.5-24.99 kg/m^2
- Blood pressure: between 110-120/75-85 mmHg

- Age: 18-50 years
- Fasting blood glucose: 4.0-5.5 mmol/L
- Fasting plasma insulin: < 20 μU/mL
- Not on prescription medication
- Non-smoking
- Not physically active at the competitive and endurance level
- No genetic or metabolic diseases

The preceding evening's dinner was provided as part of the experimental design in study 1 (chapter 3) of the thesis. Past work demonstrating that the previous evening's meal had no effect on physiological responses was subsequently found (Campbell *et al.*, 2003) and the provision of dinner was consequently omitted from the subsequent experiments. The participants were allowed to eat anything they would like for dinner but were instructed to consume a meal of similar type, size and composition every evening before test days. Water was allowed *ad libitum*. On the day prior to a test they were also advised to restrict their intake of alcohol and caffeine-containing drinks and to avoid intense physical activity (e.g. long periods at the gym, intensive swimming, running, aerobics etc). All subjects when they came in for testing each day provided details of their food intake and physical activity on the preceding evening. This information was collected to ensure compliance.

The participants arrived at the laboratory between 7:00-8:30 am following a 12-hour overnight fast. Testing was initiated before 9:00 am. Upon arrival at the lab the subjects were allowed to rest for 10 minutes before testing commenced. The participants were encouraged to keep physical activity to a minimum

during testing and remain seated. Computers, work desks, reading areas and a television were provided for their use. Snacks were provided at the end of testing in studies 1, 3 and 4. All participants maintained at least a day's gap between test sessions to minimise any carryover effects. Randomisation of participants between the treatments was carried out using an online random number generator (www.psychicscience.org/random.aspx).

2.2.1.1 Anthropometric, body composition and blood pressure measurements

Anthropometric measurements were obtained after a 12 hour overnight fast with the subject wearing light indoor clothes and no shoes. Height was measured to the nearest centimetre using a free-standing stadiometer (Seca 217, Birmingham, UK) with the subject standing erect and the head in the Frankfurt plain. Body weight was measured to the nearest 0.05 kg using calibrated anthropometric weighing scales (Seca 877, Birmingham, UK). Body mass index (BMI) was calculated as weight (kg)/ height^2 (m). Waist circumference was measured to the nearest centimetre using an anthropometric measuring tape (Seca 201, Birmingham, UK) at the mid-point between the coastal margins of the ribs and upper margin of the iliac crest. Body composition was measured using a bio-electrical impedance segmental body composition analyser (Model BC-418 MA, Tanita UK Ltd., Yiewsley, UK). Blood pressure was measured with an automatic upper-arm blood pressure monitor (UA-767, A&D Instruments Ltd., Oxford, UK) with the subject seated and the upper arm raised to mid-sternum level.

2.2.1.2 Questionnaires used to vet participants

The objective in all the studies was to use normal healthy adults not suffering from any chronic medical conditions, eating disorders and not engaged in sports at the competitive and endurance levels. Pre-validated questionnaires were therefore used to screen participants for eating disorders, physical activity and general health prior to inclusion in the experiments. Eating habits and disorders were ascertained using the Dutch eating questionnaire (Van Strien *et al.*, 1986) (appendix 1) and only those scoring less than 2.8 were included in the experiments. A score less than 2.8 signified that the participant does not intentionally restrict food intake (for reasons such as weight control and aesthetics) or suffer from eating disorders (anorexia and bulimia nervosa) (Higgs & Eskenazi, 2007). Physical activity was quantified with Baeck's physical activity questionnaire (Baecke *et al.*, 1992) (appendix 2), and those scoring a total of ≥7 (high physically active) were excluded. This ensured the omission of those engaged in intense and endurance physical activity at a competitive level. Potential participants also filled in a health questionnaire (developed by the Nutrition Research Group at Oxford Brookes University) listing details of food allergies, intolerances, food dislikes, and genetic and metabolic diseases (Appendix 3). All those allergic and/or intolerant to the foods provided in the study and having illnesses requiring prescription medicine were excluded.

2.2.2 Ethical approval

Ethical approval for all the experiments was obtained from the Oxford Brookes University research ethics committee (UREC) prior to participant recruitment

and initiation. The ethics approval covered all aspects pertaining to the study protocol, information sheets, recruitment procedures and advertisements.

All participants gave written informed consent before taking part in the study. Upon completion of the experiment they were debriefed and compensated for their time with book tokens. Examples of a UREC ethics approval letter and a participant consent form are shown in appendix 4.

2.2.3 Measurement of *in vitro* glycaemic potency

2.2.3.1 Obtaining masticated rice samples for *in vitro* digestion

Participants were given 50 g of cooked rice along with a plastic screw-cap container. Using the dessert spoon provided the subjects were instructed to eat the rice as normal and expectorate it into the plastic container when felt the need to swallow. Within five minutes of obtaining the masticated sample two sub-samples of 10 g each were measured into falcon™ tubes (BD Biosciences, Ontario, Canada). To arrest α-amylase activity the samples were immediately acidified to pH 3 with 1M HCL. Sodium azide (4%, 0.1 mL) was added into each tube as a bacteriostat and the tubes were vortexed for 15 seconds. Samples were collected over a 30 day period and analysed as one batch; therefore they were thus treated to ensure stability during storage. The treated samples were frozen (–26°C) until *in vitro* digestion.

2.2.3.2 *In vitro* digestion

The digestion technique used is a validated method that has been previously used for determining the glycaemic potency of carbohydrate foods (Englyst & Hudson, 1987; Woolnough *et al.*, 2008; Mishra & Monro, 2009). Digestion was

carried out on 10 g samples of masticated rice placed in 70 mL specimen pots inserted to their full depth in a digestion system comprising a 30 well Aluminium block maintained at 37°C using a circulating water bath (Figure 2.1). Each well was stirred independently by a 25 x 10 mm magnetic stirring bar rotating at 130 rpm. Digestion consisted of simulated gastric and pancreatic digestion stages with timed sampling during the latter phase. It involved the following specific steps (Monro *et al*., 2010).

1. Gastric digestion stage- mixed with 30 mL water + 0.8 mL 1M HCl + 1 mL 5% porcine pepsin (P7000; 800-2500 U/mL; Sigma-Aldrich, Dorset, UK) in 0.05 M HCl and incubated at 37 ℃ and pH 2.5 for 30 minutes

2. Neutralised to pH 6 with 2 mL 1M $NaHCO_3$, 5 mL 0.2M Na maleate buffer (23.2 g maleic acid+ 800 mL distilled water, adjusted to pH 6.0 with 4M NaOH), containing 0.02% Na azide and 0.15% $CaCl_2$. The volume of the contents was made up to 53 mL with water. Duplicate 0.5 mL aliquots were withdrawn (Time = 0) to measure baseline glucose concentrations.

3. Pancreatic digestion stage- amyloglucosidase (0.1 mL, from *Aspergillus niger*, E-AMGDF; 3260 U/mL; Megazyme, Wicklow, Ireland) and 1 mL of 2% porcine pancreatin (Sigma-Aldrich P7545; 8 X USP specifications) in Na maleate buffer (pH 6.0) were added in quick succession and digestion carried out for the next 120 minutes. During this period further 0.5 mL aliquots were removed in duplicate (every 20 minutes) to measure glucose concentration. The 0.5 mL digesta aliquots were each added into capped

tubes containing 2 mL of ethanol and stored at 4°C until analysed for sugar content (2.2.5.3).

Sample residues at the end of 120 minutes of *in vitro* digestion were quantitatively transferred to individual dishes and their dry weights determined after drying to a constant weight in an oven at 105°C.

Total available starch content in the rice was measured by homogenising (to a slurry) a weighed sample for 20 seconds at the end of the gastric phase of digestion using an Ultra-Turrax Homogeniser (IKA®-Werke, GmbH & Co., Staufen, Germany) with an S18N-19G dispersing element. The sample was digested for 240 minutes from the addition of pancreatin and glucose content in a 0.5 mL sub-sample obtained at the end was measured as described in section 2.2.5.3.

Figure 2.1: The in vitro digestion system

2.2.3.3 Analysis of sugar content in sample aliquots

Reducing sugar content in the ethanolic sample aliquots was analysed using the Dinitrosalycylic acid (DNS) method (Lindsay, 1973). The ethanolic phase was clarified by centrifugation (1000 g, 5 minutes; Heraeus Labofuge 300,

Thermo Fisher Scientific Inc. MA, USA). A 0.05 mL aliquot of the digesta was transferred to a glass test tube and incubated for 10 minutes at 20ºC following the addition of 0.25 mL of 1% amyloglucosidase in 0.2 M acetate buffer (pH 5.2). This completed depolymerisation of any remaining maltose and dextrins and converted all the carbohydrates into glucose (Mishra & Monro, 2009). Subsequently, 0.75 mL of DNS reagent (10 g 3,5-dinitrosalicylic acid + 16 g NaOH + 300 g Na–K tartarate in 1 L of water) was added, and the tubes were held at 100ºC for 15 minutes in a water bath (Model GD-100, Grant Instruments, Cambridge, UK). After cooling to room temperature the contents were diluted with 4 mL of distilled water and the absorbance of the resultant was read at 530 nm using a UV/Visible spectrophotometer (Model UV-1201, Shimadzu Ltd., Milton Keynes, UK) (Mishra & Monro, 2009). Glucose concentrations (mg of glucose/ g of sample) in sample aliquots were calculated using the following formula (Englyst & Hudson, 1987; Mishra & Monro, 2009).

$$\frac{(\frac{abs\ of\ sample}{abs\ of\ stand}) * glucose\ conc\ in\ stand * digestion\ pot\ vol * dilution\ in\ ethanol}{Sample\ weight}$$

where,

Abs= absorbance; stand= standard; conc= concentration; vol= volume

2.2.4 Measurement of the *in vivo* blood glucose response

The protocol used to measure the blood glucose response (GR) was adopted from that described by Brouns *et al.* (2005) and is in line with procedures recommended by the FAO/WHO (1998). The non-dominant hand was used for blood sampling. Blood was obtained by finger prick using the Unistik 3 single-use lancing device (Owen Mumford, Woodstock, UK) (Figure 2.2). To minimise

plasma dilution fingertips were not squeezed to extract blood but gently massaged starting from the base of the hand moving towards the tips. The first two drops of expressed blood were discarded and the next drop (5 µL) was used for testing. Two fasting blood samples were taken five minutes apart for baseline values and the freshly cooked test food was consumed immediately afterwards. Further blood samples were taken at specified times from the commencement of eating. Blood glucose was measured using the HemoCue® 201+ Glucose analyser (HemoCue Ltd, Dronfield, UK) (Figure 2.2). The HemoCue is a reliable method of glucose analysis (Stork *et al.*, 2005). The HemoCue instruments used in the study were calibrated against an automated glucose analyser (YSI 2300 stat, YSI Inc., Yellow Springs, Ohio, USA) which is a pre-validated instrument for the determination of blood glucose concentrations (Wolever, 2006). The unit of measurement was mmol/L.

Figure 2.2: The HemoCue® 201+ Glucose analyser and the Unistik® 3 single-use lancing device

Blood glucose response curves were plotted for each subject using change-from-baseline (CFB) values. This is the difference between the blood glucose reading at a measurement point and the baseline blood glucose value (mean of the two baseline values). The total GR (for the entire 120 minutes and for

specific time periods) was expressed as the incremental area under the curve (IAUC) ignoring the area below the baseline and was calculated geometrically (FAO/WHO, 1998).

2.2.5 Measurement of the *in vivo* blood insulin response

Blood samples were obtained using the procedure described above in section 2.2.6. At each test time point 300 µL of blood was collected into chilled Microvette® capillary blood collection tubes treated with di Potassium EDTA (CB 300 K2E; Sarstedt Ltd., Leicester, UK). The Microvette® tubes were centrifuged (6000 rpm for 10 minutes, model MC-6; Sarstedt Ltd., Leicester, UK) and 120 µL of the plasma was separated and frozen (-40 °C) until analysis. Storage time of the samples was not more than 14 days. Insulin concentrations in the plasma samples were determined by electrochemiluminescence immunoassay using an automated analyser (Cobas® E411; Roche diagnostics, Burgess Hill, UK). The Cobas® system is a reliable method of plasma insulin determination (Braden *et al.*, 1995). The unit of measurement was µU/mL.

Insulin data processing, curve construction and calculation of the IAUC were carried out in the same manner as for the GR data (section 2.2.6).

2.2.6 Measurement of gastric emptying

Gastric emptying (GE) was measured using the ^{13}C labelled sodium acetate breath test (Clegg & Shafat, 2010). Breath samples for the analysis of $^{13}CO_2$ were taken at baseline (prior to consumption of the test meal) and at specified times afterwards. Samples were collected by the subject blowing gently into a

10 mL exetainer® tubes (Labco, Buckinghamshire, UK) through a drinking straw and replacing the cap just prior to the end of exhalation. A nose-clip was worn during sampling to ensure exhaling occurred only through the mouth.

Breath samples were analysed using isotope ratio mass spectrometry (Mk 5 ABCA, SerCon LTD, Chesire, UK), and results were expressed relative to Vienna PeeDee Belemnite (V-PDB) an international standard of known ^{13}C composition. The amount of $^{13}CO_2$ was expressed as the excess amount in the breath above baseline (moles). Data were displayed as percentage of the $^{13}CO_2$ dose recovered per hour and cumulative percentage $^{13}CO_2$ recovered over time. Carbon dioxide production was assumed to be 300 mmol/m^2 body surface area per hour (Ghoos *et al.*, 1993). Body surface area was calculated using a pre-validated weight-height formula (Haycock *et al.*, 1978) which was then fitted to a GE model developed by Ghoos and colleagues (1993). For all the data, the r^2 co-efficient between the modeled and raw data was calculated and this was greater than 0.95 which indicated that the model represented the raw data to a high degree. From the model GE lag (T_{lag}) and half (T_{half}) time was calculated using the formula derived by Ghoos *et al.* (1993). Latency (T_{lat}) and ascension (T_{asc}) times were computed using the formulas of Schommartz *et al.* (1998).

2.2.7 Measurement of subjective feelings of hunger and satiety

Subjective feelings of hunger, fullness, desire-to-eat and prospective food consumption were measured with 100 mm continuous line visual analogue scales (VAS). The specific questions asked were, 'How hungry do you feel?',

'How full do you feel?', 'How strong is your desire to eat?' and, 'How much food do you think you can eat?'. The lines were anchored at the left and right ends with opposing statements for each of these questions. The participants had to fill in all four scales at each measurement time-point. The VASs were provided in the form of a booklet at each test session and the participants were instructed how to accurately complete the scales at the pre-study briefing. An example of a VAS data collection sheet is presented in Appendix 5.

The VAS ratings were quantified by measuring the distance between the left end of the scale and the point marked by the participant. The CFB was calculated using these values. The resulting data were processed in the same manner as that from the GR (section 2.2.6).

2.2.8 Measurement of oral processing parameters

Data on the number of mouthfuls taken to consume the entire portion of each test food, the number of chews per mouthful and the time taken to masticate a mouthful were collected using electromyography (EMG) (Woda *et al.*, 2006a). Electromyograms were recorded from the left and right masseter muscles using bipolar surface electrodes (Brown, 1994; Mioche & Martin, 1998). The muscles were identified by palpitation whilst the participants clenched their jaws. The electrodes were moistened with distilled water and attached lengthwise along the muscles using adhesive strips (Figure 2.3). An additional earth electrode was attached to the right-arm wrist. The electrodes were attached to a programmable data acquisition unit (DataLOG model P3X8; Biometrics Ltd., Gwent, UK).

Figure 2.3: Electrode placements on the cheeks for EMG measurement, and the data acquisition unit

Data outputs were processed using LabVIEW 2009 software (National Instruments Corporation, Austin, Texas). The number of mouthfuls to consume the test food, the number of chews per mouthful and the time taken to chew each mouthful were quantified for each data set. The average value for each attribute for each test food for each participant was calculated.

2.2.9 Measurement of particle size distribution in masticated food

The method used was adapted from previously published work (Jiffry, 1981; van der Bilt *et al.*, 2006). Each subject was provided with 100 g of the test food and 100 mL of distilled water along with a plastic screw-cap container (pre-weighed). A standard dessert spoon (12.3 mL volume) or fork (depending on the test food) was also provided. The participants were instructed to take spoonfuls/forkfuls of the test food in quantities they were habitually accustomed to and chew to the point where they felt the need to swallow. However, instead of swallowing the subjects were instructed to expectorate the food into the pre-

weighed plastic container provided. To maximise recovery the participants were advised to use the water (100 mL) and rinse their mouth with a little amount following each mouthful and also spit that into the plastic container. The participants were advised not to swallow any of the food as practically possible. The pre-weighed plastic container with the masticated sample was re-weighed immediately afterwards. Particle size analysis was carried out within 10 minutes of obtaining the samples. Total masticated fresh sample weight was calculated by difference ([weight of masticated food + container + water] – [weight of plastic container + weight of water]).

The samples were washed through a set of three standard sieves (mesh sizes: 2000 µm, 1000 µm and 500 µm; Endecotts Ltd., London, UK) under running tap water until the eluting liquid was free of rice particles (Figure 2.4). The retained particles on each sieve were quantitatively transferred into drying dishes and dried in an oven at 105^0C until a constant weight was reached. The dry matter collected on each sieve was calculated as a percentage of the total dry sample weight. Particle sizes calculated were >2000 µm, <2000 µm->1000 µm, <1000 µm->500 µm and <500 µm.

Figure 2.4: Sieves used for separating masticated bolus to different size categories

2.2.10 Test food preparation

2.2.10.1 Rice

Pure white Basmati rice of a single variety, (*Oryza sativa L.*; strain HBC-19) (Tilda Ltd., Essex, UK) was used for all the studies. All of the rice was from a single cultivated batch. The use of a single rice variety of known purity ensured that the data was not affected by varietal differences (Ranawana *et al.*, 2009). A portion was defined as the amount containing 50 g of available carbohydrates (difference between total carbohydrates and fiber) (64.8 g of rice). Compositional information (per 100 g) was obtained from the supplier (total carbohydrates 77.6 g, fiber 0.4 g, protein 8.6 g, fat 0.4 g). Each test portion was cooked separately.

The rice:water ratio for cooking was maintained at 1:8 (as per manufacturer instructions). The water was brought to the boil in a saucepan at the maximum hob setting (6, Model CK-280, Kenwood Ltd., Havant, UK). The rice was added and allowed to return to the boil. The hob setting was subsequently lowered to position 4 and the rice was simmered for exactly 10 minutes. After cooking the rice was drained and served to the participant within 5 minutes.

2.2.10.2 Spaghetti

The spaghetti used in the study was purchased as a single lot (Tesco Spaghetti, Tesco PLC, Cheshunt, UK). In order to standardise the size the spaghetti sticks were broken into 12 cm lengths before cooking. A portion was defined as the quantity consisting of 50 g of available carbohydrates (difference between total carbohydrates and fiber) (71 g of spaghetti). Compositional information (per 100 g) was obtained from the supplier (total carbohydrates 73

g, fiber 2.6 g, protein 12.5 g, fat 1.4 g). Each test portion was cooked separately. The spaghetti:water ratio for cooking was maintained at 1:14 (as per manufacturer instructions). The cooking procedure was similar to that adopted for rice (section 2.2.11.1). After cooking the spaghetti was drained and served to the participant within 5 minutes.

Leaching of carbohydrates into the water occurs during the cooking of rice and spaghetti. However, standardising the cooking procedures ensured that the losses were constant at all the test sessions and therefore did not contribute to the differences observed between treatments.

2.2.11 Measurement of salivary α-amylase activity

Saliva samples for measuring α-amylase activity were collected using the pre-validated Salivette® system (Sarstedt Ltd., Leicester, UK) (Rohleder & Nater, 2009). The participants were instructed to take a mouthful of distilled water and rinse for 30 seconds. During the ensuing 2 minutes subjects repeatedly salivated and swallowed to remove all traces of water from the mouth. The Salivette® cotton swab was subsequently placed in the mouth for exactly 2 minutes. During this time subjects were instructed to continuously move the swab around the mouth surfaces and simulate a chewing motion on the swab. After 2 minutes the saturated cotton swab was placed back in the Salivette® system and centrifuged (3000 rpm for 5 minutes, Heraeus Labofuge 300, Thermo Fisher Scientific Inc. MA, USA). The resulting saliva samples (approximately 1000 µL) were stored at -40°C until analysis.

The pre-validated Phadebas® test (Magle AB, Lund , Sweden) was used for measuring α-amylase activity in the saliva samples (Ben-Aryeh *et al.*, 1990). The method uses a water-insoluble cross-linked starch polymer carrying a blue dye. When exposed to α-amylase this substrate gets hydrolysed and releases the dye into the aqueous phase. The absorbance of the aqueous phase is measured as an indicator of α-amylase activity. Sample analysis was carried out as per manufacturer instructions. The saliva samples were defrosted and a sub-sample (200 µL) was transferred into a centrifuge tube along with 4 mL of distilled water. The tubes were incubated for 5 minutes at 37°C. One Phadebas® reagent tablet was added into each tube and mixed well by vortexing for 10 seconds. The samples were incubated for exactly 15 minutes at 37°C and the reaction stopped by the addition of 1 ml of 0.5 M NaOH. After centrifugation (5 minutes at 3500 rpm) the blue supernatant was transferred into a cuvette and its absorbance was read at 620 nm using a UV/visible spectrophotometer (Model UV-1201, Shimadzu Ltd., Milton Keynes, UK). The absorbance values were transformed to α-amylase activity (in U/ml) using a conversion table provided with the Phadebas® kit.

2.2.12 Statistical analyses

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) version 17 (SPSS Inc., Chicago, Illinois) and data and figures processed in a Microsoft Excel spreadsheet (2006, Microsoft, Reading, UK). Data are presented with descriptive statistics (mean, standard deviation [SD], standard error [SE], coefficient of variation [CV]) where appropriate. Alpha (α) was set at 0.05 for all the statistical analyses and post-hoc comparisons. All the data sets in the studies were normally distributed (determined using the

Kolmogorov-Smirnov statistic). Power analysis was carried out using the G*Power software (Ver. 3.0.10, Universitat Kiel, Kiel, Germany).

2.3 Part 2: Experiment-specific methods and protocols

2.3.1 Study 1: Glycaemic response, insulin response and satiety of liquids and solids

2.3.1.1 Subjects

Ten participants (8 females and 2 males) fulfilling the acceptance criteria (section 2.2.1) were recruited for the study (Table 2.1). The collective mean for males and females are presented because the number of males was too small to justify gender-wise values.

Table 2.1: Baseline measurements of participants in study 1 (mean ± standard error)

Number (n)		10 (2 males, 8 females)
Age (years)		28±2.5
Height (m)		1.7±0.03
Weight (kg)		62.4±3.5
Body mass index (kg/m^2)		21.3±0.01
Body fat content (%)		18.0±4.7
Fasting blood glucose (mmol/L)		4.5±0.03
Fasting blood insulin (µU/mL)		7.2 ± 3.9
Blood pressure	Systolic (mmHg)	118 ± 2.1
	Diastolic (mmHg)	79 ± 2.3

2.3.1.2 Test foods

The four test foods in the study were white Basmati rice, commercial dry durum wheat spaghetti, pure orange juice (orange juice from concentrate; Tesco PLC, Cheshunt, UK) (OJ) and a sugar-sweetened fruit (blackcurrant) drink (Ribena, Rathfarnham, Dublin) (SSD) (Table 2.2). The types and cooking procedures for rice and spaghetti are described in sections 2.2.10.1 and 2.2.10.2 respectively. The SSD was of the ready-to-drink (RTD) type and consisted of water, blackcurrant concentrate (7%) and sucrose. All four treatments were served in portions containing 50 g of available carbohydrates.

The total volume of each meal was 576 mL (this was the total volume that resulted for the beverage conditions when 100 mL of accompanying water was provided (Table 2.2). The volume of the solid meals was also matched to this total.

