DISSECTING THE EFFECTS OF SWEET TASTANTS IN THE HUMAN GUT-BRAIN AXIS

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

2013

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CHAPTER 3

The role of the gut sweet taste receptor in mediating the responses to glucose: an exploration using lactisole

Word count: 47,710

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ABBREVIATIONS

Ace-k	Acesulfame-k
AgRP	Agouti-related peptide
αMSH	α- melanocyte-stimulating hormone
ANOVA	Analysis of variance
AP	Area postrema
ARC	Arcuate nucleus
AS	Artificial saliva
AUC	Area under the curve
BBB	Blood brain barrier
BMI	Body mass index
BOLD	Blood oxygen level dependent
CART	Cocaine and amphetamine related transcript
CCK	Cholecystokinin
CNS	Central nervous system
CoM	Centre of mass
DMN	Dorsal motor nucleus
DPP4	Dipeptidyl peptidase IV
DVC	Dorsal vagal complex
EDTA	Ethylenediaminetetraacetic acid
EEC	Enteroendocrine cells
ELISA	Enzyme-linked immunosorbent assay
ENS	Enteric nervous system
Exendin-4	Exenatide
FWEc	Family wise error corrected
fMRI	Functional magnetic resonance imaging
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
GLUT2	Glucose transporter 2
GPCR	G-protein coupled receptor
IPANs	Intrinsic primary afferent neurons

IRIS	Infra-red isotope spectrophotometer
kcal	Kilocalorie
LHA	Lateral hypothalamic area
MCR3/4	Melanocortin receptor 3/4
ME	Median eminence
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
Nac	Nucleus accumbens
NG	Nasogastric
NNS	Non-nutritive sweetener
NPY	Neuropeptide Y
NTS	Nucleus of the solitary tract
OFC	Orbitofrontal cortex
OXM	Oxyntomodulin
PET	Positron emission tomography
PFC	Prefrontal cortex
physMRI	Physiological magnetic resonance imaging
POMC	Pro-opiomelanocortin
PP	Pancreatic polypeptide
PVN	Paraventricular nucleus
PYY	Peptide YY
RPM	Revolutions per minute
SD	Standard deviation
SEM	Standard error of the mean
SGLT1	Sodium-glucose transporter 1
SPM	Statistical parametric mapping
SPSS	Statistical Package for the Social Sciences
TCA	Time clustering analysis
TFEQ	Three factor eating questionnaire
VAS	Visual analogue scale
VMH	Ventromedial hypothalamus

ABSTRACT

The importance of nutrient induced gut-brain signalling in the regulation of human food intake has become increasingly apparent as the obesity epidemic progresses. Much of the caloric excess consumed comes from dietary sugars, but our knowledge about the mechanisms mediating the physiological and appetitive effects of sweet tastants in the gut-brain axis is far from complete. The comparative effects of natural sugars *vs.* artificial non-nutritive sweeteners are also poorly understood. Research in animal and cellular models has suggested a key role in the gut for the sweet taste receptors previously well described in the mechanisms of oral taste. The work presented in this thesis sought to answer key questions initially based on the hypothesis that gut sweet taste receptors also play a key role in the human gut-brain axis.

The key aims were to elucidate i) whether sweet taste receptors in the gut contribute to the effects of sweet tastants in the human gut-brain axis, and ii) whether oral sweet taste modulates gut physiology and/or gut-brain signalling.

Fifty-eight (36 males and 22 females) young (23.3 $\pm$ 3.4 years) participants were recruited into four studies. All were healthy and generally lean (BMI 22.3 $\pm$ 1.9). Key methodologies used included gastric emptying, appetite and satiety scores, food intake, blood hormone and glycaemic responses, and functional brain imaging.

In chapter 3, a sweet taste receptor antagonist, lactisole, was used as a tool to investigate the role of gut sweet taste receptors in mediating the responses to glucose. However, lactisole had no impact on gastric emptying (a proxy measure of gut-brain signalling), blood glucose, gut hormones, appetite ratings or food intake. The data outlined in chapter four revealed that ingesting non-nutritive sweeteners, (aspartame, saccharin, and acesulfame-k) in combination with glucose did not enhance glycaemic responses or affect appetite ratings. However, the studies presented in chapter five demonstrated that the pattern and rate of gastric emptying of glucose very clearly differed depending on whether it was given orally or administered intragastrically. The interaction between oral and gastrointestinal sweet stimuli on brain activation was therefore investigated using functional brain imaging, and demonstrated that an oral pre-taste of glucose had a marked impact on subsequent brain responses to an intragastric glucose load. Effects were observed in homeostatic and non-homeostatic regions.

These data offer little evidence that gut sweet taste receptors are important in humans: a non-taste pathway appears more likely to mediate the effects of glucose. However gut-brain signalling is markedly affected by oral sweet taste receptors. This has direct relevance for a better understanding of healthy human nutrition. Future studies need to investigate these interactions in more detail, using a wider panel of nutrients and tastants in health and disease.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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ACKNOWLEDGMENTS

I recognise this research would not have been possible without the financial support from the Biotechnology and Biological Sciences Research Council.

I thank both my supervisors, Professor John McLaughlin and Dr Shane McKie, for giving me the opportunity to undertake this PhD. I am very grateful to Professor McLaughlin for his time, guidance and advice during my research training and Dr McKie who has given a lot of time to mentor me through complicated imaging analysis.

Before I thank individuals I would like to acknowledge each one of the participants who took part and gave up their time to enable this research. There are many people who have assisted me in the research described herein. Firstly, I must say a big thank you to the research nurses Melanie Taylor and Ann Keene who were always on hand to assist with tricky cannulations or nasogastric tubes! I would also like to thank the Wellcome Trust Research Facility and in particular Lisa Westwood who helped to get the study set up and running there. In addition I would like to thank Barry Withnall and the radiography team at the Translational Imaging Unit for their assistance with my imaging study.

My PhD experience would have been very different and definitely not as enjoyable had I not worked alongside Dr Nerys Astbury in my first year and Dr Lucy Wasse for the remainder of my PhD. Nerys helped me at the start of my PhD and gave me the confidence to start my own research studies. Lucy has helped in the lab with studies and more than anything has been a great friend and support. I have enjoyed sharing an office, drinking endless cups of coffee and will miss working alongside her.

Finally, I can't thank my parents enough for their love and support and for always being there for me. It has been a tough four years for them and I am incredibly lucky to have an amazing family. I know this means as much to them as it does to me.

PREFACE

I graduated from Durham University in 2007 with a BSc in Health and Human Sciences and went on to complete a Masters in Human Nutrition at Sheffield University in 2008. I was appointed as a public health nutrition Research Associate by the Technical University of Denmark in 2008 until starting my PhD in 2009.

The findings within this thesis have been peer reviewed as follows:

Chapter 4

Bryant C, Wasse L, Astbury N, McLaughlin J, 2013, Non-nutritive sweeteners: no class effect on the glycemic or appetite responses to ingested glucose, European Journal of Clinical Nutrition.

Two further manuscripts are in preparation based on the work in chapters 3 and 5.

CONFERENCE PRESENTATIONS

Chapter 3

The role of sweet taste receptors in the gut and the effect on appetite and energy intake in humans, The Rank Prize Funds Symposium on Nutrient Sensing and Signalling, Windermere 2010.

Does the gut taste sweet? Biosciences KTN Food Sector Event, London 2013

Chapter 5

Nutrient induced gut-to-brain signalling in humans, Appetite Volume 57, The Society for the Study of Ingestive Behavior, Florida 2011.

Effect of sequential oral and gastrointestinal stimulation on brain BOLD responses to glucose, The 37th Congress of the International Union of Physiological Sciences, Birmingham 2013.

CHAPTER 1

Literature Review

1.1 Introduction

Obesity is a global health problem and its prevalence continues to rise, with the World Health Organisation estimating that 2.3 billion people worldwide will be overweight (body mass index (BMI) $\geq$ 25kg/m^2) and 700 million obese (BMI $\geq$ 30 kg/m^2) by the year 2015 (Source: World Health Organisation). Recognised as one of the most significant contributors to ill health, obesity, and its associated chronic diseases and economic burden, highlight the need for cost-effective strategies and therapies for prevention and to enable safe and sustainable weight loss (Trueman et al., 2010). At present, efforts to tackle the ever increasing obesity epidemic, currently restricted to only one licensed drug and gastric banding or bypass surgery, are limited by cost and risk presenting as impractical solutions for a global problem (Hussain and Bloom, 2011, Hussain and Bloom, 2013).

Studying the genetics of obesity has identified individuals' predisposition to gain weight but does not account for the exponential increase in the Westernised world (Saris and Foster, 2006) suggesting that lifestyle and environment play a huge part in driving the epidemic. If food is not scarce, eating is entirely behavioural (Blundell, 2006). In the current obesogenic environment (Lake and Townshend, 2006) it is important to piece together the physiological mechanisms and environmental pressures that ultimately translate into patterns of eating. The body's ability to modulate energy intake and match energy requirements from meal to meal is under strict control (Frayn, 2003) particularly as, in most human bodies, weight remains relatively constant despite constant variations in food intake, meal frequency, meal volume, energy density and expenditure (de Graaf et al., 2004). That said, an excess in energy intake relative to energy expenditure results in an increase in the amount of energy stored.

The brain is the key regulator in appetite control but its interaction with the gastrointestinal tract, and the signals emanating from it, forces us not to study either in isolation. The gut–brain axis and its regulation of food intake is a complex system that enables the cross-talk between peripheral and central mechanisms that influence hunger and food intake in response to environmental and behavioural stimuli.

Dietary influences are a contributing factor to the obesity epidemic. The increasing consumption of sugar and non-nutritive sweeteners (NNS) and the effect on food intake and body weight have been under debate (Mattes and Popkin, 2009). One key area warranting further investigation is the mechanisms by which sugars and NNS, present in the everyday diet, influence gastrointestinal signalling to the brain and therefore appetite and food intake.

Despite significant advances in our understanding of these mechanisms controlling food intake, our knowledge is far from complete. The precise mechanisms by which sugars and NNS are sensed within the gut, and the exact signals to the central nervous system (CNS) to modulate appetite and energy intake are poorly defined and thus form the basis of this thesis. This review focuses on what is known about the mechanisms mediating the effects of carbohydrate, in particular sugar and NNS, on appetite and food intake.

The review will start by describing appetite and feeding behaviour and the oral, gastric and intestinal factors that influence them. Thereafter, the way signals outlined in the previous sections are integrated in key neurological pathways to influence appetite and food intake are discussed. Finally, the study of feeding behaviour is reviewed. Where possible, priority is given to human studies with results from animal work referred to in the absence of human data or where major differences occur.

1.2 Feeding behaviour

Feeding behaviour can be involuntary as well as conditioned and may be triggered by both external and internal stimuli (de Graaf et al., 2004). Social, environmental and emotional state can effect intake, visual and olfactory cues that generate the

positive hedonic feeling, and reward associated with the initiation and continuation of eating (Berthoud and Morrison, 2008). The internal driving force for the search, choice and ingestion of food is appetite (de Graaf et al., 2004) and the response leading up to, during and following an eating episode is feeding behaviour. Eating episodes are defined by the amount of food and drink consumed. This is also known as energy or food intake which will be used interchangeably throughout this thesis.

1.2.1 Appetite

Although most people would believe they understand the concept of appetite, it is difficult to define scientifically and may not always be physiologically accurate. However, appetite provides a conceptual framework, for research purposes, to examine the impact of foods or nutrients on feeding behaviour. There are two main definitions of appetite in science proposed by Blundell and colleagues. The first encompasses all aspects of food intake, including selection, motivation and preference, while the second relates specifically to the response to environmental stimuli in contrast to eating in response to physiological stimuli (Blundell et al., 2010). Although appetite is likely to change the occurrence of eating episodes it does not necessarily calculate or infer the amount eaten. For this reason, appetite is difficult to quantify and measure and thus subjective sensations (i.e. fullness and hunger) are used to interpret the motivational state prior to, during and after an eating episode. Fullness and hunger are terms often used within appetite research and can be defined as a fullness sensation in the stomach (Sorensen et al., 2003) and a conscious sensation, often physical, reflecting a mental urge to eat (de Graaf et al., 2004). Hunger can also be defined as the want of food which in a broad sense reflects the feeling of desire to eat, another term often used in appetite research. Arguably the latter can be experienced in the absence of energy deficit and influenced by psychological and environmental stimuli.

1.2.2 Satiation and satiety

Although equally difficult to strictly encapsulate, ‘satiation’ has been defined as the process that leads to the termination of an eating episode to control meal size (intra-meal satiety) whilst ‘satiety’ is the process that leads to inhibition of further eating or decline in hunger (inter-meal satiety) (Cummings and Overduin, 2007). A working model for examining the impact of foods on satiation and satiety is the ‘Satiety Cascade’ (figure 1.1) proposed by Blundell more than twenty years ago, but still relevant today. The cascade demonstrates the coordination of feeding behaviours through the processes that bring about the termination of eating (satiation) and inhibit further eating (satiety) to regulate size and frequency of eating episodes (Blundell, 1991). Factors influencing the processes of satiation and satiety are considered below.

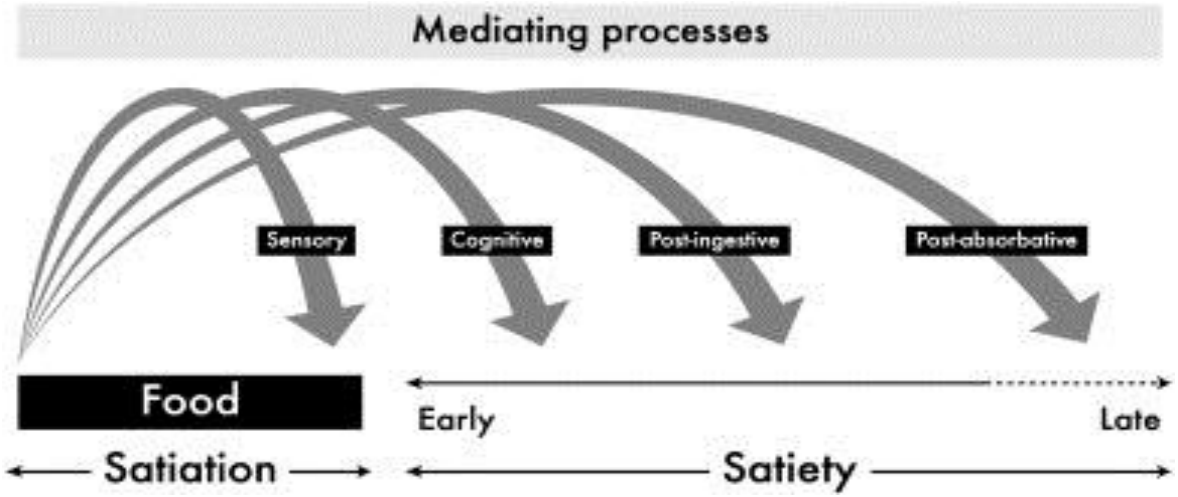


Figure 1.1 The Satiety Cascade

The control of appetite, size and frequency of eating episodes, is determined by satiation and satiety which operate together. The process of satiation occurs during an eating episode to terminate the meal while satiety arises following the episode to inhibit further eating. Both are influenced by internal physiological systems and the external environment. (Blundell, 1991)

1.3 Factors influencing appetite and energy intake

Food is sensed and processed to generate signals that are utilised for the control of appetite by way of processes including sensory, cognitive, post-ingestive and post-absorptive (figure 1.1) (Blundell et al., 1994). These processes represent a well-controlled but complex system that monitors and responds to acute changes in energy balance, and signals to reflect the body's energy status. Signals reflecting energy status can be distinguished by those that are tonic, which reflect the status of energy stored and tend to be longer term, and those that are episodic which arise from the gastrointestinal tract in response to eating episodes and fluctuate considerably within a day (Blundell, 2006). The integration of these signals by the brain, as discussed later in section 1.5, enables the coordination of feeding behaviour.

Episodic signals are synchronised with eating episodes so that during the course of an eating occasion the gastrointestinal tract can efficiently digest and absorb nutrients from ingested foods (Blundell, 2006). The gastrointestinal tract is optimised to carry out these functions via motility (particularly the regulation of gastric emptying) and neuroendocrine secretions, and in doing so can affect and homeostatically regulate the short term control of appetite and energy intake. The potential mechanisms underlying the effects of carbohydrate on food intake involve processes generated by oro-sensory and gastric stimulation preceding the interaction with receptors in the small intestine and associated satiety hormone release.

1.3.1 Oral factors

Although there are anticipatory cephalic responses to the sight or smell of food, the oral cavity is the first physical point of contact with food to be ingested. Here the sensory properties (e.g. taste and texture) are evaluated and innate physiological responses, such as secretion of saliva and gastric acid, prepare the gastrointestinal tract for receiving and processing of the food that is about to be eaten (Power and Schulkin, 2008). Oral sensations and salivary secretomotor responses are mediated via the facial and glossopharyngeal nerves, whilst gastric responses are affected by vagal pathways. The sensory properties of foods can determine palatability,

classified as how pleasant or liked the food is, and are important contributors to food selection and amount of food eaten (Sorensen et al., 2003). Increasing the palatability of a food can increase intake of that food (de Graaf et al., 1999). Furthermore, humans consume more from meals containing a variety of foods than they would from meals of a single food caused by the sensory specific satiety phenomenon defined as decreased pleasantness of an eaten food in contrast to the pleasantness of an uneaten food (Rolls et al., 1981). This relates to the hedonic aspects of the control of food intake involving the processing of sensory properties of food in the brain (discussed later in section 1.5.3.1). However, this is not an isolated process as decisions made will also be in the context of short term physical and physiological signals originating from other areas of the gastrointestinal tract.

Taste originates in the oral cavity via taste buds that house taste cells. These cells can be classified into four types depending on their features, with type II cells enabling sensing of sweet, umami and bitter foods (Iwatsuki and Torii, 2012). Investigations into the function of each cell type have led to the discovery of taste cell-specific molecules and receptors that are specific to each taste quality.

1.3.1.1 Sugars, non-nutritive sweeteners and sweet taste receptors

Sweetness is one of the five tastes that humans experience, along with sour, bitter, salty and umami, and in evolutionary terms is critical for determining whether a potential food encountered is nutritious or potentially toxic. The mechanisms for sweet taste have been well defined. Sweetness perception involves two G protein receptors (GPCR), T1R2 and T1R3, which dimerize to form the sweet taste receptor (Sclafani, 2007). Stimulation of the T1R2+T1R3 receptor by sugars or NNS, which are agonists at T1Rs, activates intracellular signalling elements such as α-gustducin. This in turn leads to activation of gustatory nerves transmitting sensory information to the brain (figure 1.2). Lactisole, the sodium salt of 2-(4-methoxyphenoxy)-propionic acid, a sweet taste receptor antagonist, blocks the sweet taste of several compounds including sugars (fructose, glucose, sucrose) and NNS (aspartame, acesulfame-K, saccharin) (Schiffman et al., 1999) by interacting with T1R3 and preventing conformational changes required to exert sweet taste upon T1R2+3

activation (Jiang et al., 2005, Schiffman et al., 1999). Lactisole is patented as a sweetness inhibiting agent, commercially available as an artificial flavour and approved for use in confectionary and snack foods (Sclafani and Pérez, 1997). Reducing the sugar content would perhaps be a wiser strategy for industry to consider in terms of health benefit.

Sweet food and drinks are highly palatable for most people and potent drivers to eat in humans (Bellisle et al., 2012) although there are individual differences in optimal levels of sweetness (Sorensen et al., 2003). Biochemically, simple sugars can be classified as monosaccharides, such as glucose, fructose, galactose and tagatose, and disaccharides such as lactose (glucose + galactose) and sucrose (glucose + fructose). The consumption of caloric sweeteners within beverages and soft drinks in particular has been associated with excess energy intake and weight gain (Vartanian et al., 2007). It is hypothesised that their high palatability can prompt overconsumption, especially when there is no adequate limitation on availability.

In an attempt to combat the consumption of caloric sugars as the main source of sweetness, there has been a marked increase in the use of NNS by the food and beverage industry albeit in the absence of compelling evidence. NNS provide no/low energy alternatives to sweetening foods and beverages with energy rich sugars, particularly sucrose and fructose which are the main simple sugar ingredients in our diet. However, the uncoupling of sweetness and energy intake has also been implicated in the obesity epidemic as it is claimed intake of NNS can dysregulate the body's metabolic systems (Pepino and Bourne, 2011) and may lead to over consumption at subsequent eating occasions regardless (Blundell et al., 1994).

The effect of sugar and NNS on peripheral and central appetite signals will be discussed in further detail in sections 1.4.2 and 1.5 respectively. In addition, sweetness and reward–relating brain signalling and the effect on appetite and energy intake will be discussed in section 1.5.3.1.

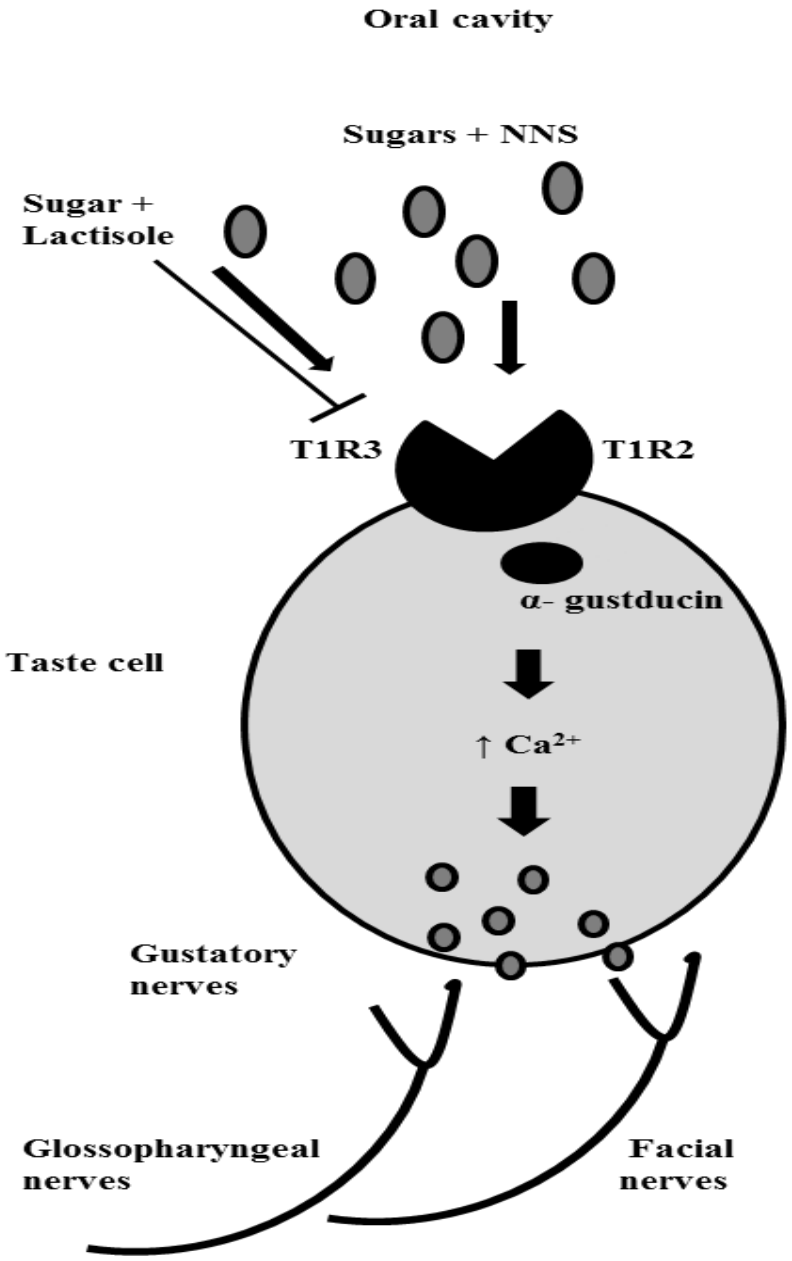


Figure 1.2 Schematic representation of an oral taste receptor cell

Adapted from (Cummings and Overduin, 2007). Sweet tastants, sugars and non-nutritive sweeteners (NNS), stimulate sweet taste receptor cells comprising of T1R2 and T1R3 of the tongue. This activates intracellular signalling pathways such as α-gustducin (others not shown) which in turn leads to an increase in intracellular calcium (Ca^{2+}). Rising intracellular Ca^{2+} triggers gustatory nerve terminals which relay sensory information to the brain. Lactisole inhibits sweet taste perception by binding to T1R3.

1.3.2 Gastric factors

The functional anatomy of the stomach can be divided into three regions; proximal, which acts as a reservoir for ingested foods, the distal compartment which mixes and grinds solid food and the pylorus which further modulates the emptying of the stomach contents into the small intestine (Rayner et al., 2001, Hellström et al., 2006). Nutrient liquids or water alone empty from the stomach from the outset of consumption, whilst emptying of solids follows once they have been processed into smaller particles, a process termed trituration (Horowitz et al., 1994). The difference between the emptying of solid and liquid food is depicted in figure 1.3.

Increasing the volume of gastric contents induces mechanical gastric distension which activates neural stretch receptors in the gastric wall and is associated with satiety and reduced food intake (Oesch et al., 2006). This is principally mediated by vagal reflexes. A role for gastric distension in inducing satiation shown was demonstrated by a reduction in food intake observed when a gastric balloon was inflated in the stomach of human subjects in the absence of any nutrients (Geliebter et al., 1988). However, this invasive technique may cause discomfort to subjects and its placement unquestionably interferes with normal physiology and feeding behaviour. A subsequent study involving the intragastric administration of preloads varying in volume, and/or energy, found a significant reduction in food intake with 400ml preloads compared to 200ml, but no effect of energy intake when volume was kept constant (Rolls and Roe, 2002) demonstrating the potent effect of gastric volume on satiation. However, the use of liquid gastric preloads cannot exclude the effects of intestinal factors. Oral, gastric and post-gastric signals are likely to occur simultaneously, with as much as 40% of a liquid meal emptied from the stomach prior to meal termination (Kaplan et al., 1992). Gastric emptying progressively reduces the volume of food in the stomach which decreases gastric distension and is thought to contribute to the return of hunger (Oesch et al., 2006). Therefore, the uncoupling of energy content and volume of a food is important. Increasing the energy density of food without an increase in volume may lead to increased energy intake as more energy is consumed compared to lower energy dense foods. Furthermore, it is thought that when consumed in a liquid form food/nutrients are less effective than solid counterparts at inducing satiety, and, since there is no

mechanism to sense ingested calories, the increased energy is often not compensated for at subsequent eating occasions leading to an increased intake (DiMeglio and Mattes, 2000).

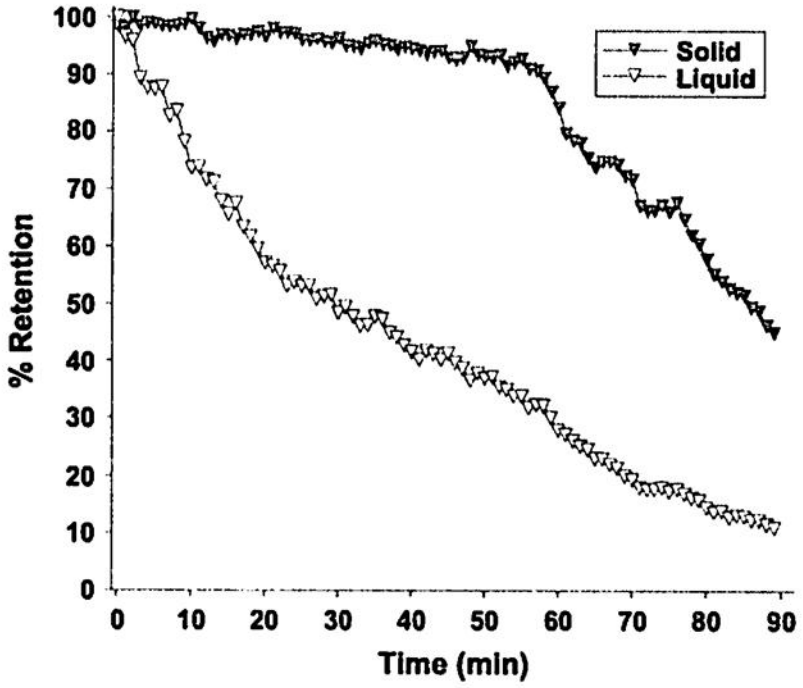


Figure 1.3 Gastric emptying curves for a solid and liquid food test meal

(Hellström et al., 2006). *Differing rates between the emptying of liquid and solid meals from the stomach show liquid meals begin emptying immediately whereas solid meals begin after a lag phase during which solid food components are broken down into smaller particles.*

The nutrient composition of gastric contents emptying into the small intestine and the effects on energy intake have also been studied using rats fitted with an inflatable pyloric cuff preventing the emptying of stomach contents into the small intestine (Ritter, 2004). Although the distension caused by the retaining of stomach contents contributed to satiation, rodents still consumed large quantities suggesting intestinal factors play a role. The hypothesis is that the rate of gastric emptying is determined by feedback from the small intestine and not by signals arising from the stomach

itself. It is believed that the nutrient composition of ingested food impacts on satiation and satiety using a post gastric feedback mechanism whereby gastric emptying will be delayed and maintain gastric distension (Phillips and Powley, 1996). The stomach is sensitive to physical changes such as tension and volume, the output of which is relayed to the brain by vagal and spinal nerves (Phillips and Powley, 2000).

1.3.3 Intestinal factors, absorption and post-absorptive mechanisms

The structure of the inner small intestinal surface is optimised for absorption due to the large surface area provided by many villi contained within it. The villi are lined with enterocytes and resident to specialised epithelial cells known as enteroendocrine cells (EEC). The small intestine responds to ingested food via mechano-sensitive pathways but is mainly reliant upon EEC in the mucosa recognising luminal content by chemosensory mechanisms and responding accordingly (Farre and Tack, 2013). The length and region of the small intestine exposed to nutrients is believed to have influence on gastric emptying, appetite and energy intake (Lin et al., 1989). The presence of nutrients in the small intestine induces a feedback mechanism that controls the rate of emptying from the stomach via the enhancement of gastric distension (Geliebter, 1988). Studies have demonstrated that intestinal carbohydrate infusions can increase perceived fullness and reduce subsequent intake (Cook et al., 1997, Lavin et al., 1996, Lavin et al., 1998) suggesting signals arising from the lumen of the small intestine impact on satiety.

In the small intestine, glucose absorption is controlled via two transporters; the active transporter sodium-glucose co-transporter (SGLT1) and the facilitative glucose transporter 2 (GLUT2) (Kellett and Helliwell, 2000). SGLT1 functions to transport glucose from the gut lumen into the enterocytes. This is particularly important at low glucose concentrations as the expression of SGLT1 is proportional to the amount of glucose in the lumen, thus glucose absorption is related to the amount available (Renwick and Molinary, 2010). GLUT2 functions to transfer intracellular glucose into the general circulation. Higher glucose concentrations in the gut lumen lead to increased GLUT2 synthesis and expression in the apical

membrane to provide a mechanism via which absorptive capacity is promptly matched to dietary intake (Kellett and Helliwell, 2000). Together these transporters enable mechanisms of glucose absorption including uptake from the gut lumen into the hepatic portal vein and the transfer from blood into tissues including the brain (Renwick and Molinary, 2010).

Substantial variations in the day to day diet lead to constant changes in glucose levels making it imperative that epithelial cells sense, respond and regulate their function appropriately. Blood glucose increases following carbohydrate ingestion and glucose uptake, and has long been related to hunger and food intake (Mayer, 1955). Mayer proposed the glucostatic theory for short term appetite regulation which postulates that feeding is initiated when blood glucose utilisation is low (Mayer, 1955).

Intraduodenally administered glucose reduces energy intake and hunger more than intravenously administered glucose, despite comparable plasma glucose concentrations. Furthermore, this effect can be abolished when gut hormone secretion is inhibited with octreotide (Lavin et al., 1996). This suggests the appetite suppressing effects of intestinal glucose are not regulated by blood glucose but more likely a result of small intestinal stimulation leading to either direct vagal stimulation and/or the release of satiety hormones.

1.3.4 Signals arising from the gastrointestinal tract

The gastrointestinal tract is the largest endocrine organ that synthesises and releases orexigenic (increasing food intake) and anorexigenic (reduces food intake) hormones to influence a number of physiologic processes and regulate gastrointestinal function (Murphy and Bloom, 2006, Wren and Bloom, 2007). Identifying the precise mechanisms by which gut hormones are stimulated and operate has received a lot of attention, in particular their role in appetite control and therapeutic potential given their influence on hunger and satiety prior to meal initiation and during the postprandial period (Murphy and Bloom, 2006). The response of the gastrointestinal tract to incoming nutrients is likely to be a coordinated response to achieve the effects on food intake particularly for those hormones that share signalling pathways

and release mechanisms. The vagus nerve, for example, expresses receptors for both orexigenic and anorexigenic hormones that are involved in long and short term control (Raybould, 2007).

Ingestion of carbohydrate increases the blood glucose concentration and stimulates the release of a number of gut hormones that have a fundamental role in food intake (Feinle et al., 2002). Cholecystokinin (CCK) was the first gut hormone to be implicated in the short term regulation of food intake (Kissileff et al., 1981) and remains one of the most extensively studied to date. However, although glucose has shown to slightly stimulate the release of CCK (Little et al., 2006a, Gerspach et al., 2011), its release and subsequent effect on gastric emptying is generally associated more with the ingestion of fat and protein rich meals (Lal et al., 2004) so will not be discussed in detail at this stage.

Ingestion of carbohydrate more classically stimulates the release of glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) and to a lesser extent peptide YY (PYY). In addition, the release of GLP-1 and GIP stimulates glucose dependant insulin secretion from beta-cells of the pancreas. Circulating insulin promotes glucose uptake into cells for utilisation and has been implicated in the long term regulation of energy balance (Suzuki et al., 2012). Furthermore, insulin acts within the CNS with receptors widely expressed in appetite regulating centres within the brain (Pliquett et al., 2006).

The specific roles of GLP-1 and PYY in food intake are considered in greater detail below. A brief overview of other gastrointestinal hormones involved in the short term regulation of appetite control is outlined in table 1.1.

1.3.4.1 GLP-1

GLP-1 is a hormone released postprandially by enteroendocrine L-cells in the distal small intestine and colon in response to ingested nutrients, particularly glucose (Herrmann et al., 1995). Following a meal, GLP-1 is released in two phases. The first rapid release occurs approximately five to ten minutes and the second extended release 30 to 60 minutes postprandially (Baggio and Drucker, 2007). It is hypothesised that the first phase of GLP-1 release is likely to be the result of a

proximal to distal signalling pathway due to GLP-1 secreting L cells not having had direct contact with ingested nutrients at this initial stage (Roberge and Brubaker, 1993). The second phase of GLP-1 release is associated with direct stimulation of the L cells by luminal contents (Lim et al., 2009). GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase IV (DPP4) to an inactive form. GLP-1 enhances glucose-stimulated insulin secretion from pancreatic islet beta cells, known as the incretin effect, and suppresses glucagon secretion from islet alpha cells (Baggio and Drucker, 2007). Thus, it has important effects on attenuating the rise in post-prandial glucose, responses not seen when glucose is infused intravenously. Furthermore, the release of GLP-1 and the subsequent rise in plasma levels following nutrient ingestion are thought to play an important part in the slowing of gastric emptying (Little et al., 2006b) and postprandial satiety (Stanley et al., 2004). Intravenous administration in humans has demonstrated a suppression in appetite and decrease in energy intake in some cases (Stanley et al., 2004, Verdich et al., 2001) but not all (Long et al., 1999). A meta-analysis of the effect of GLP-1 infusion in human subjects reported a dose dependent decrease in calorie intake (11.7%) which was effective in both lean and obese subjects (Verdich et al., 2001).

The incretin properties of GLP-1 have gathered interest particularly in the treatment of type 2 diabetes and more recently for its role in aiding weight loss (Shyangdan et al., 2010). Exenatide (exendin-4) is a GLP-1 receptor agonist that is resistant to DPP4 degradation. Its use along with other DPP4 resistant analogues have shown improved glycaemic control in type 2 diabetics and sustained weight loss (Vilsbøll et al., 2012), albeit with some subjects finding it hard to tolerate due to upper gastrointestinal symptoms including nausea.

GLP-1 mediates its effects on the CNS via GLP-1 receptors activating neurons in the area postrema (AP), nucleus of the solitary tract (NTS) of the brainstem which receives vagal afferent inputs (D'Alessio, 2008), and the arcuate nucleus (ARC) and paraventricular nucleus (PVN) of the hypothalamus (Chaudhri et al., 2006).

It has been suggested that GLP-1 may be a useful biomarker of satiation (de Graaf et al., 2004). It is possible that consuming foods which enhance GLP-1 response may reduce energy intake at that eating occasion and subsequent eating episodes.

However, such foods would need to be retained in the small intestine for longer in order to more potently stimulate distal intestinal GLP-1 release.

1.3.4.2 PYY

Peptide YY (PYY) circulates in two forms in human blood; PYY_{1-36} and PYY_{3-36} with the latter constituting the majority of total circulating PYY in both a fasted and postprandial state (Grandt et al., 1994). PYY is released postprandially from L cells in the distal gut in proportion to calories ingested and binds to the Y2 receptor in the ARC of the hypothalamus to inhibit the release of the appetite stimulant neuropeptide Y (NPY) (Batterham et al., 2002). Its levels are low in the fasted state and remain elevated postprandially for several hours.

Obese subjects have lower fasting PYY concentrations and demonstrate blunted PYY responses (Batterham and Bloom, 2003). However, obesity is not associated with a resistance to PYY as exogenous infusion leads to a reduction in intake (Doggrell, 2004). Peripheral administration of PYY has reduced food intake and corresponding subjective ratings of hunger and satiety in both obese and lean subjects (Batterham et al., 2002, Batterham et al., 2003a) the effects of which have been demonstrated to last for up to 12 hours after infusion (Batterham et al., 2004).

PYY is released predominantly following protein- and fat-rich meals but glucose also stimulates its release (Stanley et al., 2004). PYY is an important mediator of gastric emptying by the inhibition of proximal intestine and gastric motor activity following nutrient stimulation (Camilleri and Grudell, 2007). Furthermore, its elevation following gastric bypass surgery has been implicated in the mechanism of weight loss following surgery (le Roux et al., 2007) and has the potential to emerge as an anti-obesity drug.

1.3.4.3 Other gut hormones

A number of other gut hormones have been implicated in the control of appetite and food intake. For brevity an overview of these hormones is outlined in table 1.1.

Hormone	Source	Main Stimulus	Action(s)	Effect on food intake	Therapeutic potential
Amylin	Pancreatic β cells	Carbohydrate	Slows gastric emptying Inhibits gastric secretion	↓	Analogue currently associated with improved glycaemic control and weight loss in diabetics (Ratner et al., 2004). Treatment for obesity under investigation.
CCK	Intestinal I cells	Fat Protein	Stimulates gall bladder contraction Inhibits gastric acid secretion	↓	Repeated administration of an agonist failed to support long term use with no effect on weight loss (Jordan et al., 2007).
Ghrelin	Gastric A cells	Fasting	Meal initiator Promotes gastric motility Promotes pancreatic polypeptide (PP) release	↑	Antagonists have been used in preclinical studies to show decreased food intake (Beck et al., 2004)[1]. Treatment for obesity under investigation. Potential role to stimulate eating in disease associated anorexia (Guillory et al., 2013).
GIP	Intestinal K cells	Carbohydrate Fat	Enhances insulin secretion	→[2]	Antagonising GIP action has been proposed as anti-obesity therapy (Miyawaki et al., 2002)[1] but no established therapeutic potential for humans.
Oxyntomodulin (OXM)	Intestinal L cells	Fat	Suppresses ghrelin Slows gastric emptying	↓	OXM administration reduced energy intake and resulted in weight loss (Wynne et al., 2005). Treatment for obesity under investigation.
PP	Pancreatic F cells	Protein Fat	Slows gastric emptying Reduces appetite	↓	Effects on appetite prolonged over 24 hour period. (Batterham et al., 2003b). Analogue has been developed and is currently under investigation in clinical trials (Derosa and Maffioli, 2012).

Table 1.1 Overview of gastrointestinal hormones involved in appetite and food intake control
↑ Increase ↓ Decrease

[1] Animal model
[2] No evidence to support a major role in appetite and food intake control

1.4 Does the gut 'taste' sweetness?

Nutrient sensing by the cells of the gastrointestinal tract initiates a cascade of events involving hormonal and neural pathways to induce digestion, the absorption of nutrients and the control of energy intake (Rozengurt and Sternini, 2007). As previously discussed, the presence of nutrients in the small intestine is associated with decreased perceptions of hunger and decreased energy intake (Welch et al., 1988) mediated by the modulation of gastric emptying, motility and the stimulation of gastrointestinal hormones (Buchan, 1999). Furthermore, the action of specific macronutrients to limit food intake varies suggesting the intestine may be able to sense the presence of a particular nutrient. EEC are likely to be the first level of integration of input from the gut lumen (Sternini et al., 2008) acting as primary chemoreceptors to sense luminal contents and release signalling molecules. The possible mechanisms underlying the chemosensory properties of EEC are depicted in figure 1.4. However, EEC and their role in nutrient sensing are difficult to study in humans as it is not possible to gain direct access to them. Furthermore, expression of EEC is sparse and irregular along the gut and secretory responses and local interaction with vagal afferents are either indirectly measured in hormone plasma levels or not reflected at all. The initial recognition of nutrients and the subsequent signalling mechanisms involved are still largely under investigation with the majority of evidence based on *in vitro* and knockout animal models (Steinert and Beglinger, 2011). Therefore, a degree of caution is needed when interpreting the data as findings may not be representative of normal human *in vivo* EEC function.

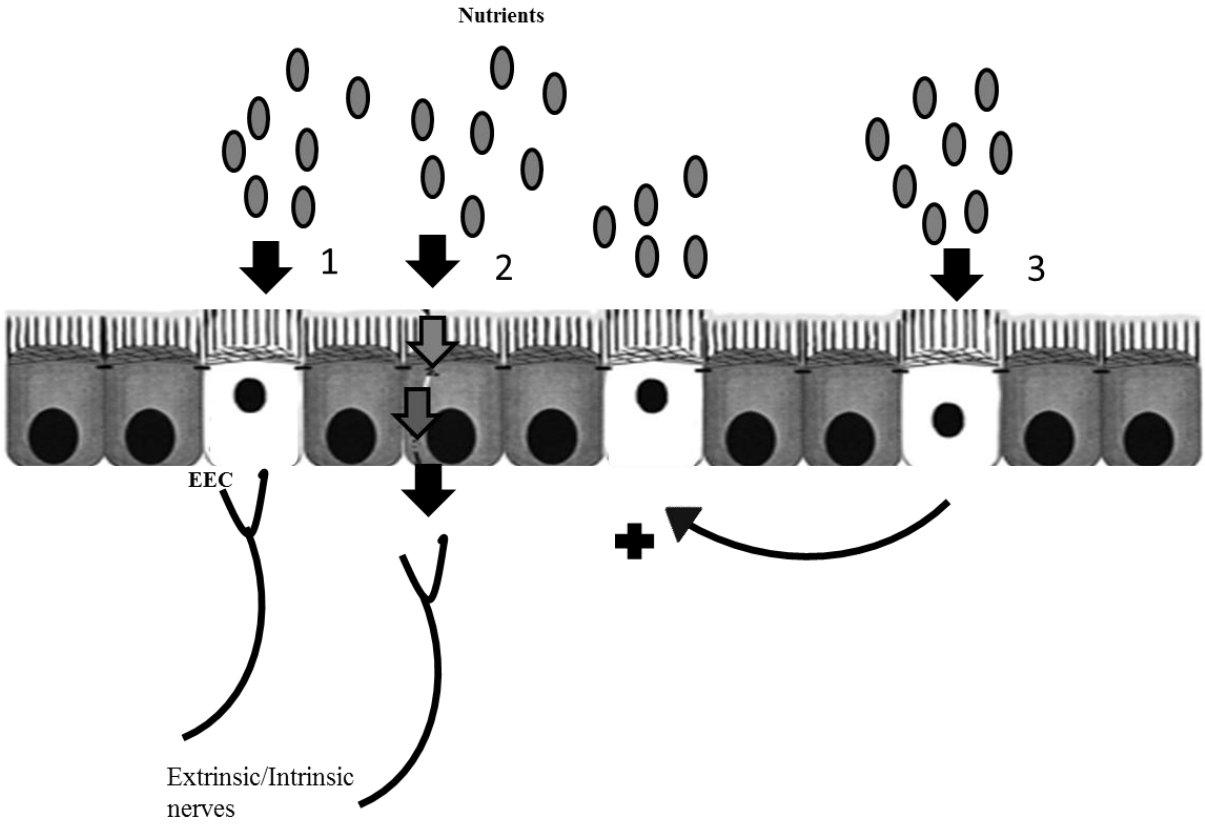


Figure 1.4 Possible pathways involved in nutrient sensing by enteroendocrine cells

Adapted from (Buchan, 1999, Sternini et al., 2008). Nutrients can interact directly with enteroendocrine cells (EEC) (1) or adjacent epithelial cells (2) to act on extrinsic and/or intrinsic afferent neurons. Finally, nutrients can interact with EEC and other cells to stimulate the release of hormones (3) which can also have a stimulatory (+) effect on neighbouring EEC and other epithelial cells.

1.4.1 Sweet taste receptors in the intestine: cell and animal models

As outlined in section 1.3.1.1, sugars and NNS are sensed in the mouth by the sweet taste receptor T1R2 + T1R3. Their functional role as "taste receptors" in the gastrointestinal tract has recently been established, at least in cell lines and rodent models. The expression of sweet taste receptors (T1R2 + T1R3), as well as the G

protein α-gustducin involved in taste-specific signalling, have been found in EEC in rats (Margolskee et al., 2007) and humans (Dyer et al., 2005, Jang et al., 2007). T1Rs and α-gustducin were found to be expressed in enteroendocrine L cells which stimulate the release of GLP-1. In addition, α-gustducin was also shown to be co-localised with GIP expressing enteroendocrine K cells and GIP and GLP-1 co-expressing enteroendocrine K/L cells (Jang et al., 2007). Rozengurt and colleagues also demonstrated that α-gustducin was expressed in enteroendocrine L cells expressing PYY and GLP-1 and co-expression with CCK in enteroendocrine I cells (Rozengurt and Sternini, 2007, Rozengurt et al., 2006). Although molecular evidence for expression may not always translate to function, the significance of these taste signalling elements in EEC has been investigated.

Evidence for a possible functional role of sweet taste receptors was established by Margolskee *et al* who demonstrated that T1R2 + T1R3 sweet receptor regulated SGLT1 expression and increased glucose absorptive capacity in response to luminal sugars and NNS in mice (Margolskee et al., 2007). Prior studies found SGLT1 expression was enhanced by glucose sensing, occurring independently of its metabolism (Dyer et al., 2003) and was confirmed by Margolskee *et al* to be the function of the T1R3 subunit (Margolskee et al., 2007). Furthermore, as apical GLUT2 insertion is inhibited if SGLT1 activity is blocked, stimulation of the T1R3 also increases GLUT2 insertion (Mace et al., 2007). Comparable to the taste receptors found in the mouth, gut expressed "taste receptors" respond to nutrients but signal and communicate via mediators such as GLP-1. These signals are detected by the enterocytes to cause an increase in SGLT1 expression and mediate glucose metabolism, gastric emptying and augment satiety.

The sensing mechanisms involved are reliant upon direct contact with EEC as intravenous administration of nutrients has shown no effect on gut hormone release (Sternini et al., 2008). Using rodent EEC lines it was demonstrated that GLP-1 and GIP secretion were enhanced when the concentration of sucralose, a NNS, was increased (Dyer et al., 2007) and in the human enteroendocrine L cell line (NCI-H716) glucose, sucrose and sucralose, all promoted GLP-1 release (Jang et al., 2007, Margolskee et al., 2007). This effect can be blocked by the sweet taste receptor antagonist, lactisole (Jiang et al., 2005) suggesting that the presence of sugars and

NNS can initiate gut-brain signalling via the release of gut hormone and concomitant activation of vagal afferents. *In vivo* studies, using knockout mice lacking α-gustducin or T1R3, observed no GLP-1 release following intragastric infusion of glucose (Jang et al., 2007) highlighting the importance of the T1R3 and α-gustducin system in sugar sensing.

1.4.2 Effects of sugars and non-nutritive sweeteners on the secretion of gastrointestinal hormones and appetite: evidence in humans

Carbohydrate is a major source of energy in the diet and the majority of studies have focused on glucose as the most important carbohydrate for human metabolism. It is known that carbohydrate ingestion stimulates satiety mechanisms (Blundell et al., 1994) but different sugars produce varying physiological responses. Sugar sensing in the intestine modulates nutrient absorption, hormone release and gastrointestinal motility (Dyer et al., 2003). The importance of intestinal glucose receptors in the control of eating behaviour has been demonstrated to show that the presence of glucose in the small intestine promotes hormone release and elicits vagal activity (Lavin et al., 1996), both of which can affect appetite. The T1R2 +T1R3 receptors in the gut would appear to be likely mediators of these effects given their role in the mouth however the function of these receptors and the effect of carbohydrate induced satiety requires further investigation. Comparable with the schematic depicting oral sweet taste mechanisms in figure 1.2, a schematic representation summarising the potential involvement of intestinal sweet taste receptor mechanisms in response to sugars and NNS is modelled in figure 1.5.