Table 2.2: Test foods used in the study investigating effects of physical state on glycaemia

	Nutritional composition (g/100 g)				Portion size (g)	Volume of water provided with meal (mL)
	Total carbohydrates	Fibre	Protein	Fat		
Orange juice	10.5	0	0.5	0	476	100
Sugar-sweetened fruit drink	10.5	0	0	0	476	100
Rice (raw)	77.6	0.4	8.6	0.4	64.8	280
Spaghetti (raw)	73	2.6	12.5	1.4	71	290

2.3.1.3 Study protocol

A randomised crossover within-subject repeated measures non-blind design was adopted. All treatments were tested once by each participant. The subjects were provided a standard dinner for the evening before each test session. This consisted of a pizza (411g; Primafresco Margherita pizza; The Pizza Factory, Nottingham, UK) and an apple (170g; variety Braeburn; Tesco PLC, Cheshunt, UK). The composition (per 100 g) of the total meal was 335.9 kcal energy, 12 g protein, 50 g carbohydrates, 12 g fat and 6 g fibre. Subjects were instructed to eat only the provided food and avoid post-dinner snacks and beverages. Water was allowed *ad libitum*. The participants also completed a 24 hour food diary.

Blood samples in the fasting state were obtained for blood insulin and glucose analysis (using procedures described in sections 2.2.5 and 2.2.4 respectively). Participants also completed VAS at this time to provide data on baseline subjective feelings (section 2.2.7). The test food and water was subsequently consumed and finished within 15 minutes. Blood samples for insulin analysis were collected 30, 45, 60, 90 and 120 minutes following the start of the meal. Blood samples for glucose analysis and VAS data were obtained at 15, 30, 45, 60, 90 and 120 minutes following the start of the meal.

The participants were not allowed any food or drink during the post-prandial testing period (120 minutes) and spent the time as described in section 2.2.1.

2.3.1.4 Statistical analyses

In vivo assessment of the GR and glycaemic index was based on 6-10 subjects as recommended by Brouns *et al.* (2005) and the FAO/WHO (1998). With a sample size of 10 and an effect size of 0.40 the study had a power of 81%.

The IAUC data for the blood GR, IR and that from VAS were analysed using the repeated measures analysis of variance (ANOVA) procedure with the IAUC data for the treatments as the within-subjects variables. Pair-wise comparisons were carried out where a significant difference was observed using the Bonferroni test. Peak GR values were also compared with the repeated measures ANOVA procedure. Bivariate correlation analysis (Pearson's correlation co-efficient) was used to assess associations between the GR, IR and VAS data. Correlations were made between the GR, IR and each of the VAS questions for each test time point and for the total response (calculated as the IAUC).

2.3.2 Study 2: The effect of carbohydrate-based energy containing beverages on satiety and short term food intake

2.3.2.1 Subjects

Twenty five males and 25 females were recruited for the study. Of the total recruited 23 men and 24 women completed the study (Table 2.3). Participant age was limited to 18-30 years as the objective of the study was to observe effects in young adults. In addition to the questionnaires described in section 2.2.1.2 the participants completed a further questionnaire on breakfast, snacking habits and food preferences. A pre-tested questionnaire developed by

Oxford Brookes University was used for this purpose (Appendix 7). The inclusion criteria were,

- Habitually consume breakfast on five or more days of the week
- Habitually consume snacks between main meals on five or more days of the week
- Habitual week-day lunch consisted of at least 50% of the foods offered in the (study) buffet lunch.

Screening for these parameters was important as the study provided breakfast, a drink preload as a snack and a buffet lunch.

Table 2.3: Baseline characteristics of participants in study 2 (mean ± standard error)

		Males	Females
Number		23	24
Age (years)		24±0.5	23±0.5
Height (m)		2.0±0.1	1.6±0.2
Weight (kg)		76±2.5	58±2.3
Body Mass Index (kg/m^2) *		24±0.5	22±0.5
Waist circumference (cm)		81±1.5	72±1.6
Blood pressure (mmHg)	Systolic	128±2.5	113±2.9
	Diastolic	73±1.9	72±2.0

2.3.2.2 Treatment beverages

The three beverages tested were orange juice from concentrate (Tesco PLC, Cheshunt, Hertfordshire) (OJ), semi-skimmed milk (Tesco PLC, Cheshunt, Hertfordshire) (milk) and a sugar sweetened fruit drink (Ribena, Rathfarnham, Dublin) (SSD). These were compared against a calorie-free artificially sweetened (aspartame) drink (Ribena, Rathfarnham, Dublin) (Table 2.4)

(control). All the beverages were presented chilled and in opaque bottles. The OJ, milk and SSD were provided in portions containing 150 kcal of metabolisable energy whilst the volume of the control was the average of the other three beverages (Table 2.4). All participants consumed the entire portions within 10 minutes.

Table 2.4: Characteristics of treatment beverages in study 2

Beverage preload	Orange juice (OJ)	Semi-skimmed milk	Sugar-sweetened fruit drink(SSD)	Artificially-sweetened fruit drink (control)
Volume per portion (mL)	319	306	349	325
Energy per portion (kcal)	150	150	150	4
Energy density (kcal/g)	0.47	0.50	0.43	0.04
Total Carbohydrates (g/100 mL)	10.5	4.8	10.5	0.5
Sucrose (g)	5.2	0	10.5	0.06
Glucose (g)	2.6	0	0	0.19
Fructose (g)	2.6	0	0	0.25
Lactose (g)	0	4.8		0
Fibre (g)	0	0	0	0
Protein (g)	0.5	3.6	0	0
Fat (g)	0	1.8	0	0
Ingredients	Orange juice from concentrate	Cow's milk	Water, blackcurrant juice concentrate (7%), sucrose	Water, blackcurrant juice concentrate (7%), Aspartame, Acesulfame K.

2.3.2.3 Test meals

The participants were provided a standard breakfast and a buffet lunch. Breakfast consisted of white and wholemeal bread (fresh or toasted), corn flakes, bran flakes, low fat spread, marmalade, jam, milk, tea and coffee (please see appendix 6-1 for compositional information). On the first day of testing the participants were informed that they could select any combination and quantity of these foods for breakfast. This was then kept standard for each individual for all the sessions.

Lunch was provided as a self-selection buffet and included a variety of foods in ample quantity (Figure 2.5). Two distinct menus were developed each adjusted to contain similar total energy contents (2679±2.4 kcal- based on energy contribution from one portion of each food item in each buffet) and was presented to the subjects at alternating test sessions and once per each test beverage (Appendix 6-2). Food energy and macronutrient composition was calculated from manufacturer information and compositional analysis software (Dietplan, Forestfield software Ltd., West Sussex). Mean protein, carbohydrate and fat percentage per portion of each food item in both menus was 4.4%, 21.3% and 7.1% respectively (Appendix 6-3). Typical portion sizes were determined from the literature (MAFF, 1993). However, the final portion sizes in the buffet were modified so that all the items contained approximately similar energy contents per portion (Appendix 6-3). At least three portions of each food item were always made available at the buffet.

Figure 2.5: An example of the buffet lunch presentation style

2.3.2.4 Study protocol

A repeated measures non-blind randomised self control design was adopted with each participant returning for eight separate test sessions. In order to minimise climatic effects participants completed the sessions within four consecutive weeks. Each beverage was tested twice by the participants, and the order of presentation was counterbalanced across sessions. The test sessions were identical in all respects except for the drink served. Each test session included a standard breakfast, the preload and an *ad libitum* buffet lunch. The time interval between breakfast and preload was three hours and that between the preload and lunch, one hour.

The participants were blinded to the principal outcomes of the study and were instead told that it, 'examined the palatability of the drinks and their effect on satiety'. They were told that the only data obtained in the study were those from the VAS. However, the primary measurement was the amount of energy consumed at the buffet meal. The study protocol is schematically presented in Figure 2.6.

Test sessions were conducted in a climate controlled food lab (ambient temperature: 22±2°C). The standard breakfast (section 2.3.2.3) was served five minutes after the participants' arrival and was consumed within 15 minutes.

Subsequently, the subjects were given in writing the times they would have to come in for the drink and lunch. No food and drinks were allowed between breakfast and the test drink except for 250 mL of water. Subjective ratings (VAS) (section 2.2.9) for hunger, fullness and desire to eat were obtained before and after breakfast, before the drink, 20 and 40 minutes following the drink and before and after lunch. Subjects also rated the palatability of the preload immediately after its consumption.

The participants were allowed free access to the lunch buffet and were allowed to eat any amount of food in any combination in as many visits to the buffet as they wished. To minimise social influences participants were seated alone whilst eating. Each participant's food intake was covertly observed and recorded during lunch. When enquired at the post-experiment de-briefing all participants admitted to not being aware that food intake was being recorded. Plate waste was collected and weighed at the end of each test session after the participants had left the laboratory.

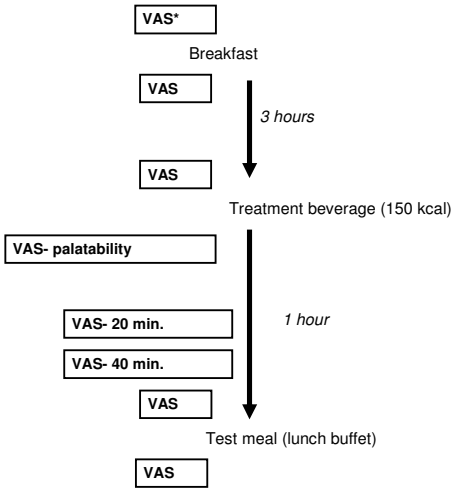


Figure 2.6: Schematic presentation of study design

2.3.2.5 Statistical analyses and data processing

Power analysis showed that the total sample size (47) and the gender-wise sub-samples (23 males and 24 females) was adequate to detect differences in compensation with a power of 92% and 83% respectively when the effect size was 0.23 (α=0.05).

The inclusion of the caloric contribution from breakfast in the statistical analyses for total energy intake (described below) did not significantly alter the outcomes. The mean (±SD) energy (kcal), carbohydrate (g), protein (g), fat (g) and fibre (g) intake at breakfast by males and females was 390±120, 71±22, 16±9, 11±2, 7±3 and 297±89, 56±21, 13±6, 8±3, 7±4 respectively. Therefore, caloric contributions from breakfast were omitted from the data analyses.

Energy compensation was defined as the change in the number of kilocalories consumed at lunch following a caloric preload, compared to energy intake after the control and calculated as the ratio between the difference in energy intake at lunch and energy content of the preload (Cecil *et al.*, 2005). Average energy intake and subjective scores (of the two repetitions per treatment) were calculated for each individual for each treatment. The mean data were used for statistical analyses.

Independent sample t-tests carried out for male and female energy intake data indicated a significant gender difference for all the treatments ($P<0.01$). Male and female energy intake data were therefore analysed separately. However, ANOVA analysis showed no significant differences in VAS data between males and females ($P>0.05$) and therefore these were collated and analysed.

Energy and nutrient intake data were analysed using the repeated measures ANOVA procedure with beverage as the within-subjects factor. Visual analogue scale data were analysed as described in section 2.2.9. One-way repeated measures ANOVA was used to analyse VAS IAUC data for treatment effects. The nutrient composition of meals was analysed separately for each macronutrient (carbohydrate, protein, fat and fibre) using one way repeated measures ANOVA. The data of both genders in this regard were pooled since a gender-wise paired t-test analysis showed no differences between the two genders for macronutrient intake. Where significant effects were observed pair-wise comparisons were made using Tukey's test.

2.3.3 Study 3: *In vitro* studies investigating the effect of food particle size, salivary α-amylase activity and habitual mastication on glycaemic potency

2.3.3.1 *In vitro* digestibility of rice of different particle sizes

White Basmati rice was cooked as described in section 2.2.10.1. The resulting rice was chopped using a hand-held food chopper (Zyliss, Zurich, Switzerland) and washed through the sieves described in section 2.2.9. Chopped rice belonging to three particle size groups were thus obtained (>2000 µm, 1000-2000 µm and <500 µm). The separated samples were washed thoroughly until all surface starch was removed. The rice fractions were gently blotted to remove excess surface moisture before weighing and digesting. In addition to these particle groups a further sample was obtained by homogenising the rice using the method described in section 2.2.3.2 (control). The final treatment

sample was whole rice. All 5 samples were then subjected to *in vitro* digestion in the method described in section 2.2.3.

2.3.3.2 *In vitro* digestibility of rice habitually masticated by individuals

2.3.3.2.1 Subjects

Fifteen participants (10 female and 5 male) fulfilling the acceptance criteria (section 2.2.1) were recruited for the study (Table 2.5). Only those having a full set of natural and unbroken dentition were included.

Table 2.5: Baseline characteristics of participants in study 3 (mean ± standard error)

		Males	Females
Number (n)		5	10
Age (years)		47.5 ± 14	30.3 ± 9
Height (m)		1.8 ± 0.1	1.6 ± 0.1
Weight (kg)		78.7 ± 15	60.1 ± 11
Body mass index (kg/m^2)		19 ± 5.3	24.1 ± 3.1
Fasting blood glucose (mmol/L)		4.6 ± 1.1	4.8 ± 1.2
Blood pressure	Systolic (mmHg)	117 ± 12	121 ± 18
	Diastolic (mmHg)	76 ± 8	79 ± 11

2.3.3.2.2 Treatment food

The test food used was Basmati rice. Its type and preparation are described in section 2.2.10.1.

2.3.3.2.3 Study protocol

Individuals provided masticated rice samples for particle size distribution analysis (section 2.2.9) and *in vitro* digestion (section 2.2.3) within one session.

The participants also provided two samples of saliva (120 minutes apart) for measuring α-amylase activity (section 2.2.11). Sample collection from all the participants was completed within 30 days. *In vitro* digestion of the samples was carried out as one lot in a single digestion block (section 2.2.3.2).

2.3.3.3 Statistical analyses

The *in vitro* method used in this study has a high degree of precision and reproducibility (CV of less than 5%) (Mishra & Monro, 2009). With a sample size of 15 and a medium effect size the design had a power of 82%.

The weights of masticated rice samples used for *in vitro* analysis were corrected for saliva dilution by 23%. This is based on a previous study which found a 23% mean increase in weight following normal mastication of boiled rice (Watanabe & Dawes, 1988).

In vitro digestion data are presented as glucose release (mg/ g of rice) and percentage carbohydrate digested values. The IAUC of the *in vitro* digestion curves were calculated using the trapezoidal method (section 2.2.4). Rapidly digestible starch (RDS) content in individuals' masticated samples was defined as the amount of carbohydrate digested in the first 20 minutes of *in vitro* digestion (Mishra & Monro, 2009). The remaining starch was defined as slowly digestible starch (SDS). Data were analysed using the one-way analysis of variance (ANOVA) procedure with IAUC or percentage carbohydrates digested and the subjects as dependent and factor variables respectively. Salivary α-amylase activity data were also analysed with the one-way ANOVA procedure with enzyme activity (in U/ml) and subjects as the dependant and factor

variables respectively. Correlations were made using linear regression with particle size as the independent factor and the IAUC or the percentage of digested starch the dependant factor. Pair-wise comparisons were made using the Tukey test where significant differences were observed.

2.3.4 Study 4: Habitual mastication and its impact on the *in vivo* glycaemic response

2.3.4.1 Subjects

Twelve participants (8 females and 4 males) fulfilling the acceptance criteria (section 2.2.1) were recruited for the study (Table 2.6). All those included had a full set of natural and unbroken dentition. Of the total recruited 8 females and 3 males completed the study.

Table 2.6: Baseline characteristics of participants in study 4 (mean ± standard error)

		Males	Females
Number (n)		3	8
Age (years)		35.3 ± 4.8	26.9± 2.1
Height (m)		1.7 ± 0.01	1.6 ± 0.03
Weight (kg)		66.6 ± 4.5	58.4 ± 3.6
BMI (kg/m^2)		22.7 ± 1.3	21.6 ± 0.7
Body fat content (%)		14.3 ± 4.6	28.7 ± 2.0
Fasting blood glucose (mmol/L)		4.4 ± 0.02	4.3 ± 0.04
Blood pressure	Systolic (mmHg)	116 ± 3.6	118 ± 1.6
	Diastolic (mmHg)	79 ± 2.9	78 ± 1.4

2.3.4.2 Test foods

Rice and spaghetti were used as the model foods. Details regarding type and preparation are outlined in sections 2.2.10.1 and 2.2.10.2 respectively.

2.3.4.3 Study protocol

A randomised within-subject repeated measures non-blind design was adopted. The participants came to the laboratory on 6 days and each food was tested on three random days. While the participants tested the *in vivo* GR (section 2.2.4) on all three days oral processing parameter data and samples for particle size analysis were collected on two random days for each food. Testing began in the mornings after subject preparation (section 2.2.1). Blood glucose measurements were obtained at baseline and at 15, 30, 45, 60, 90 and 120 minutes following the consumption of the test food (section 2.2.4). Data from VAS were also collected at the same times (section 2.2.7). In the test sessions where oral processing parameters were measured (as described in section 2.2.8) the EMG electrodes were attached to the participants' cheeks before they consumed the test food and after the baseline blood measurements were taken. Masticated food samples for determining the particle size distribution (section 2.2.9) were obtained at the end of the 120 minutes of GR testing.

2.3.4.4 Statistical analyses

Studies on *in vivo* assessment of glycemic response and glycemic index have been based on 6-10 subjects as recommended by Brouns *et al* (2005) and the FAO/WHO (1998). With an effect size of 0.42, a sample size of 11, and each treatment tested in triplicate, the experimental design had a power of 81%.

Only the percentages of particles larger than 2000 μm and smaller than 500 μm were used to determine correlations with the GR. These two groups represented the greater proportion of the masticated food. The intermediate

particle size groups accounted for less than (mean± SD) 15 (±3.7) and 5 (±2.2)% for rice and spaghetti respectively.

Correlations were made using the linear regression procedure with the percentage of particles (of different size groups), number of chews, number of mouthfuls, chewing time as the independent and the IAUC and GR as the dependant factors. The results were expressed as r (Pearson correlation coefficient), R^2 (regression coefficient) and *P* values. Between-individual variations in mastication rate were analysed with the one-way ANOVA procedure using mean values from each replicate for each individual. Within-individual variations in the mastication parameters and the GR for rice and spaghetti were analysed using the paired t-test procedure. Individual differences in the particle size distribution of masticated food were also analysed using the paired t-test procedure.

2.3.5 Study 5: Between-individual variations in post-mastication digestion aspects and effects of ingested food particle size on glycaemic response, insulin response and gastric emptying.

2.3.5.1 Subjects

Thirteen healthy males fulfilling the acceptance criteria (section 2.2.1) were recruited for the study. Of the 13 the full data sets of 12 were obtained (Table 2.7).

Table 2.7: Baseline characteristics of participants in study 5 (mean ± standard error)

Number (n)		12
Age (years)		27 ± 5
Height (m)		1.8 ± 0.02
Weight (kg)		75.4 ± 2.6
BMI (kg/m^2)		23.3 ± 0.6
Body fat content (%)		13.1 ± 3.2
Fasting blood glucose (mmol/L)		4.8 ± 0.1
Fasting blood insulin (µU/ml)		7.7 ± 1.1
Blood pressure	Systolic (mmHg)	116 ± 3.2
	Diastolic (mmHg)	78 ± 2.1

2.3.5.2 Treatments

The test food used was white Basmati rice (section 2.2.10.1) and the two treatments were large (>2000 µm) and small (500-1000 µm) rice particles. The large particle rice consisted of entire grains. The measurement of 100 randomly selected grains indicated a mean (±SD) length and thickness of 11868±607 µm and 1653±200 µm respectively. For preparing the small particle treatment the whole rice was ground in a burr mill (Cuisinart ® Model DBM8U, Hampshire, UK) and passed though a sieve with a mesh size of 1000 µm (Endecotts Ltd, London, UK). The fraction that passed through was sieved through another mesh (500 µm) (Endecotts Ltd, London, UK) and the retained particles were collected.

The treatments were prepared as a vegetable flavoured risotto. To ensure a standardised degree of cooking the cooking times for the two treatments were different. Since the particle size of the small treatment was smaller than that of the large treatment the cooking time of the former was shorter. The volume of cooking liquid was adjusted so that all of it was absorbed into the rice by the end of cooking and also resulted in similar volumes of cooked risotto.

Accordingly, the large particles were cooked in 200 mL of vegetable stock (Knorr liquid Vegetable stock, Unilever, Merseyside, UK) and 180 ml of water. The small particle treatment was cooked in 200 mL of vegetable stock (Knorr liquid Vegetable stock, Unilever, Merseyside, UK) and 80 mL of water.

The rice and cooking liquid were placed in a pan and 100 mg of sodium [^{13}C] acetate was added (to measure gastric emptying (section 2.2.8)). Adjusting the cooking liquid volume (so that none remained at the end of cooking) also ensured that the sodium [^{13}C] acetate was completely absorbed into the cooked rice. The cooking procedure for large particles was as described in section 2.2.10.1. The small particles were cooked in the same manner with the exception that the simmering time after bringing to the boil was 2 minutes.

2.3.5.3 Study protocol

A randomised crossover within-subject repeated measures non-blind design was adopted. Following the consumption of the treatment the GR, IR and gastric emptying were measured. Baseline blood samples for GR (section 2.2.4) and IR (section 2.2.5) and breath samples for gastric emptying analysis (section 2.2.6) were obtained and the treatment was consumed immediately afterwards. To ensure that the predetermined particle sizes in the treatments reached the stomach without further breakdown the participants were instructed to swallow the food without chewing. The participants were also provided with 200 mL of water which had to be consumed in entirety. After consuming the food further blood samples for GR measurement was taken at 15, 30, 45, 60, 90 and 120 minutes. Blood samples for IR measurement were obtained at 30, 45, 60, 90 and 120 minutes. Breath samples for gastric

emptying measurement were obtained every 15 minutes from the commencement of the meal up to 240 minutes.

2.3.5.4 Statistical analyses

Studies on *in vivo* assessment of glycemic response and glycemic index have been based on 6-10 subjects as recommended by Brouns *et al.* (2005) and the FAO/WHO (1998). A sample size of 12 and a medium effects size gave the design a power of 79%.

Differences in total GR, IR, gastric emptying (calculated as IAUC) between large and small particles were analysed with the one-way paired t-test procedure. Correlations between the GR and IR at each time point were made using Pearson's bivariate correlation coefficient.

Chapter 3: Glycaemic response, insulin response and satiety of liquids and solids

3.1 Introduction

Sugars provided in liquid form have been implicated in encouraging 'passive over-consumption' of energy (Gibson & Neate, 2007). It has been speculated that energy delivered in liquid form does not trigger satiety (DiMeglio & Mattes, 2000; St-Onge *et al.*, 2004) and that only solids are detected and activate satiety mechanisms (DiMeglio & Mattes, 2000; Almiron-Roig *et al.*, 2004; Mattes, 2006a; Mourao *et al.*, 2007). Several studies have investigated the comparative effects of liquid and solid carbohydrate based foods on satiety and short-term food intake and reported equivocal findings (chapter 1).

The glucostatic hypothesis (Mayer, 1953) states that food intake is induced when the blood glucose concentration declines, and this has been demonstrated using both animals and humans (Smith & Campfield, 1993; Campfield *et al.*, 1996; Melanson *et al.*, 1999c). Similarly, an inverse association has been observed between food intake and insulin concentrations (Bolton *et al.*, 1981; Wolever, 2006).

A limited number of studies have comparatively observed glycaemic and hormonal responses to liquids and solids. Studies comparing the whole, pureed and liquid forms of foods have shown that the physical state affects the glycaemic response (GR) (chapter 1). However, no previous studies have attempted to address the speculated differences in satiety between liquids and solids from a glucostatic theory perspective. Although some studies have

investigated the physical state of food and its effects on either the GR, insulin response (IR) or subjective feelings of hunger (chapter 1), none have combined all these factors into one comparative trial. The current study was initiated to fill this gap in the literature.

Using a balanced design the specific objective of the study was to determine the GR, IR and subjective feelings of hunger and satiety to two solid (rice and spaghetti) and two liquid (orange juice [OJ] and a sugar-sweetened fruit drink [SSB]) foods (Table 2.2). The OJ was included to represent a fruit juice and the SSB to typify beverages sweetened with extrinsic sucrose. Rice and spaghetti were selected because they are popular starchy staples. It was hypothesised that the GR and IR to liquids and solids will be significantly different, and that this will result in correspondingly distinct satiety responses.

3.2 Materials and methods

The materials and methods related to the study are described in section 2.3.1.

3.3 Results

3.3.1 The glycaemic response

The incremental areas under the curve (IAUC) for the four treatments were significantly different ($F[3,27]=4.485$, $P=0.032$). Subsequent post-hoc comparisons showed that the significant difference was only between OJ and rice (Table 3.1). The between-individual variations for the GR IAUC for the two solid treatments were considerably greater than that of the liquids (Table 3.1). The largest variation was observed in rice.

The GR to all four treatments peaked at 30 minutes (Figure 3.1). Although the statistical model comparing the peak glycaemic responses of the four treatments was significant ($F[3,27]= 5.860$, $P=0.003$) post-hoc comparisons showed that only the peak responses for SSB and spaghetti, and rice and spaghetti were significantly different (Table 3.1).

The shape of the mean GR curves for OJ and SSB were similar as were those for the rice and spaghetti (Figure 3.1). The GR following OJ and SSB reached baseline at 45 and 54 minutes respectively (Table 3.1). After 120 minutes the GR to the two liquids had fallen below baseline (Figure 3.1). The rice and spaghetti in contrast showed positive and sustained GR values at 120 minutes.

Table 3.1: Peak and IAUC for the blood glucose and Insulin responses (mean ± SE)

	IAUC of the GR (mmol.min/l)	Peak GR (mmol/l)	Time taken for GR to revert to baseline (min)	IAUC of the IR (µU.min/ml)	Peak IR (µU/ml)	Time taken for IR to revert to baseline (min)
Rice	96.0 ± 15.9*	1.8 ± 0.3$^{\#}$	> 120	1140.9 ± 224.4	19.6 ± 4.5	>120
Spaghetti	57.6 ± 12.1	1.1 ± 0.1$^{*\#}$	>120	948.2 ± 212.5	18.8 ± 4.5	>120
Orange juice (OJ)	45.0 ± 6.2*	1.7 ± 0.2	45	869.3 ± 114.5	31.5 ± 5.7	80
Sugar-sweetened fruit drink (SSB)	65.6 ± 7.1	2.1 ± 0.2*	54	850.8 ± 72.1	27.9 ± 3.8	74

SE=Standard error; IAUC= Incremental area under the curve; GR= Glycaemic response; IR= Insulin response; Values with the same superscript symbols within a column are significantly different (Repeated measures ANOVA, Post-hoc Bonferroni test, $P<0.05$).

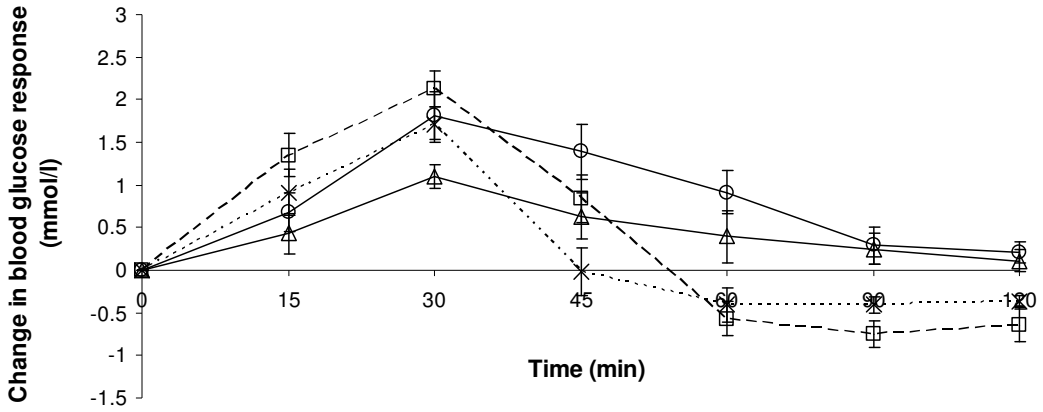


Figure 3.1: Mean temporal blood glucose response curves for rice (○), spaghetti (Δ), orange juice (X) and the sugar-sweetened fruit drink (□). Curves represent the GR for 120 minutes following the ingestion of the test food

3.3.2 The insulin response

The IR and GR data at each time point for all four treatments showed a significant association (P<0.001). The correlation co-efficients between the GR and IR for OJ, SSB, rice and spaghetti were 0.97, 0.97, 0.97 and 0.99 respectively.

The IAUC for the IR for OJ, SSB, rice and spaghetti were not significantly different (F[3,27]=0.756, *P*=0.529). The peak IR for all four treatments was observed at 30 minutes (Figure 3.2) and was not significantly different between the four treatments (F[3, 27]= 2.499, *P*=0.081). The IAUC for the IR showed considerably higher between-individual variations and were relatively larger than that observed for the GR (Table 3.1). Similar to the GR the variations in the solids were greater than that in the liquids.

The shape of the IR curves for OJ and SSB were similar, as were those for rice and spaghetti (Figure 3.2). Similar to the trends observed with the GR, the IR to OJ and SSB fell below baseline before 120 minutes (at 80 and 74 minutes respectively) (Figure 3.2 and Table 3.1). The IR to rice and spaghetti remained above baseline during the entire testing period.

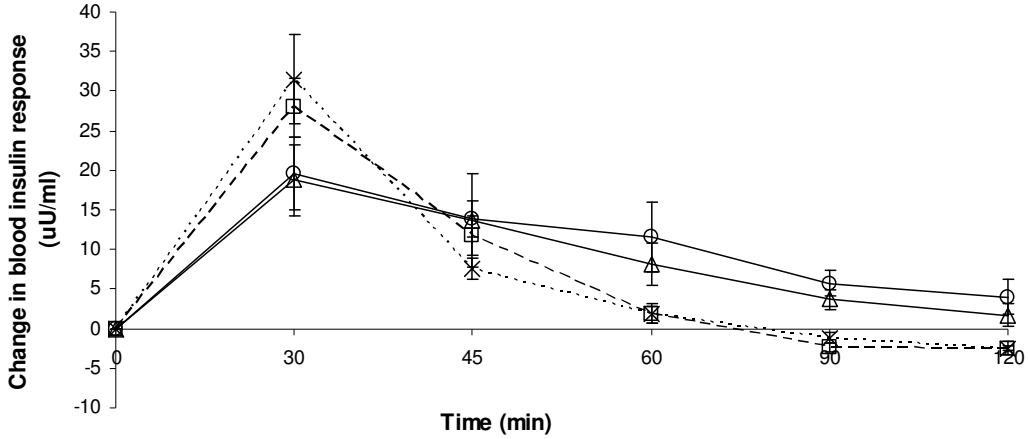


Figure 3.2: Temporal insulin response (IR) curves for basmati rice (○), spaghetti (Δ), orange juice (X) and the sugar-sweetened fruit drink (□). Curves represent the IR for 120 minutes following the ingestion of the test food.

3.3.3 Subjective feelings of hunger

All four test foods were most satiating at 15 minutes after consumption (Figure 3.3). Feelings of hunger and fullness decreased thereon and the desire to eat increased. The statistical models individually comparing the VAS IAUC for hunger, fullness, desire to eat and prospective food consumption were significant ($F[3,27]=3.529$, $P=0.028$; $F[3,27]= 6.679$, $P=0.002$, $F[3,27]=3.865$, $P=0.020$, $F[3,27]=3.409$, $P=0.032$ respectively) (Figure 3.3, Table 3.2).

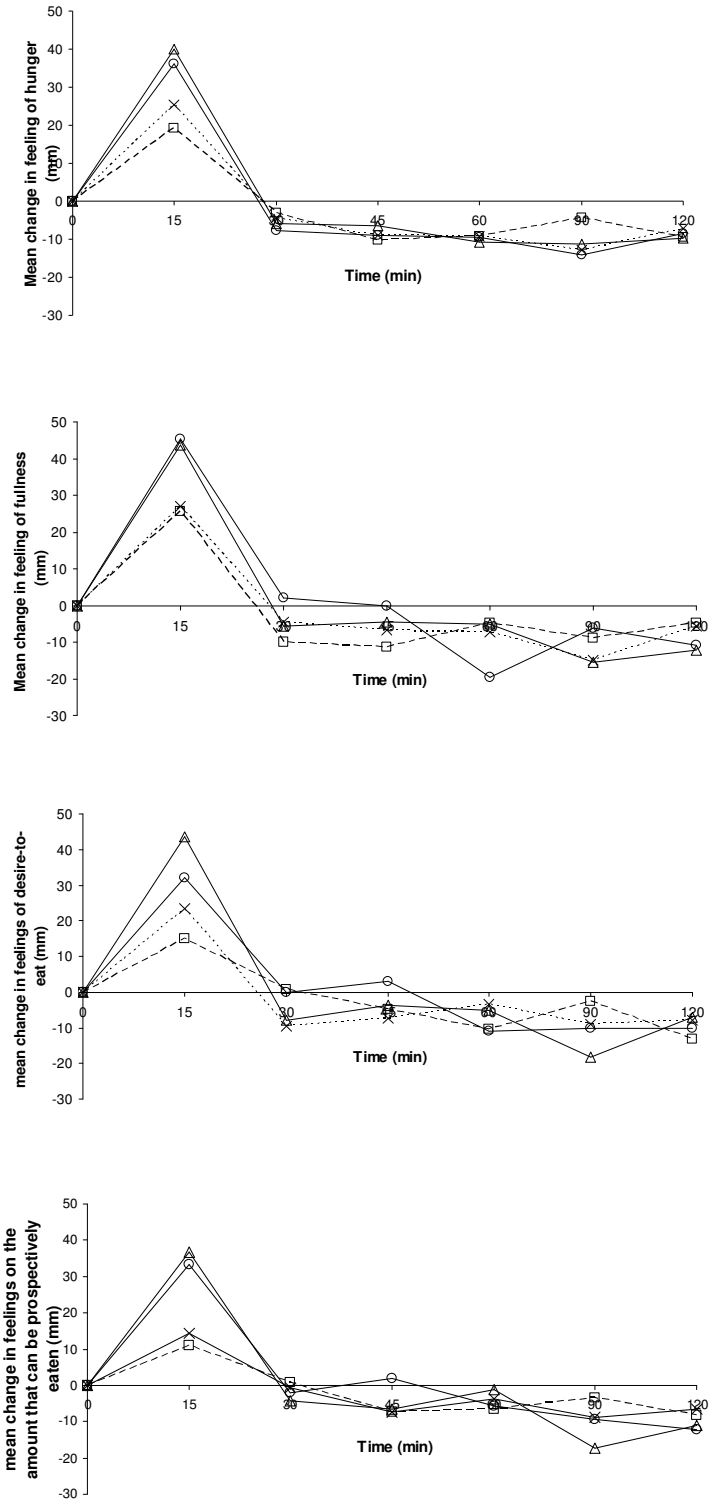


Figure 3.3: Temporal curves for the subjective feelings of hunger, fullness, desire to eat and prospective food consumption for basmati rice (○), spaghetti (Δ), orange juice (X) and the sugar-sweetened fruit drink (□).The curves illustrate the change in the subjective feeling during the 120 minutes following the consumption of the test foods.

However, post-hoc comparisons showed that only rice and SSD were significantly different in all four models. All four subjective feelings had fallen back to baseline by 30 minutes for all the treatments (Figure 3.3).

No significant correlations were observed between the GR/IR and the VAS data both for the IAUC and at any of the test time points.

Table 3.3 Incremental areas under the curve for subjective feelings (mean ± SE)

	Feeling of Hunger (mm.min)	Feeling of Fullness (mm.min)	Desire to eat (mm.min)	Prospective food consumption (mm.min)
Rice	662.2 ± 87.5*	979.9± 155.8*	688.8 ± 81.4*	728.6 ± 132.7*
Spaghetti	619.1 ± 108.0	720.6 ± 90.9	688.4 ± 103.2	599.3 ± 117.9
Orange juice (OJ)	408.8 ± 88.7	438.2 ± 101.3	466.3 ± 133.8	342.7 ± 108.3
Sugar-sweetened fruit drink (SSB)	348.8 ± 75.4*	404.8 ± 97.8*	315.4 ± 67.6*	292.8 ± 65.2*

IAUC= Incremental area under the curve; SE= Standard error; Values with asterisks within a column are significantly different to each other (Repeated measures ANOVA, Post-hoc Bonferroni test, $P<0.05$).

3.4 Discussion and conclusion

Using a balanced and controlled design this was the first study to investigate the comparative effects of iso-caloric volume-matched portions of liquid and solid foods on the GR, IR, and subjective feelings of hunger. The GR is influenced by food volume also when the available carbohydrate content is kept constant (Wolever, 2006). The total volume of food consumed was therefore

equalised for all 4 treatments by adjusting the quantity of water served with the food. This also ensured that stomach distention and resulting post-ingestive satiety signals were equalised for all four treatments (Blundell & Tremblay, 1995).

The results showed that the total IAUC for the GR significantly differed only between OJ and rice. In comparison the IAUC for blood insulin showed no significant differences between the treatments. The peak blood glucose responses for rice and spaghetti were significantly different, although there were no notable differences in the peak blood IR between the four foods. The largest between-individual variation in the IR and GR was observed for the solid treatments and rice in particular. The subjective feelings for hunger, fullness, desire to eat and prospective eating were significantly different between rice and SSB.

The IAUC for the GR observed in this study for rice and spaghetti (96 and 57 mmol.min/l respectively) are comparable to previous findings (94 and 60 mmol.min/l) (Bornet *et al.*, 1990; Ranawana *et al.*, 2009). The current study appears to be the first instance where GR (and IR) values for OJ and SSD were published and therefore no previous data were found for comparisons. However, one previous study observed a peak GR for OJ that was similar to that in the current study (Bolton *et al.*, 1981).

The between-individual variations were relatively greater for the solids than for the liquids and this pattern agrees with previous reports (Lee & Wolever, 1998). This suggests that a greater number of variables affect the GR of solids and

whole grains (like rice) in particular. Factors such as starch structure (Behall *et al.*, 1988; Vansteelandt & Delcour, 1999; Vandeputte & Delcour, 2004), degree of processing, particle size and fibre content (Jenkins & Jenkins, 1985; Vosloo, 2005; Wolever, 2006) have been shown to influence the GR of solids (Vosloo, 2005). However there may also be other contributors to this variation that are yet undetermined. Reasons for the greater variations in solids require further investigation.

The low GR properties of spaghetti have been attributed to its compact structure which makes the starch less accessible to digestive enzymes (Jenkins *et al.*, 1983). This was demonstrated in a study that compared the GR and IR to bread, spaghetti, and linguine of different thicknesses, all made from the same durum wheat based ingredients (Granfeldt *et al.*, 1991). The authors found that the bread elicited a significantly greater GR and IR compared to the pasta. Similarly, thin linguine produced greater responses than the thick form, which suggested that the size and density of food affected digestibility. Mourot *et al.* (1988) observed that gastric emptying of spaghetti was relatively slow and that its rate correlated significantly with the GR. The low GR properties of spaghetti therefore appear to be due to its compact structure and slow gastric emptying. The different GRs to OJ and SSB may be due to the varying amounts of glucose, fructose and sucrose they contained. These sugars have been shown to differentially affect the GR (Anderson, 1995; Anderson & Woodend, 2003a).

Independent of nutrient and chemical composition the shape of the blood glucose response curves for rice and spaghetti showed a strong concordance

as did those for OJ and SSB. This suggests that the physical state influences the GR pattern. For the two liquid preloads the peak response was followed by a rapid drop to below baseline. The solid foods in comparison showed a positive and sustained GR throughout. These patterns have been previously recorded, and are reported to influence satiety and food intake (Anderson & Woodend, 2003a; Bornet *et al.*, 2007). Transient and dynamic declines in the GR lead to hunger and food intake (Campfield & Smith, 2002) and the acute diminution observed for liquids suggest that they induce hunger at an earlier stage compared to solids. Based on the glucostatic theory (Mayer, 1953) these observations support the thesis that liquids are less satiating (DiMeglio & Mattes, 2000; Gibson & Neate, 2007). The greatest peak and dip was seen for SSB and this suggests that it would be the most satiating in the short-term (during the first 60 minutes) but also induce the greatest hunger afterwards (Bornet *et al.*, 2007).

For all four treatments the correlation coefficient (r) between the GR and IR curves was greater than 0.95 which indicated that the changes in the IR closely followed that of the GR. The IAUC for the GR and IR for the four treatments also showed a correlation co-efficient of 0.86 which implied that the relative total responses were similar. However, correlation co-efficients do not make indications regarding the magnitude of the responses and do not prove causal effects or the direction of causality. Although not significantly different the IAUC for the IR showed larger mean values for the solids. The peak IR values conversely showed higher mean values for liquids. It appears that solids produce marginally larger total insulin responses while liquids elicit greater insulin surges.

The magnitude and shape of the insulin response curves showed distinct differences between liquids and solids and comparable trends have been previously observed (Lee & Wolever, 1998; Tieken *et al.*, 2007). Since the total IAUC for the IR was similar for all the treatments these trends indicate that the liquids elicited a greater initial insulin response compared to solids. Insulin secretion occurs in a biphasic pattern (Nesher & Cerasi, 2002). The first phase lasts approximately between 4-10 minutes and is proportional to the initial glucose load. In the second phase insulin levels show more gradual increases. The initial entry of a large amount of glucose into the blood would therefore produce a greater first phase insulin secretion. The slower digestion rate of solids produces a subdued initial GR and corresponding IR that both sustain above baseline for longer. The trends observed in the current study are consistent with these patterns. Since elevated insulin levels have been associated with increased satiety (Holt & Miller, 1995; Flint *et al.*, 2000; Anderson, 2006), the observed trends suggest that solids will be satiating for longer.

The insulin data in this study were associated with considerably large between-individual variations. The variations were greater for the solids compared to the liquids and these trends have been previously observed (Doyle *et al.*, 1997). The larger variations in the IR for solids may be as a result of the greater GR variations. However, the variations observed in the IR in general were notably greater in magnitude than GR variations. This indicates that the physical state affects the IR in a relatively more varied manner. Peracchi and colleagues (2000) observed differing blood incretin concentrations when the same meal was consumed in solid and liquid forms. Varying secretion of these other

secretagogues may also influence the IR and contribute to the observed variations.

The speed of gastric emptying of solids is slower than that of liquids (Collins *et al.*, 1991) which suggests a decreased digestion rate of solids. The rapid gastric emptying of liquids causes a larger initial influx of food into the duodenum and a correspondingly large GR and IR. Indeed, Berry *et al.* (2003) showed that the early phase of gastric emptying significantly influenced total insulin release and glycaemia. Similarly, Mourot *et al.* (1988) observed that gastric emptying of spaghetti was slower than an equal portion of rice, and that the GR and IR of the two foods correlated significantly with their gastric emptying rate. Therefore, the differential response patterns observed in the current study for solids and liquids may have been mediated through their effects on gastric emptying. Gastric emptying also correlates negatively with incretin concentrations (Drucker & Nauck, 2006) and appears to therefore have indirect effects on insulin secretion. A faster gastric emptying rate also results in a brief gastric holding time and consequent post-ingestive satiety phase (Blundell & Tremblay, 1995).

Only rice and SSB produced significantly different subjective feelings of satiety in the current study. Therefore, chemical composition and digestibility appear to play greater roles in stimulating subjective satiety than the physical state. Some previous studies observed no differences in subjective feelings for liquid and solid preloads (Tournier & Louis-Sylvestre, 1991; DiMeglio & Mattes, 2000) while others found liquids to be less satiating than solids (Haber *et al.*, 1977;

Bolton *et al.*, 1981). Although not significant, the trends in the current study also suggested that liquids may be less satiating than solids.

In the current study satiety for all the treatments peaked immediately after consumption (at 15 minutes) and declined thereon. Despite modest dissimilarities the differential subjective feelings lasted only 30 minutes for all treatments. This suggests that both liquids and solids were subjectively perceived in an analogous manner by individuals and that the satiating duration of both liquids and solids was similar. This trend is comparable with previous observations made for a variety of solids and beverages (Merrill *et al.*, 2002; Almiron-Roig *et al.*, 2009; Hlebowicz *et al.*, 2009). Since the GR and IR to the four test foods peaked at 30 minutes, data from the current study suggest that post-ingestive satiety mechanisms influence subjective feelings to a greater degree than the GR and IR. The absence of significant associations between the GR/IR and VAS data further strengthen this hypothesis. The satiating effects of the GR and IR (Bolton *et al.*, 1981; Smith & Campfield, 1993; Campfield *et al.*, 1996; Melanson *et al.*, 1999b; Wolever, 2006) may therefore have a greater influence on the post-absorptive satiety phase (Blundell & Tremblay, 1995). Appetite and food intake could have been more accurately measured if an *ad libitum* test meal was offered at the end of the 120 minutes of testing, the omission of which is a limitation of the current study in hindsight.

The VAS data in the current study showed wide between-individual variations which indicated that subjects perceived subjective feelings in considerably different ways. However it is uncertain if these variations were due to between-individual differences in subjective feelings or individuals' inability to accurately

record their sensations on VAS. Visual analogue scales are currently the most accepted measurement method for subjective hunger and appetite sensations (Flint *et al.*, 2000; Stubbs *et al.*, 2000). However, their reproducibility and sensitivity depend on methodological and biological factors (Flint *et al.*, 2000) (chapter 1).The large variations also suggest that the sample size used in the current study may have been inadequate to discern differences in subjective feelings of satiety. Flint *et al.* (2000) reported that a minimum sample size of 18 was required to detect a appetite sensation difference of 10 mm in VAS.

In conclusion, the results do not agree with the study hypothesis that the physical state of food affects its total GR, IR and perceived satiety. However, differential response patterns in the GR and IR to liquids and solids can be discerned, notably with regards to the slope and amplitude of the curves. The liquids elicit a greater early phase GR and IR. The physical state does not affect the total IAUC. Differences in the pattern of the response curve may have an impact on the satiogenic properties to liquids and solids, and practical significance in food intake and weight control. Based on the glucostatic theory the results suggest that liquids induce hunger at an earlier stage compared to energy and volume matched solids. It is well established that humans detect and compensate for solid caloric preloads (DiMeglio & Mattes, 2000; Tieken *et al.*, 2007; Stull *et al.*, 2008). Further studies are however required to ascertain the compensatory effects to carbohydrate-based liquids in the short-term.

The main conclusions of the study can be summarised as follows:

- The physical state of food does not affect the total glycaemic response or the insulin response.
- Liquids produce a greater early phase blood glucose and insulin response
- The between-individual variations in the GR and IR are greater for solid compared to liquid foods.
- There are notable differences in the blood glucose and insulin response patterns between liquids and solids. Based on the glucostatic theory these differences may indicate that liquids elicit hunger at an earlier stage compared to solids.

Chapter 4: The effect of carbohydrate-based energy containing beverages on satiety and short-term food intake

4.1 Introduction

Results from the previous study examining the effects of food physical state on physiological parameters (chapter 3) showed that the glycaemic response (GR), insulin response (IR) and subjective feelings of hunger were not different between liquids and solids. A distinct difference in the blood glucose and insulin response patterns was however observed between liquids and solids, independent of food composition. Based on the glucostatic theory the results suggested that beverages induce satiety for a shorter period of time following their consumption compared to energy and volume matched solids. These and previous observations have also led to the speculation that energy in a liquid media is relatively less satiating and therefore poorly compensated for in the short term. Studies have conclusively shown that humans compensate for a solid caloric load in the short term (DiMeglio & Mattes, 2000; Mourao *et al.*, 2007; Tieken *et al.*, 2007; Stull *et al.*, 2008). However, it is still uncertain if carbohydrate-based energy containing liquids induce similar calorie compensatory effects.

Outcomes from longitudinal and cross-sectional studies suggested that caloric liquids encourage a 'passive over-consumption' of energy (Gibson & Neate, 2007), and that energy regulatory systems in the body did not detect energy provided in a liquid media. Results from short-term randomised controlled studies (RCS) conversely indicated that liquid calories are indeed compensated

for in the short term, and therefore do not lead to a 'passive over-consumption' of energy chapter 1). However, a limited number of RCS have focused on this area and they too vary considerably in terms of methodology. This emphasises the need for data from more experiments before robust conclusions can be made.

Outcomes of preload studies are greatly influenced by design aspects. These include sample size, subject, preload and test meal characteristics. Some limitations in previously published work are notable. These include the use of small sample sizes (<20), restricted test-meal variety and inadequate gender-based distinctions. These aspects were given particular consideration in the current study.

The variety of foods offered at the post-preload meal was limited in previous work. While food intake increases with palatability and variety (Sorensen *et al.*, 2003) repetitive presentation of a small selection produces a monotony effect (Siegel & Pilgrim, 1958) that could considerably affect outcomes in preload trials. Although the presentation of a large variety of food may cause overeating (Rolls, 1986) it is possible that physiological compensation still occurs. This aspect has not been previously investigated and is addressed in the current study.