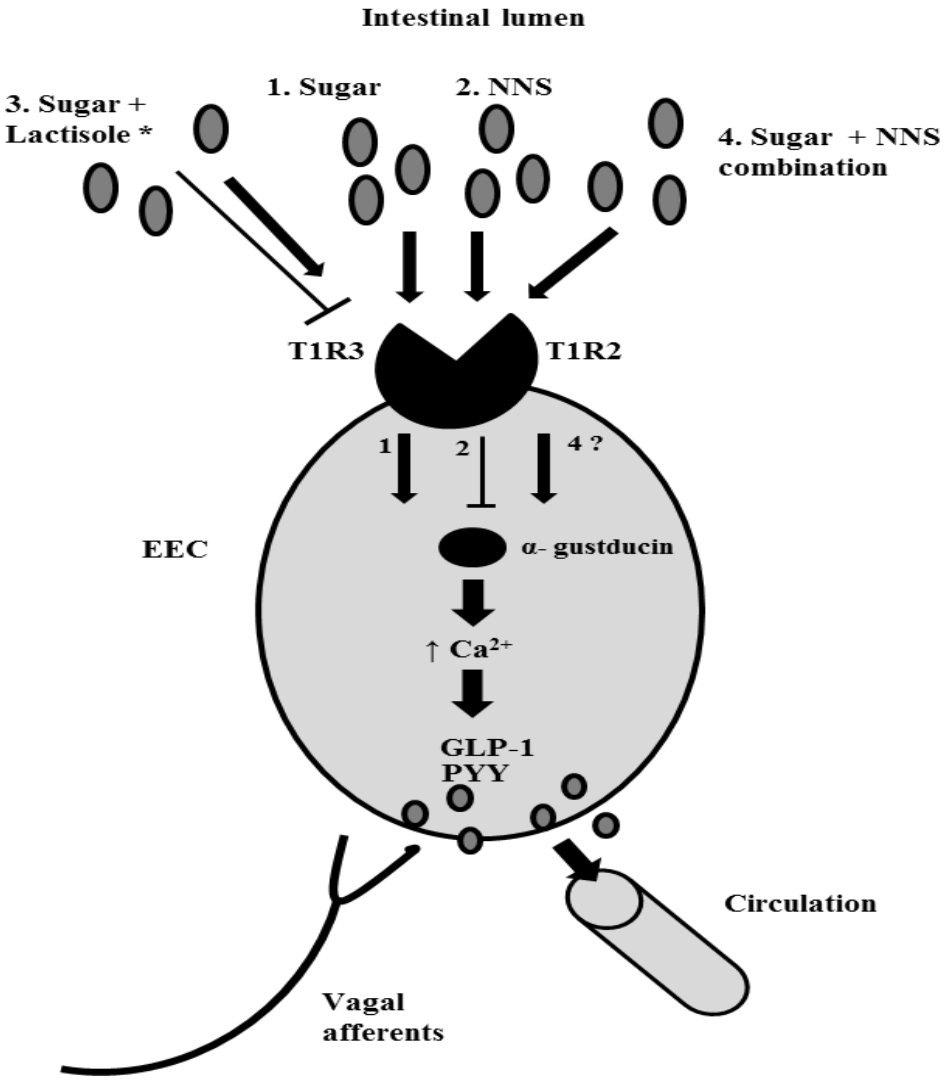


Figure 1.5 Schematic representation of intestinal sweet taste receptor mechanisms

Adapted from Cummings and Overduin, 2007. Sugars activate intracellular signalling pathways such as α-gustducin (others not shown) leading to an increase in intracellular calcium (Ca^{2+}). Rising intracellular Ca^{2+} leads to the release of hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) which enter the circulation or interact with vagal afferents to relay information to the brain (1). In comparison, non-nutritive sweeteners (NNS) have no effect on gut hormone release (2). The sweet taste receptor antagonist, lactisole, inhibits glucose stimulated GLP-1 release (3) (limited observations in vitro). The effect of sugars in combination with NNS on gut hormone release has yielded inconsistent results (4). EEC-enteroendocrine cell.*

The differential effects of ingested sugars and the role of osmolality on gastric emptying have been reported (Little et al., 2010b), and propose that a hexose sugar-specific effect may mediate gastric emptying, rather than sweetness *per se*. Gastric emptying is a brainstem mediated effect that can be used as an indirect proxy measure of gut to brain signalling. The poorly absorbed hexose sugar, tagatose, an epimer of fructose, slows gastric emptying more potently than either equi-osmolar solutions of glucose or fructose (Little et al., 2010a). This highlights the possibility for novel poorly absorbed sugars to maximise gut to brain signalling responses and thereby suppress food intake. The sweetness of ingested sugars and NNS, and the associated different effects on gastric emptying and gut hormone secretion, has also been reported but curiously failed to show any change with equally sweet solutions with only glucose potently stimulating the secretion of GLP-1 (Little et al., 2009, Steinert et al., 2011). A potential problem was an unavoidable difference in osmolality despite being matched for sweetness. However, all the sugars were given at a concentration below that at which saline starts to slow gastric emptying (~1M). Nonetheless this may suggest that sweet taste *per se* is not the underpinning mechanism by which sweet tasting molecules delay gastric emptying in humans. Furthermore it is possible that measuring gastric emptying, which occurs primarily via a CCK1 receptor mediated pathway may not be a suitable measure particularly as fatty acids are a potent stimulator of CCK (Lal et al., 2004), more so than carbohydrates (unpublished data from this department). However, the CCK1 receptor antagonist dexloxiglumide completely abolishes the effects of glucose, fructose and tagatose on gastric emptying (Little et al., 2010a). Therefore measuring peripheral blood levels of CCK apparently overlooks physiologically active effects of CCK, presumably operating by paracrine actions on adjacent vagal afferents that do not translate to elevated plasma levels.

At the point in time that the studies in this thesis commenced, the data were limited, with no published evidence that the effects of sweet tastants on human gastric emptying are mediated via the sweet taste receptor. If this were the case it was hypothesised that NNS should replicate the effects of glucose. However, given the convincing data from cell and animal models, experimentally addressing this possibility further in healthy humans substantially formed the part of the work presented this thesis, and underpinned the funding received from BBSRC.

Subsequent publications that appeared during the latter course of my studies will be introduced and discussed in the relevant chapters.

1.4.2.1 Potential significance of non-nutritive sweeteners to diet and health: a conflicting and confused landscape

Whatever the cellular basis for the response, the recognition that EEC, in response to luminal sugars, secrete anorectic gut hormones, such as GLP-1, is of increasing importance in the face of the obesity epidemic. In particular the possible target of sweet taste signalling would present a valuable opportunity for intervention by nutritional manipulations. Not only do sweet tasting foods stimulate eating in humans via hedonic mechanisms, but the increased consumption of processed food products is frequently linked to the surge in obesity. As a consequence, the food industry has increased their use of NNS in replace of simple sugars on the assumption they are nutritionally inert (Kellett et al., 2008). The effect of NNS on hormone secretion has been investigated using different models with inconsistent results (Table 1.2). Jang *et al* (Jang et al., 2007) and Margolskee *et al* (Margolskee et al., 2007) demonstrated the NNS, sucralose, stimulated GLP-1 secretion from a human L-cell line (NCI-H716 cells) and mouse EEC line (GLUTag cells) respectively. However, the majority of *in vivo* human data have failed to confirm the effects of NNS on hormones secretion observed *in vitro* (Ma et al., 2009, Steinert et al., 2011). This supports the consensus that NNS, at least in isolation, are not capable of stimulating hormone secretion. It is also worth noting that the concentrations of NNS used in these *in vitro* studies were excessive (19.8 grams vs. 0.06 grams *in vivo* in humans).

NNS consumption and the effect on appetite and food intake have raised concerns with suggestions NNS may increase appetite and lead to weight gain (Egan and Margolskee, 2008). However, the majority of data suggests NNS do not have undesirable effects on appetite, food intake, and blood glucose or insulin levels (Anton et al., 2010, Bellisle and Drewnowski, 2007, De La Hunty et al., 2006). The diversity of the human diet ensures consumption of NNS in isolation, usually in the form of diet beverages, is uncommon. The regulation of intestinal glucose absorption

via both SGLT1 and the GLUT2 has shown sensitivity to NNS. This has been demonstrated, albeit again only in animal models, by an up regulation of SGLT1 and an increase in GLUT2 insertion following supplementation of the diet with sucralose, acesulfame-k (ace-k) and saccharin (Mace et al., 2007, Margolskee et al., 2007). Using a proxy measure of intestinal glucose absorption this effect was not replicated in humans (Ma et al., 2010). One human study showed oral ingestion of sucralose and ace-k (amounts not specified) caused an increase in GLP-1 after subsequent glucose ingestion but no change in blood glucose or insulin (Brown et al., 2009). Other GLP-1 effects such as satiety were not measured. In contrast Ma *et al* (Ma et al., 2009) found no difference in GLP-1 concentrations following intragastric infusion of a sucralose and glucose combination arguing against a significant effect. The biological significance of the different findings is unclear but interestingly the studies used different methods of administration (oral vs. intragastric). There remains a lack of clarity about whether NNS in combination with caloric sweeteners could alter appetite and/or glucose metabolism, not least due to considerable variations in experimental protocols and outcome measures.

		Model					
		In vitro		*In vivo* -animals		*In vivo*- humans	
		Method	**Effect**	**Method**	**Effect**	**Method**	**Effect**
NNS	**Ace- k**	Rat intestinal and human cell line Mouse pancreatic cell line	↑ glucose absorption (Zheng and Sarr, 2013) ↑ insulin secretion (Nakagawa et al., 2009)	Gastric gavage in rats Ad libitum drinking water x 14 days in mice Perfused into small intestine of rats	No effect on GIP or GLP-1 (Fujita et al., 2009) ↑ SGLT1 expression (Margolskee et al., 2007a) ↑ glucose absorption (Mace et al., 2007a)	Intragastric infusion Oral (ace-k + Sucralose)	No effect on GLP-1 or PYY (Steinert et al., 2011) ↑ glucose stimulated GLP-1 secretion (Brown et al., 2009)
	Sucralose	Human intestinal cell line Mouse intestinal cell line	↑ GLP-1 secretion (Jang et al., 2007) ↑ GIP and GLP-1 secretion (Margolskee et al., 2007a)	Gastric gavage in rates Ad libitum drinking water x 14 days in mice Perfused into small intestine of rats	No effect on GIP or GLP-1 (Fujita et al., 2009) ↑ SGLT1 expression (Margolskee et al., 2007a) ↑ glucose absorption (Mace et al., 2007a)	Intragastric infusion Intragastric infusion Intraduodenal infusion	No effect on GLP-1 or PYY (Steinert et al., 2011) No effect on GIP or GLP-1 (Mace et al., 2009) No effect of glucose stimulated GLP-1 secretion (Ma et al., 2010)
	Aspartame	No evidence		Ad libitum drinking water x 14 days in mice[1]	No effect on SGLT1 expression (Margolskee et al., 2007a)	Intragastric infusion	No effect on GLP-1 or PYY (Steinert et al., 2011)
	Saccharin	Mouse pancreatic cell line	↑ insulin secretion (Nakagawa et al., 2009)	Ad libitum drinking water x 14 days in mice Perfused in small intestine of rats	↑ SGLT1 expression (Margolskee et al., 2007a) ↑ glucose absorption (Mace et al., 2007a)	No evidence	

Table 1.2 Summary table showing effects of non-nutritive sweeteners on gut hormone secretion and glucose absorption

↑ Increase ↓ Decrease

[1] Aspartame is not recognised as sweet in rodents

1.5 Gut-brain communication and the pathways relating to appetite and food intake

Multiple neural systems are involved in the control of food intake. The signals arising from the gastrointestinal tract integrate in the brain, via the vagus nerve or directly through an incomplete blood brain barrier (BBB), and the regulation of food intake lies in the gut-brain axis. The hypothalamus and brainstem are at the core of this homeostatic function receiving hormonal and neural input from the endocrine system and gastrointestinal tract (Berthoud, 2008). The remainder of this section focuses firstly on the way EEC sugar sensing and associated signals, outlined in previous sections, interact with visceral afferents, and how they are integrated in the brain to influence appetite and energy intake, and secondly how these homeostatic functions are strongly influenced by non-homeostatic factors relating to the environmental factors, discussed in 1.2 and 1.3.1, and processed via the limbic system. Relevant brain areas involved in the control of appetite and food intake are shown in figure 1.6.

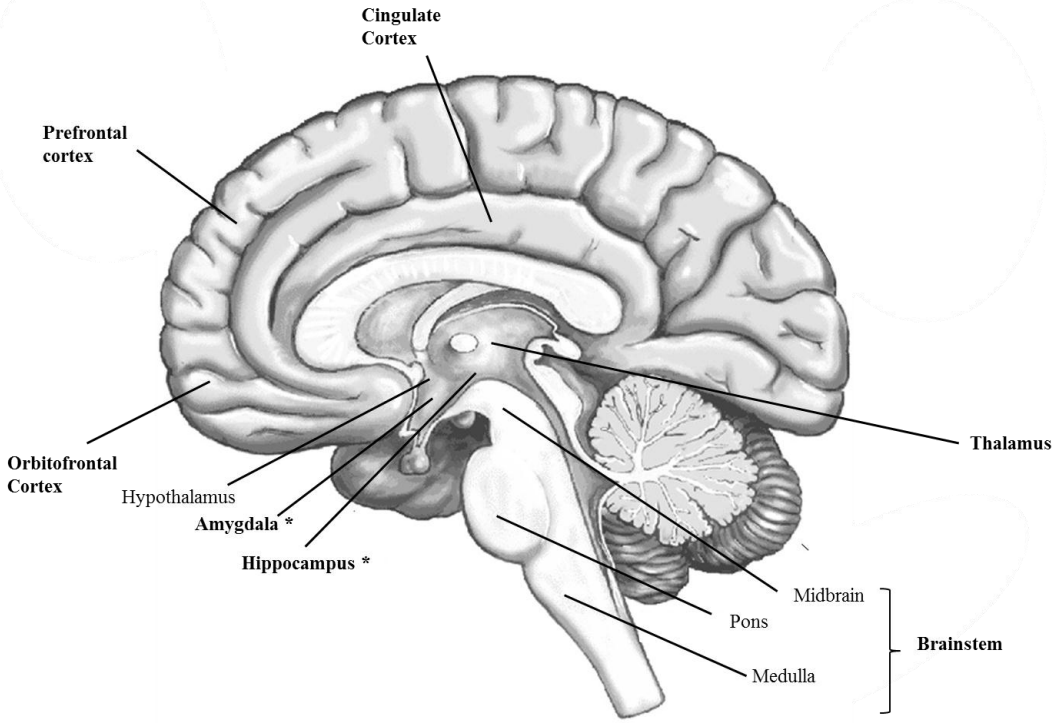


Figure 1.6 Brain structures involved in appetite and food intake

Adapted from *http://www.emc.maricopa.edu/faculty/farabee/biobk/biobooknerv.html#TheBrain*.

*The hypothalamus is at the core of homeostatic control but also receives and integrates input, via the brainstem, from limbic systems (highlighted in bold) involved in eating behaviour (e.g. taste, pleasure and reward). The brainstem, although not regarded as a limbic area, receives taste signals from taste receptor cells via gustatory nerves (Small, 2012). *Structures not visible in this view of the brain.*

1.5.1 Innervation of the gastrointestinal tract and interaction with enteroendocrine cells

The accurate coordination of regulatory functions of the gastrointestinal tract, such as absorption, secretion and motility, are modulated by intrinsic and extrinsic innervation (Farre and Tack, 2013) which monitor the mechanical and chemical gut environment in order to coordinate appropriate responses (Blackshaw et al., 2007). Primary afferent nerves of the gastrointestinal tract are divided into intrinsic and extrinsic categories. The connections and cell bodies of the intrinsic primary afferents neurons (IPANs) lie completely within the gut wall and the extrinsic primary afferents lie within vagal and spinal afferent neurons (Furness et al., 1998) (figure 1.7). IPANs are situated in the enteric nervous system (ENS) and are principally involved in the control and coordination of gastrointestinal motility and secretion. The system comprises neurons of myenteric and submucosal plexuses resembling those found in the CNS (Phillips and Powley, 2007) and are able to perform in the absence of extrinsic innervation but coordinate and relay information from both pathways. IPANs are stimulated by the presence of food in the gastrointestinal lumen and convey to neurons in the ENS which control digestion (Kunze and Furness, 1999).

The entire length of the gastrointestinal tract is innervated by spinal primary afferent neurons in the distal gut and vagal primary afferent neurons predominating in the proximal gut (Brookes et al., 2013). Vagal afferent nerves are able to detect chemical and mechanical changes in the gut, and gastrointestinal function is thus modulated when vagal afferents integrate with vagal efferents known as the vagovagal reflex (Aziz and Thompson, 1998, Rogers et al., 1995). The circuitry for these reflexes lies within the nucleus of solitary tract (NTS), where vagal afferents terminate, and the dorsal motor nucleus (DMN) of the brainstem region (Konturek et al., 2004). Activation of these afferent neurons is thought to depend on pre- and post-absorptive mechanisms as they do not protrude into the gut lumen itself (Raybould, 1998). Vagal chemoreceptors including glucoreceptors have been described and as discussed previously gut hormone receptors (CCK, GLP-1 and Y2) have been identified on afferent fibres (Nakagawa et al., 2004, Koda et al., 2005). Furthermore, the ability of EEC, possibly via the sweet taste receptor system, to respond to incoming carbohydrates and the concomitant stimulation of vagal afferents and gut

hormones, which also act to stimulate vagal afferents in close vicinity, provides the interface between the gastrointestinal lumen and the nerve terminals (Höfer et al., 1999, Steinert and Beglinger, 2011).

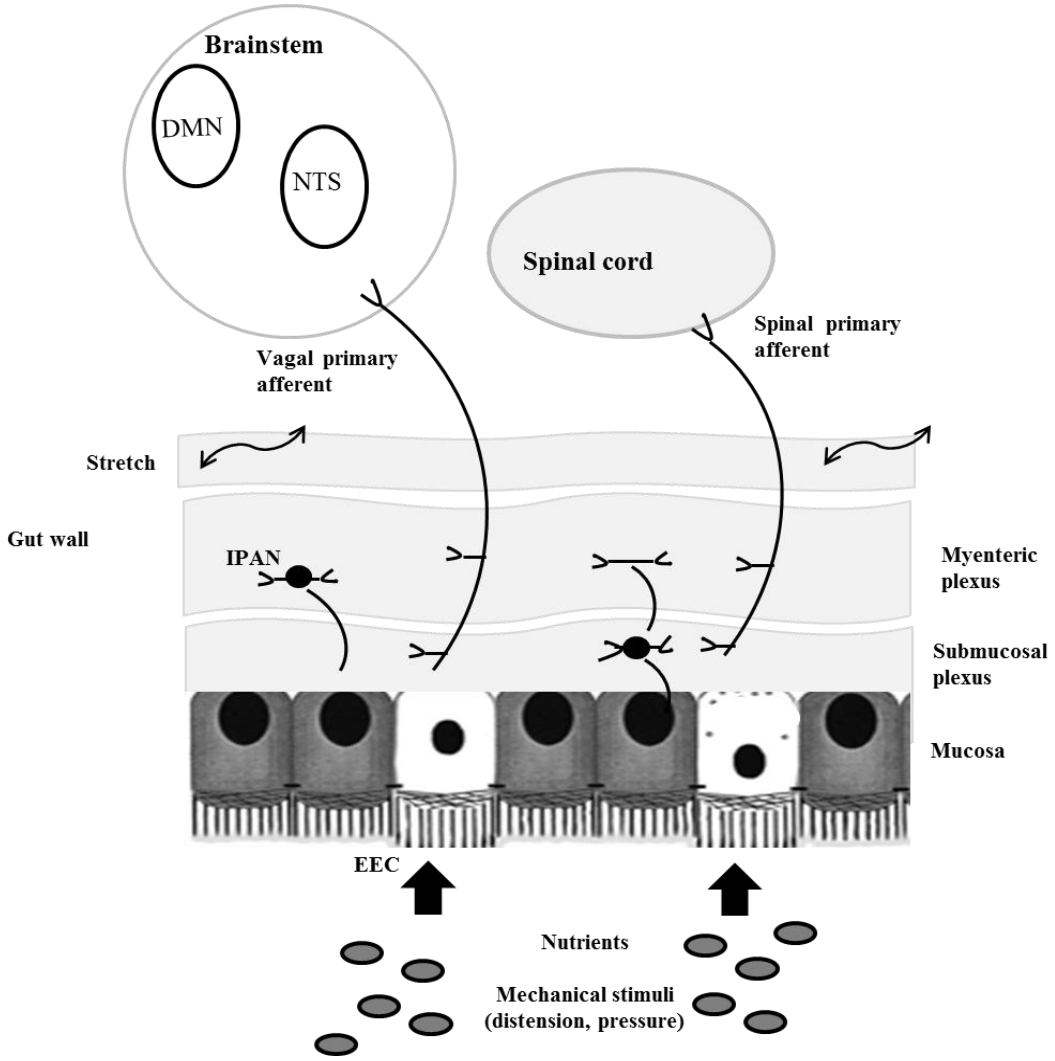


Figure 1.7 Arrangement of vagal, spinal and intrinsic primary afferents

Adapted from (Mayer, 2011). Mechanical and nutrient stimuli can activate extrinsic (spinal and vagal) and intrinsic primary afferent neurons (IPANs). Vagal and spinal afferent neurons relay information to the nucleus of the solitary tract (NTS) and dorsal motor nucleus (DMN) of the brainstem and spinal cord respectively with networks running into myenteric regions. IPANs are confined within the gut wall and relay information locally to regulate gut function independent of extrinsic innervation.

1.5.2 Areas of the brain involved in appetite and energy intake control

1.5.2.1 Hypothalamus

The hypothalamus receives and integrates tonic signals from adipocytes reflecting energy stores (e.g. leptin), episodic signals from the gut, including hormones (e.g. GLP-1), and circulating nutrients including glucose. The output of this integration of tonic and episodic signals is energy homeostasis. These signals are processed in the hypothalamic ARC in which two distinct pathways exist; the lateral hypothalamic area (LHA) termed the "appetite centre" expresses anorectic factors, the other involves the ventromedial hypothalamic area (VMH) also known as the "satiety centre", expressing orexigenic factors (Konturek et al., 2004). Early animal studies in support of this demonstrate lesions in the LHA or VMH significantly alter feeding behaviour, the latter causing a decrease in energy intake leading to cachexia and anorexia and the former resulting in hyperphagia and obesity (Konturek et al., 2004). ARC neurons, involved in the appetite inhibiting pathway, function through the release of α-melanocyte–stimulating hormone (αMSH) derived from pro-opiomelanocortin (POMC), which acts on melanocortin receptors (MCR3 and MCR4) to inhibit food intake. Conversely ARC neurons, expressing the neurotransmitter NPY and agouti–related peptide (AgRP), signal to stimulate feeding. Alterations in the release of these neuropeptides to control feeding behaviour occurs via NPY-POMC interactions (Broberger and Hökfelt, 2001). For example, the release of AgRP has an antagonistic effect on MC3 and MC4 receptors involved in the POMC pathway. Similarly, the peptides that stimulate the POMC act as a brake on hypothalamic NPY signalling (Broberger and Hökfelt, 2001). A critical target of the ARC neurons is the PVN which signals to higher brain centres and other hypothalamic nuclei to increase or decrease appetite (Murphy and Bloom, 2006).

1.5.2.2 Brainstem

The hypothalamus is not exclusive in the homeostatic control of energy intake. A network of neural circuits involved in tasks such as the digestion and absorption of nutrients are contained within the brainstem and do not require input from

hypothalamic structures (Berthoud, 2008). Studies have demonstrated this using the decerebrate rat (forebrain surgically disconnected) which showed the brainstem capable of responding to gastrointestinal hormones necessary for the short term regulation of energy intake (Broberger and Hökfelt, 2001). The dorsal vagal complex (DVC) facilitates communication between the hypothalamus and periphery to control food intake. The reciprocal connections between the hypothalamus and brainstem enable the integration of signals to generate efferent signals that coordinate food intake and gastrointestinal function (Hussain and Bloom, 2013, Schwartz, 2000). Furthermore, vagal afferent nerves terminate in the NTS, making neurons in this area the terminus for many signals arising from the gut in order to limit food intake.

1.5.3 Signalling and central control of food intake

The gut-brain axis involves the coordination of hypothalamic, brainstem and vagal signalling. The presence of ingested nutrients in the gastrointestinal tract stimulates mechanoreceptors and chemoreceptors to signal via vagal afferents to control energy intake. Gut hormone signals are also relayed via the median eminence (ME) and AP, structures which have an incomplete BBB, to the ARC of the hypothalamus and NTS of the brainstem respectively (Banks, 2006). The role of the BBB in mediating communication between the brain and signals from the gastrointestinal tract is believed to be an important mechanism underlying gut-brain interaction. Mechanisms suggested include a direct transfer allowing hormones to act directly upon neurons within areas of the brain involved in appetite and food intake (Chaudhri et al., 2008, Rogers et al., 1995) and the ability of hormones to alter the functions and/or secretions from the BBB to have an effect on feeding (Banks, 2006). A representation of these systems operating in the gut-brain axis control of food intake and appetite is shown in figure 1.8.

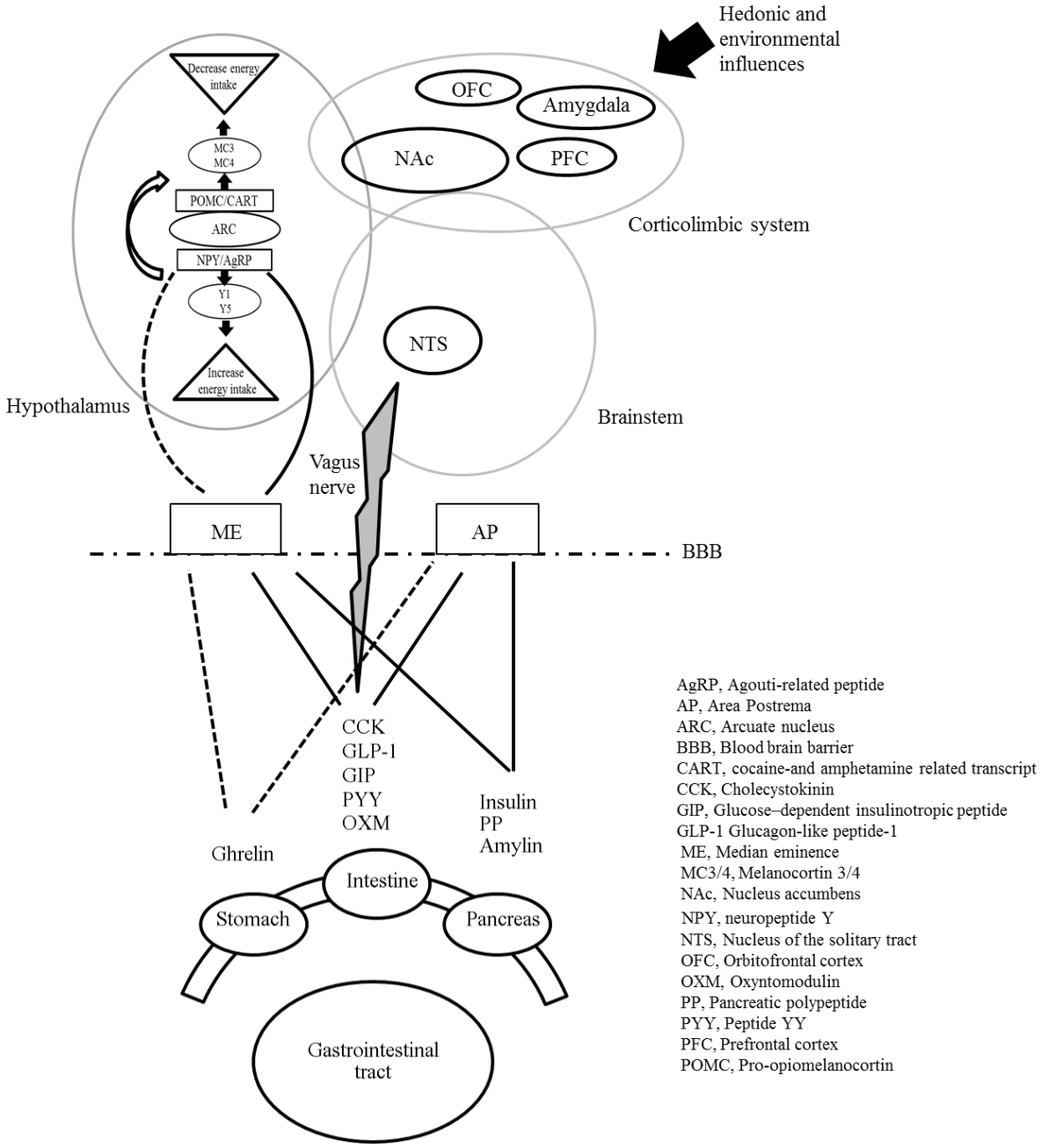


Figure 1.8 Gut-brain pathways involved in the control of appetite and food intake

Adapted from (Hussain and Bloom, 2013, Suzuki et al., 2010). The hypothalamus, brainstem and corticolimbic system integrate signals emanating from the gut and external stimuli (e.g. emotional cues, taste). The vagus nerve conveys neural signals from the gut to nucleus of the solitary tract in the brainstem. Gut hormones also act on the arcuate nucleus in the hypothalamus via the median eminence which forms an incomplete blood brain barrier and relays directly to the brainstem via area postrema. Solid black lines indicate inhibitory effects and dashed lines indicate stimulatory effects. Hedonistic and environmental influences are processed by the corticolimbic system which modulates hypothalamic feeding centres.

1.5.3.1 Hedonic mechanisms affecting appetite and energy intake

Alongside the homeostatic mechanisms mentioned above, the control of food intake is strongly influenced by higher brain centres which process the sensory pleasure and reward aspects of eating via the corticolimibic system. This system includes areas such as the insula, amygdala, hippocampus, cingulate cortex, orbitofrontal cortex (OFC), prefrontal cortex and ventral and dorsal striatum (Schloegl et al., 2011). When food is presented, visual and olfactory information is processed via olfactory fibres which relay to the DVC and corticolimbic system to determine palatability, a principal determinant of food intake (Rolls, 2005, Rolls, 2006). At the time of ingestion, gustatory, olfactory and somatosensory systems are stimulated, taste is conveyed, via gustatory and sensory fibres to the DVC and corticolimbic system (Rolls, 2006, Small, 2012) including the primary gustatory cortex which comprises of the insula and frontal operculum (Frank et al., 2008) and the OFC, prefrontal cortex and dorsal and ventral striatum which integrate with other cognitive inputs to further characterise the reward value of the food ingested (O'Doherty et al., 2001, O'Doherty et al., 2002). This is thought to constitute learning, underpinning subsequent phenomena of 'liking and wanting' which motivate subsequent feeding behaviour (Berthoud, 2006). This is integrated with homeostatic mechanisms to alter food intake with environmental cues dominating homeostatic regulation (Berthoud, 2006) and gut hormones modulating neuronal activity in brain regions associated with reward processing (Grill et al., 2007, Batterham et al., 2007).

1.6 Methodologies used to study feeding behaviour

In light of the current obesity epidemic the mechanisms controlling appetite and food intake have been a major focus of research in recent years. *In vitro* research and *in vivo* animal studies constitute the majority of work on mechanisms involving intestinal sweet taste receptors and often focus on single processes in isolation. However, it is evident from the literature presented in this chapter that there are many complex pathways and mechanisms which may interact to influence feeding behaviour making it difficult to translate and apply findings to humans particularly

as psychological and environmental processes are profound determinants of human feeding behaviour.

Biomarkers of satiation and satiety in human physiology studies require markers that are not only feasible but are sensitive and specific measures of appetite. Methods employed include that of functional neuroimaging techniques to measure human brain responses that relate to appetite. Furthermore, given the evidence supporting gastric volume as a determinant of meal size, the use of physical measures such as those relating to gastric distension and gastric emptying are also utilised. Gastric emptying is considered to be a good surrogate marker for activation in the gut-vagus-brain regulatory axis (Little et al., 2013, In review). Hormonal and biochemical (i.e. glucose, insulin and GLP-1) measures are available as biomarkers of mechanisms that regulate satiation and satiety and most human physiology studies are likely to use a combination of these measures in conjunction with behavioural or subjective appetite measures to understand not only the physiology of appetite and intake but also the causal factors.

Visual analogue scales (VAS) are a commonly used tool to assess subjective feelings of appetite (e.g. hunger and fullness) in studies examining the impact of various foods. The reproducibility and validity of using VAS in appetite research was confirmed (Flint et al., 2000) and correlates well with food intake. Measuring actual food intake, either free-living outside the laboratory environment via self-reported measures (e.g. diaries) and/or *ad-libitum* test meals (e.g. buffet) is also a commonly used method. Both have inherent problems as they are subject to bias and underreporting or do not accurately reflect intake due to the laboratory environment. However, measuring food intake in the laboratory enables the intake of meals, with a fixed macronutrient composition, to be quantified and has proved a reproducible assessment of food intake (Gregersen et al., 2008).

1.6.1 Functional brain imaging

As previously discussed, nutrient sensing and the associated gut hormone release result in neuronal responses in the brainstem and hypothalamus to mediate the inhibitory effects of nutrient intake on gastric emptying, appetite and food intake.

The intricate connection of the gastrointestinal tract to the CNS is fundamental to these mechanisms. Our knowledge of the CNS regions involved in maintaining energy homeostasis has been significantly advanced by experimental studies in animals (Fraser et al., 1995, Kuo et al., 2007). For example, nutrient induced c-fos activation in the brainstem regions where gut vagal afferents terminate has furthered our understanding of the role of vagal afferents in the gut-brain axis and demonstrated how critical the vagal gut-bran axis is in controlling food intake as evidenced by vagotomised animals (Schwartz, 2000). Furthermore, functional brain imaging in intact and vagotomised rats has demonstrated the importance of circulating factors such as insulin in addition to vagal pathways for the brain responses to glucose (Tsurugizawa et al., 2009). However, the clinical relevance to humans is restricted by species differences, not least in the degree of brain development between the two. Studies using animal models are limited by their inability to fully explore the more highly developed areas of the brain involved in eating behaviour that are found in humans but not animals. Furthermore, subjective perceptions cannot be measured in animals.

Unravelling the neuroanatomical sites of eating behaviour has been facilitated by the development of neuroimaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Both techniques have advantages and limitations associated with their use. However, fMRI is currently the best tool for exploring brain function (Logothetis, 2008) and has the potential to provide the most accurate measurement of the brains response to nutrient ingestion.

The tracking of neuronal activity in response to nutritional stimuli has begun to be characterised *in vivo* through the use of fMRI (Lassman et al., 2010, Jones et al., 2012). The MRI technique involves the use of a powerful magnetic field causing protons to rotate about the axis. Upon their return to the original position in the magnetic field they emit detectable radiofrequency energy (Howseman and Bowtell, 1999). fMRI enables the observation of changes in neural activity over time. Increased neural activity leads to increased regional cerebral blood flow and an associated increase in oxygenated blood; for this reason, increased neuronal activity alters the concentrations of oxyhaemoglobin and deoxyhaemoglobin, which are detected using MRI (Tataranni and DelParigi, 2003). fMRI measures changes in the

blood oxygen level dependant (BOLD) signal. The BOLD signal is a proxy measure of neural activity and reflects the concentrations of deoxyhaemoglobin, an intrinsic paramagnetic contrast agent, relative to the concentrations of oxyhaemoglobin thus influencing the MR signal (Logothetis and Wandell, 2004). The major advantage of fMRI is its relatively high spatial and temporal resolution with advances enabling hypothalamic and brainstem imaging (Liu et al., 2000). That said, the hypothalamus is positioned deep in the midbrain which makes imaging technically difficult. Furthermore, the BOLD signal is a crude marker of neural response and imaging lacks the specificity to identify the contribution of particular nuclei within hypothalamic circuitries.

The most frequently used paradigm in fMRI studies examines brain responses to different stimuli (visual food cues i.e. images of high vs. low calorie food) presented in a block or event related design (Amaro and Barker, 2006). These approaches explore primarily hedonic responses and often miss fundamental brain regions (e.g. hypothalamus) which are key to homeostatic regulation.

A study by Liu *et al* was one of the first to use fMRI analysis to show neuronal activation following the ingestion of glucose. A decrease in hypothalamic BOLD signal occurred 7-12 minutes after oral ingestion of glucose (Liu et al., 2000). In this instance, the relationship between glucose ingestion and brain activation was demonstrated using a technique called time clustering analysis (TCA). Subsequent studies have reinforced these findings showing a dose-dependent and prolonged decrease in BOLD signal in the hypothalamus following glucose ingestion (Smeets et al., 2005b). In a further study by Smeets *et al* they showed no hypothalamic BOLD decrease following equally sweet (aspartame) or calorific (non-sweet maltodextrin) solutions suggesting activity is glucose sensitive requiring both sweetness and energy content (Smeets et al., 2005a). In addition, a more pronounced response following oral glucose as opposed to intravenous administration was demonstrated (Smeets et al., 2005b). However, these results may be flawed by the hedonic responses to sugar ingestion as it is impossible to exclude the pre-gastric effects relating to sight, smell and taste. Furthermore, the movement of the head and neck during the swallowing and consumption of test meals can result in imaging artefacts. Nutrients administered directly into the gut via oro- or naso-gastric tube

eliminates these confounding issues and theoretically enable the study of gut to brain signalling independently. More recently, more detailed imaging of the brain responses to glucose have been investigated within this department using physiological MRI (physMRI) which enables the exploration of BOLD signal response to nutrients over time with a high spatial and temporal resolution across the whole brain (Jones et al., 2012, Little et al., 2013, In review). In particular, detailed imaging of the brainstem, a region that is the first point of contact with signals transmitted from the gastrointestinal tract via vagal afferents along with other regions of interest was investigated following an intragastrically administered bolus of glucose (Little et al., 2013, In review). In line with observations previously discussed, a decrease in BOLD signal was demonstrated in the hypothalamus but also in brainstem regions such as the medulla and pons. However, using a CCK receptor antagonist the study demonstrated these responses were not solely dependent on the activation on vagal afferents suggesting additional actions of circulating glucose and gut hormones such as GLP-1 which were temporally related to the observed decrease in BOLD signal.

physMRI differs from conventional fMRI in two ways; i) there is no predefined model and ii) the setup in conventional fMRI usually consists of a task being switched between separate conditions in an on/off manner. This is not the case for physMRI where temporal changes in the BOLD signal are investigated from a predefined baseline, usually the average of the images just before the infusion is administered into the gut with no predefined model present and no switching between on and off states.

The overall response to ingested nutrients will however not exclusively depend on gut-to-brain signals. In particular, no work has ever been undertaken to integrate brain responses to oral and gastrointestinal nutrient sensing, which more closely resembles consumption.

1.7 Summary and aims

In summary, a cascade of gut derived and metabolic signals converge within the CNS to control appetite and food intake. The role of gastrointestinal sweet ‘taste’

sensing in humans is unclear, and in particular, the important role of feedback signalling from oral and gastrointestinal stimulation to the brain in response to sugar and NNS ingestion is of great importance. Sugar and NNS consumption are commonplace and the overall aim of this thesis is to better understand their effects in the gut-brain axis.

The underpinning hypothesis is that sweet taste receptors in the gut contribute to the effects of sweet tastants in the gut-brain axis.

All studies were conducted in healthy young adult humans.

Chapter 3: The role of the gut sweet taste receptor in mediating the responses to glucose: an exploration using lactisole

Nutrient sensing in the gut is a rapidly evolving research field, albeit mostly in *in vitro* and animal models, but it is tempting to speculate that sweet taste signalling mechanisms expressed in the gut may function to mediate the responses to sugar and be therapeutic targets for appetite related disorders. The objective of this study was to use the sweet taste receptor antagonist lactisole as a tool to investigate the role of the gut sweet taste receptors in mediating the physiological response to glucose.

Chapter 4: Non-nutritive sweeteners do not enhance the glycaemic or appetitive responses to ingested glucose

NNS consumption has increased considerably and in most cases the putative mechanisms by which they operate within the gastrointestinal system are studied in isolation. However, consumption in isolation is rare in a diverse Westernised diet, and concerns have been raised about the synergistic effect of NNS and sugars potentially increasing glucose uptake via upregulation and insertion of transporters. The objective of the study was therefore to determine the effects of a panel of three commonly consumed NNS (aspartame, saccharin, ace-k) given in real-life doses in combination with glucose on glycaemic and appetite responses.

Chapter 5: Gastric emptying and CNS responses to orally and intragastrically administered sweet tastants

This chapter details two studies which aimed to further our understanding of the influences of sugars and NNS in mediating mouth/gut to brain signalling and appetite responses. Most studies investigate satiety mechanisms in isolation yet the synergy between sequential oro-sensory and gastrointestinal factors is more indicative of real life consumption, and may be essential for a particular effect to become fully apparent. The first study aimed to establish whether sweet tastants have equivalent effects on gastric emptying (again used as a proxy measure of gut-brain signalling) and appetite responses when consumed orally or administrated intragastrically. The second study aimed to define the areas in the human brain, using fMRI, where oral and gastrointestinal 'sweetness' interact. This enabled the dissection of the guts response to ingested sugar and the cerebral activation that ensues and, the interplay with the hedonic system activated by oral taste. This is a highly robust and non-subjective way to assess sensory and signalling mechanisms.

CHAPTER 2

General Methods

Two or more of the studies described in this thesis have the following methods, protocols and measurements in common. Methods and protocols used in specific studies are detailed in each experimental chapter.

2.1 Ethical Approval

Ethical approval was granted by the National Health Service North West Research Ethics Committee and The University of Manchester prior to commencing study recruitment. Specific references are given in each study chapter.

2.2 Participants

All participants were recruited from the staff and students of Salford Royal Foundation Trust Hospital and The University of Manchester via poster and website advertisement. Those who expressed an interest in volunteering were invited to attend a pre-study screening. The screening assessed that participants met the inclusion criteria for each particular study.

2.2.1 General inclusion criteria

The criteria for participation in studies were as follows, unless stated otherwise:

- Aged between 18-45 years
- BMI between 18-25kg/m^2
- General good health - no history of gastrointestinal disorders, metabolic disorders or eating disorders
- Non-smokers

- Female participants neither pregnant or lactating
- Not currently taking any medications (other than females taking oral contraceptives)
- No self-reported reported weight change +/- 3kg in previous six months or currently dieting
- No self-reported intolerance or allergy to foods supplied during the study
- Low score for dietary restraint (section 2.2.2.2)
- No participation in other scientific research in three months prior to study

Studies with additional or different inclusion criteria are specified in the methods section of the specific experimental chapter.

2.2.2 Screening

Each participant was given a study specific information sheet to read prior to the screening visit. During the screening, details of the study were explained to each participant and they were given the opportunity to ask any questions. If they were happy to take part, informed written consent was obtained. During the screening visit anthropometric measurements (section 2.2.2.1) were collected and participants completed the following questionnaires:

General information (Appendix I)

Medical screening (Appendix II)

Three factor eating questionnaire (TFEQ) (Appendix III)

2.2.2.1 Anthropometry

Measurements for height, weight and BMI were made for all studies in this thesis as follows:

- Weight was measured to the nearest 0.1kg using a flat scale (Seca, Hamburg, Germany). Participants removed footwear and removed any heavy items while being weighed.
- Height was measured to the nearest 0.01m using a stadiometer (Seca, Hamburg, Germany). Participants removed footwear and stood flat-footed with their heels against a back plate.
- BMI was calculated using the following formula:

$$\text{BMI (kg/m}^2\text{)} = \frac{\text{Body mass (kg)}}{\text{Height}^2\text{ (m}^2\text{)}}$$

2.2.2.2 Three factor eating questionnaire

TFEQ is a tool that evaluates dietary restraint, disinhibition and hunger; three dimensions of eating behaviour (Stunkard and Messick, 1985) and is a commonly used tool to evaluate eating behaviour in appetite research (Blundell et al., 2010). Dietary restraint theory is an approach that encompasses internalised behavioural and cognitive processes determining eating behaviour. That is, cognitive processes override physiological hunger and satiety cues (Bond et al., 2001). Restrained eaters may restrict food consumption for a period of time to achieve certain goals such as weight loss. However, studies have shown disinhibition experiences (emotional stress) interfere with self-control and may result in overeating (Lowe, 1993). A concept of one of the studies in this thesis (chapter three) relies upon participants responding to internal cues that initiate meal termination at a test meal. Furthermore, in all studies in this thesis participants are required to report subjective feelings of appetite pre- and post-intervention making it essential to measure dietary restraint in prospective participants.

The questionnaire consists of fifty one questions, divided into three sections, each relating to one of the three dimensions of eating behaviour. Participants completed all fifty one questions but the responses pertaining to dietary restraint only were analysed and are reported herein. The first twenty one questions assess dietary

restraint in which responses are scored either zero or one and totalled to give a total restraint score.

For example:

	True	**False**
I deliberately take small helpings as a means of controlling my weight	☐	☐

Higher scores indicate higher levels of dietary restraint (above ten) while low dietary restraint is generally accepted as a score of five or lower. (Bellisle et al., 2009). The median split of scores (high/low) is often used to determine a score which indicates a high degree of dietary restraint (Rideout and Barr, 2009, Steere and Cooper, 1993). Participants were categorised as restrained eaters according to whether they fell above the median (score of six) determined from two previous studies involving females (Yeomans et al., 2003) and male and female volunteers (Lesdama et al., 2012). Therefore, any participants with a dietary restraint score above six (one participant in chapter three *part III*) were therefore excluded from taking part in the studies outlined herein.

2.3 Experimental protocols

2.3.1 Pre-study standardisation and fasting

Twenty four hours prior to each study visit participants were asked to refrain from consuming alcohol or taking part in vigorous physical exercise. They were advised to consume and complete their evening meal before 22:00 hours the night before each visit following which they were instructed not to consume any other food or drinks (excluding water) until they arrived at the laboratory the next day. Participants were asked to consume similar foods for their evening meal the night before each study visit.

2.3.1.2 Female participants

Hormonal fluctuations during the menstrual cycle can influence appetite and eating behaviour (Dye and Blundell, 1997). It is therefore important to control the menstrual cycle phase in which female participants are studied, particularly as it may obscure responses to experimental conditions (Buffenstein et al., 1995). Female participants not taking the oral contraceptive pill were therefore studied on days six to twelve following menstruation.

2.3.2 Subjective appetite and taste ratings

VAS are tools that rate experience on a continuous dimension between two possibilities such as 'Not at all' and 'Extremely'. VAS are commonly used to measure a variety of subjective sensations including in the field of appetite research. Appetite ratings were collected in three out of four studies in this thesis using VAS presented on single A4 paper sheets (Appendix IV). Each rating was presented as a question with a 100mm horizontal line underneath and the terms 'Not at all' and 'Extremely' anchored at either end.

For example:

How strong is your desire to eat?

Not at all Extremely

Eleven questions were asked at specific time points to measure ratings of 'clear-headed', 'desire to eat', 'energetic', 'friendly', 'full', 'happy', 'hungry', 'jittery', 'nauseous', 'relaxed' and 'thirsty'. The VAS included subjective ratings of mood (e.g. relaxed and friendly) to distract participants from focusing on current motivational state and are not reported herein. Each VAS was presented to the participant at the necessary time point and they were asked to place a mark on the line corresponding to their response. Ratings were then scored on a scale from zero

(not at all) to one hundred (extremely) using a ruler to measure, to the closest millimetre, where the mark intersected the horizontal line. Higher scores implied a greater subjective sensation. Participants were not permitted to review previously completed VAS questionnaires.

Taste ratings were presented in a similar VAS format (Appendix V). After sampling each test drink (chapter three and four), participants were asked 'How (rating) is the drink' which included 'creamy', 'pleasant', 'fruity', 'salty', 'strong', 'sweet', 'bitter' and 'sour'. Each rating was presented as a question with a 100mm horizontal line underneath and the terms 'Not at all' and 'Extremely' anchored at either end. Participants were asked to place a mark on the line corresponding to their response which was then scored on a scale from zero (not at all) to one hundred (extremely) using a ruler to measure, to the closest millimetre, where the mark intersected the horizontal line.

Studies investigating the validity and reliability for measuring appetite using this tool have investigated the reproducibility of VAS questionnaires and their relationship to food intake and feeding behaviour producing inconclusive results (Raben et al., 1995, Flint et al., 2000, Stubbs et al., 2000, Parker et al., 2004). The validity of VAS as a measure of appetite is multifaceted. Ratings of appetite are subjective and should therefore be interpreted with caution or used in conjunction with other methods such as measuring food intake. The VAS have shown motivational appetite ratings such as 'hunger' and 'desire to eat' are related to subsequent food intake (Parker et al., 2004) and best used in within subject, repeated measures designs (Stubbs et al., 2000) as is used in studies within this thesis.

2.3.3 Gastric emptying - ^{13}C breath test

Assessment of gastric emptying rate was performed using the ^{13}C-labelled breath test. Each test meal/drink was labelled with 100mg ^{13}C sodium acetate (CK Gas Products, Hampshire, UK). The principle of this method is that acetate is predominantly absorbed in the small intestine and then rapidly metabolised by the liver to CO_2 which is taken to the lungs via the pulmonary circulation where it is exhaled in the breath. Hence the rate of $^{13}CO_2$ appearance in expired air represents

the rate of its absorption, which in turn reflects its rate of emptying from the stomach. A series of end expiratory breath samples were collected in air tight aluminium bags and sealed with a plastic stopper to be analysed the same day. Samples were collected from participants seated in an upright posture in accordance with data suggesting gastric emptying may be affected by body posture (Moore et al., 1988). A baseline exhaled breath sample was collected on arrival and then end expiratory breath samples were collected at study specific time intervals following the intervention (test meal/drink). Breath samples collected were analysed by non-dispersive infrared spectroscopy using an isotope ratio mass spectrophotometer (IRIS, Wagner Aanlysen Technik, Bremen, Germany) (figure 2.1). This method uses infra-red light to calculate the composition of gases it is presented by determining the ^{13}C to ^{12}C ratio in each breath sample. Results are presented as the change in $^{13}CO_2$ to $^{12}CO_2$ ratio over time. The machine was calibrated using room air and exhaled air as reference values prior to use. This technique, although only a proxy measure of gastric emptying, is a non-invasive, safe and repeatable method. Furthermore, its reliability has been proven against the gold standard scintigraphy method (Braden et al., 1995, Chew et al., 2003).

Figure 2.1 IRIS machine

2.3.4 Blood sampling: collection and processing

The collection and processing of blood samples is described below. Samples collected in chapters three and four were analysed for blood glucose the method of which is described in section 2.3.4.1. Samples collected in chapter three *part III* were analysed for blood glucose, insulin, GLP-1, GIP, PYY and PP the details of which are outlined in the chapter.

A cannula (Venflon, Helsingborg, Sweden) was inserted into a forearm vein for repeated blood collection. Samples were collected from a three way tap using a 10ml syringe (Plastipak, Becton, Dickinson UK. Limited, UK) (figure 2.2). Following each sample collection the cannula was flushed with ~ 10ml of sterile saline solution (0.9%w/v sodium chloride, Baxter Healthcare Ltd, Thetford, UK) to ensure the cannula remained patent. A 2ml sample was collected and discarded before each blood sample collection to avoid any contamination with saline. A small sample was drawn off from each blood sample for the immediate determination of blood glucose and remaining samples were distributed between ethylenediaminetetraacetic acid (EDTA) coated anticoagulant vacutainers (Sarstedt, Leicester, UK) containing 75µL aprotinin (Trasylol, Sigma-Aldrich, Munich, Germany) and serum separating vacutainers (Sarstedt, Leicester, UK). Tubes were centrifuged (Haraeus Labofuge 400R) immediately for fifteen minutes at 3000 revolutions per minute (RPM) and 4^{o}C with the exception of serum separating tubes which were left to clot for thirty minutes at room temperature before centrifuging. After centrifuging, serum for the determination of insulin and plasma for the determination of gut hormones was removed, aliquoted into labelled Eppendorph tubes on ice and stored at -80^{o}C for future analysis as detailed in chapter three.