This experiment also limited its participants to an age between 18-30 years in an attempt to specifically observe compensatory effects within a young adult group. Previous work with this age group showed a greater proclivity towards overeating when presented with *ad libitum* food (Levitsky & Youn, 2004; Rolls

et al., 2004). A previous study also reported possible gender variations in energy intake (Davy *et al.*, 2007). The authors observed a precise compensation for a 418 kcal yogurt preload at a buffet meal 30 minutes later by males but not females. No studies have attempted to confirm these findings, nor has any work been initiated in this respect using liquid caloric preloads.

The objective of the current study was to observe short term calorie compensation effects to carbohydrate-based energy containing beverages following their consumption. This was determined within a design that included a larger variety of treatment drinks, greater food selection at the test meal and larger sample size compared to previous studies. The study also focused more on gender-based distinctions. The treatments were also tested in replicate by all participants to increase precision, a design aspect not adopted in previous preload experiments. The study hypothesised that young adults would compensate for the liquid caloric preloads (containing 150 kcal of energy) at the *ad libitum* buffet lunch provided 60 minutes later.

4.2 Materials and methods

The effects of three caloric beverages (orange juice [OJ], semi-skimmed milk (milk), and a sugar-sweetened fruit drink [SSB]) and a calorie-free aspartame-sweetened fruit drink (control) on subjective feelings of hunger and subsequent food intake (60 minutes later) were determined. The selection of this time interval was based on results of previous studies which also observed significant compensations in energy intake at the meal eaten 60 minutes after a preload (containing 30-300kcal) (Birch *et al.*, 1989; Rogers *et al.*, 1995; Anderson, 2002). The treatment drinks were served in portions containing 150

kcal of energy. The basis of a 150 kcal energy load was that most soft drinks are marketed in portions approximating this calorie content (Holland *et al.*, 1991). The aspartame-sweetened drink was selected as the control because it was similar in appearance and sensory properties to caloric beverages but with no energy. Previous reports have shown that aspartame and other artificial sweeteners do not influence short term food intake and hunger ratings (Rodin, 1990; Black *et al.*, 1991; Canty & Chan, 1991; Drewnowski *et al.*, 1994; Holt *et al.*, 2000). All four beverages were commercially available drinks of the ready-to-drink (RTD) type. Participant, design and experiment details are described in detail in section 2.3.2.

4.3 Results

The four test beverages were liked equally by the participants as measured by 100mm visual analogue scales (VAS). Mean (±SE) preference for the drinks were, OJ (67± 4), milk (57±5), SSB (60±4) and control (55±5) ($F[3,129]=2.38$, $P=0.073$). There were no significant differences in preference ratings between the two genders ($P>0.05$).

4.3.1 Energy intake

Energy intakes at lunch by males and females were significantly different for all four drink conditions as analysed by independent-sample t-tests ($P<0.01$) (Figure 4.1). Male participants consistently ate more food at lunch at all the test sessions (Table 4.1). Mean (±SE) energy intake at lunch (for collated data from all four drink conditions) of males was 1199±11 kcal, while for females it was 843±60 kcal.

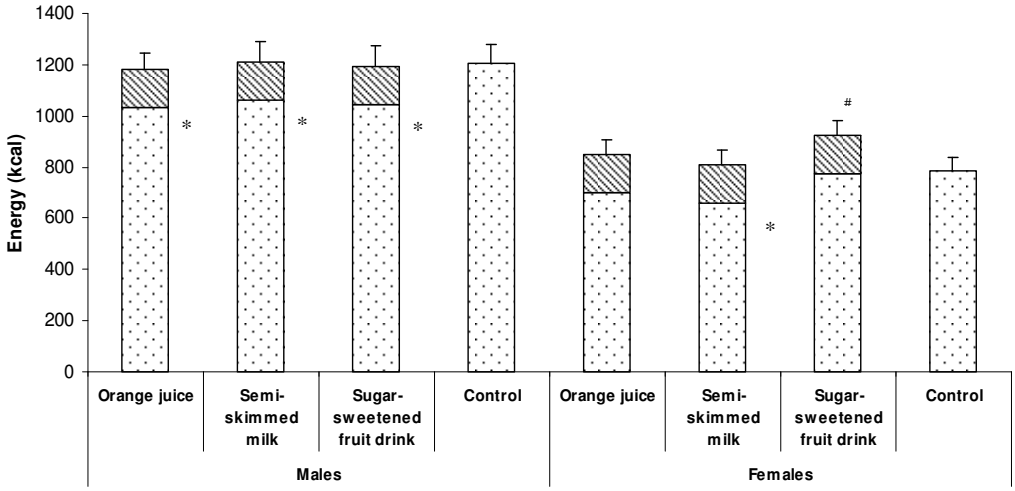


Figure 4.1: Gender-wise (males n=23; females n=24) comparison of lunch (spots) and total (lunch [spots] + preload [stripes]) energy intakes for the four beverages. Columns (spotted area of the column) with an asterisk represent significantly different energy intakes at lunch compared to the control within each gender group. # (sugar-sweetened drink) represents a significantly greater total energy intake compared to the control by females. Error bars are standard errors. $P<0.05$

Males consumed significantly less at lunch following the energy containing preloads compared to the control ($F(3,22)=3.30$; $P=0.03$) (Table 4.1). Post-hoc Tukey analysis indicated that food intake subsequent to all three treatments (OJ, milk, SSB) was significantly lower than that following the control. Females also demonstrated a significantly different energy intake following the caloric beverages compared to the control ($F(3,69)=3.50$; $P=0.02$) (Table 4.1). Post-hoc analysis however indicated that they consumed significantly less only following milk, relative to the control (Figure 4.1). As a percentage, males demonstrated compensations of 116%, 99% and 108% for the OJ, milk and SSB, whilst females showed 57%, 85% and 7% respectively.

Table 4.1: Energy intakes by males and females at lunch following preload and mean total energy intakes (mean ± standard error)

Beverage preload	Energy content in preload (kcal/ portion)	Males		Females	
		Mean energy intake at lunch (kcal)	Mean total energy intake (kcal) #	Mean energy intake at lunch (kcal)	Mean total energy intake (kcal) *
Control	4	1207 ± 70	1207 ± 70	786 ± 52	786 ± 52
Orange juice (OJ)	150	1033 ± 62 *	1184 ± 62	701 ± 56	851 ± 56
Semi-skimmed milk	150	1059 ± 82 *	1209 ± 82	658 ± 57 *	808 ± 57
Sugar-sweetened fruit drink (SSB)	150	1045 ± 77 *	1195 ± 77	776 ± 54	926 ± 54 *

Values with asterisks (*) are significantly different from the control within a column (repeated measures ANOVA, post-hoc Tukey test, $P<0.05$); # corresponds to energy intake from lunch and preload; Control= Artificially-sweetened fruit drink

Mean total energy intakes (energy in preload+ energy intake at lunch) by males following all four beverages were statistically similar [$F(3,66)=0.069$, $P>0.05$] (Table 4.1). However, mean total energy intakes for females were significantly different for the beverages [$F(3,69)=3.53$, $P<0.02$]. Females demonstrated a trend of greater total energy intake following all three caloric beverage conditions compared to the control. However, post-hoc Tukey comparisons showed that a significantly greater total energy intake compared to the control in this group was seen only for SSB.

The nutrient composition of lunch following the four beverages were not significantly different between males and females and the four drink conditions (P>0.05). Participants intra-individually consumed a lunch of similar composition at all 8 test sessions. Mean (±SE) percentage energy contribution from protein, carbohydrate and fat at lunch in males was 13±1.2%, 44.4±3.3% and 42.6±1.9%, and in females, it was 12.7±0.9%, 44.2±4.0% and 43.1±1.3% respectively.

4.3.2 Subjective feelings of hunger

The relative VAS scores for feelings of hunger, fullness and desire to eat correlated significantly (P<0.05) with each other at each point of measurement. Therefore the response pattern for all three attributes was similar. The mean VAS scores at each measured point for hunger, fullness and desire to eat following the four drinks was not significantly different between males and females. When adjusted to the pre-breakfast rating all four treatment conditions demonstrated similar hunger patterns throughout the study sessions (Figure 4.2). Feelings of hunger decreased until 20 minutes after the preload and then gradually increased until the initiation of lunch. The feelings of fullness demonstrated an inverse pattern.

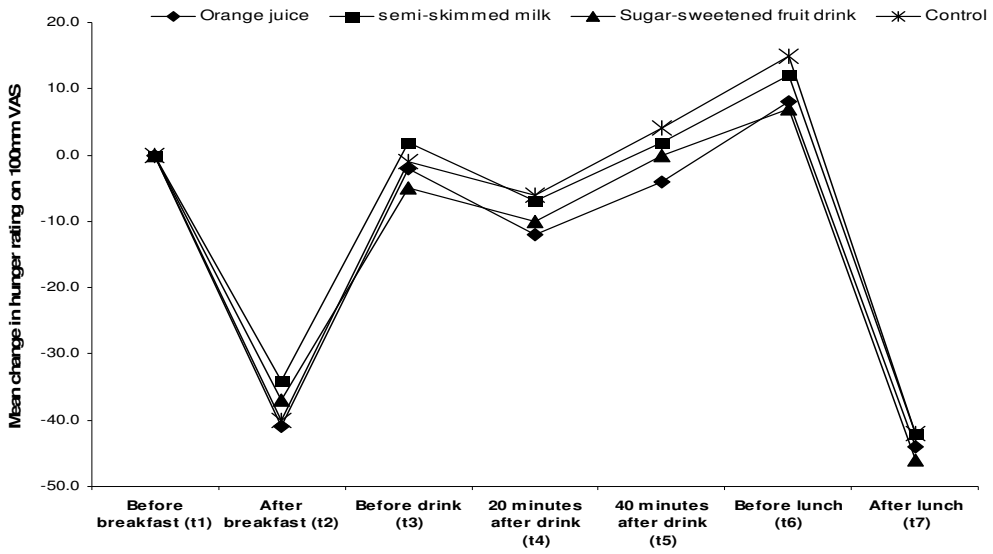


Figure 4.2: Change in (mean) hunger sensations over time for each beverage.

VAS= Visual analogue scales; Values adjusted for t1 (subjective feeling before breakfast). Intensity of hunger increases toward the positive end of the scale. Time points are numbered (t1 to t7) Control= Artificially-sweetened fruit drink.

When the motivational ratings were however adjusted to the pre-drink level (t3) (Figure 4.2) and the IAUC calculated for the time period, t3-t6 (i.e. from before consuming the test beverage until the start of lunch), a significant difference in satiety ratings for the four drinks was observed ($F[2,77]=99$, $P=<0.001$) (Figure 4.3). Post hoc Tukey analysis indicated that OJ and milk were significantly more satiating than SSB and the control. The OJ and milk elicited the highest suppression of hunger and were not significantly different to each other. The SSD induced a significantly lower satiety compared to the former two. The least satiating drink was the control and it was significantly so compared to OJ and milk (Figure 4.3). Food intake at the test lunch did not reflect these trends in subjective feelings of hunger as determined by VAS.

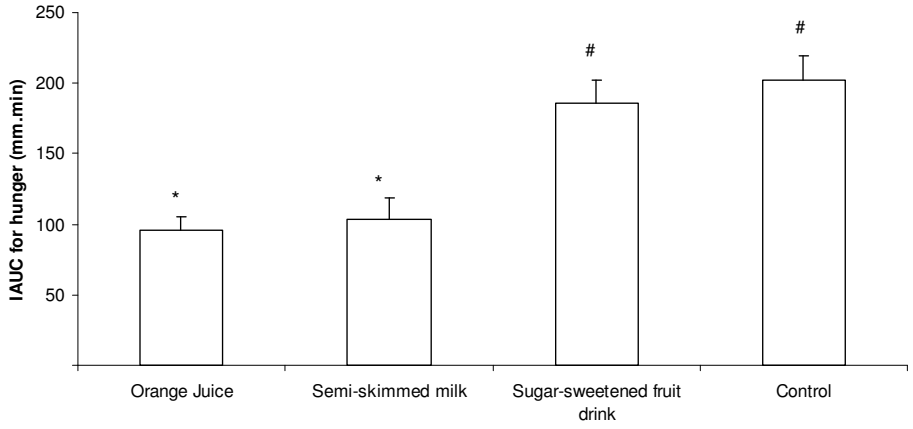


Figure 4.3: Incremental area under the curve (IAUC) for subjective feelings of hunger from t3 to t6 (figure 4.2) i.e. before the drink until the initiation of lunch. Values adjusted for t3 (hunger level before consuming the test beverage). Columns with different symbols are significantly different (*P*=0.05); Control= Artificially-sweetened fruit drink.

4.4 Discussion and conclusion

The two buffets were presented to the subjects on an alternating basis and each individual was allowed a maximum of two tests per week. These design aspects ensured that any monotony effects associated with having the same foods at all the sessions was minimised.

The findings of this study showed an energy compensation for liquid calories by young adults, also in the presence of ample quantity and variety at the test meal. Compensation was observed in both males and females, although it was more exact in the former group. Compared to the control, males demonstrated a relative compensation of 100% for the OJ, milk and SSB, while females displayed 57%, 85% and 7% respectively. Relative compensation is defined as the percentage difference in test meal intake relative to the energy content in the preload (Gray *et al.*, 2002; Cecil *et al.*, 2005). These levels of compensation

resulted in the males consuming similar amounts of total energy in all four test drink conditions. The weaker compensation by females however led to a larger total energy intake following the treatment drinks compared to the control in general, and a significant increment when SSB was consumed. Therefore, ingesting a beverage containing 150 kcal did not affect total energy intake in males. However, in females the SSB significantly increased total energy intake compared to the control. The nutrient composition of lunch was similar in all the treatment conditions for both genders which suggest that the drink characteristics did not influence subsequent meal constitution.

A significant gender difference in energy intake was observed in the study. Males consistently ate more at all the test sessions than females. The caloric contribution of the beverage to total energy intake was therefore higher in females. Whilst one drink contributed approximately 13% to the total energy intake of males, it accounted for 18% in females. The results of the current study are in agreement with those of Davy *et al.* (2007) who also observed a precise compensation for a caloric preload by males but not females. Similar to the findings of the current study they further reported no significant gender differences in subjective feelings of hunger and satiety (determined by VAS). These outcomes were observed by Davy and colleagues in a protocol that comprised of a larger energy preload (360-475 kcal), a time lag of 30 minutes between preload and lunch, a sample size of 12 per gender and a relatively small variety of foods at the test meal. The current study in comparison observed similar effects with a smaller energy preload (150 kcal), longer time lag (60 minutes), larger sample size (n>20 per gender) and a larger selection of foods at the subsequent test meal. A more precise mean (±SD) compensation

was observed in males in the current work (107±10%) compared to that observed by Davy *et al.* (2007) (86±5%), and a weaker and more varied compensation by females (50±39% vs. 74±5%). Whereas one semi-solid treatment was evaluated by Davy *et al.* (2007) the current study provided data on three liquid treatments and their differential effects. The similar results obtained in the two studies therefore indicate gender differences in short-term energy compensation independent of the physical state of food.

The study offers further evidence using a preload paradigm differing in protocol to those previously reported, of a possible energy compensation dysregulation in females compared to males. Food intake has been shown to be influenced by the menstrual cycle in females (Lissner *et al*., 1988; Buffenstein *et al*., 1995; Bryant *et al*., 2006). As the design did not correct for this its effects may have arguably interfered with the food intake of females. Davy *et al.* (2007)however showed energy compensation dysregulation in females also when the study was adjusted for the menstrual cycle, suggesting this phenomenon was independent of hormonal influences. The literature suggests some possible reasons for sex differences in energy regulation independent of the menstrual cycle. Del Parigi *et al.* (2002) used positron emission tomography and showed that different areas of the brain in men and women are activated in response to hunger and satiety signals. This suggests cognitive differences in the way males and females process hunger and satiety cues which may consequently result in different eating responses. Concentrations of satiety related hormones such as leptin and ghrelin also differ between genders. Females have shown higher concentrations for both hormones compared to BMI matched males (Havel, 2001; Greenman *et al*., 2004). Woods and colleagues (2003)

suggested that females were more receptive to catabolic processes resulting from low leptin, and males more sensitive to low insulin levels. These differences in hormonal sensitivity may also differentially influence food intake by the two genders. Hagobian *et al.* (2009) reported significant male and (menstrual cycle controlled) female differences in appetite, insulin and ghrelin responses to exercise, suggesting that physical activity affected male and female appetite responses in different manners. Animal studies have also shown sex differences in physiological mechanisms associated with appetite (Gayle *et al.*, 2006; Diaz *et al.*, 2009).

Davy *et al.* (2007) suggested gender differences in the glycaemic response (GR) as a possible reason for differences in compensation. However, previous research has shown that there are no gender differences in the GR to carbohydrate loads (Wolever, 2006) and it is therefore unlikely that the GR is responsible for the observed discrepancies. However, it may be possible that females are physiologically adapted to overeat, and therefore have relatively suppressed energy intake regulatory mechanisms. Excess energy reserves in the body would be advantageous during pregnancy and lactation (Harris & Ellison, 1997) and females inherently have a greater amount of fat storage cells compared to males (Sjostrom *et al.*, 1972). This may be suggestive of their physiological proclivity towards a positive energy balance from an evolutionary perspective. Females due to cultural and social demands have also been reported to show more cognitive dietary restraint than males (Rolls *et al.*, 1991; De Castro, 1995). Therefore, when used in preload studies they may not always consume food in quantities reflective of their physiological cues.

Previous reports investigating the effect of caloric beverages on subsequent food intake observed good compensation when the time between preload and test meal was sixty minutes or less (Birch *et al.*, 1989; Anderson, 1995), but not at greater time intervals (DiMeglio & Mattes, 2000; Almiron-Roig & Drewnowski, 2003; DellaValle *et al.*, 2005). In another study (Canty & Chan, 1991) caloric drinks caused a relative reduction in subjective hunger in adults up to 45 minutes after the preload. There were no significant differences in food intake between treatments and the water control at the test meal 60 minutes later. However, the energy content in their sugar preload was approximately 80kcal and may have been insufficient to elicit a physiological response 60 minutes later. Rolls and colleagues (1990) observed no compensation by male participants at a meal 60 minutes following the consumption of a sucrose drink containing 166 kcal of energy. Their study used a relatively smaller sample size (n=14) compared to the current experiment (>20 per gender). Based both on previous reports and findings of the current study it seems apparent that liquid calories are indeed detected and compensated for by the body in the short term. However, the energy content of the preload and the time between preload and the subsequent meal appear to be two key factors influencing the observation of a compensatory effect. Depending on the energy content in the beverage there appears to be a specific time frame within which compensation occurs.

Characteristics of the *ad libitum* test meal need careful consideration when designing a preload study. There is good evidence to indicate a positive correlation between energy intake and food variety (Sorensen *et al.*, 2003; Levitsky & Youn, 2004; Rolls *et al.*, 2004; Norton *et al.*, 2006). A limited

selection of food will conversely produce a monotony effect which could influence measurements in short-term repeated measures preload studies (Siegel & Pilgrim, 1958). The portion size also influences how much is eaten (Levitsky, 2008). Previous studies provided a relatively limited variety of foods at the test meal and consisted of either a single dish or a selection of sandwiches, sweets and fruit. Investigating compensatory effects when individuals are also exposed to a large food selection is crucial to determine how energy regulatory systems function under conditions conducive for overeating. This study provides evidence that compensatory mechanisms function in normal-weight young adults also when food variety and quantity are not limiting factors.

The pattern of change in hunger ratings observed in this study was similar to that observed in the earlier study (chapter 3) and in a previous study by Almiron-Roig & Drewnowski (2003). Maximal satiety was observed immediately following the consumption of the preload and this may have been due to post-ingestive satiety resulting from gastric distension (Blundell & Tremblay, 1995). The IAUC for hunger corrected to the pre-drink level showed a notable post absorptive satiety effect for the caloric beverages. Compared to milk and OJ the control was significantly less satiating. The control elicited the greatest level of hunger of all four drinks and the SSD produced an insignificant but lesser degree of hunger. The data thus suggested that energy content differences between caloric and non-caloric drinks were subjectively perceived and reflected in VAS. However, this appears to marginally occur when the drinks are sensorially analogous. Sensory attributes may be notably influencing perceived satiety to a food and governed by past experiences. Previous

research has indeed shown that the sensory aspects affect individuals' perceptions regarding a food (Spence *et al.*, 2010). The subjective outcomes from VAS did not correspond with energy intake at the subsequent test meal. This further demonstrates that VAS data are not always reflective of actual food intake (Stubbs *et al.*, 2000) and highlights the importance of including more than one satiety measurement type in food intake studies.

In conclusion, the overall results agree with the study hypothesis that calories in a liquid media are physiologically detected and compensated for. These effects were observed in a design where the drinks contained 150 kcal of energy, the gap between the treatment and test meal was 60 minutes and the variety and quantity of food at the test meal was maximised. The degree of compensation differs between the two genders. Males compensate accurately for calories provided in a liquid media whilst females compensate more poorly in comparison. Therefore, consumption of sugar-sweetened drinks may lead to excess energy intake in females. The study shows for the first time, dissimilarities in compensation levels to liquid calories by the two genders, in a pattern similar to that previously observed with semi-solids (Davy et al., 2007).

The main conclusions of the study can be summarised as follows:

- An energy load of 150 kcal provided in a liquid media is detected and compensated for by young adults at an *ad libitum* buffet meal 60 minutes later.
- Compensation mechanisms appear to be functioning even when food variety and quantity are maximised at the test meal.
- There is a notable gender difference in compensation capabilities. Males are able to detect and accurately compensate for liquid calories at the following meal. Females in comparison compensate poorly. Sugar-sweetened drinks may cause an excess energy intake in females.

Chapter 5: *In vitro* studies investigating the effect of food particle size, salivary α-amylase activity and habitual mastication on glycaemic potency

5.1 Introduction

Results of the study investigating the effects of food physical state on physiological responses and satiety (chapter 3) showed that the between-individual variation in the glycaemic response (GR) was considerably greater for solid foods than for liquids. This suggests that a greater number of factors influence the GR of solid foods. An aspect associated exclusively to solid foods is the oral mastication stage. The major objective of mastication is to break down food to a degree that a cohesive bolus which is safe to swallow can be formed. During mastication the food is fragmented into small particles and mixed with saliva.

It is well established that food particle size significantly impacts both *in vitro* and *in vivo* digestion rates (Snow & O'Dea, 1981; Heaton *et al.*, 1988; Bjorck *et al.*, 1994). As particle size decreases the surface area exposed to digestive enzymes increase, leading to an increased rate of digestion.

Previous studies have shown that the degree of habitual mastication differed considerably between individuals (Woda *et al.*, 2006a), and that this subsequently resulted in food being broken down to different extents in terms of particle size (Jiffry, 1981). Since particle size reduction during normal mastication differs between individuals it is possible that the digestibility of the

resultant boluses also differs and influences the GR. These differences may also be contributing to the observed between-individual variations seen in the GR to a solid carbohydrate food. Salivary α-amylase has been shown to initiate starch breakdown in the mouth (Granger *et al.*, 2007). However, its importance in overall starch digestion and influence on the GR is yet uncertain due to limited research. It is possible that salivary α-amylase activity (SAA) differs between individuals and impacts on starch digestion and contributes to differences in the GR. However, this hypothesis remains to be confirmed. The degree of physical breakdown during mastication and the extent of starch digestion as a result of exposure to α-amylase may both affect the glycaemic potency of the swallowed food.

Previous studies have suggested possible associations between mastication and GR (Read *et al.*, 1986; Suzuki *et al.*, 2005). However, these studies were conducted under controlled mastication conditions and did not investigate the effects of habitual chewing on the GR. Investigating this aspect forms the basis of this study.

The current study was conducted as a preliminary experiment to discern the effect of habitual mastication on the GR. Using a pre-validated *in vitro* model the study observed the impact of habitual mastication on the glycaemic potency of swallowed food boluses. As secondary objectives the study also investigated the effects of food particle size on *in vitro* starch digestion rate and SAA levels in different individuals. Therefore, the study had three hypotheses: that food particle size inversely correlates with *in vitro* digestion rate, that SAA significantly differs between individuals, and that those who broke down the

food to relatively smaller particles during mastication would demonstrate a greater *in vitro* starch digestion rate.

5.2 Materials and methods

Rice was used as the model. The study comprised of three experiments:

1. Cooked rice of different particle sizes were digested in an *in vitro* digestion system. The relationship between rice particle size and *in vitro* digestibility was determined.
2. Saliva samples were obtained from 15 healthy participants in duplicate (120 minutes apart) and were analysed for salivary α-amylase activity.
3. Habitually masticated rice boluses were obtained from the same 15 individuals and its particle size distribution and *in vitro* digestibility were determined.

Participant, design and experiment details are described in detail in section 2.3.3.

5.3 Results

5.3.1 Effect of rice particle size on *in vitro* digestibility

In vitro digestion of the different sized rice particles revealed a significant effect of particle size on digestion (calculated as the total area under the curve [AUC]) during the first 60 minutes of digestion ($F[4,5]= 506.35$, $P <0.001$) (Figure 5.1). Post-hoc comparisons showed that digestion of whole rice was significantly

slower compared to other particle sizes. Particles larger than 2000 µm and between 1000 µm- 2000 µm were significantly different to all but each other. Similarly, the digestibility of particles smaller than 500 µm and homogenised rice were not significantly different to each other but differed from all other particle sizes. The rapidly digestible starch (RDS- the starch that digests within the first 20 minutes of digestion) content also demonstrated an inverse relationship with particle size (Table 5.2). The greatest amount of RDS resided in the smallest particle size category (<500 µm) (Table 5.2). Complete digestion of all the samples had however occurred by the end of 180 minutes. This was ascertained by measuring the sample residue remaining in the digestion pots at the end of 180 minutes of digestion, which was less than 1% for all the treatments.

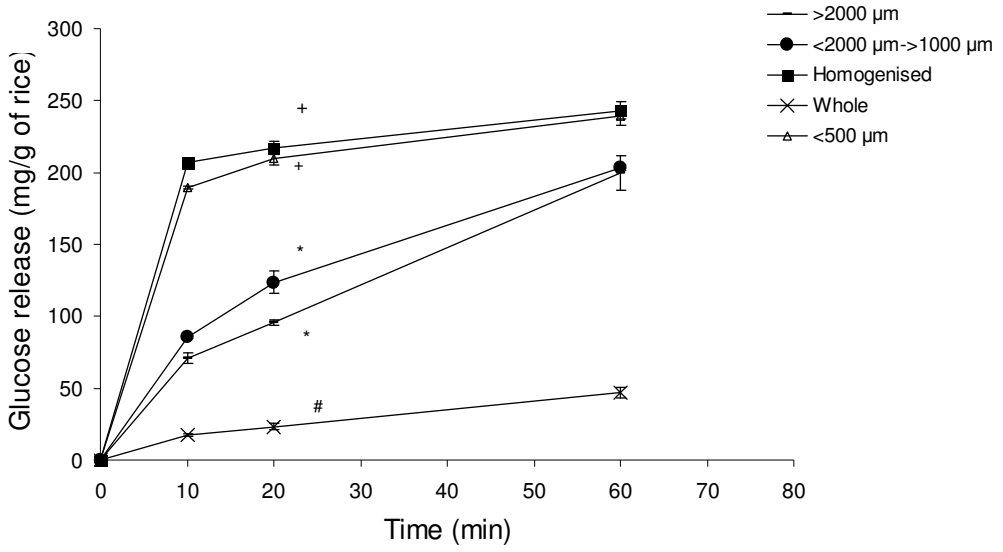


Figure 5.1: Glucose release curves during 60 minutes of in vitro pancreatic digestion of cooked rice of different particle sizes. Standard errors are represented by vertical bars. Curves with different symbols (+, *, #) have significantly different areas under the curve (AUC) (One way ANOVA [p<0.001]; post hoc Tukey test [P<0.05]).

5.3.2 Salivary α-amylase activity

Salivary α-amylase activity was not significantly different between the subjects (F[14,15]=0.992; *P*=0.558) (Table 5.1). The within-individual variation in SAA was low and ranged between 0-5% (CV). The mean between-individual standard error was also small (0.01) (Table 5.1).

Table 5.1 Salivary α-amylase activity of the 15 participants

Subject	SAA Rep 1 (U/ml)	SAA Rep 2 (U/ml)	Mean of the 2 reps (U/ml)	CV (%)
1	1.61	1.65	1.63	1.61
2	1.65	1.57	1.61	3.29
3	1.61	1.65	1.63	1.61
4	1.57	1.65	1.61	3.29
5	1.61	1.57	1.59	1.69
6	1.65	1.65	1.65	0
7	1.57	1.65	1.61	3.29
8	1.68	1.68	1.68	0
9	1.57	1.65	1.61	3.29
10	1.68	1.68	1.68	0
11	1.57	1.68	1.63	4.82
12	1.57	1.65	1.61	3.29
13	1.65	1.65	1.65	0
14	1.65	1.65	1.65	0
15	1.65	1.65	1.65	0
Mean	1.62	1.64	1.63	1.75
SD	0.04	0.03	0.03	1.69
SE	0.01	0.01	0.01	0.44

SAA=Salivary α-amylase activity; Rep=Repetition; CV= Coefficient of variation; SD= Standard deviation; SE= Standard error

5.3.3 Particle size distribution in chewed rice

The particle size distribution of rice masticated by the 15 individuals showed a significant between-individual variation (F[14,15]=529, *P*=<0.001) (Figure 5.2). The percentage of rice disintegrated into particles <500 µm ranged from 53.1-88.9 % of the total, and the amount larger than 2000 µm (intact and partially broken) ranged from 3.5 to 37.4 % of the total (Table 5.2). Therefore, the largest degree of between-subject variation was observed in the smaller than 500 µm and larger than 2000 µm particle size categories (Figure 5.2). The intermediate categories together accounted for approximately 10% of the total.

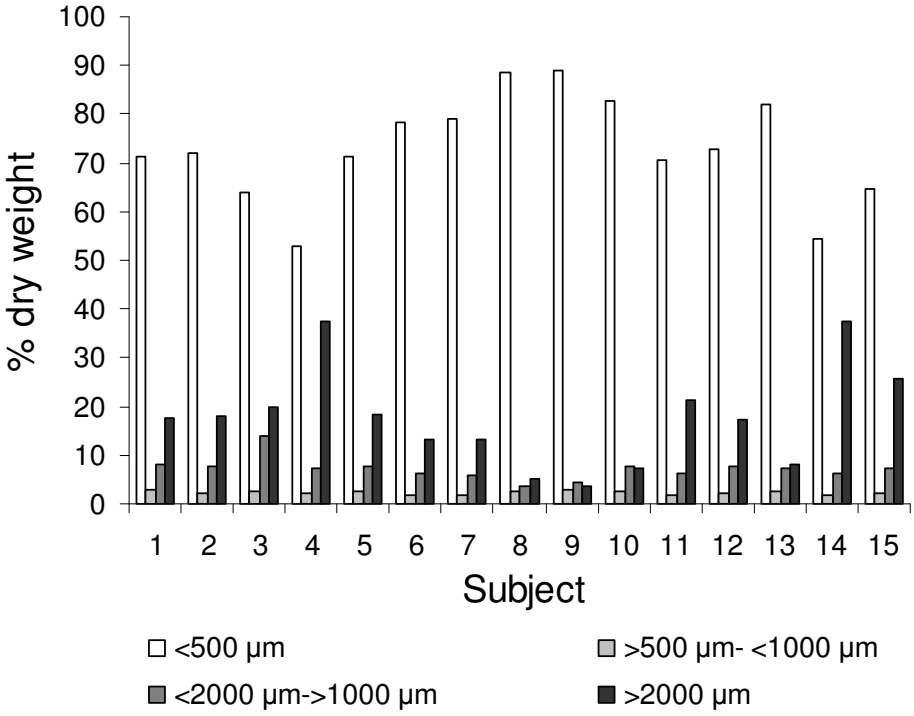


Figure 5.2 Particle size distribution of cooked rice masticated by 15 subjects.

Table 5.2 Percentage of rice particles and rapidly digestible starch content in each size category, in rice boluses masticated by 15 individuals to the point of swallowing (mean ± standard deviation).

	>2000μm	<2000>1000μm	<1000>500μm	<500μm
Mean (%)	17.0 ± 10.1	7.5 ± 2.2	2.4 ± 0.4	73.1 ± 10.6
Range (%)	33.90	9.30	1.6	35.8
	(3.5-37.4)	(4.5-13.8)	(1.8-3.4)	(53.1-88.9)
RDS mg/g of rice	95.7 ± 2.3	123.7 ± 10.9	143.9 ± 15.1	217.6 ± 6.5

RDS= Rapidly digestible starch

The RDS content in each particle size category (Table 5.2) was multiplied by the amount of rice in each particle category (Figure 1) to obtain a total RDS content value for each particle size group for each subject (Figure 5.3). These values showed that more than 80% of the RDS resided in the smallest particle size category (<500 μm).

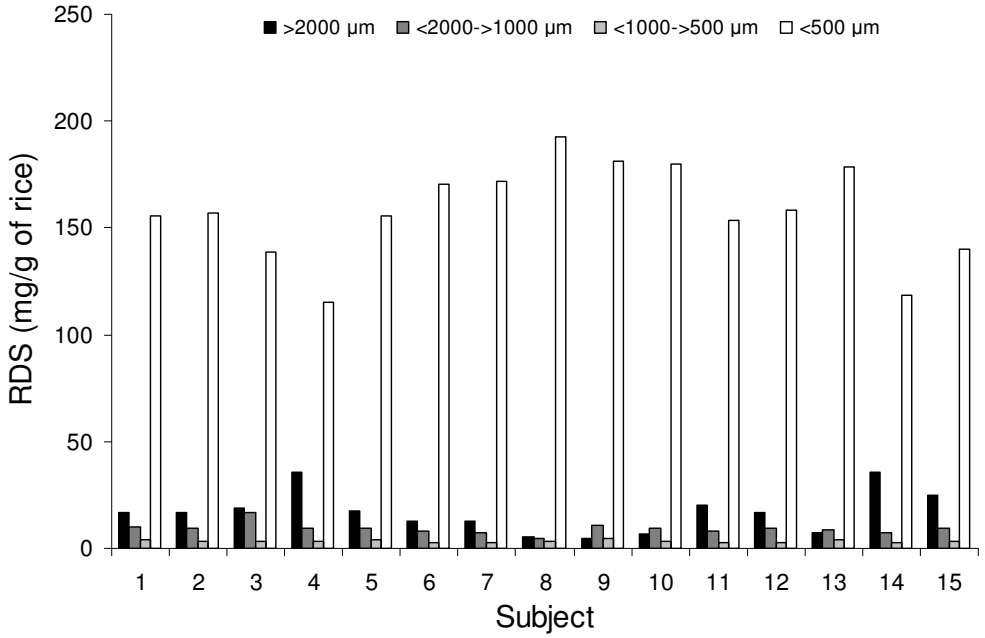


Figure 5.3 Rapidly digested starch (RDS) contribution by the particle size fractions of rice chewed to point of swallowing by 15 subjects.

5.3.4 *In vitro* digestibility of chewed rice

The amount of starch that had digested to free sugars and oligosaccharides by the end of the oral (chewing) and gastric (HCl-pepsin) phases (time 0 minutes in Figure 5.4), and prior to pancreatic digestion was significantly different between subjects (F[14,15]= 5.58, *P*=0.001). The quantity ranged between 28-82%. However, from the start of pancreatic digestion (immediately after 0 minutes) the curves began to gradually converge (Figure 5.4). The slope of the curves indicated an inverse relationship between the degree of breakdown during mastication and the time taken to digest all the starch in the sample.

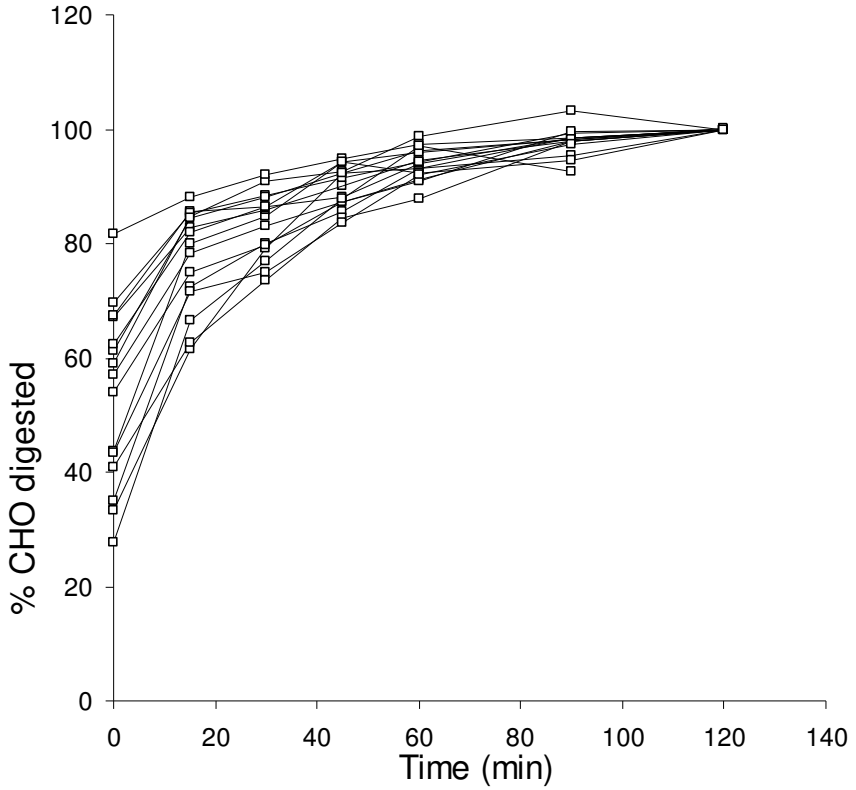


Figure 5.4 In vitro digestion curves for rice after chewing to point of swallowing by fifteen subjects. CHO= starch

The degree of oral breakdown (expressed as the percentage of particles smaller than 500 μm in the masticated bolus) demonstrated a significant

correlation with *in vitro* digestion rate (Figure 5.5). The percentage of digested starch at 0 minutes was significantly associated with the percentage of particles smaller than 500 µm in individuals' food boluses (F[1,13]= 27.23, *P*=<0.001, R^2=0.68). The correlation diminished with time as digestion progressed (F[1,13]= 16.956, *P*= 0.001, R^2= 0.57 at 15 minutes; F[1,13]= 17.615, *P*= 0.001, R^2= 0.543 at 30 minutes and R^2= 1.00 at 120 minutes).

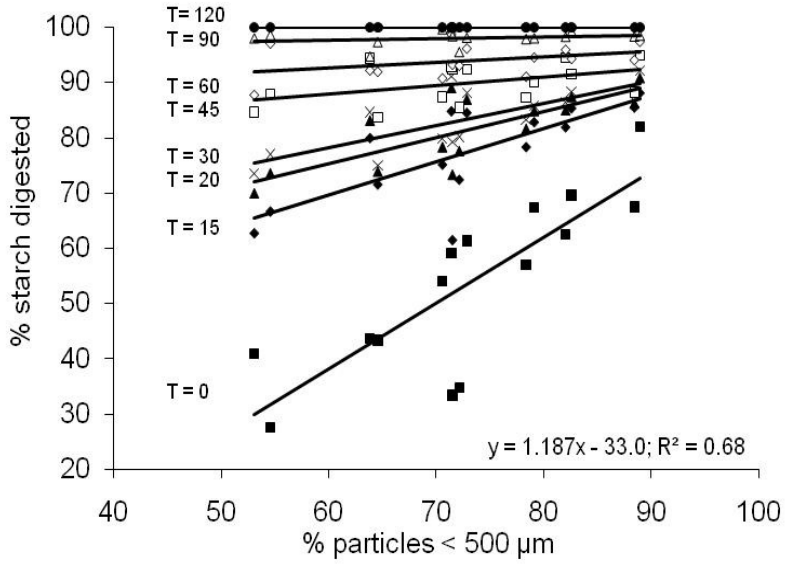


Figure 5.5 Relationship between the percentage of particles smaller than 500 µm and the proportion of starch digested in vitro at increasing times after initiation of pancreatic digestion. Equation represents the trend line for time 0 (T=0)(P<0.001).

The percentage of particles larger than 2000 µm in the masticated food boluses correlated significantly with the quantity of undigested sample remaining at the end of 120 minutes of *in vitro* digestion (F[1,13]=24.79, p=<0.001, R^2=0.66) (Figure 5.6). Individual food boluses with a relatively higher percentage of particles larger than 2000 µm had a greater percentage of undigested sample matter remaining at the end of digestion.

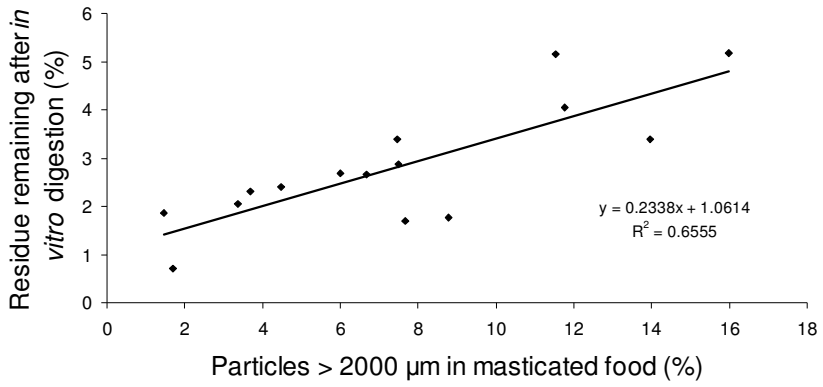


Figure 5.6 Correlation between the percentage of particles >2000 µm in the masticated food boluses of individuals and the percentage of undigested masticated rice remaining at the end of 120 minutes of in vitro digestion

5.4 Discussion and conclusion

The *in vitro* model used in the study produces quick and accurate indicative data on starch digestibility (Mishra *et al*., 2008; Mishra & Monro, 2009; Monro *et al*., 2010). The study therefore produced reliable data on the potential effects of mastication on *in vivo* GR and variability. The method used to measure SAA was also a pre-validated method (Rohleder & Nater, 2009). By obtaining all the samples in one test session the study was able to minimise within-individual variations caused by environmental and physiological variables.

The study showed a significant particle size effect on *in vitro* digestibility. Whole grains digested at a slower rate compared to smaller particles. The digestion rates of homogenised rice and particles smaller than 500 µm were similar which indicates that particle size is not a digestion rate limiting factor for fragments smaller than 500 µm. Collier and O'Dea (1982) observed that ground rice and glucose produced a similar GR which further suggests that ingesting particles smaller than 500 µm generates a GR comparable to that when

glucose is consumed. The intermediate sized particles demonstrated a medium rate of glucose release compared to the whole and homogenised rice. These findings confirm the inverse relationship between food particle size and digestibility reported in previous studies (Snow & O'Dea, 1981; Heaton *et al.*, 1988). The experiment also confirmed the sensitivity of the *in vitro* model in discerning differences between the particle size groups.

Salivary α-amylase activity appears to be similar between individuals. The activity was also comparable within individuals in samples taken two hours apart. These findings concur with previous reports which also showed that SAA does not significantly differ between- and within-individuals (Rohleder *et al.*, 2006). Another study similarly showed that mastication increased saliva secretion rates but did not affect α-amylase concentrations (Mackie & Pangborn, 1990). The authors demonstrated that α-amylase secretion varied in response to different foods but was relatively constant when eating the same food. They also showed that salivary flow rate increased when chewing parafilm, celery and bread respectively which confirm that the properties of the food determines secretion rate. Salivary α-amylase activity is affected by factors such as age, gender, time of day, smoking, alcohol, caffeine and prescription drugs (Rohleder & Nater, 2009). Whilst the current study was controlled for all these aspects, no significant differences between the two genders were observed. Humphrey and Williamson (2001) argue that saliva's role in starch breakdown is limited. Instead, it is believed that salivary α-amylase's primary functions are maintaining oral hygiene and bacterial clearance by adhesion (Scannapieco *et al.*, 1993). However, results of the current study showed that a significant amount of starch had been broken down

to simple sugars during mastication in some individuals. Although SAA does not differ between individuals a greater oral residence time may cause a sizable amount of starch to hydrolyse during mastication (due to the prolonged exposure to α-amylase). Salivary α-amylase may therefore be having a considerable influence on the GR in those who spend a longer time chewing.

The degree of breakdown during mastication varied significantly between subjects in the current study and this concurs with previous observations (Jiffry, 1981; Jiffry, 1983; Peyron *et al.*, 2004). The number of chewing episodes required to prepare a specific food type for swallowing is relatively constant within individuals, but varies considerably between individuals (Woda *et al.*, 2006a). The primary purpose of mastication is the disintegration of food to particles small enough to form a cohesive bolus that can be swallowed (van der Bilt *et al.*, 2006). The differential degrees of breakdown observed in the current study suggest that the extent of disintegration required to make a food suitable for swallowing differs between individuals. These differences appear to be independent of saliva volume as previous work has shown no correlation between salivary flow rate and the number of mastication cycles (Gaviao *et al.*, 2004).

The study showed that the degree of habitual mastication and resulting particle size breakdown influenced the digestibility of the food bolus. This suggests that habitual mastication may impact on the *in vivo* GR. Indeed Collier and O'Dea (1982) fed normal and diabetic subjects whole and ground brown rice and observed significantly lower glycemic and insulinaemic response following whole rice compared to the ground rice in both groups. Read *et al.* (1986) also

observed that the *in vivo* GR was lower when subjects swallowed sweetcorn, rice, apple and potato, compared to when they chewed and swallowed them. However, both these studies demonstrated particle size effects on *in vivo* GR by manipulating the particle size of food (prior to mastication) and did not consider habitual mastication and its effects on the GR. The results of the current study convincingly suggest that the degree of breakdown during mastication may have its own independent effects on the *in vivo* GR.

The results showed that those who broke the rice to a greater degree had a higher percentage of RDS in the masticated food bolus. The initial rate of digestion therefore was greater in those who broke down the bulk of the rice to particles smaller than 500 µm. The study conclusively showed an inverse relationship between food particle size and RDS content. In contrast, a higher percentage of residual matter remained at the end of 120 minutes of *in vitro* digestion in the samples containing a greater percentage of particles larger than 2000 µm in their chewed food (i.e. in those who broke down the rice relatively less). This suggests that complete digestion of available carbohydrates may not be occurring within the first 120 minutes of digestion when the swallowed food contains a greater proportion of large particles. Meyer (1980) observed that the stomach does not release food into the duodenum until it is fragmented into particles smaller than 1000 µm. Swallowing a food bolus consisting of a greater proportion of large particles could both delay gastric emptying and digestion rates. Conversely, complete digestion occurs before the completion of 120 minutes if the food consists predominantly of particles smaller than 500 µm. Therefore, the time taken to complete digestion of a food appears to differ between individuals depending

on the degree of mastication. It is likely that these differences in digestion rate may consequently contribute to between-individual differences observed in the *in vivo* GR (chapter 1).

In conclusion, the results showed that the particle size of food influences *in vitro* starch digestion rate to a significant degree. There is an inverse relationship between food particle size and digestion rate. Salivary α-amylase activity is similar between individuals. However, a longer time spent chewing may cause a greater amount of starch to hydrolyse in the mouth (due to lengthier exposure times) and have a significant impact on glycaemic potency. The findings also agree with the third hypothesis that the degree of habitual mastication affects individuals' *in vitro* starch digestion rate. Individuals who break down food to smaller particles elicit a greater initial rate of starch digestion. Therefore, the degree of habitual mastication could be influencing individuals' *in vivo* GR to a carbohydrate food, and potentially contribute to the between-individual variations observed in the GR to solid foods. The findings justify extending the study to a clinical phase to ascertain effects of habitual mastication on *in vivo* GR.

The main conclusions of the study can be summarised as follows:

- *In vitro* starch digestion rate correlates inversely with food particle size.
- Under standardised conditions, salivary α-amylase activity is similar between individuals when consuming a single food type. Salivary α-amylase may be considerably contributing to the starch digestion process and the initial GR especially in those masticating for a longer time.
- When eating the same food, the degree of breakdown during mastication differs significantly between individuals.
- The degree of breakdown during mastication significantly affects *in vitro* starch digestion rate and glycaemic potency of the chewed food. Therefore, due to differences in mastication efficiency, the *in vitro* starch digestion rate of masticated food is different between individuals.

Chapter 6: Habitual mastication and its impact on the *in vivo* glycaemic response

6.1 Introduction

The previous study (chapter 5) showed that the degree of food breakdown during habitual mastication was different between individuals, and that this influenced the *in vitro* starch digestion rate. The findings suggested an influence of individuals' habitual mastication on *in vivo* glycaemic response (GR) to a starchy solid food and a potential contribution to the observed between-individual variations in the GR (Wolever, 2006). Regulating the GR is important in those with impaired glucose tolerance and also from a satiety perspective.