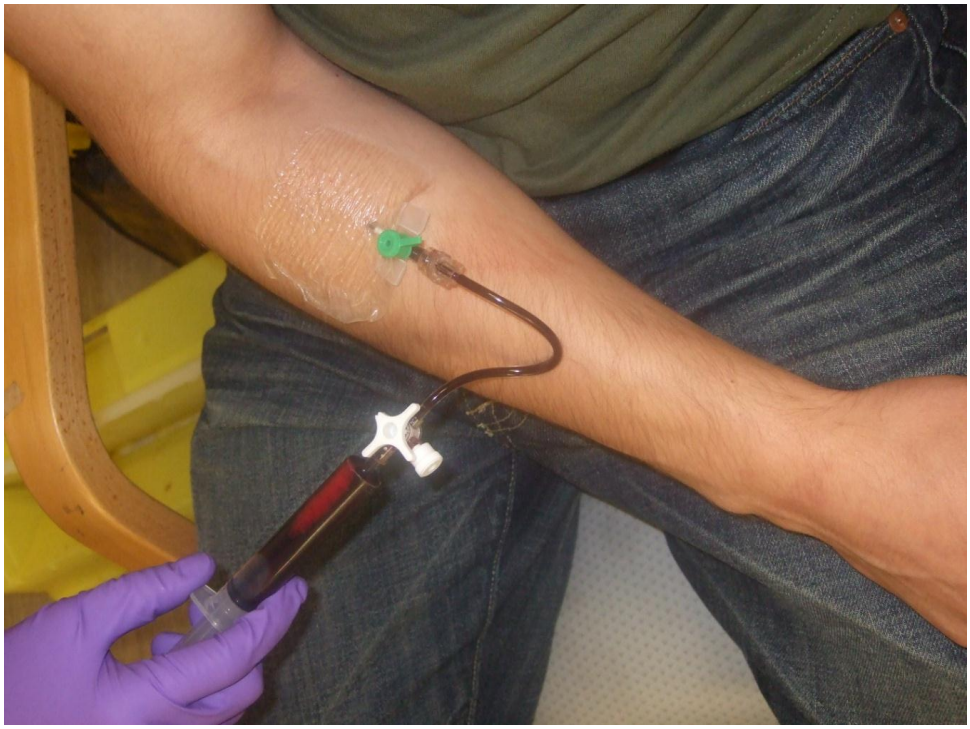


Figure 2.2 Blood sample collection via a three way tap

2.3.4.1 Blood glucose

Blood glucose was analysed immediately using a HemoCue Glucose 201^{+} Analyser (HemoCue, Angelholm, Sweden). The HemoCue analyser uses a dual wavelength photometer to measure glucose in whole blood. Following the collection of each sample a HemoCue cuvette was placed into a droplet of whole blood which was absorbed through capillary action. The cuvette was wiped clean and placed in the cuvette holder to be measured. Results were displayed and are presented in mmol/L.

2.4 Statistical analysis

Descriptive statistics were used for demographic variables such as age, weight, height and BMI. Data were analysed using the Statistical Package for the Social Sciences (SPSS) software (v20.0 for Windows, SPSS, Chicago, IL). Results are reported as means ± standard error of the mean (SEM) unless stated otherwise. Differences in fasting blood glucose, gut hormones and VAS score values were

determined using Student's t-tests. Blood glucose, gut hormone profiles and gastric emptying data are presented as raw values over time and areas under the curve (AUC) calculated using the trapezoidal rule. Blood glucose, gut hormone profiles, gastric emptying data and VAS scores were analysed using two–factor repeated measures analysis of variance (ANOVA) with time and experimental trial as factors, or for the AUC data, with trial, as a factor (chapters three, four and five study I). In chapter five (study I), three-factor repeated measures ANOVA was used to examine differences in variables between trials with the three main effects being condition, route of administration and time. Where there were significant main effects, *post-hoc* analysis using the Bonferroni correction for multiple comparisons were performed. In addition, differences in gastric emptying AUC values for oral and intragastric administration were determined using paired t-tests. Statistical significance was accepted at $P<0.05$. Analysis of data collected from methods not outlined in this section is discussed in the corresponding chapter.

CHAPTER 3

The role of the gut sweet taste receptor in mediating the responses to glucose: an exploration using lactisole

3.1 Introduction

Taste cells in the oral cavity are the first point of contact and initial evaluation of ingested nutrients, contributing not only to the selection of food but the specific satiation and reward value of food. Sweet taste in the mouth has been well characterised (Lindemann, 2001) and is a highly subjective experience involving higher forebrain centres, but by what mechanism/s would the gut determine sugars/sweetness in the absence of the psychological-physical interaction that occurs within the mouth? Fundamental biological similarities between oral taste signalling and 'taste' signalling in the gut have led to the suggestion that cells of the gastrointestinal tract may be involved in 'sugar sensing' by pathways analogous to those mediating and occurring in taste cells of the oral cavity. Beyond tasting at ingestion, it is evident the gastrointestinal tract plays a major role in appetite and food intake control. This is mediated by factors including gastric emptying and the production and release of hormones, including GLP-1, GIP and PYY, from EEC that signal to the CNS, operating via the blood stream and vagus nerve, to induce satiation and thereby limit food intake (Verdich et al., 2001, Degen et al., 2005, Lavin et al., 1996, Lavin et al., 1998).

It is known that glucose, when given orally or administered directly into the gastrointestinal tract, elicits a much greater incretin hormone response than intravenous glucose implying it is sensed from the gut itself (Lavin et al., 1998, Schirra et al., 1996) to induce satiety and suppress intake (Feinle et al., 2002). Following the demonstration of α-gustducin expression, a taste signalling protein involved in taste signal transduction, (Hofer et al., 1996) and sweet taste receptor (T1R2 and T1R3) expression in EEC of the human gut (Dyer et al., 2005, Margolskee et al., 2007) the functional role of the gut sweet taste receptor in gut nutrient sensing has to be experimentally explored. Enteroendocrine L and K cells

are well characterised for their secretion of hormones GLP-1 and GIP respectively but are also thought to express the sweet taste receptor (Jang et al., 2007).

Studies in mice, using knockout models for α-gustducin or the sweet receptor subunit, T1R3, have shown deficiencies in GLP-1 secretion providing evidence for the involvement of GPCRs in nutrient stimulated hormone secretion (Jang et al., 2007, Kokrashvili et al., 2009). Glucose stimulates the secretion of GLP-1 from the human L cell line (NCI-H176) (Jang et al., 2007), a response that is blocked by the sweet taste receptor antagonist, lactisole (Jiang et al., 2005). Furthermore, *in vivo* lactisole suppresses sweet taste perception on the tongue in humans (Schiffman et al., 1999). These observations suggest the gut sweet taste receptor could be involved in the secretion of satiation hormones and that lactisole could be an ideal tool to investigate the functional involvement of gut sweet taste receptors in humans. Therefore, if glucose was sensed by the gut sweet taste receptor, then infusion of a glucose and lactisole solution, administered directly into the stomach to bypass oral taste receptors, should have a lesser effect than the responses to glucose alone. A recent study in humans demonstrated that a glucose solution, administered following lactisole, resulted in a lower mean peak blood glucose (Simpson et al., 2009). It is however, unknown what the effects on gastric emptying, appetite and food intake would be.

The above findings illustrate some of the advances in our knowledge of nutrient sensing; however the exact contribution of the human gut sweet taste receptor system is unclear and a better understanding of its role in hormone release, gastric emptying and the subsequent effect on appetite and food intake presents as a target for therapeutic intervention in obesity. It is not viable to study directly nutrient sensing mechanisms operating within the gut of human subjects; therefore, I determined whether the effect of lactisole on oral sweet taste receptors would have equivalent effects on sweet taste receptors in the gut to modulate gastric emptying, appetite perceptions, food intake and metabolic responses.

The objective was to use lactisole as a tool to investigate the role of gut sweet taste receptors with the hypothesis that if the regulation of gastric emptying, appetite and therefore subsequent food intake are dependent, at least in part, on gut sweet taste receptors then lactisole would attenuate the responses to glucose.

3.2 Method

3.2.1 Design

The study consisted of three experimental parts, each performed as a single-blind randomised cross-over design. Participants attended the laboratory on one occasion in *part I* and on two or four separate occasions (plus a screening visit) for *part II* and *III* respectively with at least five days between each visit (*part II* and *III*). Ethical approval for the study was granted by the National Health Service North West Research Ethics Committee (ref. 10/H1016/11).

3.2.2 Participants

Seven participants (four female, three male) participated in *part I*, nine in *part II* (all male) and seven in *part III* (three female, four male). Two participants were involved in both *part II* and *III*. All participants met the inclusion/exclusion criteria as detailed in chapter 2.2.1.

3.2.3 Experimental protocol

Part I

This preliminary study was undertaken to identify the amount of lactisole necessary to block sweet taste sensing in the study population. Participants were asked to attend the Gastrointestinal Physiology research lab on one occasion prior to which they were asked to refrain from eating, drinking and smoking for a minimum of one hour. Participants were presented with four test drinks including 1M (45g, 180 kcal) glucose, 1M glucose with 250 ppm (25mg/100ml) of lactisole, 1M glucose with 500 ppm (50mg/100ml) of lactisole and 500 ppm lactisole dissolved in tap water in a randomised design. Each test drink consisted of 20ml solution in a plastic cup coded with a letter for identification. Participants were instructed to swirl each sample around their mouths for approximately 20 seconds and then to spit. After sampling each test drink, participants were asked to rate the taste of the drink (chapter 2.3.2).

Between tasting test drinks each participant would thoroughly rinse their mouth with deionised water and wait ~ 10 minutes for any previous taste to dispel.

Part II: pilot study

Participants were studied on two separate occasions and instructed to follow the pre-study standardisation protocol (chapter 2.3.1). On the day of each study participants arrived at the Gastrointestinal Physiology research lab at approximately 9.00 hours following an overnight 11 hour fast. Upon arrival at the laboratory, an intravenous cannula was inserted into a vein in the forearm to allow repeated blood sampling (chapter 2.3.4) and participants were intubated with a nasogastric (NG) feeding tube (Ryles tube 12FG) (figure 3.1).

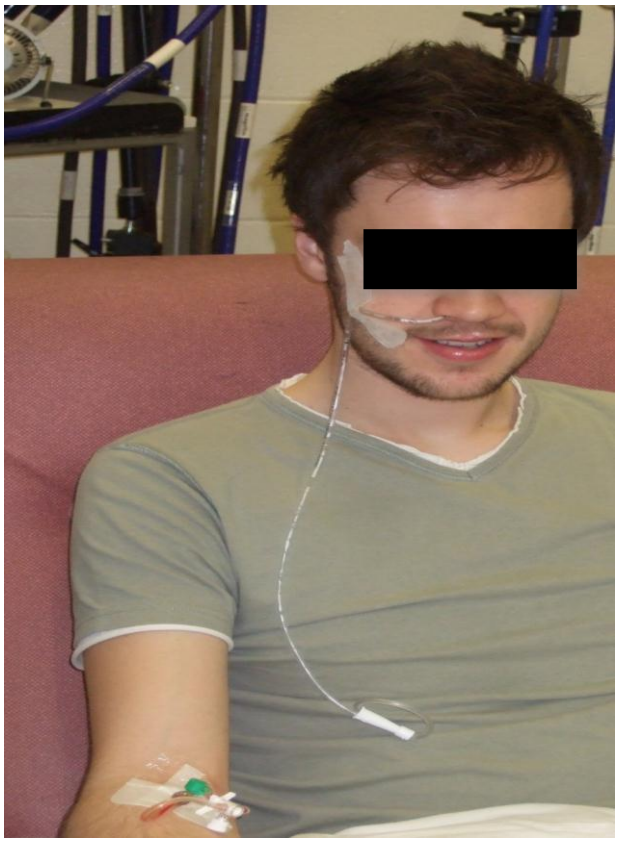


Figure 3.1 Participant in the laboratory following cannulation and nasogastric tube placement

A fasting baseline blood sample was collected and participants were asked to provide a baseline, end-expiratory breath sample (chapters 2.3.4 and 2.3.3 respectively). A baseline assessment of appetite was collected using VAS (chapter 2.3.2). All participants received an intragastric infusion over 2 minutes (t= -2 -0min) of either a) 1M glucose or b) 1M glucose together with 250 ppm lactisole dissolved in tap water to a total volume of 250ml. All infusions were prepared on the morning of the trial at room temperature and were presented in transparent beakers as colourless liquids with no visible differences. Infusions were administered through the NG tube to conceal any olfactory and gustatory differences between infusions which could influence appetite while also ensuring participants remained blind to the nature of the infusions. All infusions were labelled with 100mg of [^{13}C] sodium acetate for determination of gastric emptying rate, which was the primary end point based on previous observations that gastric emptying is potently delayed by glucose. End-expiratory breath samples and blood samples (10ml) were collected immediately before the infusion (t= -5) and at t= 5, 10, 15, 30, 45, 60, 90, 120 and 150 min. Subjective appetite perceptions of hunger, fullness and desire to eat were collected using VAS immediately before and after the infusion (t= -5 and 0) and at 15 minute intervals (t= 15, 30, 45, 60, 90, 120 and 150 min). The NG tube was removed immediately following the infusion and after 150 min, the intravenous cannula was removed. Participants were presented with an *ad-libitum* pasta based test meal the composition of which is described below (chapter 3.2.5). Following the termination of the test meal participants were free to leave the laboratory. The study protocol is presented in figure 3.2.

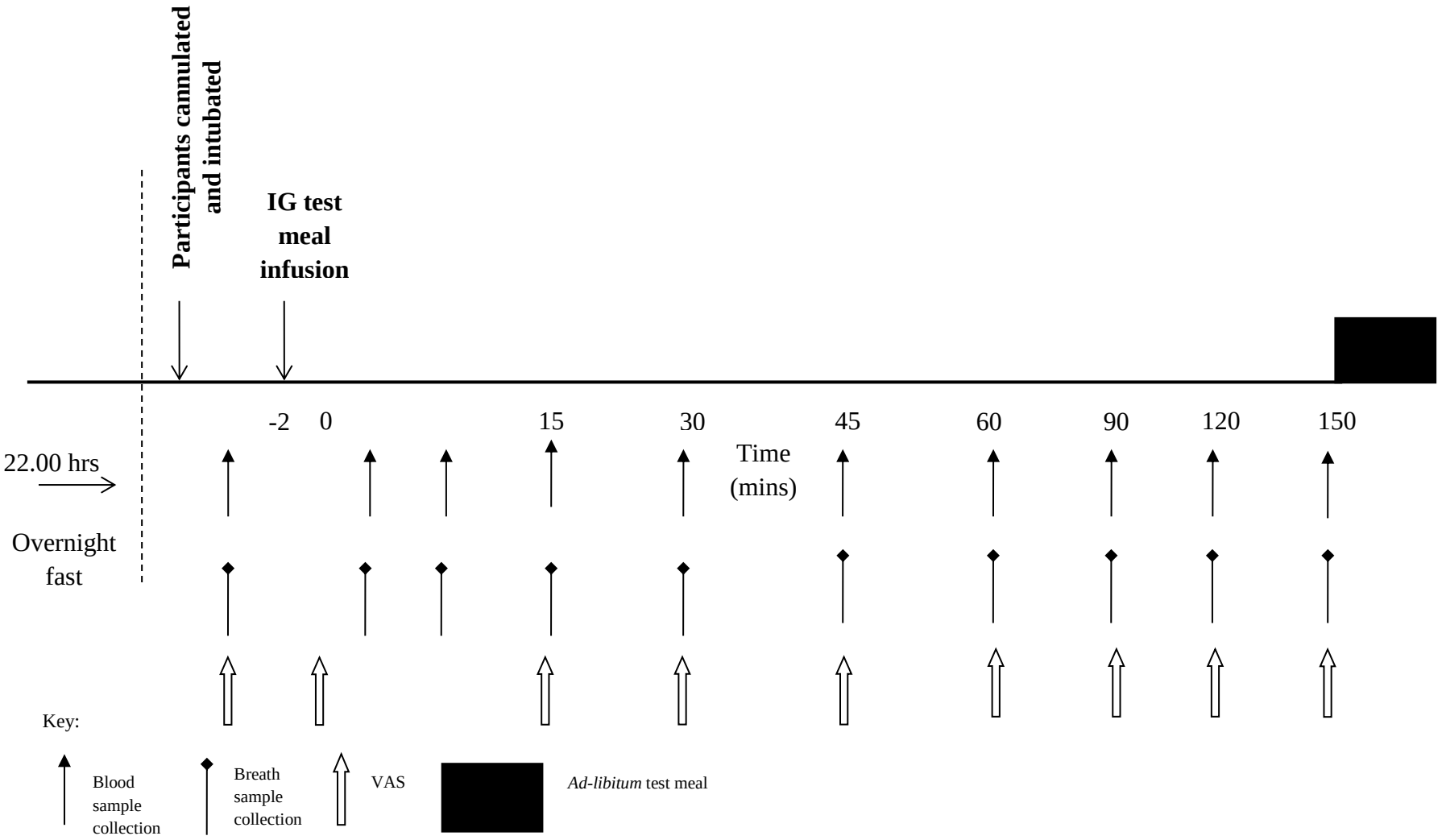


Figure 3.2 Schematic representation of study protocol (*part II*)

IG- intragastric

3.2.4 Blood glucose

Blood samples were collected and processed as described in chapter 2.3.4. Blood glucose was determined immediately using HemoCue Glucose 201^{+} Analyser as outlined in chapter 2.3.4.1.

3.2.5 Assessment of energy intake

3.2.5.1 *Ad-libitum* test meal composition

Each test meal bowl consisted of 125g penne pasta (dry weight), 40g mature cheddar cheese, 15g olive oil (J Sainsbury, London UK) and 170g Dolmio tomato and basil sauce (Masterfoods, Slough UK) providing 814 kilocalories (49% carbohydrate, 14% protein and 37% fat). The composition of one portion is displayed in table 3.1 and a photograph shown in figure 3.3.

	Amount (g)	Energy (kcal)	Protein (g)	CHO (g)	Fat (g)
Dolmio (1 sachet)	170	88.4	2.6	13.4	2.7
Mature Cheddar	40	164	10.0	0.04	13.6
Olive oil	15	134.9	0	0	15
Dry weight pasta	125	427.5	15	92.6	2.3
Total		814.7	27.5	106	33.7
Percentage energy			14%	49%	37%

Table 3.1 Nutrient composition of test meal

Figure 3.3 *Ad-libitum* test meal

3.2.5.2 Test meal preparation and energy intake calculation

Test meal portions were prepared in advance and heated in a microwave oven when required to minimize any food related smells in the laboratory prior to the serving of the meal, which could influence participants' appetite (Yeomans, 2006). The *ad-libitum* test meal was presented to participants in a bowl which was removed and replaced with another following consumption until participants indicated they were satiated. Calculation of total energy intake was determined by the weight of food consumed. The homogeneous nature of the test meal enabled the calculation of energy and macronutrient intake by the weight of food consumed. This method has been verified as a reproducible method for assessing energy intake (Gregersen et al., 2008).

3.2.6 Materials and test foods

D- (+)- Glucose (Sigma-Aldrich, Munich, Germany). The concentration of glucose was based upon previous work showing 1M glucose to have a potent effect on gastric emptying (Little et al., 2010a).

Lactisole (Endeavour Chemicals, Northamptonshire, UK). The chosen dose of lactisole was derived from earlier work demonstrating a reduced sweet intensity rating of a panel of sugars and NNS in mixtures with lactisole (Schiffman et al., 1999).

[^{13}C] sodium acetate (CK Gas Products, Hampshire, UK).

3.2.7 Statistical analysis

Data are presented and analysed as described in chapter 2.4. Gastric emptying AUC values and energy intake, presented in grams (g) and kilocalories (Kcal), were analysed using a paired t-test (*part II*).

3.3 Results -*Part I*

3.3.1 Participants

Participant characteristics are displayed in table 3.2

	mean ± SD (*n* =7)
Age (years)	27.7 ± 2.4
Height *(cm)*	173.9 ± 10.7
Weight *(kg)*	64.1 ± 9.5
BMI (kg/m^2)	21.9 ± 1.8

Values represent mean ± SD (n =7)

Table 3.2 Participant characteristics

3.3.2 Visual analogue scales - taste

All participants scored the glucose test drink as being significantly sweeter yielding a higher mean score (82.1 $\pm$ 6.3) than the other test drinks containing the same concentration of glucose mixed with 250 ppm (9.4 $\pm$ 4.3 P <0.001) or 500 ppm lactisole (8.7 $\pm$ 3.7, P < 0.001), (figure 3.4A). Participants scored the lactisole alone test drink as being significantly more bitter than when presented with glucose, (P< 0.01), however this did not quite reach significance for the glucose and lactisole mixtures (P= 0.053) (figure 3.4B).

Therefore, the extremely oral sweet taste of 1M glucose was effectively abolished by 250-500 ppm lactisole, and it was hypothesised that any of the well characterised gastrointestinal effects of glucose exerted via T1R would also be attenuated by lactisole co-administered with glucose.

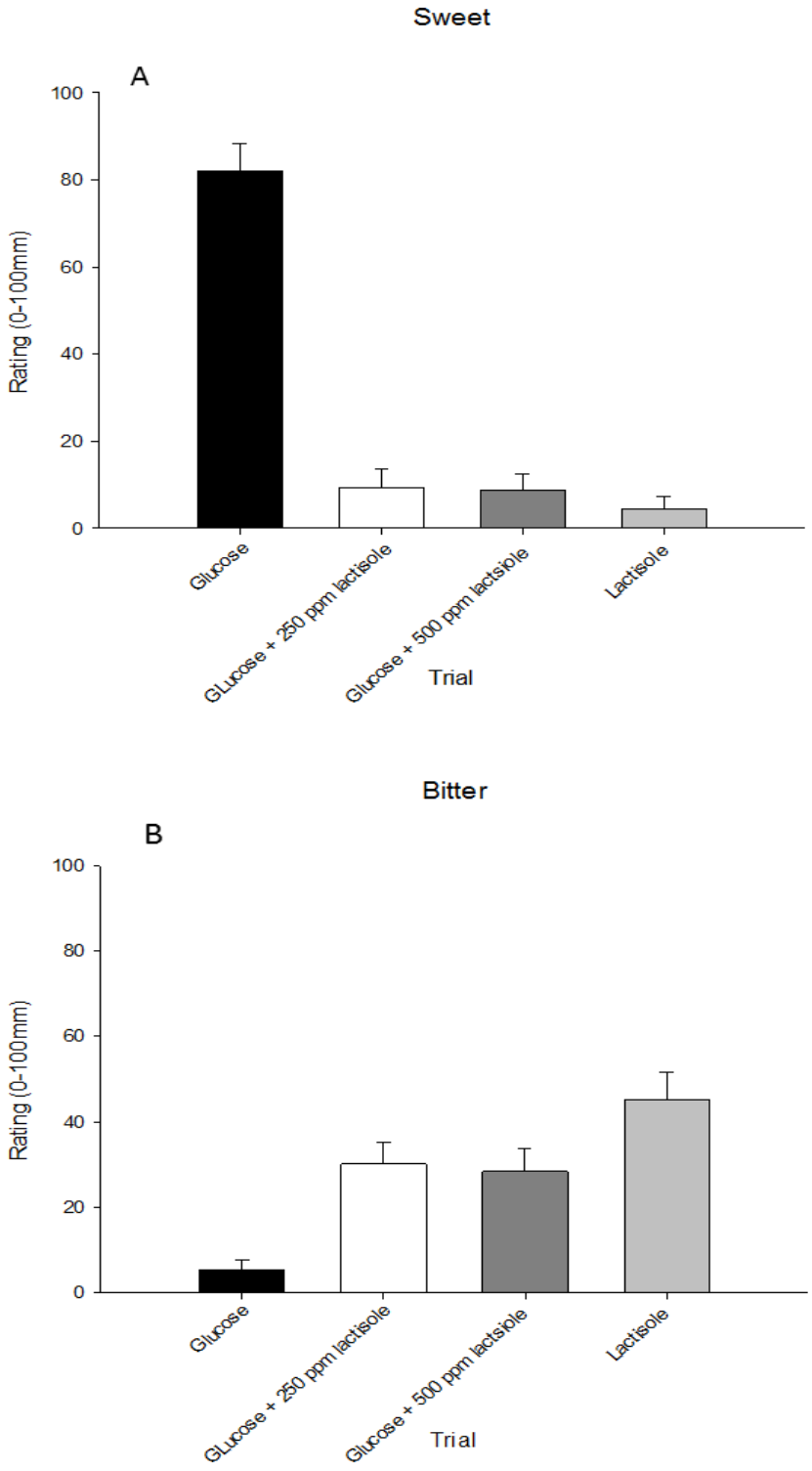


Figure 3.4 Sweetness and bitterness ratings

Mean sweetness (A) and bitterness (B) ratings for glucose (■), glucose + 250 ppm lactisole (□), glucose + 500 ppm lactisole (▩) and lactisole (▨) test drinks. Values represent mean ± SEM, (n=7).

3.4 Results -*Part II*

3.4.1 Participants

Participant characteristics are displayed in table 3.3

	mean ± SD (*n* =9)
Age (years)	24.9 ± 2.5
Height *(cm)*	179.1 ± 6.9
Weight *(kg)*	72.7 ± 7.0
BMI (kg/m^2)	22.7 ± 2.3
Restraint Score[1]	2.3 ± 1.8

Values represent mean ± SD (n =9)

[1] Score of restraint using the three factor eating questionnaire (Stunkard and Messick, 1985)

Table 3.3 Participant characteristics

3.4.2 Gastric emptying

Following both infusions, there was a rise in $^{13}CO_2$: $^{12}CO_2$ with peak values reaching 40.3 $\pm$ 3.3 and 43.6 $\pm$ 1.6 in the glucose + lactisole and glucose conditions respectively (figure 3.5A). Peak values were reached at 45 minutes after which $^{13}CO_2$: $^{12}CO_2$ values began to decrease and by 60 minutes the plots converged indicating the rates of gastric emptying were similar from that point forward. There was a main effect of time ($P < 0.001$) but there was no significant effect of lactisole nor a trial x time interaction on the rate of gastric emptying ($P= 0.51$ and $P= 0.63$ respectively) or the area under the curve ($P= 0.54$) (figure 3.5B).