The objective of the present study was to confirm the *in vitro* findings of the previous chapter using an *in vivo* model. The study hypothesised that individuals' degree of habitual mastication (and consequent degree of food breakdown) will influence their *in vivo* GR. It was speculated that those who habitually broke down food to a greater degree during mastication will elicit a larger initial and total GR.

6.2 Materials and methods

Participant, design and experimental details are outlined in detail in section 2.3.4. The test foods were basmati rice and spaghetti. The experiment measured oral processing parameters (number of mouthfuls taken to consume the standard portion of the test food, time taken to chew one mouthful of food,

and the number of chews per mouthful), the particle size distribution of habitually masticated food, and the *in vivo* GR of the test foods during 120 minutes. Correlations were made between these aspects to discern relationships between oral processing parameters, degree of breakdown and the *in vivo* GR.

The study produced three repeated sets of data per participant for the GR (for each test food) and two sets of data per participant for oral processing parameters and particle size distribution. Repeated measurements were obtained to make allowances for variability. Unlike for the GR a single repetition for oral processing parameters and particle size analysis was adequate as previous studies have reported a low within-individual variation for mastication when consuming a single food type (van der Bilt *et al.*, 2006).

6.3 Results

6.3.1 Mastication parameters

For both rice and spaghetti, the mastication rate (chews per mouthful) varied significantly between individuals ($F[10,11]=3.51$; $P=0.025$ and $F[10,11]=6.35$; $P=0.003$ respectively) (Figure 6.1). The within-individual variation in mastication rates (number of chews per mouthful of food) for rice and spaghetti were not significantly different ($t[10]=1.252$, $P=0.239$, 95% CI= -2.30-8.21). The chewing time per mouthful did not differ between the two test foods ($t[10]=0.599$, $P=0.563$, 95% CI= -3.10-5.37). The number of mouthfuls taken to consume the entire portions of rice and spaghetti were significantly different ($t[10]=3.950$, $P=0.003$, 95% CI=1.57-5.62) (Table 6.1). Whilst it took a mean 19 mouthfuls to

eat the portion of rice only 15 were required to finish the spaghetti. The three oral processing parameters; the number of mouthfuls taken to finish the test food, the number of chews per mouthful and the chewing time per mouthful correlated significantly with each other, for each test food (r= 0.88; *P*=0.002 and r= 0.97; *P*=<0.001; for rice and spaghetti respectively). There were however, no significant relationships between these mastication parameters and the particle size distribution of the masticated food.

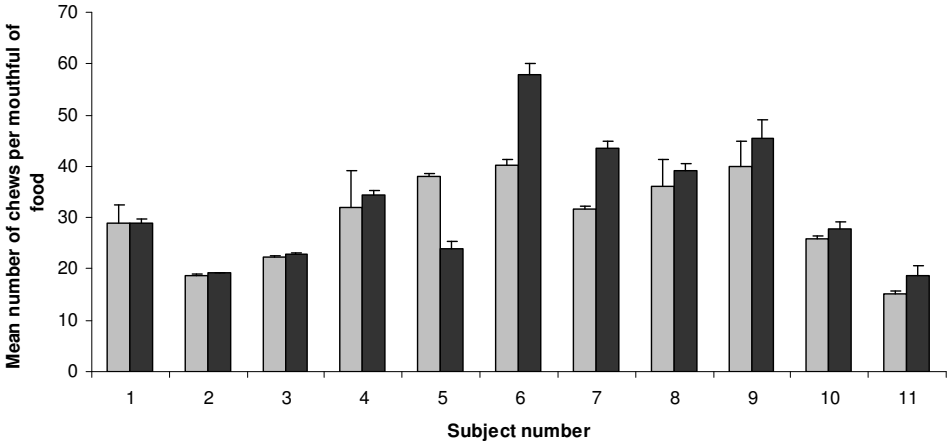


Figure 6.1 Mean number of chews per mouthful of rice (light columns) and spaghetti (dark columns) made by individuals.

Error bars represent standard deviations.

Table 6.1: Mastication dynamics data and particle size distribution of rice and spaghetti masticated by 11 participants (mean ± standard error)

	Oral processing parameters			Particle size distribution in masticated food (%)			
	Number of mouthfuls taken to eat the entire portion of food	Number of chews per mouthful	Chewing time per mouthful (s)	>2000 µm	>1000-2000 µm	>500-<1000 µm	<500 µm
Rice	18.6 ± 1.5 *	29.9 ± 2.6	23.0 ± 1.9	19.8 ± 3.4*	10.5 ± 1.1*	3.8 ± 0.2*	65.9 ± 4.1*
Range (95% CI)	15.1-25.7	24.2-35.7	18.4-26.9	14.6-25.0	8.6-12.2	3.2-4.1	59.8-72.5
Spaghetti	15.1 ± 1.1 #	33.0 ± 3.7	24 ± 3.0	53.0 ± 3.6#	3.6 ± 0.4#	1.0 ± 0.1#	42.4 ± 3.2#
Range (95% CI)	12.4-17.3	24.6-41.1	17.1-30.5	47.6-58.4	2.8-4.3	0.8-1.2	37.3-47.5

CI= Confidence interval; s= Seconds; values with different superscript symbols within a column are significantly different (paired t-test, $P<0.05$)

6.3.2 Particle size distribution in masticated rice and spaghetti

The mean particle size distributions of masticated rice and spaghetti were significantly different (F[10,11]=8.40; P=0.001) (Figure 6.2). The percentage of particles larger than 2000 µm was greater in masticated spaghetti, whilst the proportion of particles <500 µm was more in rice (Figure 6.2). The intermediate particle size groups (>1000-<2000 µm and >500-<1000 µm) accounted for a mean (± SD) 15 (±3.7) and 5 (±2.2)% of the total masticated rice and spaghetti respectively. Within each particle size category, the percentage amount of rice and spaghetti differed significantly (P <0.001) (Figure 6.2).

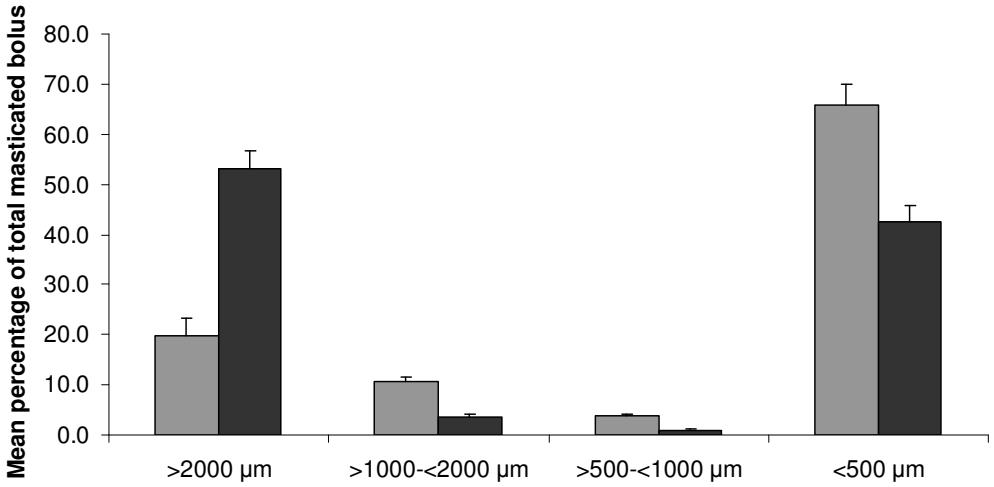


Figure 6.2 Mean particle size distribution of masticated rice (light columns) and spaghetti (dark columns).

Error bars are standard errors

6.3.3 The glycaemic response

Rice produced a significantly higher mean total IAUC compared to spaghetti (t[10]= 7.239, P<0.001, 95% CI= 29.04-54.88) (Table 6.2). The IAUC for the first 45 and 60 minutes were also significantly different between rice and spaghetti (P<0.001). Whilst the mean GR for rice peaked at 45 minutes, spaghetti maximised at 30 minutes. The total incremental areas under the curves for the two test foods showed a significant intra-individual relationship (F[1,9]=24.71; R^2=0.733; *P*=0.001;) where each individual demonstrated similar total glycemic responses to both foods in relation to those of others (Figure 6.3). This indicated that the GR to rice and pasta for each individual fell within a subjective and specific range.

Table 6.2: Glycemic responses for rice and spaghetti (mean ± standard error)

	Total IAUC (mmol.min/l)	IAUC for first 45 min (mmol.min/l)	IAUC for first 60 min (mmol.min/l)	Mean change in GR at 30 min (mmol/l)	Mean change in GR at 45 min (mmol/l)
Rice	125.2 ± 11*	24.3 ± 2.4*	50.4 ± 5.1*	1.9 ± 0.2*	2.0 ± 1.2*
Spaghetti	83.3 ± 10.6#	17.3 ± 2.6#	36.8 ± 5.0#	1.4 ± 0.2#	1.3 ± 0.2#

GR= Glycaemic response; IAUC= Incremental area under the curve for the GR; Values with different superscripts within a column are significantly different (paired t-test, $P<0.05$)

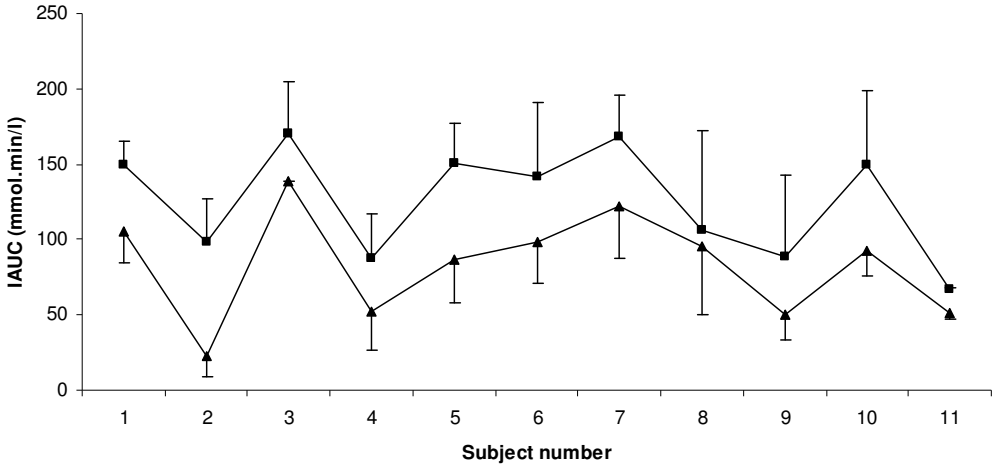


Figure 6.3 Comparative presentations of individuals' mean total incremental areas under the curve (IAUC) for the blood glucose response for rice (■) and spaghetti (▲). Error bars are standard deviations

6.3.4 Correlations between mastication parameters and glycaemic response

Significant correlations were observed between individuals' particle distribution in masticated food and their GR for rice, but not for spaghetti. The mean percentage of particles larger than 2000 µm in chewed boluses showed a significant inverse correlation with the respective total IAUC of individuals for

rice (r= -0.72; *P*=0.012), but not spaghetti (r= -0.43; *P*=0.186). The particle size distribution of masticated rice also demonstrated a significant relationship with the peak GR, where the percentage of particles larger than 2000 μm showed a significant inverse relationship with the GR at 45 minutes after consumption (r= -0.82; *P*=0.002). The percentage of particles smaller than 500 μm also showed a significant direct correlation with the peak GR at the same time (r= 0.71; *P*=0.014). No other significant relationships with particle groups and GR at individual times for rice were observed. The particle size distribution of masticated spaghetti showed no significant correlations with its peak GR (i.e. at 30 minutes) (>2000 μm: r= -0.52; *P*=0.103 and <500 μm: r= 0.53; *P*=0.096).

The percentage of particles larger than 2000 and smaller than 500 μm in the masticated rice also showed significant relationships with the IAUC for the first 45 and 60 minutes following consumption (r= -0.74; *P*=0.010, r= -0.75; *P*=0.008 and r=0.73; *P*=0.010, r= 0.67; *P*=0.023 respectively). No similar significant relationships were observed for spaghetti (*P*>0.05). Therefore, the degree of breakdown during mastication significantly affected the initial GR for rice, but not spaghetti.

6.4 Discussion and conclusion

The previous study (chapter 5) showed that the degree of mastication significantly influenced the *in vitro* glycemic potency of rice. The results of the current study confirmed these findings *in vivo.* In the previous study, a greater *in vitro* starch digestion rate was observed in individuals whose masticated rice boluses contained a larger percentage of particles smaller than 500 μm. Consistent with this, the percentage of particles smaller than 500 μm showed a

significant relationship with the peak *in vivo* GR for rice in the current experiment. The degree of breakdown of rice during mastication influenced the magnitude of the *in vivo* GR. The previous study (chapter 5) also showed that the extent of fragmentation influenced the initial *in vitro* digestion rate. The current study confirmed this effect *in vivo*.

The significant between-individual variation in mastication observed in the current study is consistent with previous findings (Jiffry, 1981; Jiffry, 1983; Hoebler *et al.*, 1998; Fontijn-Tekamp *et al.*, 2004; Peyron *et al.*, 2004). The number of chewing cycles is relatively constant within individuals when eating a single food type (Lassauzay *et al.*, 2000; Woda *et al.*, 2006a) and this was confirmed in the current study from the results obtained for both rice and spaghetti. However, the within-individual degree of mastication differed significantly when different food groups such as nuts, raw vegetables and legumes are eaten (Jiffry, 1981; Peyron *et al.*, 2004). Foods that are firm and dry were chewed for longer compared to those that were soft and moist. The former food types required more oral processing before a bolus suitable for deglutition could be formed (van der Bilt *et al.*, 2006). The comparable number of chews per mouthful for rice and spaghetti observed in this study may have been due to the similarity of the two foods in terms of texture and hardness.

Although the number of chews per mouthful for rice and spaghetti were similar, the degree of breakdown of the two foods was significantly different. Particle size analysis of the masticated boluses showed that approximately 66% of chewed rice fell into the smallest particle category (<500 μm) and conversely, 53% of the spaghetti was in the largest particle group (>2000 μm). Cooked

spaghetti strands are considerably larger than rice grains. An equal number of chews therefore would result in the spaghetti invariably breaking down to a lesser extent. These differences between rice and spaghetti demonstrate that particle size reduction before swallowing does not have to be equal for all foods. The primary purpose of mastication is to convert the food to a cohesive bolus that can be swallowed (Peyron *et al.*, 2004; van der Bilt *et al.*, 2006). Therefore, rice appears to require a greater degree of breakdown for bolus formation compared to spaghetti.

The extent of breakdown significantly influenced the total IAUC and the peak GR for rice. The habitual degree of mastication (and particle size breakdown) therefore affected individuals' blood glucose response for rice and may be a factor contributing to the between-individual variations previously observed in the GR for rice (SE ranging between 14-21 in normal subjects) (Panlasigui & Thompson, 2006). As an intact grain the starch of rice resides within the storage cells of the seed (Vandeputte & Delcour, 2004). Disruption of the cell structure and release of starch therefore is entirely reliant on mastication for such foods. The degree of breakdown subsequently determines the surface area exposed to digestive enzymes and hence the digestion rate. Therefore, the extent of particulation during mastication may be more important when ingesting intact foods such as cereals, legumes, seeds and nuts compared to foods made from flour and that's undergone processing. Mastication was not a rate limiting factor for the digestion of spaghetti. The GR to cereal foods correlate directly with the severity of milling (Collier & O'Dea, 1982; Brand *et al.*, 1985; Ross *et al.*, 1987; Vosloo, 2005) and the degree of gelatinisation (Ross *et al.*, 1987; Vosloo, 2005). Pasta is produced from intensively milled

wheat (milled to particles 200-400 microns in size) and is pre-gelatinised during processing (Marchylo & Dexter, 2001). The intense milling breaks the cellular structure and releases the starch as granules which enable easier subsequent gelatinisation and digestion. Whilst the low GR properties of spaghetti have been attributed to a slowed digestion due to its compact structure (Jenkins *et al.*, 1983) and effects on gastric emptying (Mourot *et al.*, 1988), the degree of mastication and resulting breakdown does not appear to be a rate limiting factor.

The particle size of masticated rice influenced the magnitude and the IAUC for the first 45 and 60 minutes for rice. Those who chewed rice to a relatively lesser extent elicited a correspondingly smaller peak and total GR. The extent of chewing therefore affected both the shape and amplitude of the glycaemic response curve. Previous studies observed that the stomach did not empty food into the duodenum until it was broken down into particles smaller than 1000 μm (Meyer *et al.*, 1981). The effects of mastication on the GR may have therefore been mediated by particle size effects on gastric emptying. A greater amount of particles smaller than 1000 μm in the masticated bolus would cause faster gastric emptying and a larger GR within a shorter time period.

The total IAUC for rice and spaghetti showed a strong concordance within subjects, where the GR to both foods were relatively similar within each individual compared to those of others. Using repeated measures for each test food this is the first instance where a within-individual similarity in the GR to two different starchy foods was shown. The mean within-individual CVs for rice and spaghetti (29% and 30% respectively) were less than the acceptance cut-off

limits for the GR (40%) (Wolever, 2006) which demonstrates the consistency of the repeated responses by subjects to the two foods. Relative to that of others, a subject's GR to starchy foods appears to lie within a narrow individual-specific range. Whilst some consistently showed high glycaemic responses for both foods, others showed low values. Therefore, the data suggests that all individuals can be categorised as 'high' and 'low' glycaemic responders. The between-individual variations in the GR to different foods appear to be greater than within-individual variations. These trends in variability were previously reported when subjects were given a single food (glucose or bread) (Wolever, 2006). The current study for the first time showed these same patterns also when comparing the GR to two foods. These observations were unanticipated but an interesting outcome from the experiment. However, before firm conclusions can be reached, future studies need to test a larger number of foods to determine if these trends are independent of food type.

The absence of mastication force measurements was a potential limitation in the current study. Mastication parameters (the number of mouthfuls, number of chews per mouthful chewing time per mouthful) did not show useful correlations with the particle size distribution and the GR. This suggests that chewing force is an important parameter when determining the effects of oral processing. Although considered at the design stage, a practically feasible method to accurately measure the force of habitual chewing could not be determined. This did not however affect the objectives or outcomes of the study since the particle size distribution in the masticated food was used to make associations with the GR. The particle size of chewed food is the final outcome of mastication and is the most reliable indicator of mastication efficiency

(Hoebler *et al*., 2000). The study showed that chewing time, number of chews and number of mouthfuls were not reliable predictors of the actual degree of breakdown and hence the GR.

In conclusion, the results for rice agree with the study hypothesis that the degree of habitual mastication influences the *in vivo* GR of individuals. The extent of breakdown during chewing affects the initial GR, total GR and the peak GR, and therefore, the magnitude and shape of the GR curve. The hypothesis however does not hold true for spaghetti. The degree of habitual mastication may therefore be a considerable contributor to between-individual variations in the *in vivo* blood glucose response to foods comprising of intact grains (such as rice) but not highly milled and pre-gelatinised starchy foods (such as spaghetti). The current study must be extended to evaluate other food types (pulses, nuts, cereals and their products, and foods made from highly processed/milled starches such as flour based products) and the impact of mastication on their GR. Forthcoming studies also need to encompass measurements relating to gastric emptying, digestion rate and the insulin response to further elucidate mechanisms by which mastication influences the GR.

The main conclusions of the study can be summarised as follows:

- Habitual mastication differs significantly between individuals. As a result, particle size of masticated food differs significantly between individuals.
- Oral processing parameters; the number of chews per mouthful, chewing time per mouthful and volume per mouthful are not reliable predictors of the degree of breakdown during mastication.
- The extent of breakdown during chewing significantly influences the early phase, peak and total GR of rice, but not spaghetti.
- Mastication appears to be a factor contributing to between-individual variations in the GR of rice.

Chapter 7: Between-individual variations in post-mastication digestion aspects and effects of ingested food particle size on glycaemic response, insulin response and gastric emptying.

7.1 Introduction

A central theme of the thesis has been to examine factors affecting the glycaemic response (GR). The amplitude and pattern of the GR are both important as they impact on glucose homeostasis and have practical relevance in the management of impaired glucose tolerance. More recently, it has been reported that wide blood glucose excursions and peaks significantly influence the risk of developing type 2 diabetes (Wolever, 2006). The GR also affects satiety through glucostatic mechanisms (Bornet *et al.*, 2007) and consequently has implications in food intake and weight control. Therefore, managing the GR is important in many respects.

A consistent observation in GR studies is the wide within- and between individual variations (Crapo *et al.*, 1977; Wolever *et al.*, 2006; Wolever, 2006; Wolever *et al.*, 2008). These account for approximately 16% and 62% of the total variation respectively (Wolever, 2006). Therefore between-individual differences are the larger contributor to total variability. This is in agreement with the results of the study described in chapter six. Variation in the GR between individuals was demonstrated by Vega-Lopez and colleagues (2007) in a study using white bread and glucose. The authors observed that the GR elicited by 23 healthy subjects varied significantly both in terms of total

response and pattern (Figure 7.1). The between individual CV was considerably high (55%) and the within-individual CV (for three repeated tests) was relatively lower (35%). For example, a comparison of the GR of subjects one and four (Figure 7.1) showed that the former produced a low and flat response for bread compared with the latter who produced an augmented response and greater peaks (Figure 7.1). These differences occurred despite the study being controlled for participant characteristics, protocol and study conditions. Therefore, there appear to be also other factors contributing to the glycaemic variability between individuals.

Figure 7.1: Individual temporal glycemic response curves to white bread (▲) and glucose (○) for 23 normal male (m) and female (f) subjects. (Source: Vega-Lopez *et al.*, 2007)

The GR to a carbohydrate food is influenced by both intrinsic and extrinsic factors (Figure 7.2). Whilst extrinsic factors relate to the food, intrinsic aspects involve the digestion process and participant characteristics (disease status, medication, physical activity and insulin secretion) (Bjorck *et al.*, 1994; Vosloo, 2005; Wolever, 2006). The GR to any food is consequential to all these factors (Figure 7.2). The cornerstone of successful GR management is having a comprehensive understanding of all these variables affecting it.

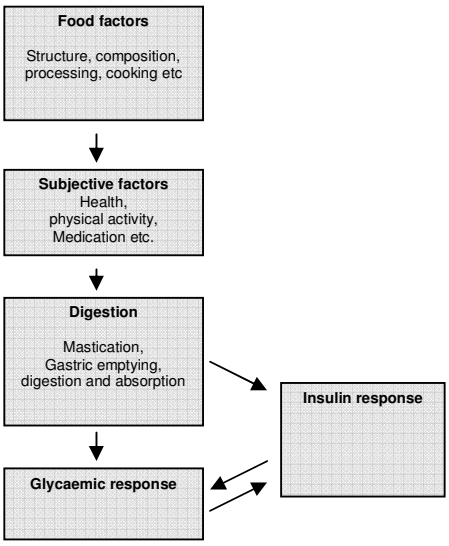


Figure 7.2: Schematic of factors determining the glycaemic response and insulin response to solid carbohydrate foods

The study comparing the GR and insulin response (IR) to liquid and solid carbohydrate foods (chapter 3) showed greater between-individual variations for solids compared to liquids. Chewing is the first step in the digestion process of solids and the work described in the preceding sections (chapters 5 and 6) showed that the degree of habitual mastication affected the GR of rice. Subjects who broke down the food to a greater degree showed a larger GR compared with those who chewed less. These results suggest that the particle

size distribution in masticated rice affects an individual's GR and is therefore a significant contributor to between-individual variations in the GR. The studies also indicated that the exposure time to salivary α-amylase may influence the GR.

However, no work has been carried out to determine the contribution of digestive steps after the mastication phase on between-individual variations in the GR. It is possible that there are between-individual variations in gastric emptying and intestinal digestion rates even when there are no influences from food particle size (i.e. mastication) and external variables. The current study was initiated to address this gap in knowledge.

The specific objective of the current study was to observe between-individual variations in the GR, IR and gastric emptying when external variables, study protocol, participant characteristics and mastication were standardised. The hypothesis was that between-individual variations can be observed in gastric emptying, GR and IR even when there are no influences from particle size (mastication), food system and external variables in healthy young males. The current study is the first instance where ingested food particle size effects on gastric emptying, GR and IR have all been determined within a single design.

7.2 Materials and methods

Thirteen male participants were fed two rice treatments differing only in particle size (large [>2000 μm] and small [between 500-1000 μm]) and the subsequent GR, IR and gastric emptying was measured under standardised conditions. Of

the 13 partiipants, the full data sets of 12 were obtained for statistical analyses. One participant dropped out after one session due to personal reasons. The two particle size treatments mimicked low and high degrees of breakdown during mastication respectively. Participant, design and experimental aspects are described in detail in section 2.3.5.

7.3 Results

7.3.1 Glycaemic response

Considerable between-individual variations in the GR were observed for both treatments (Table 7.1). The variations for the small particles were substantially greater. The total IAUC for the GR for small particles varied 45% more compared with large particles. The peak GR for small particles also showed a between-individual variation that was 57% greater compared with that of large particles.

The total IAUC for the GR differed significantly between the two treatments ($t[11]= -4.50$; $P<0.001$) (Table 7.1). The small particles elicited a significantly greater total IAUC than the large particles. The peak GR for both treatments was observed at 30 minutes. The small particles demonstrated a significantly greater peak GR compared with large particles ($t[11]= -4.17$; $P= 0.001$) (Table 7.1).

Table 7.1: Glycemic and insulin responses following the ingestion of large and small rice particles (mean ± standard error (standard deviation))

	Total IAUC for the GR	Peak GR	Total IAUC for the IR	Peak IR
Large particles	65.4±10 (36.6)	1.6±0.2 (0.7)	525.3±90 (310.6)	14.4±3 (9.4)
Small particles	119.0±15 (53.3)	2.6±0.3 (1.1)	1115.0±178 (579.3)	31.6±6 (22.5)

IAUC=Incremental Area Under the Curve for 120 minutes; GR= Glycemic Response; IR= Insulin Response; SE= Standard error; SD= Standard deviation; The peak GR and IR for both treatments were observed at 30 minutes; There was a significant difference between large and small rice particles for all variables (Paired t-test, $P<0.05$)

The temporal GR patterns for large and small particles differed considerably (Figure 7.3). Whilst the large particles produced a low and sustained GR during the entire 120 minute period the small particles produced a greater initial GR that dropped below baseline by 120 minutes. The mean (±SE) difference between the peak and nadir GR was therefore significantly greater ($P=0.001$) for the small (2.7 ±0.3 mmol/l) compared with the large (1.8 ±0.2 mmol/l) particles.

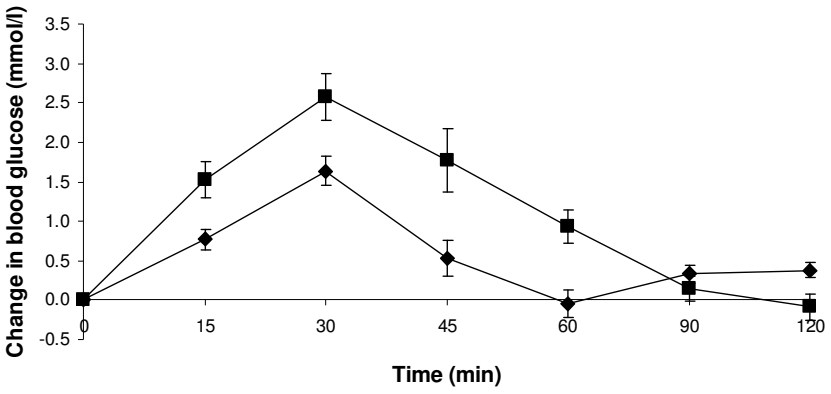


Figure 7.3: Temporal blood glucose response curves for large (♦) and small (■)rice particles. The total incremental area under the curve (IAUC) and the peak glycaemic responses were significantly different between the two treatments (Paired t-test, $P<0.05$).

7.3.2 Insulin response

The between-individual variations in the IR were larger than those observed for the GR (Table 1). The variations were again greater for the small particles compared with the large particles. The standard deviations associated with the IAUC for the IR for small particles was 140% greater than that of the large particles. The standard deviation for the peak insulin response for small particles was 139% greater than that of large particles.

The total IAUC for the IR was significantly different between the two treatments (t[11]= -2.53; *P*= 0.014) (Table 7.1). The small particles elicited a greater total IR. The peak IR was observed at 30 minutes after initiation of consumption for both treatments and the small particles produced a significantly greater peak compared with large particles (t[11]= -2.96; *P*= 0.007).

Similar to the GR the IR patterns differed notably for the two treatments. Compared with the insulin response of large particles which showed a low and sustained positive response the small particles elicited an augmented peak response which then dropped below baseline by 120 minutes. The mean (±SE) absolute difference between peak and nadir IR was significantly greater (*P*= 0.006) for the small (35 ±7.5 µU/ml) compared with large (14.6 ±2.6 µU/ml) particles.

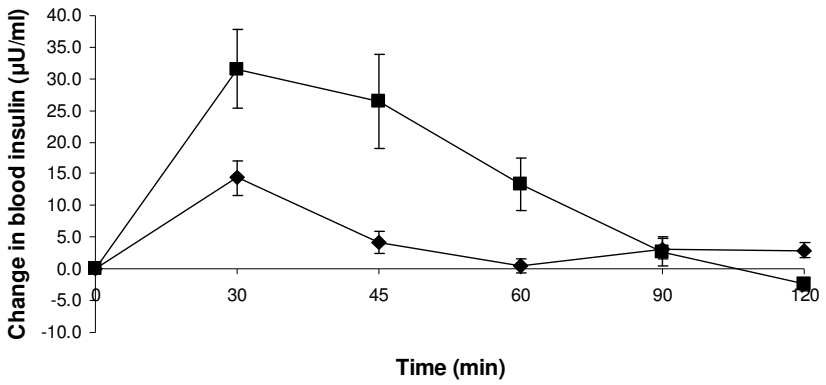


Figure 7.4: Temporal blood insulin response curves for large (♦) and small (■) rice particles. The total incremental area under the curve (IAUC) and the peak insulin responses were significantly different between the two treatments (Paired t-test, $P<0.05$).

The temporal insulin response pattern for both treatments mirrored that of the GR (Figure 7.4). The correlation between the IR and GR at each time point for the small and large particles was very significant (r= 0.989; $P< 0.0001$ and r= 0.996; $P< 0.0001$ respectively).

7.3.3 Gastric emptying

The between individual variations associated with the gastric emptying latency (T_{lat}), lag (T_{lag}), ascension (T_{asc}) and half time (T_{half}) data were similar for both treatments (Table 7.2). The latency phase showed the smallest between-individual variations. The lag phase and half time demonstrated similar variations.

The gastric emptying T_{lat}, T_{lag} and T_{half} were significantly different between the two treatments. The small particles had a significantly shorter T_{lat} (t[11]= -2.045; P= 0.032), T_{lag} (t[11]= -2.199; P= 0.025) and T_{half} (t[11]= -1.825; P= 0.042). There was no difference in gastric emptying for T_{asc} ($P>0.05$) (Table 7.2). The

data showed that initial emptying was faster for the small compared with large particles. The time of peak emptying (lag phase) was where this was most evident as there was an average difference between the treatments of approximately 12 minutes (Table 7.2).

Table 7.2: Gastric empting parameters for large and small rice particles (mean ± standard error (standard deviation))

	T_{lat} (min)	T_{lag} (min)	T_{half} (min)	T_{asc} (min)
Large particles	11.3±2.0 (6.9)*	46.1±4.9 (16.9)*	96.2±5.3 (18.3)*	84.8±4.4 (15.4)
Small particles	7.3±1.8 (6.4)$^{\#}$	34.6±5.6 (19.5)$^{\#}$	87.0±5.0 (17.3)$^{\#}$	79.7±3.5 (12.2)

T_{lat} = Gastric emptying latency phase; T_{lag} = Gastric emptying lag phase; T_{half} = Gastric emptying half-time Tasc = Gastric emptying ascension time; SE= Standard error; SD= Standard deviation; Values with different superscripts within a column are significantly different (Paired t-test, $P<0.05$).

7.4 Discussion and conclusion

Instructing the participants to swallow the treatments without chewing ensured that the rice treatments reached the stomach without further breakdown. It was thus possible to observe subsequent (post-mastication) metabolic responses independent of influences from chewing. Since the treatments were prepared in the style of a risotto the moist nature made it easy to swallow without chewing. The study was also carried out under standardised conditions and used consistent procedures and instruments for data collection. The participant cohort was carefully vetted for homogeneity in terms of age, gender, health, physical activity and dietary habits. These design aspects ensured that the influence of external and participant variables and mastication on between-individual variations was minimised. In evaluating two different particle sizes

the study also generated comparative data on the effects of ingesting large and small particles on the GR, IR and gastric emptying.

The between-individual variations in gastric emptying half time, lag, ascension and latency phases were considerably lower than previously reported values using the same analytical method (Clegg *et al.*, 2007; Clegg & Shafat, 2010; Clegg *et al.*, 2010). These studies tested pancake meals and did not account for particle size breakdown during mastication. The lower variations in the current study suggest that the degree of breakdown during habitual mastication may be a significant contributor to between-individual variations in gastric emptying of solids. In agreement, Brophy *et al.* (1986) observed that the standard deviation in gastric emptying half time was greater following a solid (beef stew) (17.7) compared with when the same subjects consumed a liquid (orange juice) (8.5). The degree of mastication and particle size of food reaching the stomach may therefore be a significant contributor to variations in gastric emptying. This will subsequently also impact on glucose appearance rate in the blood and contribute to variations in the GR.

The current study was the first to show considerable between-individual variations in the GR and IR in healthy males even when the effects from food, mastication and external environment were standardised. The greater variations in the GR and IR compared with that of gastric emptying suggest that gastric emptying may not be entirely accountable for the variability of the GR and IR. Post-gastric emptying aspects associated with digestion and absorption in the small intestine may therefore be differing between individuals and contributing to the observed variations. To date, no studies have attempted to

specifically determine between-individual differences in small-intestine digestive aspects such as enzyme secretion, enzyme activity, transit time and absorption rates. However, there is evidence to suggest that oro-caecal transit time, pancreatic enzyme secretion and glucose absorption vary between individuals when the same food is consumed (Modigliani & Bernier, 1971; Layer *et al.*, 1988; Van Den Driessche *et al.*, 2000). Therefore, it is possible that the carbohydrate digestion and absorption rate in the small intestine varies between individuals and subsequently affects both the GR and IR. The between-individual variations associated with the GR and IR data were noticeably greater for the small particles which suggest that these factors may vary more when small particles are ingested compared with large particles. These hypotheses can be confirmed only after the extension of the current study to a trans-pyloric intubation phase (Cresci & Martindale, 2003) where the gastric phase can be bypassed and the food placed directly in the duodenum.

Since the mastication phase was standardised in the current study and both treatments were consumed within an equal time period the effects of the cephalic phase on the GR and IR were equalised. The cephalic phase is associated with the secretion of insulin and other factors (glucagon, pancreatic polypeptide) which significantly influence the total glycaemic and insulin responses (Teff, 2000; Zafra *et al.*, 2006). In addition to the standardised conditions described above the study further demonstrated that the GR and IR showed variations also when influences from the cephalic phase were equalised.

The observed inverse relationship between food particle size and GR agrees with previous data obtained using whole, cracked, coarse and fine flour forms of wheat, maize and oats (Heaton *et al.*, 1988), ground and unground forms of white and brown rice (O'Dea *et al.*, 1980) and with results of the studies described in chapters 5 and 6. The observed inverse relationship between particle size and the IR was also in agreement with previous findings (O'Dea *et al.*, 1980; Heaton *et al.*, 1988). The concordance between the GR and IR patterns observed in the current study was striking and this suggests that the GR closely predicted the IR for the two treatments. Similar evidence was found in the first study forming this thesis (chapter 3). A substantial amount (nearly half) of post-meal insulin secretion has been shown to be due to incretins (GIP, GLP-1 and PYY) (De Leon *et al.*, 2006; Drucker, 2006). In addition to their effects on insulin release high concentrations of GLP-1 and PYY slow gastric emptying and transit time (Savage *et al.*, 1987; Naslund *et al.*, 1999). Since food particle size affects gastric emptying it is possible that ingested food fragments will also influence secretion levels of incretins. However, no studies have attempted to determine food particle size effects on this group of hormones. Irrespective of the specific mechanisms the data showed that ingestion of small particles elicited a greater and more varied insulin response. Therefore, swallowing small particles incur greater demands on the hormone's regulatory mechanisms. Chewing less and ingesting large particles may be beneficial for those with impaired insulin activity.

The current study is the first instance where particle size effects on gastric emptying was measured using a stable isotope method. Using a different analytical method the study confirms the previously reported inverse correlation

between food particle size and gastric emptying (Meyer *et al.*, 1988; Mourot *et al.*, 1988; Vincent *et al.*, 1995). However, previous studies only manipulated particle size in the food system and did not consider changes occurring during mastication. By delivering food of exact particle size to the stomach the current study showed that the degree of breakdown during mastication may have its own effects on gastric emptying. In the gastric antrum solids are ground to particles smaller than 1000 µm before release into the small intestine (Meyer *et al.*, 1981). In agreement results of the current study showed that large particles (>2000 µm) were initially held in the stomach for a longer time period and emptied at a slower rate compared with small particles (500-1000 µm). The significantly lower T_{half} for small particles indicated quicker emptying of the stomach when particulation was greater. Food chewed to a greater degree will therefore provoke a shorter holding time and a more rapid emptying rate. These differences in gastric emptying dynamics could explain the differential GR patterns observed for large and small particles. Indeed, previous studies have shown that the GR to starchy foods correlates closely with gastric emptying dynamics (Mourot *et al.*, 1988)

In conclusion, the results agree with the study hypothesis. Between-individual variations can be seen in gastric emptying even under conditions where mastication (particle size), food factors, study conditions and participants are standardised. However, these variations are relatively small and independent of particle size. The between-individual variations in the glycaemic and insulin responses are notably greater when small particles are in the stomach. Post-gastric digestive factors may be contributing significantly to between individual variations in the GR and notably so when ingesting small particles. Small

particles cause faster gastric emptying and produce greater glycaemic and insulin responses. Ingested food particle size therefore influences the magnitude and pattern of the glycaemic and insulin responses.

The main conclusions of the study can be summarised as follows:

- Variations in the GR and IR can be observed also when experimental conditions, cephalic phase, food characteristics, participant characteristics, and mastication (particle size) are standardised
- There are between-individual variations in gastric emptying even under the above standardised conditions. However, these variations are relatively small.
- It is possible that factors associated with digestion and absorption in the small intestine varies between individuals even when all preceding variables are standardised.
- Ingesting small particles produce greater between-individual variations in the glycaemic and insulin responses.
- Smaller particles elicit a greater gastric emptying rate, and glycaemic and insulin response.

Chapter 8: Overall summary, conclusions and recommendations for future work

8.1 Overall summary and conclusions

Following the recognition of its importance in energy balance regulation (Campfield & Smith, 2002; Bornet *et al.*, 2007) and health (Clement *et al.*, 2004) the past two decades have seen a significant rise in glycaemic response (GR) related research. Whilst a large volume of studies have examined food and physiological factors influencing the GR, some aspects remain little explored. Two such aspects, notably the physical state (liquid-solid) of food and the ingested particle size (mastication) were studied in this thesis.

One objective of the thesis was to compare the GR, insulin response (IR) and satiety of liquid and solid carbohydrate foods. This was fulfilled in the first experimental chapter described in chapter 3. It was apparent that the physical state of food (liquid-solid) did not influence the total GR and the IR. The chemical and compositional characteristics of a food seem to be their greater influencer. However, the physical state significantly affects the amplitude and shape of the GR and IR curves. Compared to solids, liquids elicit a considerably larger early phase (possibly due to a more rapid gastric emptying rate (Glasbrenner *et al.*, 1993; Chen *et al.*, 2009)) and a quicker drop back to baseline for GR and IR values. It has been shown that such dynamic fluctuations between peak and nadir GR and IR values exert more demands on the physiological regulatory mechanisms and also increase the risk of developing type 2 diabetes (Wolever, 2006). The glucostatic theory states that

food intake is suppressed when the GR is high but is induced when it falls below baseline (Bornet *et al.*, 2007). The more dramatic fall back to baseline of the GR and IR suggests that liquids are also less satiating than solids from a glucostatic perspective. Visual analogue scale (VAS) data showed that subjective satiety for all the treatments had reached baseline by 30 minutes which indicated that the satiating properties of both liquids and solids lasted for a similar period of time. However, this finding requires cautious interpretation as the study may have been underpowered for the VAS data.

Compared to the study described in chapter 3 which tested iso-carbohydrate portions of the test food (50 g available carbohydrates), the study in chapter 4 tested treatment portions varying in carbohydrate content (orange juice 33.5 g, Sugar-sweetened drink 36.7 g, milk 14.7 g) but were iso-caloric (150 kcal). This was because studies investigating compensatory effects are required to be carried out on an iso-energy basis compared to GR studies which are based on iso-carbohydrate levels. Previous studies have shown that the GR to carbohydrate loads ranging from 25-100 g did not differ significantly (Lee & Wolever, 1998; Wolever, 2006). Therefore, the properties and magnitude of the GRs elicited by the common treatments in the two studies (chapter 3 and 4) were similar which allowed for comparisons across studies.

The study described in chapter 4 showed that carbohydrate containing beverages induced an energy compensatory effect in the short-term. These findings provide further evidence that carbohydrate-based caloric beverages do not contribute to a passive overconsumption of energy as previously suggested (Bes-Rastrollo *et al.*, 2006; Striegel-Moore *et al.*, 2006; Gibson & Neate, 2007;

Harrington, 2008). The study further showed that males are capable of compensating more precisely than females for carbohydrate-based caloric liquids. Therefore, consuming carbohydrate-based drinks do not appear to lead to excess energy intake in males, although it may do so in females. Similar outcomes have also been observed with semi-solid foods (Davy *et al*., 2007) indicating that gender differences in energy compensation are independent of the physical state of food. These observations highlight the need for policy makers to consider gender differences in energy compensation when making public health recommendations. However, it is important to note that energy compensation occurs only if the subsequent meal is consumed within the compensatory time frame (the length of which depends on the carbohydrate/energy content in the beverage). The GR data (chapter 3) suggests that liquids provoke a shorter compensation period compared to solids. Therefore, the shorter compensation time and potential for faster consumption rates (Kissileff, 1985) may predispose excess energy intakes when caloric beverages (compared to solids) are consumed (Chen *et al*., 2009).

The energy compensatory effects observed in the study in chapter 4 agree with the glucostatic hypothesis. Studies observing the relationship between the GR and food intake have found that a GR decline of 6-12% below baseline triggered voluntary food intake (Smith & Campfield, 1993; Campfield *et al*., 1996; Campfield & Smith, 2002; Bornet *et al*., 2007). The study in chapter 3 showed that the GR of the treatments had reached baseline at 60 minutes but had not dropped below it. This suggests that the satiety exerted by the preloads still manifested when the subsequent meal was consumed in the study in

chapter four. However, the more rapid drop to baseline (of the GR and IR) observed with liquids suggest that they lose their satiating properties more swiftly than solids.

In conclusion, the data from the two studies (chapter 3 and 4) collectively showed that the physical state affects the shape and amplitude of the GR and IR curves but not the total metabolic response. Carbohydrate containing liquids appear to be less satiating than solids but are nevertheless detected by the physiological regulatory mechanisms and compensated for in the short term. However, the gender-wise variations in compensation signify that glucostatic mechanisms are not the only regulators of short-term energy intake regulation and that other factors also influence it.

It was observed in the study in chapter 3 that solid foods elicited larger between-individual variations in the GR and IR. Hence, a greater number of physico-chemical factors seem to influence the GR and IR of solids compared to liquids. Since mastication is a step exclusively associated with solid foods its effects on the GR were studied in this thesis. The studies described in chapters 5 and 6 collectively showed that the degree of habitual mastication and resulting particle size breakdown significantly affected the GR. Therefore, the degree of habitual mastication seems to be a significant contributor to between-individual differences in the GR.

Mastication seems to be an influential factor only when consuming intact grains such as rice but not foods such as spaghetti which are highly processed, pre-gelatinised and made with refined flour. Indeed, the first study forming this

thesis (chapter 3) showed that between-individual variations in the GR and IR were also greater for rice than spaghetti. This further suggests that differences in mastication may be the causal factor for the greater variations in the GR and IR of rice. However, more foods of similar type need to be evaluated to confirm these hypotheses. The results show that masticating less and ingesting larger particles may be a simple intervention strategy for the modulation of the GR in those with impaired glucose tolerance.

The results in the current thesis confirmed previous findings that mastication parameters (number of chewing cycles, time spent chewing) (Lassauzay *et al.*, 2000; van der Bilt *et al.*, 2006) and the particle size following habitual mastication (Jiffry, 1981; Jiffry, 1983; Peyron *et al.*, 2004) varied significantly between individuals. However, the number of chewing cycles, time spent chewing and amount of food per mouthful does not predict particle size breakdown. Thus, contrary to previous suggestions (Lassauzay *et al.*, 2000; van der Bilt *et al.*, 2006; Woda *et al.*, 2006a) these factors are not effective measures of mastication efficiency. Directly measuring particle size distribution in the chewed food is the best method of quantifying mastication efficiency.

Slowly digestible starch (SDS) has been shown to produce a subdued early phase and sustained late phase GR due to its lower digestibility (Normand *et al.*, 2001) and this pattern was confirmed in the *in vitro* study described in chapter 5. The study further showed that SDS content was more in individuals whose masticated boluses contained a greater proportion of large particles (large particles digest slowly and thus contribute to SDS). Therefore, based on the degree of breakdown during mastication the same food will produce a low

GR in some and a high GR in others, and this too was confirmed in the study described in chapter 6. Hence, the structure and its susceptibility to disintegration during mastication appear to be a key determinant of the SDS content and thereby the GR. Indeed, high glycaemic index (GI) foods (white bread, baked potato, cornflakes) have an easily disintegrable structure that requires little or no mastication compared to low GI foods (whole grains, pulses, nuts). This was demonstrated by Jenkins and colleagues (1983) when they compared the GR to bread and pasta made with the same ingredients and found that the former elicited a significantly higher GR. In agreement O'Dea *et al.* (1980) stated that the physical structure was more important towards the GR and IR of a food than even the fibre content. Indeed, the benefits of insoluble fibre on the GR could also be through its effects on maintaining structural integrity and resistance to disintegration. The observation that only naturally present (and not externally added) insoluble fibre reduced the GR of a food strengthens this argument (Wolever & Miller, 1995). Results from the studies forming this thesis further suggest that the glycaemic potency of a food with a relatively hard structure may further depend on the degree of mastication. The low GR benefits associated with firm structured foods may be less in those who habitually masticate to a greater degree.

The results of the studies in the thesis collectively showed that ingesting liquids and small food particles produced comparable glycaemic and insulin responses. Surprisingly the small food particles produced slightly larger total responses (incremental areas under the curve) (chapter 7) than the beverages (chapter 3). The shape of the response curves and the amplitude of the peak responses were also similar for beverages and small particles. Thus the GR

and IR pattern and magnitude appears to depend more on the digestibility of the carbohydrate food rather than its physical state (liquid-solid). Beverages have been suggested to encourage impaired glucose tolerance due to their high rapidly absorbable sugar content and resulting GR (Schulze *et al.*, 2004). However, data from the studies forming this thesis showed that ingesting highly digestible solid carbohydrate foods may also induce metabolic responses similar to when beverages are consumed. Therefore, intervention strategies geared at glycaemic control need to focus more on the digestibility of carbohydrates in the food rather than the physical state.

The thesis further demonstrated that ingested particle size correlated inversely with gastric emptying, GR and IR (chapter 7). The uniqueness of this study was that it combined measurements for gastric emptying, GR and IR and showed that the size of ingested food particles affected all of these attributes. Whilst previous studies have shown that food related factors affected the GR (Vosloo, 2005) the work described in chapter 5 and 6 showed that habitual mastication and the degree of particle size breakdown also contributed to between-individual variations in the GR. The study in chapter 7 went on to show that between individual-variations in the GR can be seen also when external, food and mastication related variables were standardised. This indicates that post-mastication aspects associated with digestion vary between subjects. The study further showed that between-individual variations in gastric emptying were similar for both large and small particles and relatively small. All known factors influencing gastric emptying were controlled for in the study (particle size, viscosity, volume, nutritional composition, energy content, pH) (Low, 1990; Hellstrom *et al.*, 2006) and this may have reduced variations to a

minimum. However, interestingly, the GR and IR still showed considerable between-individual variations also under these standardised conditions, and the deviations were greater following the ingestion of small particles. This suggests that post-gastric aspects associated with digestion in the small intestine vary between individuals and cause the differences in the GR and IR. These factors appear to vary more when small particles are ingested.