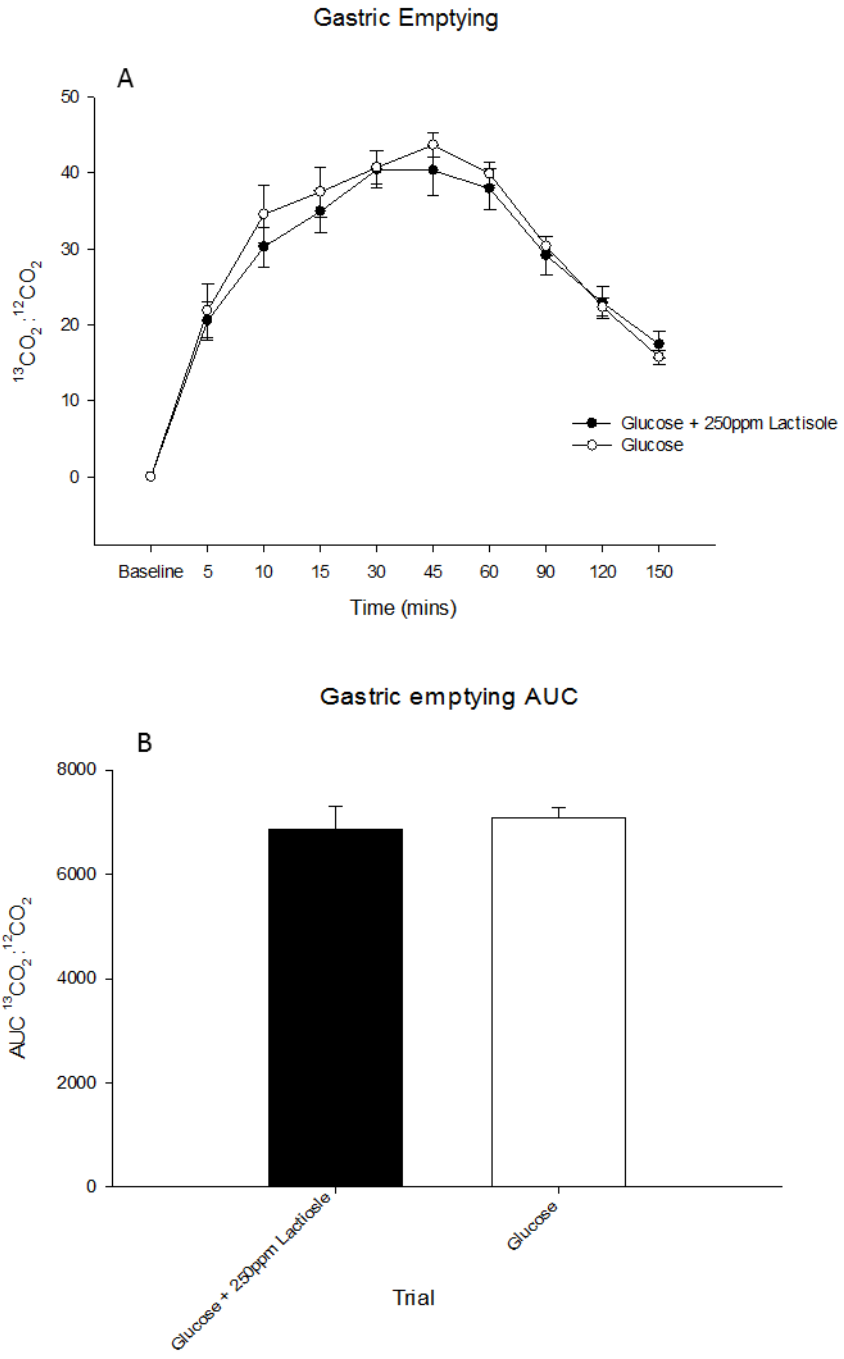


Figure 3.5 Gastric emptying rate

Gastric emptying rate for glucose + 250 ppm lactisole (⊸ / ■) and glucose (⊸/□) trials displayed over time (A) and as area under the curve (B). Values represent mean ± SEM, (n=9).

3.4.3 Blood glucose

There was no significant difference in fasting blood glucose concentrations between the two trials (P= 0.21). In both trials blood glucose increased and peaked at 30 minutes after infusion reaching 7.7 ± 0.6 mmol/L and 7.3 ± 0.4 mmol/L for the glucose + lactisole and glucose trials respectively (Figure 3.6). Blood glucose responses to the infusion showed a significant main effect of time (P < 0.001) but there was no significant effect of lactisole (P= 0.41).

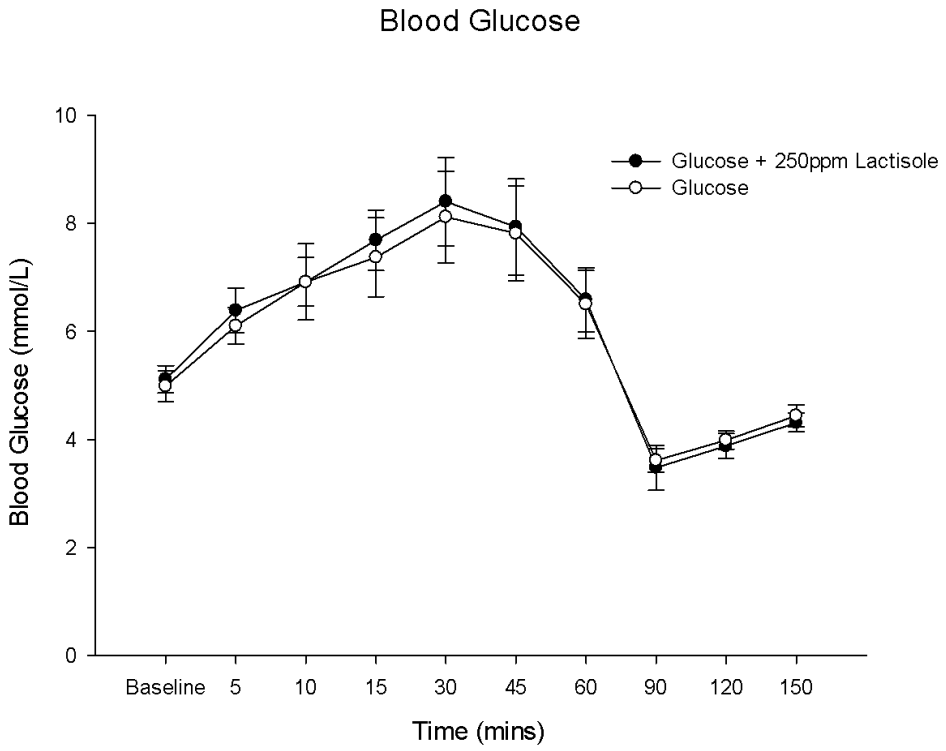


Figure 3.6 Blood glucose

Blood glucose concentrations for the glucose + 250 ppm lactisole (⊸) and glucose (⊸) trials displayed over time. Values represent mean ± SEM, (n=9).

3.4.4 Appetite perceptions

Baseline ratings of desire to eat, hunger, fullness and nausea were not significantly different between the trials ($P > 0.05$). Ratings of desire to eat and hunger decreased following the infusion while ratings of fullness and nausea increased in both trials (figure 3.7). Ratings of desire to eat tended to be higher and ratings of fullness lower in the glucose + lactisole trial compared to the glucose only trial however these observations were not significant. There was a main effect of time ($P < 0.001$) for each of the appetite perceptions assessed (desire to eat, hunger and fullness) but there were no trial or trial x time interaction effects for desire to eat or fullness. For hunger, there was a significant trial effect ($P= 0.04$) showing that ratings tended to be higher in the glucose + lactisole trial compared to glucose alone. Following their decline, ratings of hunger and desire to eat increased until the test meal. Similarly, following the increase, ratings of fullness gradually decreased until the test meal. There were no trial or interaction main effects for nausea and following a slight increase post infusion, ratings decreased until the test meal.

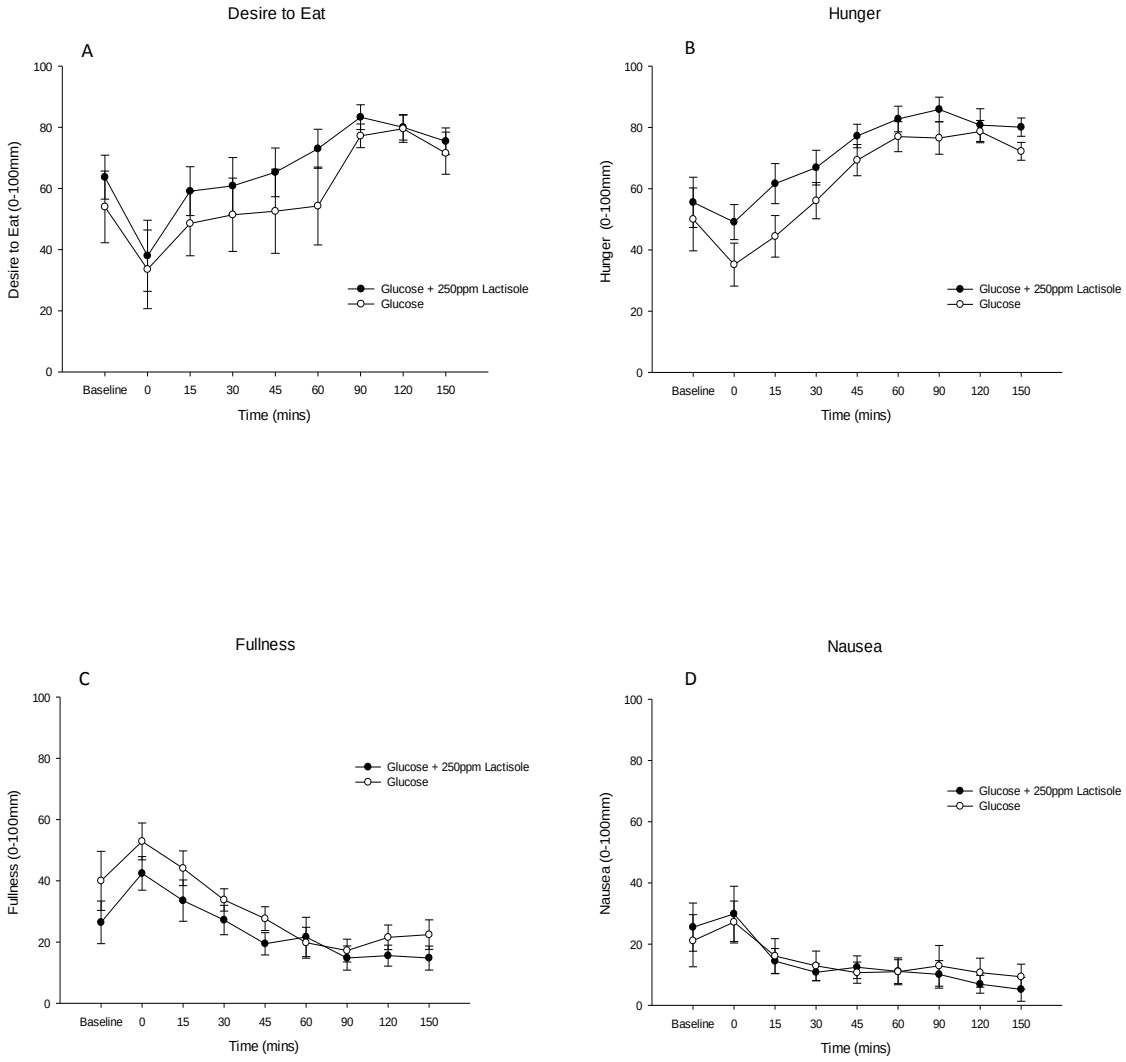


Figure 3.7 Subjective appetite ratings

Subjective ratings for desire to eat (A), hunger (B), fullness (C) and nausea (D) for the glucose + 250 ppm lactisole (●) and glucose (○) trials displayed over time. Values represent mean ± SEM, (n=9).

3.4.5 Test meal intake

There was no significant difference in the amount of food consumed during the *ad-libitum* test meal between trials, 826.3 ± 55.6 vs. 765.3 ± 57.8g (1295.3 ± 87.1 vs. 1200.0 ± 90.7 Kcal) in the glucose + lactisole and glucose trials respectively (P= 0.19) (figure 3.8)

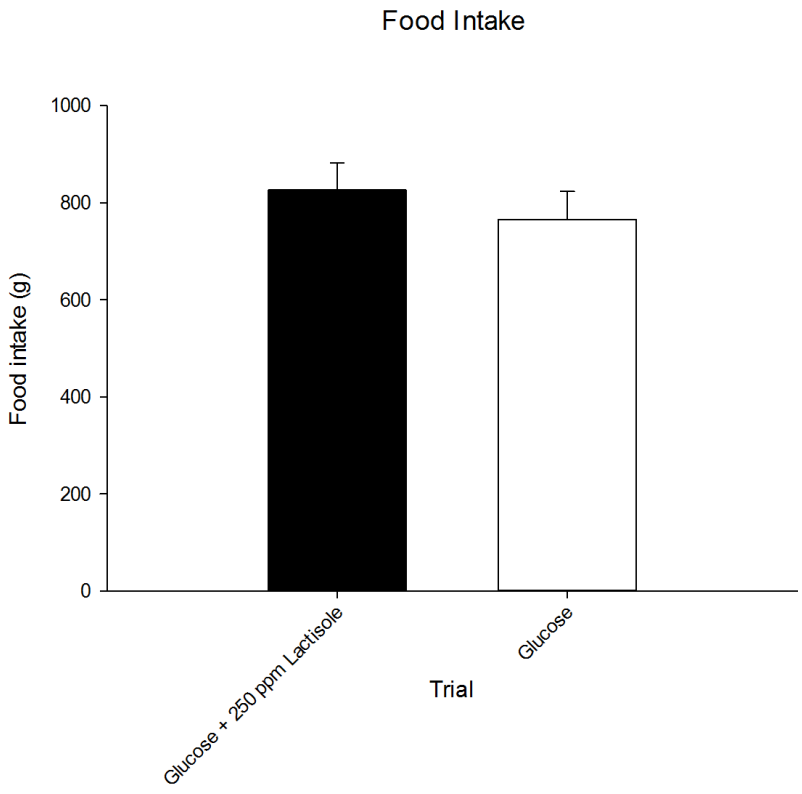


Figure 3.8 Food intake

Food intake at the *ad-libitum* test meal following the glucose + 250 ppm lactisole (■) and glucose (□) trials. Values represent mean ± SEM (n=9).

3.5 Discussion *Part I* and *II*

Although the sample size of nine subjects was small in this proof of concept study, interim analysis at this point made it clear that no significant difference in the primary end point would be identified even with an extremely large study, so further recruitment was discontinued. The study was halted at this stage and a new protocol devised. A more controlled cross over study was initiated, but using the higher dose of lactisole in case any dilutional effect in the stomach had dropped the 250 ppm lactisole below an effective concentration.

The initial evaluation of nutrients being ingested takes place within the mouth. Sweetness, one of five basic taste qualities that is distinguished, is mediated by GPCRs, and is dependent on what has been identified as the sweet taste receptor, comprising of the two subunits T1R2 and T1R3 (Nelson et al., 2001). Glucose, along with other caloric sugars, is a known activator of sweet taste receptors in the oral cavity enabling sweet taste perception, an effect which is suppressed by lactisole (Schiffman et al., 1999) via its antagonistic binding to T1R3 (Jiang et al., 2005). In the preliminary study (*part I*), lactisole at both 250 ppm and 500 ppm, when mixed with glucose, significantly suppressed the sweetness of the mixture. This is in line with previous work that showed lactisole blocking the sweetness intensity for a panel of sugars and NNS (Johnson et al., 1994, Schiffman et al., 1999). The experience is really quite striking when sampled by the investigative researchers during preliminary development: 1M glucose is extremely sweet and this simply disappears.

Food intake is not only determined by the sense of taste but subsequently by cells of the gastrointestinal tract. Following the identification of the same sweet taste receptors expressed in the human gastrointestinal tract as in the mouth, it raises the question of whether this system plays a functional role in sugar sensing in the small intestine, and the physiological responses that ensue. Results from *Part I* formed the basis for the design of *Part II* of the study. Lactisole at a concentration of 250 ppm successfully blocked sweet taste receptors in the mouth presenting a tool to investigate the role of the gut-expressed receptor counterparts. The aim of the study (*part II*) was to investigate whether the responses to glucose were mediated, at least in part, by the sweet taste receptors in the gut. Following an intragastric infusion of glucose with or without the lactisole, the effects on gastric emptying, blood glucose,

appetite perceptions and food intake were measured. It is of course not feasible to directly study taste receptor activation in the human gut; therefore, lactisole was used as a tool. The current data demonstrate that, when administered directly into the gut to bypass oral taste receptors, the addition of lactisole does not affect the rate of gastric emptying, blood glucose or food intake in response to glucose.

To my knowledge this is the first study that has used an intragastric infusion of lactisole in human subjects and measured the responses to a glucose solution involving both physiological and subjective and actual measures of appetite and food intake respectively. There were no differences in blood glucose between the two conditions, unlike that found by Simpson and colleagues who found that lactisole slightly attenuated postprandial hyperglycaemia (Simpson, Nixon et al 2009). However, as lactisole is known as a competitive inhibitor (Schiffman et al., 1999) it is not known how a pre-infusion of lactisole, as used by Simpson and colleagues, would alter the blood glucose responses, since in our developmental work prior to the experiments inhibition of oral taste was observed only when lactisole is mixed with glucose prior to tasting.

There is also some evidence from animal models that glucose transport may be increased by sweet taste receptor activation in the gut. However, lactisole did not affect blood glucose levels in the current study. Although not currently significant, inspection of the data suggests that a very minor difference may arise (in a larger powered study) to show gastric emptying rate to be trivially faster for the first 60 minutes in the glucose + 250 ppm lactisole condition compared to glucose alone. However, it is unlikely that such minor differences would have any biomedical relevance, and the need to study large groups to detect small differences would not be pragmatic or worthwhile using the current study protocol, requiring NG intubation.

Furthermore, while food intake at the lunchtime test meal did not display any significant differences between the two conditions, subjective appetite ratings indicated increased hunger and desire to eat, and decreased fullness in the glucose + 250 ppm lactisole condition compared to glucose alone. The fact that results did not reach statistical significance, except for hunger, may be due to low power, since data for nine participants only were tested. The inability to detect differences in

subjective appetite scores could be due to the nature of subjective ratings that gives rise to large between participant and within participant variability. This is an innate problem with VAS, and as gastric emptying was the primary endpoint it was not considered worthwhile to pursue this protocol further.

From the current data it appears the effect of glucose was preserved in the presence of lactisole, and, although small differences may perhaps emerge with larger sample sizes, 250 ppm lactisole appears to be having little effect. This does not support the current hypothesis, but does accord with the earlier findings of Little *et al* who showed no role for the sweetness of a meal in mediating gastric emptying or appetite responses, in that responses induced by ingested sugar were not replicated by NNS (Little et al., 2009). It may be therefore that the sweet taste receptors are not implicated in these responses to glucose in humans and blocking will confer no alteration in responses. However, given that the stimulation of T1R2 and T1R3 by sugars and NNS, can increase uptake of an oral glucose load in animal models (Mace 2007), and thus the rate of glucose uptake is reported to be regulated by these receptors (Dyer, Daly et al, 2007), it was thought that stimulation would factor in blood glucose levels and gastric emptying. Factors which determine postprandial blood glucose concentrations include the nutrient composition of meals, the rate of gastric emptying, which influences the rate at which nutrients are delivered to the small intestine from the stomach and the rate of glucose absorption in the small intestine (Horrowitz, 1993). Furthermore, stimulation of these receptors has been shown to promote the release of gut hormones, such as GLP-1 and GIP, which control gastric motility and influence appetite and food intake.

The current data, taken with previously published work, suggest that in humans T1R2 and T1R3 do not mediate the physiological effects of glucose on gastric emptying, and may only play a small role in appetite. However, a number of alternative possibilities may explain these observations: (i) the dose of lactisole is insufficient to block the sweet taste receptors in the gut, (ii) limitations of the protocol and method used. In particular, it is not possible to include a positive experimental control for the effect of lactisole in blocking gut sweet taste receptors, since there is no established physiological response to block. The use of oral sweet taste abolition is the closest proxy I could use to demonstrate that the agent does

exert an effect in the study group, but clearly is limited in validity. Including a no-glucose control to show that glucose itself had an effect in these subjects might have been worthwhile, although this is extremely well established in multiple published studies so was not considered essential in this early phase study.

In considering the first possibility, the dose of lactisole used in this study is based on results from *Part I* in which 250 ppm lactisole successfully blocked sweet taste receptors in the mouth and previous observations showing lactisole at the same concentration, was able to lower the postprandial rise in blood glucose following a glucose infusion (Simpson et al., 2009). Furthermore lactisole has been shown to inhibit sweet taste at even lower concentrations (150 ppm) (Schiffman et al., 1999), so it is unlikely that any lack of effect was purely dilutional. However, the present study assumes that the sweet taste receptors found in the gut, function and signal similarly to those in the mouth and thus the dose of lactisole which inhibits sweet taste in the mouth would be sufficient enough to interact with domain of T1R3 receptor in the gut to block the effect of glucose. While T1R2 +T1R3 receptors and intracellular signalling elements have been found in taste cells in the mouth and EEC in the human gut (Margolskee, 2002), there may be differences in the level of expression. Dyer and colleagues demonstrated, in mice, the expression of T1R receptors and the signalling element, α-gustducin are expressed along the length of the small intestine (Dyer 2005). However, they found expression levels were low and equivalent to those detected on the tongue suggesting that either the level of receptors expressed in cells is low or expression is limited to a subpopulation of cells rather than all cells along the villus.

The second possibility relates to a number of limitations in the study design and methods. The duration of the study may affect the sensitivity of the *ad-libitum* test meal to detect any differences in food intake. Plots for gastric emptying converge after 60 minutes and it is arguable that the test meal is being be served too late and may be more sensitive if it was served between 40 and 60 minutes post-infusion. Some of the neuroendocrine effects of a meal are however sustained for several hours, and there is evidence that for example vagal sensitivity to gut hormones is altered for a significant time period (le Roux and Bloom, 2005).

Taking into account these possible limitations, the protocol in *part III* was designed to address these methodological issues: the hypothesis and aims remain the same. To maximise the likelihood of finding effects, the dose of lactisole was doubled to 500 ppm. The effect of carbohydrate on food intake is dependent on the rate of gastric emptying and in turn blood glucose and gut hormone release. Owing to this, the study duration was shortened. In healthy subjects, blood glucose usually peaks at or before 60 minutes following a carbohydrate test meal (Feinle et al., 2002) and given the convergence of gastric emptying plots after 60 minutes it is thought that decreasing the study duration and serving the *ad-libitum* test meal at 60 minutes may impact on food intake. In keeping with this, potential effects that might have been present in *part II* were noticed in the first hour.

Although blood was collected and processed for the determination of gut hormones it was decided, based on the current observations, that further analysis would not be worthwhile or economically justified. However, given the significance of gut hormone secretion to gastrointestinal function and food intake, *part III* has investigated the role of the human gut sweet taste receptor in regulating insulin, GLP-1, PYY, GIP and PP release. Furthermore, the addition of two control conditions, lactisole and water, were added to the protocol.

3.6 Experimental protocol - *Part III*

Participants were studied on four separate occasions and instructed to follow the pre-study standardisation protocol (chapter 2.3.1). On the day of each study participants arrived at the Gastrointestinal Physiology research lab at approximately 9.00 hours following an overnight 11 hour fast. The experimental protocol was identical to the protocol described in *part II* with the exception of the lactisole dose which was increased from 250 ppm to 500 ppm and the duration of the study was changed to 60 minutes. These changes were based on the results of the pilot work in *part II*. Furthermore, two control conditions were added to the protocol to take the total number of visits, excluding the screening visit, up to four. All participants therefore received an intragastric infusion over 2 minutes (t= -2-0min) of either a) 1M of glucose, b) 1M glucose together with 500 ppm lactisole, c) 500 ppm lactisole or d) tap water in a total volume of 250ml. In comparison to the all male participation in *part II, part III* involved the inclusion of female participants who if not on the oral contraceptive pill were studied during days 6-12 only of the menstrual cycle (follicular phase) to avoid any potential effects of the menstrual cycle on appetite, food intake and gastrointestinal function (Dye and Blundell, 1997, Brennan et al., 2009) (chapter 2.3.1.2). The study protocol is presented in figure 3.9.

3.6.1 Gut hormone concentrations

Samples collected were analysed for blood glucose, insulin and GLP-1. Blood glucose was determined immediately using HemoCue Glucose 201^{+} Analyser (Hemocue, Angelholm, Sweden) (chapter 2.3.4.1). Serum samples were defrosted and insulin concentrations determined using an ELISA (enzyme-linked immunosorbent assay) kit (Alpco Diagnostics, Massachusetts, USA). Plasma samples from whole blood collected in EDTA coated tubes containing aprotinin were defrosted and total GLP-1 concentrations were determined by ELISA immunoassay (Alpco Diagnostics, Massachusetts, USA) according to the manufacturer's instructions. To ensure precision of analysis, quality controls supplied with each assay kit were run on each plate. All samples were measured in duplicate (VersaMax, Molecular Diagnostics, Berkshire, UK) but the five and 60

minute time point samples were excluded to ensure all samples from the same participant could be analysed on the same assay to avoid inter-assay variation.

Further analysis was conducted to establish a fuller gut hormone profile. In the light of the broadly negative physiological data in the preceding study, actual assays were only undertaken using the two main experimental trials, i.e. glucose with and without 500 ppm lactisole. Total GIP, PP and PYY concentrations were determined in duplicate using a Multiplex assay kit (Millipore Corporation, Missouri, USA) and plate reader (Luminex 200, Luminex Corporation, Texas, USA) according to the manufacturer's instructions.

3.6.2 Statistical Analysis

Data are presented and analysed as described in chapter 2.4. Energy intake, presented in grams (g) and kilocalories (Kcal), was analysed using repeated measures ANOVA. The two main experimental trials of interest referred to are glucose + 500 ppm lactisole and glucose.