Considerable biological variability in the GR is a consistent observation even when individuals are given the same carbohydrate food under standardised conditions. The literature review showed that between-individual variations are the greater contributor to GR deviations (chapter 1). A thorough understanding of this biological variability will enable better control of the GR and its effective use as a tool in clinical nutrition interventions. Unlike data from exact sciences (such as physics) variations in biological data are inevitable (Sebastian-Gambaro *et al.*, 1997) and measures can only be taken to minimise them through understanding all its determinants. Studies in this thesis showed that the physical state and particle size both influenced the magnitude of variations. The results further suggested that the oral processing phase, gastric emptying and subsequent digestive aspects all contributed to GR variations when a solid is ingested. Therefore, between-individual variations in the GR are the collective result of deviations occurring throughout the digestion process. The greater variations observed with small particles suggest that the extent of breakdown during mastication may be significantly influencing the magnitude of the variations at the subsequent digestion stages. Properties of the food and those of the swallowed bolus will both have significant impacts on the magnitude of between-individual variations.

In synthesising the observations made in the thesis it is speculated that ingested food particle size may also impact on satiety and food intake. Since elevated blood glucose and insulin levels suppress food intake (Campfield & Smith, 2002; Bornet *et al.*, 2007; Flint *et al.*, 2007) the GR and IR trends observed in the studies herein indicate that the ingestion of small particles will produce a greater satiety in the short term but also induce a greater degree of hunger afterwards, compared to large particles which would elicit a lower but consistent level of satiety for a longer time period. The presence of food in the stomach also suppresses hunger by inducing post-ingestive satiety (Blundell & Tremblay, 1995) through gastric distension (Geliebter *et al.*, 1988). Ingesting large particles may therefore suppress hunger for longer both through glucostatic and post-ingestive satiety mechanisms. This has practical implications in food intake control and in the management of obesity. However, no work has been carried out to determine the effects of food particle size on satiety and the above hypotheses remain to be verified.

The study in chapter 5 showed that salivary α-amylase activity does not differ between individuals when consuming the same food. Contrary to previous assertions that salivary α-amylase had little impact on starch digestion (Scannapieco *et al.*, 1993; Humphrey & Williamson, 2001) work forming this thesis showed that a considerable amount of starch was broken down to simple sugars in some during the mastication process and that this affected the initial glycaemic potency (chapter 5). Therefore salivary α-amylase may have a notable role in carbohydrate digestion and the GR, at least in those who spend a longer time masticating (chapter 6). However, these conclusions are based on *in vitro* work and *in vivo* effects remain to be determined.

The GR influences short-term food intake (Anderson & Woodend, 2003b; Bornet *et al.*, 2007) and therefore energy balance and obesity (Ludwig *et al.*, 1999; Brand-Miller *et al.*, 2002). Regulating the GR is also critical in the management of diabetes (Salmeron *et al.*, 1997a; Salmeron *et al.*, 1997b; Kayode *et al.*, 2009) and coronary heart disease (Liu *et al.*, 2000; Marks & Raskin, 2000) two conditions which are in turn exacerbated by obesity. Good glycaemic control has also been shown to prevent the onset of chronic diseases (Jenkins *et al.*, 2002). The work presented in this thesis demonstrated that food and physiological factors influence the GR. A good understanding of the determinants of the GR will enable the development of better strategies and foods for the management of obesity and diabetes. The escalating incidence of these two conditions justifies further research in this area.

8.2 Recommendations for future work

- The study described in chapter four showed that males and females compensate differently to carbohydrate-based drinks in the short term. It appears that females are less able to compensate for energy in the short term. Conclusive reasons for these differences are yet to be established. Research in this area is important as it has practical relevance both from a public health perspective and when formulating dietary guidelines. Further research investigating physiological and neurological differences in compensatory and satiety mechansims between males and females need to be initiated.

- The study described in chapter six showed that the degree of mastication affected individuals' GR to rice but not spaghetti. This suggests that the extent of habitual mastication affects the GR of intact cereals but not processed starchy foods. However, this speculation can be confirmed only after more types of starchy foods (cereals, legumes, nuts and flour-based processed products). Future studies should also evaluate other shapes of pasta to investigate if the absence of a relationship between the degree of mastication and GR of pasta is independent of shape. The findings will in total produce a clearer picture regarding the relationship between mastication and the GR.

- The study described in chapter 7 demonstrated that between-individual variations in the GR and IR can be observed even when all known influential factors up to and including gastric emptying were controlled for.

This suggests that post-gastric carbohydrate digestion and absorption aspects might be varying between individuals. To ascertain this, future studies must use post-gastric intubation methods to place carbohydrate foods directly in the duodenum and subsequently measure the GR, IR and their variations. These studies should compare intestinal glucose uptake rates, hepatic glucose output rates, digestive enzyme secretion and digestion rates. The findings will help to further identify internal factors contributing to glycaemic response variability. It will allow for both better public health recommendations and better controls in future experiments involving GR measurements.

- Food particle size has been shown to inversely impact on gastric emptying. The studies in this thesis showed that the particle size distribution in food swallowed after habitual mastication differed significantly between individuals. It is therefore possible that individuals' degree of breakdown will influence their rate of gastric emptying. Differential rates of gastric emptying will result in different rates of delivery of chyme to the duodenum and this will therefore impact on the rate of glucose appearance in the blood. This has not been investigated. A study must be therefore initiated to determine if differences in particle size breakdown during habitual mastication also influence the gastric emptying rate and its variations.

- Food particle size has also been shown to inversely affect satiety. Therefore, a comprehensive series of studies must be initiated to elucidate the effects of ingested food particle size (following habitual mastication) on satiety. These studies should include subjective satiety (VAS), biochemical

(satiety hormones, blood glucose) and food intake measurements. The findings will highlight the importance of ingested food particle size on satiety and have significant applications when formulating future public health recommendations and intervention strategies for the control of chronic diseases.

Appendices

Appendix 1: The Dutch eating behaviour questionnaire

Subject number:

Dutch Eating Behaviour Questionnaire

Please tick the response which applies best to each of the questions. **All of the results will be strictly confidential and will be available only to the researcher**. This is a previously validated questionnaire. Please answer each question carefully.

Thank-you

1	If you have put on weight, do you eat less than you usually do?	Never	Seldom	Sometimes	Often	Always
2	Do you try to eat less at mealtimes than you would like to eat?	Never	Seldom	Sometimes	Often	Always
3	How often do you refuse food or drink because you are concerned about your weight?	Never	Seldom	Sometimes	Often	Always
4	Do you watch exactly what you eat?	Never	Seldom	Sometimes	Often	Always
5	Do you deliberately eat foods that are slimming?	Never	Seldom	Sometimes	Often	Always
6	When you have eaten too much, do you eat less than usual the following days?	Never	Seldom	Sometimes	Often	Always
7	Do you deliberately eat less in order not to become heavier?	Never	Seldom	Sometimes	Often	Always

8	How often do you try not to eat between meals because you are watching your weight?	Never	Seldom	Sometimes	Often	Always PTO
9	How often in the evening do you try not to eat because you are watching your weight?	Never	Seldom	Sometimes	Often	Always
10	Do you take into account your weight with what you eat?	Never	Seldom	Sometimes	Often	Always
11	Do you have the desire to eat when you are irritated?	Never	Seldom	Sometimes	Often	Always
12	Do you have the desire to eat when you have nothing to do?	Never	Seldom	Sometimes	Often	Always
13	Do you have a desire to eat when you are discouraged or depressed?	Never	Seldom	Sometimes	Often	Always
14	Do you have a desire to eat when you are feeling lonely?	Never	Seldom	Sometimes	Often	Always
15	Do you have a desire to eat when someone lets you down?	Never	Seldom	Sometimes	Often	Always
16	Do you have a desire to eat when you are cross?	Never	Seldom	Sometimes	Often	Always
17	Do you have a desire to eat when you are approaching something unpleasant to happen?	Never	Seldom	Sometimes	Often	Always
18	Do you get the desire to eat when you are anxious, worried or tense?	Never	Seldom	Sometimes	Often	Always
19	Do you have a desire to eat when things are going against you or when things have gone	Never	Seldom	Sometimes	Often	Always

wrong?

20	Do you have a desire to eat when you are frightened?	Never	Seldom	Sometimes	Often	Always
21	Do you have a desire to eat when you are disappointed?	Never	Seldom	Sometimes	Often	Always
22	Do you have a desire to eat when you are emotionally upset?	Never	Seldom	Sometimes	Often	Always
23	Do you have a desire to eat when you are bored or restless?	Never	Seldom	Sometimes	Often	Always
24	If food tastes good to you, do you eat more than usual?	Never	Seldom	Sometimes	Often	Always
25	If food smells and looks good, do you eat more than usual?	Never	Seldom	Sometimes	Often	Always
26	If you see or smell something delicious, do you have a desire to eat it?	Never	Seldom	Sometimes	Often	Always
27	If you have something delicious to eat, do you eat it straight away?	Never	Seldom	Sometimes	Often	Always
28	If you walk past the baker, do you have the desire to buy something delicious?	Never	Seldom	Sometimes	Often	Always
29	If you walk past a snack bar or a café, do you have the desire to buy something delicious?	Never	Seldom	Sometimes	Often	Always

PTO

30	If you see others eating, do you also have the desire to eat?	Never	Seldom	Sometimes	Often	Always
31	Can you resist eating delicious food?	Never	Seldom	Sometimes	Often	Always
32	Do you eat more than usual, when you see others eating?	Never	Seldom	Sometimes	Often	Always
33	When preparing a meal you are inclined to eat something?	Never	Seldom	Sometimes	Often	Always

Appendix 2: The habitual physical activity questionnaire

Subject number:

Habitual Physical Activity Questionnaire

Please circle the response most applicable to each of the statements or answer the questions. **All of the results will be strictly confidential and will be available only to the researcher.** This is a previously validated questionnaire.

Thank You

1.	What is your main occupation?					
2.	At work I sit	never	seldom	sometimes	often	always
3.	At work I stand	never	seldom	sometimes	often	always
4.	At work I walk	never	seldom	sometimes	often	always
5.	At work I lift heavy loads	never	seldom	sometimes	often	always
6.	After work I'm tired	very often	often	sometimes	seldom	never
7.	At work I sweat	very often	often	sometimes	seldom	never
8.	In comparison with others of my own age, I think my work is physically	much heavier	heavier	as heavy	lighter	much lighter
9.	Do you play sport?	Yes	No			
	If Yes: Which sport do you play most frequently ……					
	How many hours a week?	<1	1-2	2-3	3-4	>4
	How many months a year?	<1	1-3	4-6	7-9	>9

If you play a second sport:
Which sport is it?

……..

	How many hours a week?	<1	1-2	2-3	3-4	PTO >4
	How many months a year?	<1	1-3	4-6	7-9	>9
10.	In comparison with other of my own age I think my physical activity during leisure time is	much more	more	the same	less	much less
11.	During leisure time I sweat	very often	often	sometimes	seldom	Never
12.	During leisure time I play sport	never	seldom	sometimes	often	very often
13.	During leisure time I watch television	never	seldom	sometimes	often	very often
14.	During leisure time I walk	never	seldom	sometimes	often	very often
15.	During leisure time I cycle	Never	seldom	sometimes	often	very often
16.	How many minutes do you walk and/or cycle per day to and from work, school and shopping?	<5	5-15	15-30	30-45	>45

Appendix 3: The health questionnaire

Subject number:

HEALTH QUESTIONNAIRE

All of the results will be strictly confidential and will be available only to the researcher

(Please circle as appropriate)

- Date of birth ……..

- Are you allergic to any foods? Yes No
  If yes, which food(s)? .....
  .....

- Do you have intolerance to foods? Yes No
  If yes, which food(s)? .....
  .....

- Do you have a genetic or metabolic disease? Yes No

- Are you taking any medication? Yes No
  If yes, which ones(s)? .....
  .....

- Are you a smoker? Yes No
  If yes, how many cigarettes/day: .....

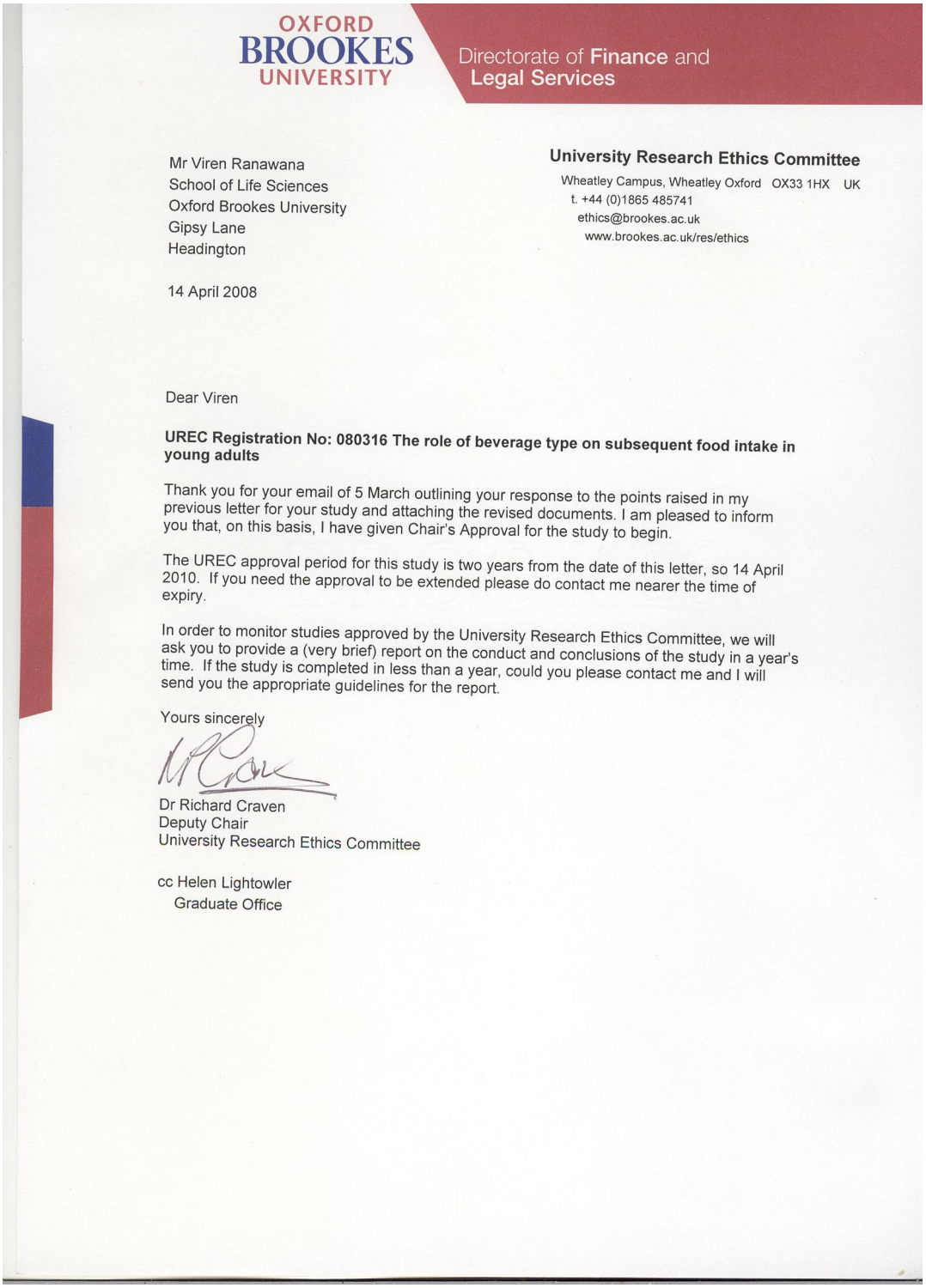
- Are you following a special diet? Yes No
  If yes, which diet(s)? .....
  .....

- Do you exercise or participate in any sports? Yes No
  If yes, times/week:_____ Duration:_____ Intensity:_____

- Are there any foods you dislike? Yes No
  If yes, which food(s)? .....
  .....

Appendix 4: Ethics and consent documents

4.1. Example of a University Research Ethics Committee (UREC) approval letter



OXFORD BROOKES UNIVERSITY

Directorate of **Finance** and **Legal Services**

Mr Viren Ranawana
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Oxford Brookes University
Gipsy Lane
Headington

University Research Ethics Committee
Wheatley Campus, Wheatley Oxford OX33 1HX UK
t. +44 (0)1865 485741
ethics@brookes.ac.uk
www.brookes.ac.uk/res/ethics

14 April 2008

Dear Viren

UREC Registration No: 080316 The role of beverage type on subsequent food intake in young adults

Thank you for your email of 5 March outlining your response to the points raised in my previous letter for your study and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so 14 April 2010. If you need the approval to be extended please do contact me nearer the time of expiry.

In order to monitor studies approved by the University Research Ethics Committee, we will ask you to provide a (very brief) report on the conduct and conclusions of the study in a year's time. If the study is completed in less than a year, could you please contact me and I will send you the appropriate guidelines for the report.

Yours sincerely

Dr Richard Craven
Deputy Chair
University Research Ethics Committee

cc Helen Lightowler
Graduate Office

4.2. Example of a consent form

Consent form

Influence of oral dynamics on the glycaemic response to a food

Contacts:
Professor Jeya Henry, Professor of Human Nutrition
Dr Helen Lightowler, Senior Lecturer in Nutrition
Viren Ranawana, Researcher

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Oxford OX3 0BP
Tel: 01865 483818 / 483245 / 483283
Email: jhenry@brookes.ac.uk / hlightowler@brookes.ac.uk / vranawana@brookes.ac.uk

Please ***INITIAL*** the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	☐	☐
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	☐	☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, or to withdraw any unprocessed data previously supplied.	☐	☐
4. I understand that confidentiality of information provided can only be protected within the limits of the law.	☐	☐
5. I agree to take part in the above research.	☐	☐

Name of Participant ..... Date .....
(block capitals)

Signature .....

Contact number: ..... email:.....

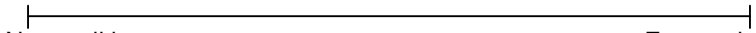
Name of Researcher ..... Date .....
(block capitals)

Signature .....

Appendix 5: Example of visual analogue scales (VAS)

0:00

How hungry do you feel?

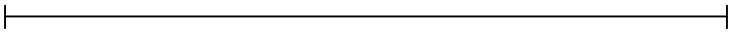


Not at all hungry — Extremely hungry

How full do you feel?

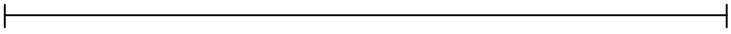
Not at all full — Extremely full

How strong is your desire to eat?



Not at all strong — Extremely strong

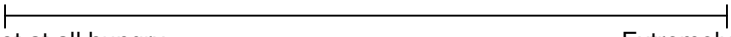
How much food do you think you can eat?



A large amount — Nothing at all

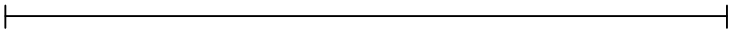
0:15

How hungry do you feel?



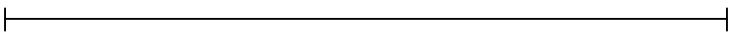
Not at all hungry — Extremely hungry

How full do you feel?



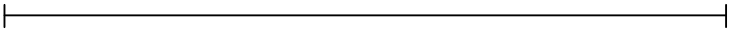
Not at all full — Extremely full

How strong is your desire to eat?



Not at all strong — Extremely strong

How much food do you think you can eat?



A large amount — Nothing at all

Appendix 6: Details of test meals in experiment 2

6.1. Details of food served at breakfast

Food	Portion size (g)	Protein per portion (g)	Carbohydrates Per Portion (g)	Fat Per Portion (g)	Fibre Per Portion (g)	Energy Per Portion (kcal)
Wholemeal bread	40	4	14.1	0.8	2.4	80
White bread	40	9	17.2	2	2.7	83
Corn flakes	40	2.8	34	0.36	1.2	149
Bran flakes	40	4	27	0.8	6	130
Low fat spread	15	0.02	0.42	5.7	0	53
Strawberry jam	15	0	9.6	0	0	39
Marmalade	15	0.03	9.3	0	0	38
Milk	210	8	10	4	0	105
Tea	3	0	0	0	0	
Coffee	5	0.35	1.8	0.01	1.4	3.15

6.2. Food selection in the two menus served for lunch

Menu 1	Menu 2
Beef Lasagne	Spaghetti Bolognese
Baked Potato, baked beans and cheese	Margherita pizza
Fish cakes and chips	Cheese and onion quiche
Egg Mayonnaise sandwiches	Cheese and tomato sandwiches
Houmous and cucumber sandwiches	Tuna mayonnaise sandwiches
Alfresco salad and French dressing	Alfresco salad and French dressing
Yoghurt- 4 flavours	Yoghurt- 4 flavours
Crisps- 4 flavours	Crisps- 4 flavours
Apples	Apples
Oranges	Oranges
Banana	Banana
Plums	Plums
Cadbury brunch bars	Cadbury brunch bars
Alpen strawberry and yogurt bar	Alpen strawberry and yogurt bar
Special K original bars	Special K original bars
Cadbury Milk chocolate	Cadbury Milk chocolate
Twix chocolate bars	Twix chocolate bars
Snickers chocolate bars	Snickers chocolate bars
Total energy: 2678 kcal*	Total energy: 2681*

6.3. Nutrient and energy profiles of foods served at lunch

Food	Portion size (g)	Protein per portion (g)	Carbohydrate Per portion (g)	Fat Per portion (g)	Fibre Per Portion (g)	Energy Per portion (kcal)
Pizza	42	4.3	10.5	3.2	0.5	120
Lasagne	125	5.4	16.8	5.6	1	144
Quiche	50	3.5	8.8	8.8	0.4	125
Fish cakes	42	2.9	9.1	5	0.7	95
Potato chips	100	2.5	28.5	3.8	2.3	172
Alfresco salad	50	0.4	1.8	0.2	0.9	10
Dressing	10	0	0.6	0.3	0	4.6
Baked potato, baked beans and cheddar cheese	185	8.9	25.7	5.6	9.4	170
Spaghetti Bolognese	120	11.8	30.9	4.9	1	319
Egg mayonnaise sandwiches	37.5	2.2	3.1	16.2	1.3	95
Houmous and cucumber sandwiches	37.5	1.5	4.1	3.6	2.4	77
Cheese and tomato sandwiches	40	3.8	3.3	9.3	1.4	93
Tuna mayonnaise	33.8	0.7	3.1	18	1.3	93

sandwiches						
Yogurt (4 flavours)	125	4.6	16	4.0	2	118
Apple	133	0.4	14.4	0.3	2.3	61
Banana	100	1.2	23.2	0.3	1.1	105
Clemantines	80	1.3	12.4	0.1	1.7	30
Plum	60	0.4	5.3	0.1	0.9	22
Cadbury brunch bar	35	2.5	21.4	7.6	0	160
Alpen strawberry and yogurt cereal bar	29	1.7	21	3.3	0.7	119
Special K original cereal bar	23	2	17	2	0.5	90
Twix bar	25	3	18	17	0.4	123
Cadbury's milk chocolate	40	1.3	9.9	5.3	0.28	205
Snickers bar	42	2.8	24	9.7	0.5	215
Crisps (4 flavours)	25	1.6	12.3	8.5	1.8	131

Appendix 7 Questionnaire for determining breakfast and snacking habits, and lunch preferences

Habitual mid-morning snack consumption questionnaire

All of the results will be strictly confidential and will be available only to the researcher

Subject number:

Date:

(1) How often do you have breakfast?

Everyday □
1-2 days per week □
3-4 days per week □
5-6 days per week □
Never □

(2) How often do you have a snack between breakfast and lunch?

Everyday □
1-2 days per week □
3-4 days per week □
5-6 days per week □
Never □

(3) What foods do you usually have as snacks in the mid-morning? Please tick all that are appropriate and state the quantity you usually have.

□ Crisps _____ packs

□ Chocolate _____ bars/pieces/packs

□ Biscuits _____ numbers

□ Fruits
Banana ____
Apples/Pears ____
Orange/Citrus ____
Grapes ____
Plums ____
Others (please specify) _____ ____

□ Cereal bars _____ numbers

□ Muffins/cake/flapjacks etc. _____ numbers

□ Juice/ Soft drinks _____ bottle/can

□ Other (please specify)

(4) What do you usually have for lunch on weekdays?

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