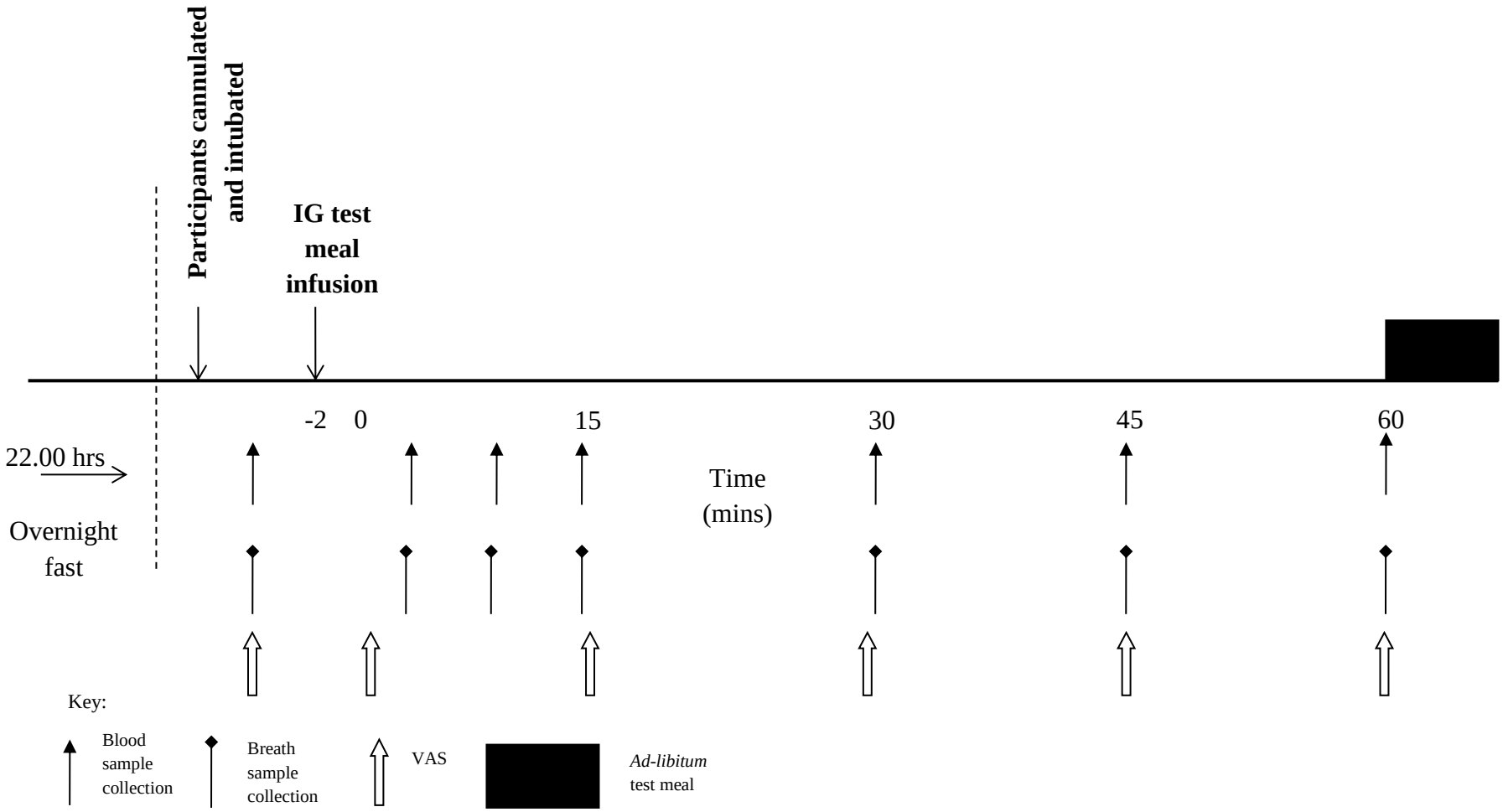


Figure 3.9 Schematic representation of study protocol (*part III*)

IG-intragastric

3.7 Results- *Part III*

3.7.1 Participants

Participant characteristics are displayed in table 3.4

	mean ± SD (*n* =7)
Age (years)	19.9 ± 1.1
Height *(cm)*	173 ± 10.7
Weight *(kg)*	66.7 ± 7.0
BMI (kg/m^2)	22.1 ± 1.5
Restraint Score[1]	3.7 ± 2.3

Values represent mean ± SD (n =7)

[1] Score of restraint using the three factor eating questionnaire (Stunkard and Messick, 1985)

Table 3.4 Participant characteristics

3.7.2 Gastric emptying

Gastric emptying was the primary end point in this study. There was a main effect of time ($P < 0.001$). Following all infusions, there was a rise in $^{13}CO_2$: $^{12}CO_2$ with peak values reaching 40.2 $\pm$ 1.5, and 35.1 $\pm$ 4.0 in the glucose + lactisole and glucose trials respectively (figure 3.10A). In the control trials, lactisole and water, values peaked at 49.9 $\pm$ 2.9 and 55.9 $\pm$ 3.5 respectively. Peak values were reached at 30 minutes after which $^{13}CO_2$:$^{12}CO_2$ values began to decrease. As expected, glucose slowed gastric emptying when compared with the lactisole and water trials. For gastric emptying rate and AUC (figure 3.10B), there was a main effect of trial ($P< 0.01$), however post-hoc analysis revealed the lactisole + glucose trial had no differential effect on gastric emptying rate or AUC compared with the glucose trial (3080.3 $\pm$ 159.1 vs. 2743.2 $\pm$ 275.0).

Around the time that this work was being undertaken, Gerspach *et al* published a large study involving 35 participants showing that lactisole had no effect at all on gastric emptying when added in a similar dose (450ppm) to an even higher glucose load (75g) or a mixed nutrient meal (Gerspach et al., 2011). Given the lack of any effect on my primary endpoint at this stage a decision was made to terminate this study rather than recruit more participants, on ethical and pragmatic grounds. However the additional data are presented below and will be discussed in light of Gerspach's data.

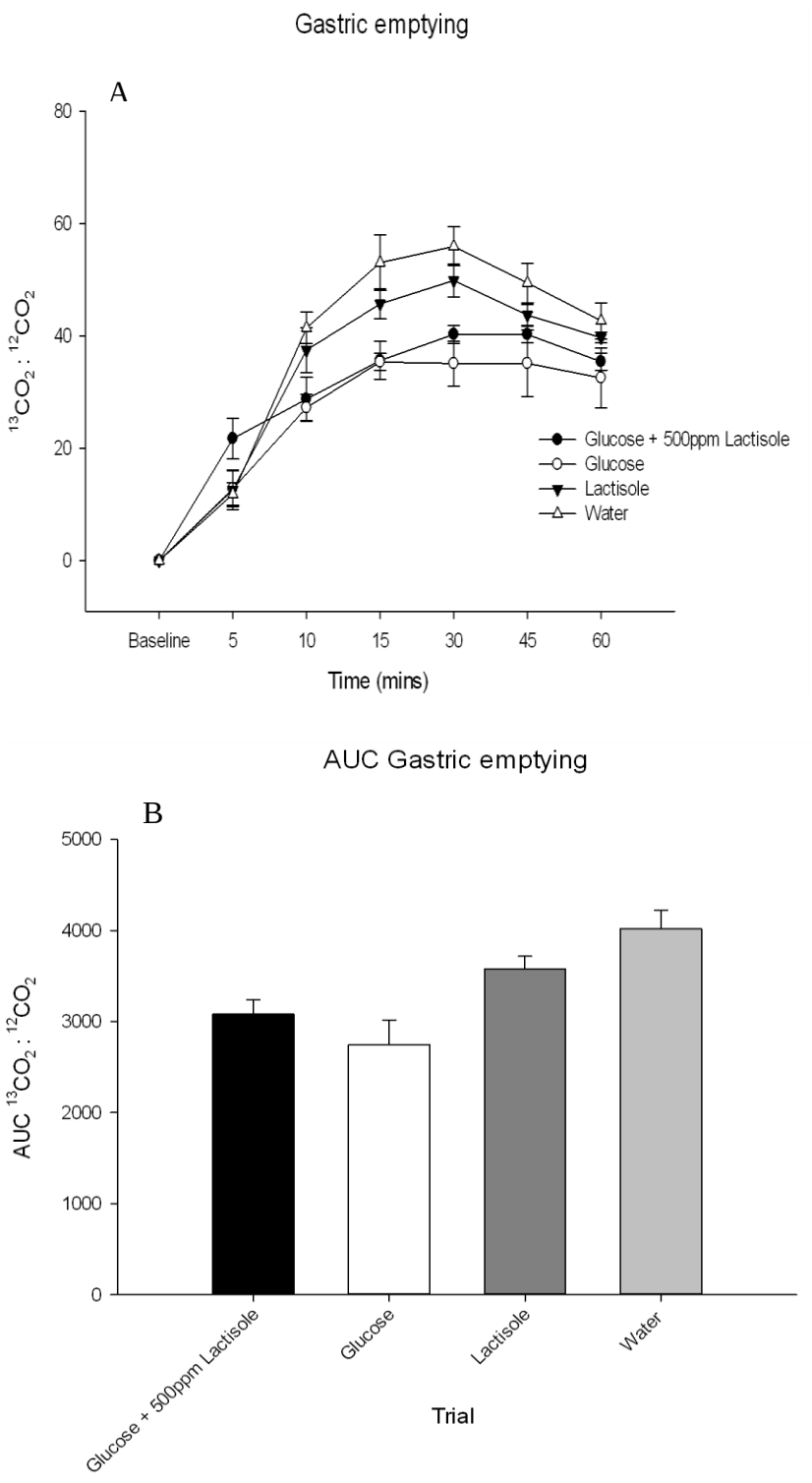


Figure 3.10 Gastric emptying rate

Gastric emptying rate for glucose + 500 ppm lactisole (✦ / ■), glucose (-○- / □), lactisole (-★- / ■) and water (-△- / □) trials displayed over time (A) and as area under the curve (B). Values represent mean ± SEM, (n=7).

3.7.3 Blood glucose

There was no significant difference in fasting blood glucose concentrations between the four trials (P= 0.82). Following the glucose + lactisole and glucose trials blood glucose increased and peaked at 30 minutes after infusion reaching 8.4 ± 0.8 and 8.1 ± 0.8 mmol/L respectively (figure 3.11). As expected, the two control trials, lactisole and water, had no effect on blood glucose. Blood glucose responses to the infusions showed a significant main effect of time (P < 0.001) and trial (P < 0.001), but post-hoc analysis did not indicate there was any difference between the two main experimental trials.

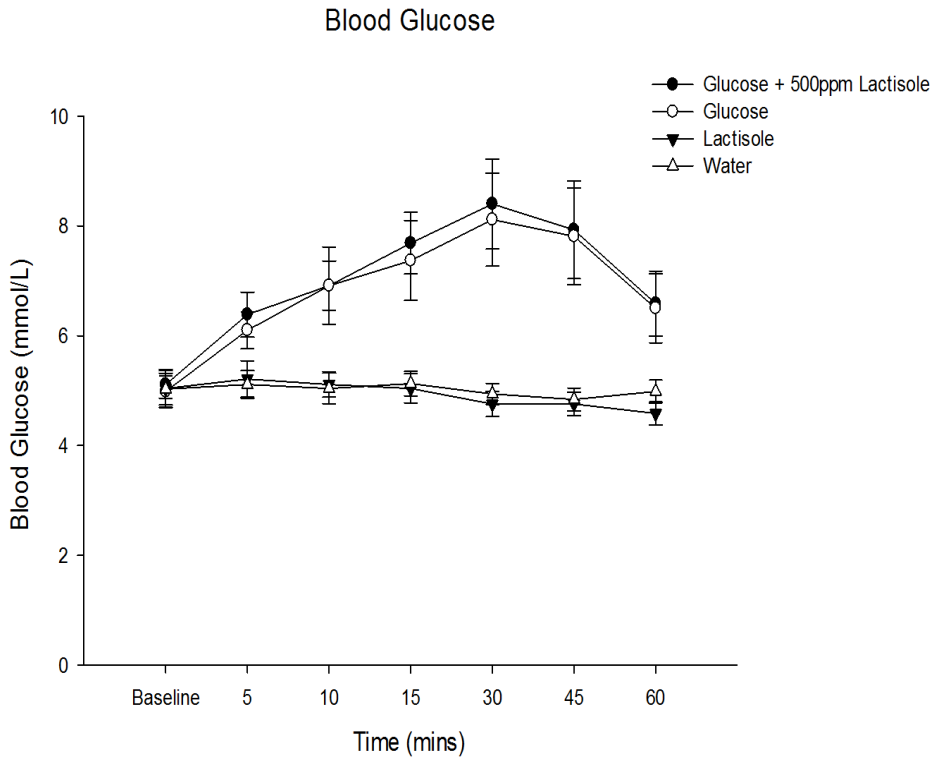


Figure 3.11 Blood glucose

Blood glucose concentrations for the glucose + 500 ppm lactisole ( ), glucose ( ), lactisole ( ) and water ( ) trials. Values represent mean ± SEM, (n=7).

3.7.4 Gut hormone concentrations

There were no significant differences in fasting concentrations between the four trials for any of the gut hormones ($P > 0.05$). Following the glucose + lactisole and glucose trials, serum insulin (figure 3.12) and plasma GLP-1 concentrations increased and peaked at 30 and 15 minutes respectively in both trials (figure 3.13A). As expected, the two control trials, lactisole and water, had no effect on either serum insulin or plasma GLP-1. Serum insulin and plasma GLP-1 responses to the glucose infusions showed a significant main effect of time ($P < 0.001$) and trial ($P < 0.001$), but post-hoc analysis did not indicate there was any difference between the two main experimental trials for either hormone ($P > 0.05$).

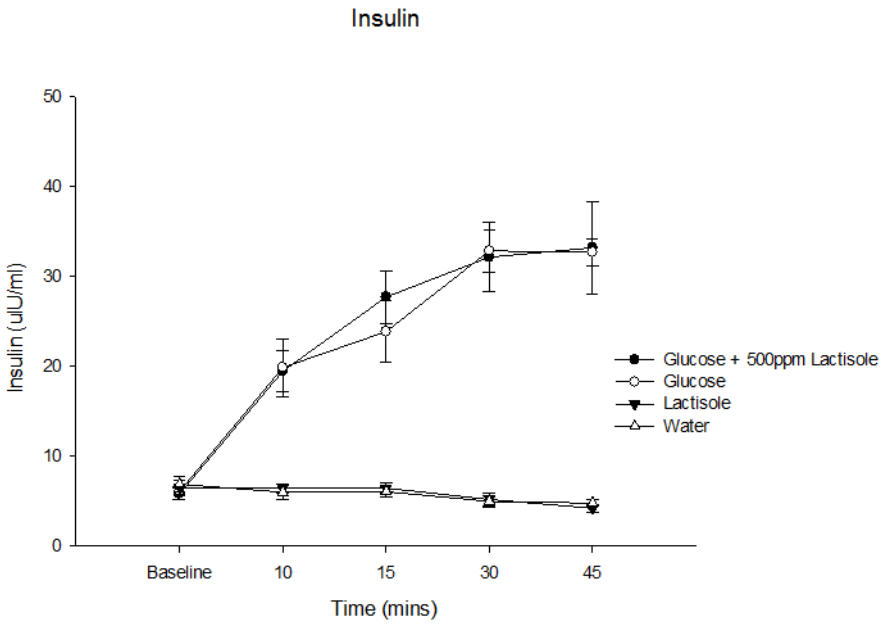


Figure 3.12 Insulin concentrations

Insulin concentrations displayed over time from baseline to 45 minutes for the glucose + 500 ppm lactisole ( ), glucose ( ), lactisole ( ) and water ( ) trials. Values represent mean ± SEM, (n=7).

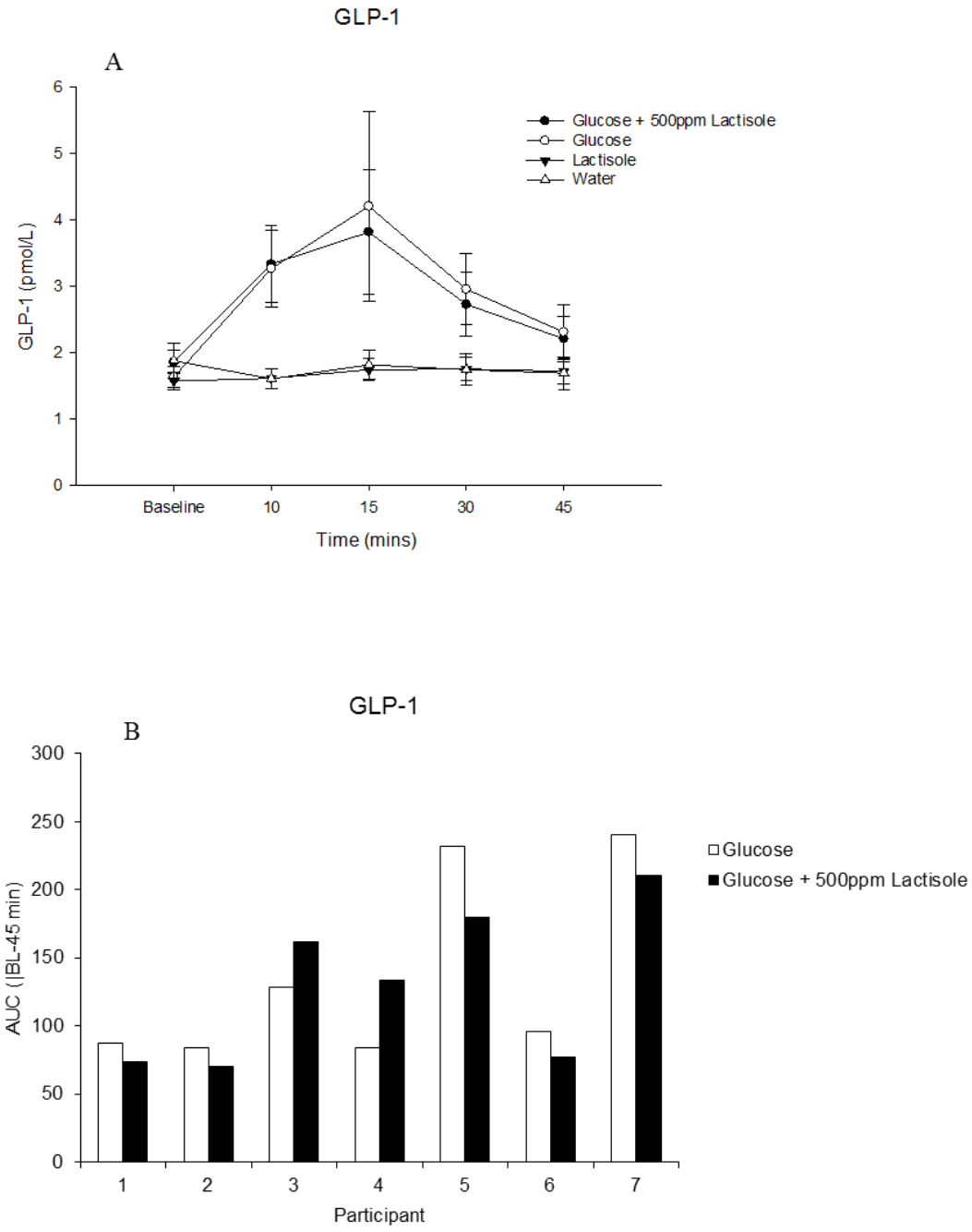


Figure 3.13 GLP-1 concentrations

GLP-1 concentrations displayed over time for the glucose + 500 ppm lactisole ( ), glucose ( ), lactisole ( ) and water ( ) trials (A). Values represent mean ± SEM, (n=7). Area under the curve from baseline (BL) to 45 minutes for each participant for glucose ( ) and glucose + 500 ppm lactisole ( ) (B).

Following the glucose + lactisole and glucose trials, plasma GIP (figure 3.14A) and PYY (figure 3.15A) concentrations increased. Plasma GIP concentrations increased to 21.6 $\pm$ 3.4 and 22.9 $\pm$ 3.3 pmol/l and peaked at 15 and 30 minutes for the glucose + lactisole and glucose trials respectively. There was a main effect of time ($P < 0.001$) but no significant effect of trial ($P= 0.6$). Similarly, plasma PYY concentrations increased to 93.0 $\pm$ 13.3 and 101.2 $\pm$ 26.0 pg/ml for the glucose + lactisole and glucose trials respectively peaking at 15 minutes. There was no effect of time or trial ($P > 0.05$) indicating lactisole had no effect on the plasma PYY responses to glucose. Plasma PP (figure 3.16) concentrations increased to 115.3 $\pm$ 39.6 pg/ml following the glucose infusion and peaked at 10 minutes compared to 84.6 $\pm$ 12.1 pg/ml in the glucose + lactisole trial. There was a main effect of time ($P < 0.05$) but no significant effect of trial ($P= 0.3$). Individual AUCs are shown for each gut hormone to illustrate the variability between subjects.

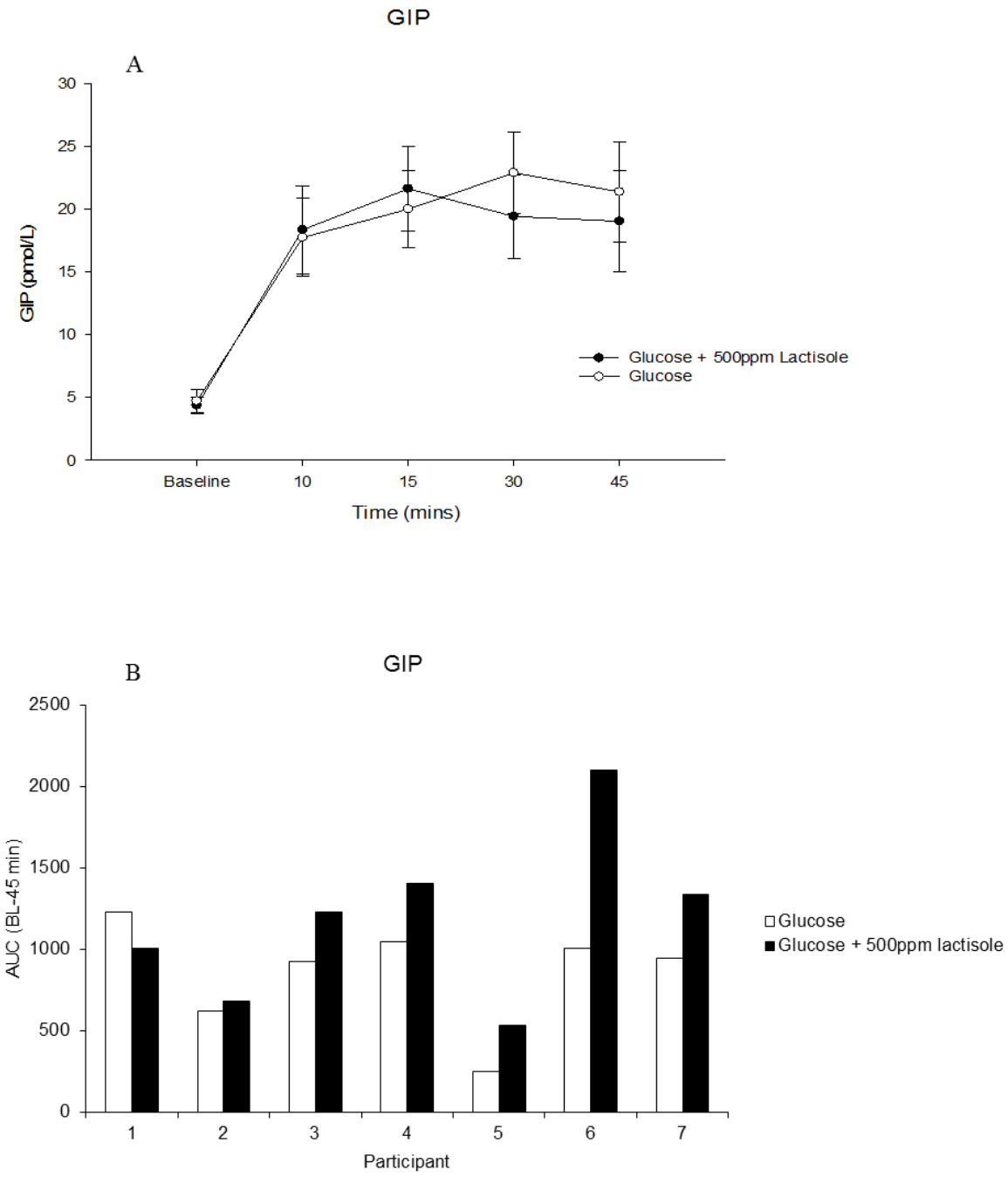


Figure 3.14 GIP concentrations

GIP concentrations displayed over time for the glucose + 500 ppm lactisole (●) and glucose (○) trials (A). Values represent mean ± SEM, (n=7). Area under the curve from baseline (BL) to 45 minutes for each participant for glucose (□) and glucose + 500 ppm lactisole (■) (B).

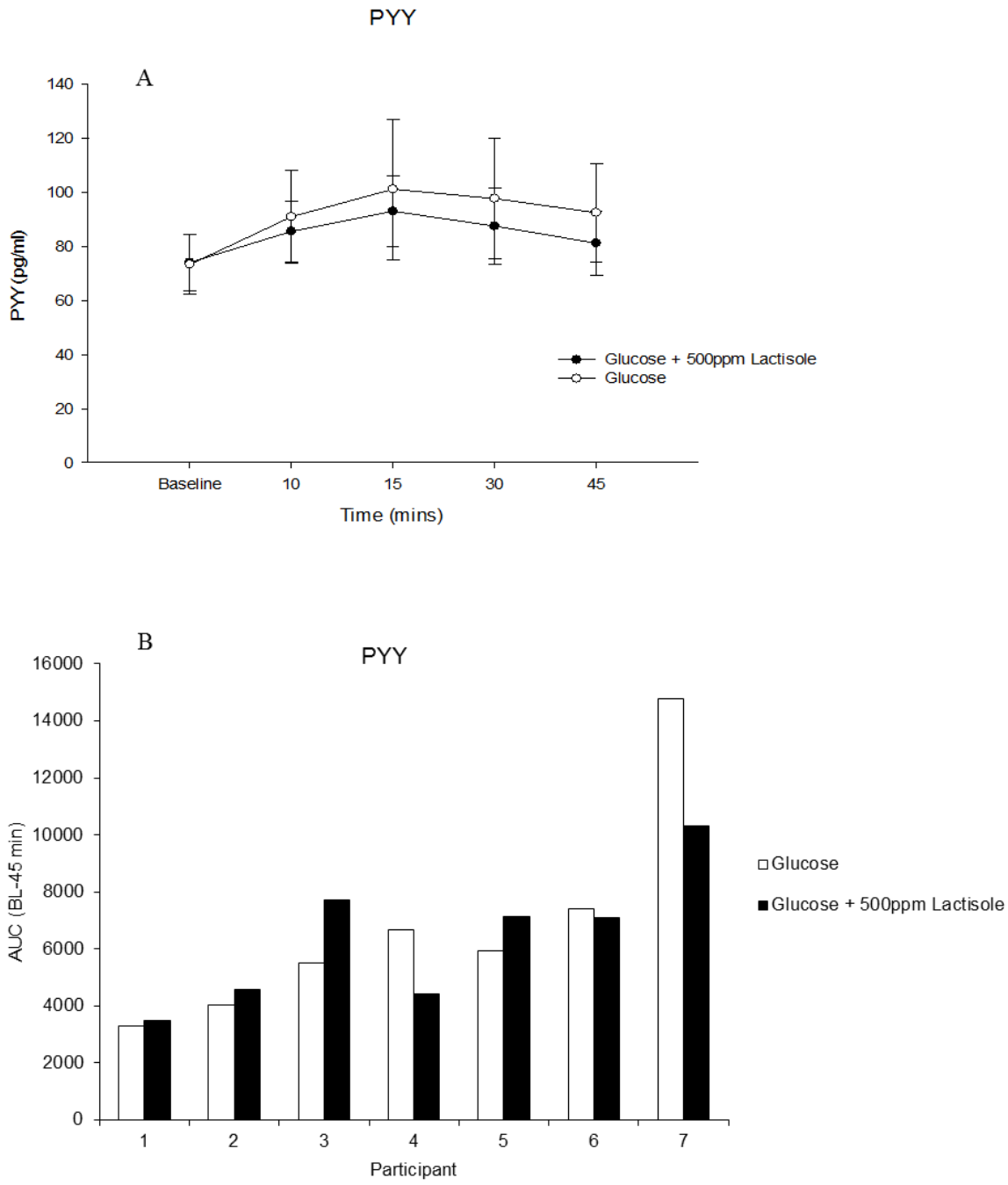


Figure 3.15 PYY concentrations

PYY concentrations displayed over time for the glucose + 500 ppm lactisole (⊸) and glucose (⊸) trials (A). Values represent mean ± SEM, (n=7). Area under the curve from baseline (BL) to 45 minutes for each participant for glucose (□) and glucose + 500 ppm lactisole (■) (B).

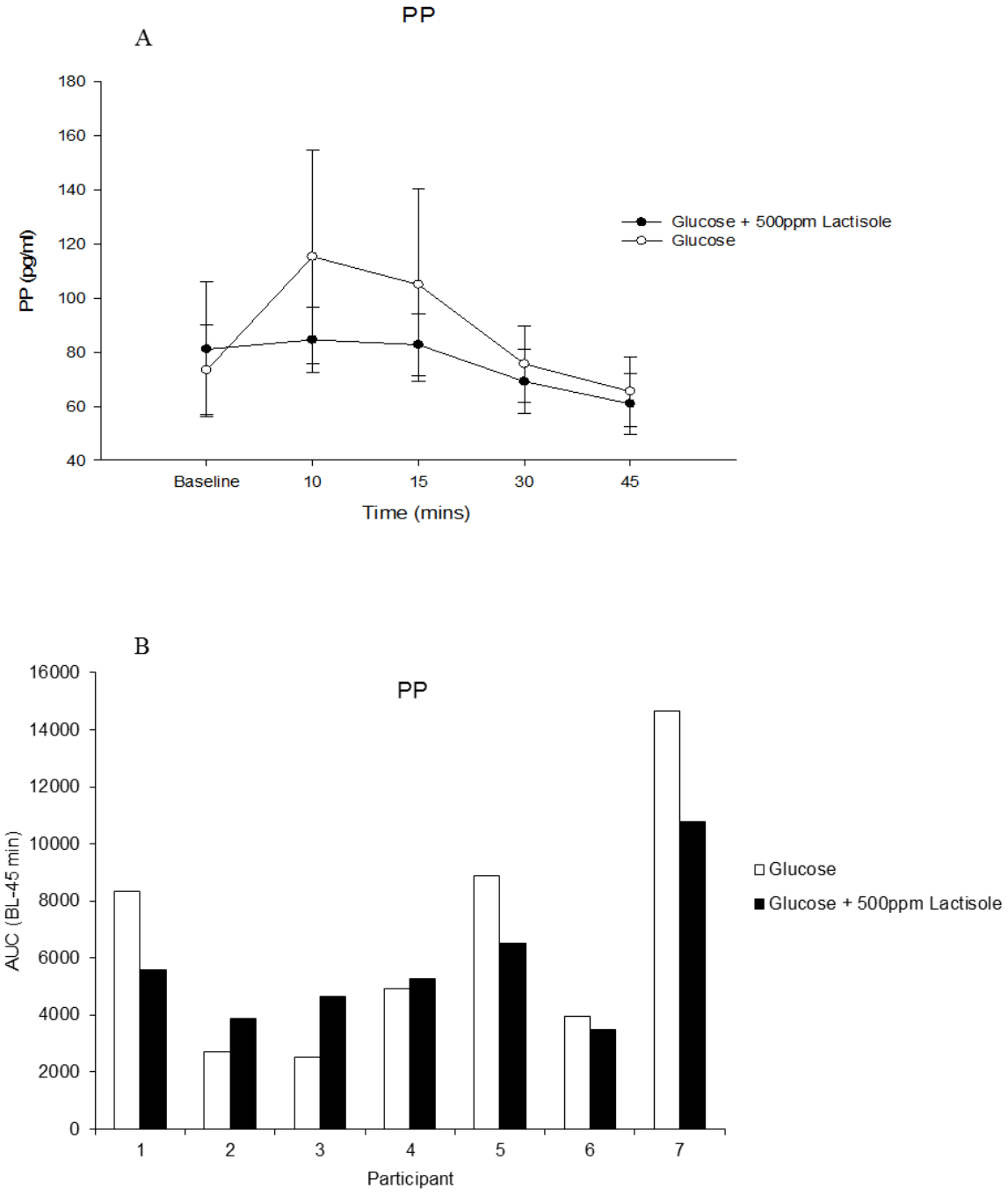


Figure 3.16 PP concentrations

PP concentrations displayed over time for the glucose + 500 ppm lactisole (⊸) and glucose (⊸) trials (A). Values represent mean ± SEM, (n=7). Area under the curve from baseline (BL) to 45 minutes for each participant for glucose (□) and glucose + 500 ppm lactisole (■) (B).

3.7.5 Appetite perceptions

Baseline ratings of desire to eat, hunger, fullness and nausea were not significantly different between the trials ($P > 0.05$). Ratings of desire to eat and hunger decreased following all infusions while ratings of fullness and nausea increased in all trials (figure 3.17). Indeed, the rise in nausea was quite marked in this study, and considerably higher than in *part II*. Ratings of desire to eat tended to be higher in the glucose + lactisole trial compared to the glucose only trial however these observations were not significant. There was a main effect of time ($P < 0.001$) for each of the appetite perceptions assessed (desire to eat, hunger and fullness) but there were no trial or trial x time interaction effects for desire to eat or fullness. For hunger, there was a significant trial effect ($P= 0.04$) showing that ratings tended to be higher in the glucose + lactisole trial compared to glucose alone. Following their decline, ratings of hunger and desire to eat increased until the test meal. Similarly, following the increase, ratings of fullness gradually decreased until the test meal. There were no trial or interaction main effects for nausea with ratings decreasing until the test meal.

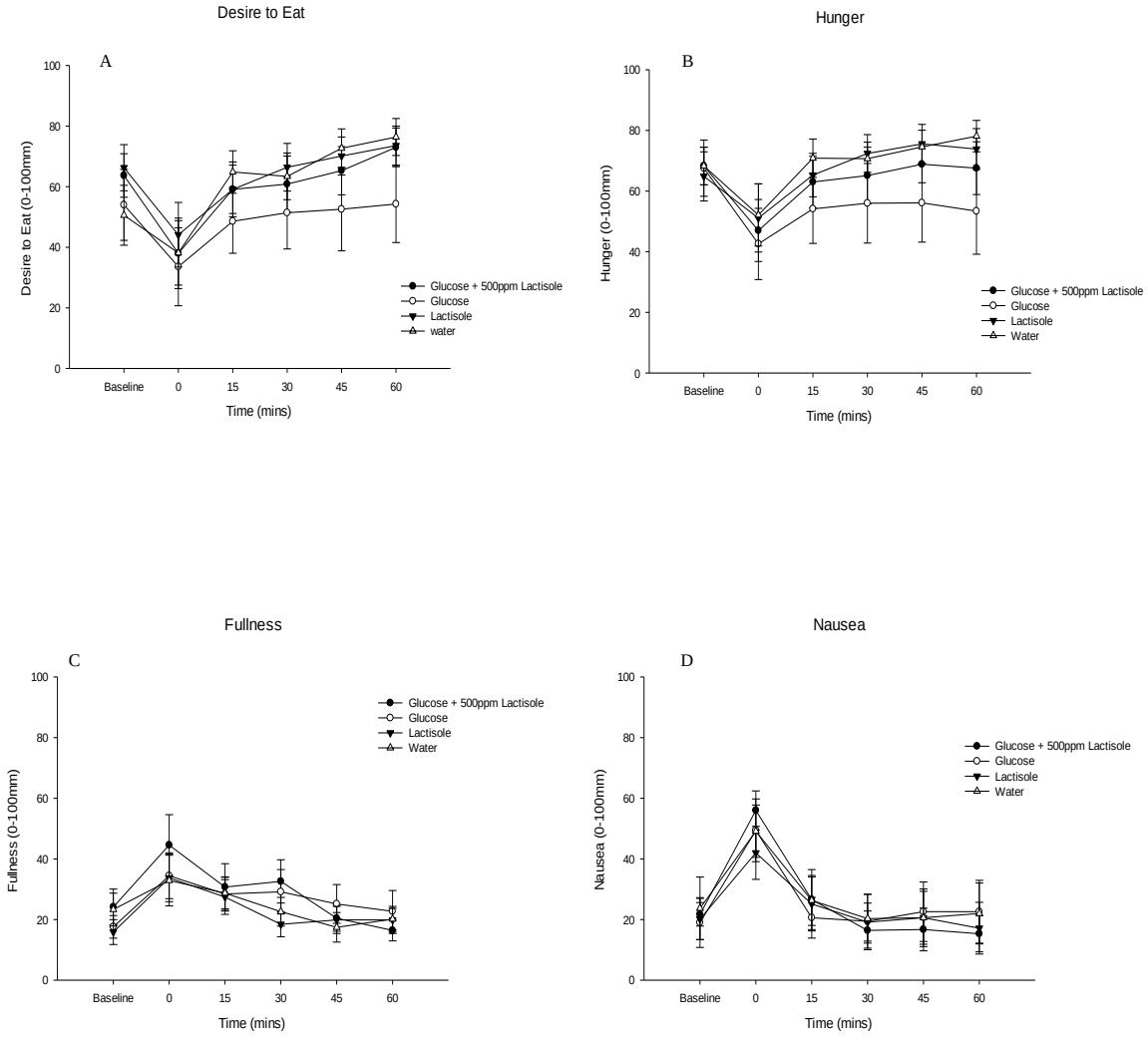


Figure 3.17 Subjective appetite ratings

Subjective ratings for desire to eat (A), hunger (B), fullness (C) and nausea (D) for glucose + 500 ppm lactisole (—●—), glucose (—○—), lactisole (—▲—), and water (—△—) trials. Values represent mean ± SEM, (n=7).

3.7.6 Test meal intake

Food intake at the *ad-libitum* test meal was significantly lower following the glucose trial compared to lactisole and water only conditions ($P < 0.05$) but did not quite reach statistical significance between the two main trials 441 $\pm$ 20.9 vs. 295 $\pm$ 25.5 g (691.5 $\pm$ 32.8 vs. 462.5 $\pm$ 40.0 kcal, $P= 0.06$) (figure 3.18).

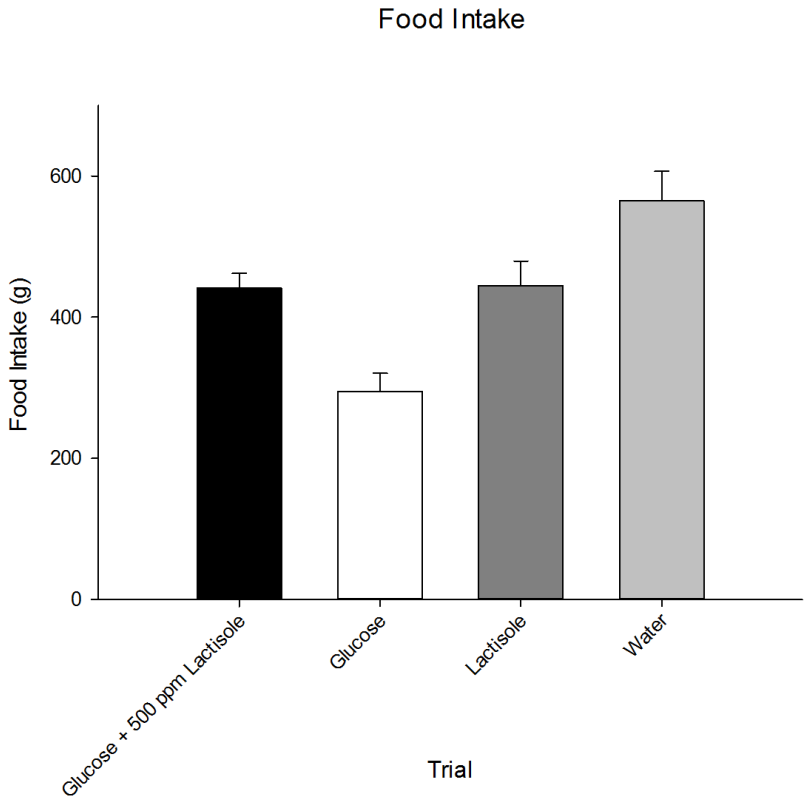


Figure 3.18 Food intake

Food intake at the *ad-libitum* test meal after the glucose + 500 ppm lactisole (■), glucose (□), lactisole (■), and water (□) trials. Values represent mean $\pm$ SEM, (n=7).

3.8 Discussion

As discussed in section 3.7.2 following the commencement of *part III*, a larger similar study was published by Gerspach *et al*. Using a comparable dose of lactisole (450 ppm vs. 500 ppm herein), Gerspach *et al* investigated the hormone responses, appetite perceptions and gastric emptying rates in response to an intragastric administration of glucose (1.35M vs. 1M herein) or a mixed nutrient meal. Taken together, the results can be summarised as follows. i) In both studies, gastric emptying was unaffected by lactisole. Importantly lactisole had no effect on CCK secretion in the Gerspach *et al* study (Gerspach et al., 2011) which is known to modulate the effects of glucose on gastric emptying as evidenced by the administration of the CCK1 receptor antagonist, dexloxiglumide (Little et al., 2010a). ii) In the current study lactisole had no effect on GLP-1 or PYY secretion. These results are not in line with the work by Gerspach *et al* who showed a significant reduction in both hormones following an intragastric load of lactisole and glucose. However, they demonstrated a much larger gut hormone response probably as a consequence of the higher dose of glucose used. In addition, there was no effect in the current study on the secretion of the hormones PP or GIP. Although there was a trend for GLP-1, PYY and PP concentrations to be lower in the presence of lactisole, the results were very variable between participants with no discernible patterns. iii) In contrast to Gerspach *et al*, insulin was unaffected by lactisole in the current study. As a likely consequence of the reduction they found in GLP-1 secretion, Gerspach *et al* found a reduction in insulin in the first 30 minutes following infusion although this was not apparent in the latter half of the study. Furthermore, and again as a likely consequence of the reduction in GLP-1 seen by Gerspach *et al*, the AUC for blood glucose was increased by lactisole. A similar but non-significant trend was apparent in the current study. iv) There was a minimal effect on appetite perceptions in the current study. A trend for reduced feeling of fullness were reported by Gerspach *et al* in line with a trend toward increased feelings of hunger and desire to eat comparable to glucose alone in the current study. However, the nausea, which was not reported in the Gerspach study, was quite marked in the current study. v) The amount of food eaten at the *ad-libitum* test meal was higher in the presence of lactisole compared to glucose alone, although this did not quite reach significance. Food intake was not measured by Gerspach *et al*.

On the basis of the data and that published by Gerspach *et al* it was not considered practical or economical to pursue the study any further than the seven participants already recruited as large numbers would be required.

The lack of effect of lactisole on gastric emptying in the present study and that by Gerspach *et al* is not consistent with the attenuated GLP-1 response they documented if a mechanism of GLP-1 is to mediate gastric emptying in response to glucose as suggested (Deane et al., 2010). However, this is consistent with a study by Nicolaus *et al* who showed no effect of the GLP-1 receptor antagonist on gastric emptying rate in response to a mixed meal (Nicolaus et al., 2011). Although not measured in the current study, CCK secretion was unaffected by lactisole in Gerspach's study suggesting its release is not mediated via the sweet taste receptor and reinforcing its role with respect to glucose control to delay gastric emptying (Little et al., 2010a).

One discrepancy between the two studies is the difference in gut hormone responses observed. A number of possibilities could account for this. The higher dose of glucose used in the Gerspach study caused a greater hormone response overall with GLP-1 and PYY concentrations peaking at approximately 10pgmol/L and 160pg/ml respectively following intragastric glucose compared to 4pgmol/L and 101pg/ml in the current study. Large variability and small sample size also noted, and the assays presented here were undertaken in house using commercial kits, which may produce different absolute values.

Despite observing significantly different gut hormone responses in the Gerspach study, and a trend for a difference in *ad-libitum* food intake at the test meal in the current study, in both studies subjective appetite ratings were not significant. An innate problem with VAS as proxy measure for food intake is the lack of sensitivity and large between participant and within participant variability. Voluntary food intake was determined by providing a standard test meal for participants to consume *ad libitum.* This meant food intake could be calculated from the weight of food consumed, and the macronutrient composition was fixed as opposed to buffet style test meals which can compromise the measure of food intake by stimulating participant's appetite through the provision of a variety of foods (Feinle et al., 2002). While food intake at the *ad-libitum* test meal did not quite reach significance, there was a difference between the two main trials which may be evident in a larger

powered study. Although food intake for the control trial, lactisole, was similar to the glucose + 500 ppm lactisole trial and thus the effect of lactisole itself cannot be ruled out. Since there is evidence that sweet taste receptor expression can be modulated by the agonist glucose, albeit *in vivo* animal models (Young et al., 2009), it is reasonable to propose a possible comparable antagonistic effect of lactisole. The effect of lactisole on sweet taste receptor expression is not known.

Due to the limited number of participants in the current study the results have to be interpreted with caution as the study is obviously in part underpowered.

Speculations about the functional role of gut sweet taste receptors in humans stems from *in vitro* and *in vivo* animal studies showing their involvement in glucose stimulated GLP-1 and GIP secretion (Jang et al., 2007, Kokrashvili et al., 2009). Based on these data the hypothesis raised was that:

(i) Sweet agonists such as glucose stimulate the sweet taste receptor on EEC.
(ii) This causes an increase in the secretion of gut hormones such as GLP-1, GIP and PYY which can be blocked/diminished by the sweet receptor antagonist lactisole.
(iii) Attenuating the secretion of these hormones with lactisole would alter glucose metabolism, insulin release, modulate gastric emptying and augment satiety.
(iv) Thus, gut sweet taste receptors present as potential therapeutic targets.

Although Gerspach *et al* found an effect of lactisole on GLP-1 and PYY secretion, it was only a partial effect. The effect of the mixed meal, which was a composition of protein, fat and carbohydrates, on all parameters was resistant to lactisole suggesting the involvement of alternative mechanisms. Furthermore, the effect of lactisole appeared to be offset by these other mechanisms questioning the functional importance of the sweet taste receptor in regulating hormone release. In support of this, but in contrast to *in vitro* experiments, studies have shown activation of sweet taste receptors by equisweet solutions (sugars and NNS) is not enough to stimulate hormone secretion and delay gastric emptying (Little et al., 2009, Steinert et al., 2011, Fujita et al., 2009). It is clear that findings from *in vitro* studies are not always representative of human physiology and in this case additional mechanisms are in

play. Sugar sensing independent of the sweet taste receptor mechanism may exist and given the significance of glucose as a major fuel for the body, a glucose specific mechanism has been proposed (Geraedts et al., 2012). Examples of a glucose specific mechanism have been investigated by Sclafani *et al* who demonstrated flavour conditioning in animal models (Sclafani, 2007, Sclafani and Ackroff, 2012).

Aside from the proposed functional role of sweet taste receptors in mediating the physiological responses to glucose evidence suggests intestinal glucose absorption may be mediated by gut sweet taste receptors (Margolskee et al., 2007). The expression of SGLT1 was increased in mice models following a sugar and/or NNS diet, an effect not seen in T1R3 knockout deficient mice (Margolskee et al., 2007).

At present, much remains to be discovered about the mechanisms of post-ingestive glucose sensing, which appears to be different in whole human physiology when compared to data from animal and cell line studies. Access to primary gut tissue to culture and study EEC response will be required to achieve this, but this is not currently feasible. In addition, the data presented here are in lean, healthy, young subjects. It is possible that differences may arise if studied in obese or type 2 diabetic individuals, in whom manipulation of sweet sensing in the gut may have possible utility.

Although the data using the antagonist were broadly negative, multiple experimental approaches are needed to confirm or refute potential mechanisms. Therefore, further studies were undertaken using NNS as potential agonists of the sweet receptors, and will be outlined in the next chapter.

CHAPTER 4

Non-nutritive sweeteners do not enhance the glycaemic or appetitive responses to ingested glucose

4.1 Introduction

NNS consumption has increased considerably with a catalogue of data underlining the safety and efficacy of NNS consumption leading to the general consensus that they are metabolically inert (Anton et al., 2010, De La Hunty et al., 2006, Bellisle and Drewnowski, 2007). Although this may be intrinsically true, recent data suggest NNS may have physiological effects that alter appetite and/or glucose metabolism. Following the identification of sweet taste receptors in the gut (Dyer et al., 2005) it is logical to hypothesise that NNS could activate these receptors causing comparable metabolic effects to their caloric sweet counterparts. Jang *et al* (Jang et al., 2007) and Margolskee *et al* (Margolskee et al., 2007) demonstrated that the NNS, sucralose, stimulated GLP-1 secretion from a human L-cell line (NCI-H716) and a murine EEC line (GLUTag) respectively but the majority of *in vivo* human data have failed to confirm the effects of NNS on hormone secretion observed *in vitro* (Ma et al., 2009, Steinert et al., 2011), supporting the consensus that NNS, at least in isolation, are not capable of stimulating hormone secretion in humans.

The data in chapter three, alongside others (Gerspach et al., 2011), demonstrated that sweet taste receptors in the gut may only be partially responsible for glucose mediated responses. Furthermore, equally sweet sugars and NNS (glucose, fructose, ace-k, saccharin) did not have comparable effects on gut hormones responses and gastric emptying (Little et al., 2009, Steinert et al., 2011) suggesting activation alone is not enough and other mechanisms independent of the sweet taste receptor may be crucial. Following reports that NNS could enhance sweet receptor activation in the rodent gut concerns were raised about the synergistic effect of NNS and sugars, potentially increasing small intestinal glucose absorption via upregulation and insertion of transporters (Mace et al., 2007, Margolskee et al., 2007).

The diversity of the human diet ensures that consumption of NNS in isolation is uncommon except in the form of diet beverages. This leads us to consider whether

NNS in combination with sugars could alter appetite and/or glucose metabolism. The regulation of intestinal glucose absorption via both SGLT1 and the facilitative transporter GLUT2 (Kellett et al., 2008) has shown a sensitivity to NNS. This has been demonstrated by the upregulation of SGLT1 and the increase in GLUT2 insertion to the apical membrane following supplementation of the diet with sucralose, albeit in animal models (Mace et al., 2007, Margolskee et al., 2007). Using a proxy measure of intestinal glucose absorption this effect was not replicated in humans (Ma et al., 2010).

The notion that consuming NNS in combination with sugars could potentially alter glucose absorptive capacity and affect postprandial blood glucose is of great clinical and nutritional relevance. One human study showed that oral ingestion of sucralose and ace-k caused an increase in GLP-1 after subsequent glucose ingestion but no change in blood glucose or insulin (Brown et al., 2009). Other GLP-1 effects such as satiety were not measured. In contrast *Ma et al* (Ma et al., 2009) found no difference in GLP-1 concentrations following infusion of a sucralose and glucose combination arguing against any significant effect. There is also a lack of clarity about the effects of NNS on appetite (Mattes and Popkin, 2009) when given in combination with sugars (Brown et al., 2009). What is completely unknown is whether they exert any effect in humans if ingested together at dietetically relevant doses.

The objective of the present study was to examine the effects of a panel of commonly used NNS, aspartame, saccharin, and ace-k, given in combination with glucose, on glycaemic responses and appetite in healthy humans. The hypothesis is that, if a sugar and NNS have a synergistic effect and enhance glucose absorption large enough to be dietetically relevant, then the addition of a NNS to a glucose solution would increase blood glucose more than glucose alone.

4.2 Method

4.2.1 Design

This study was conducted as a single-blind randomised four-way cross-over study. Participants attended the laboratory on four separate occasions plus one screening

visit with at least five days between each visit. Ethical approval for the study was granted by the National Health Service North West Research Ethics Committee (ref. 10/H1017/52).

4.2.2 Participants

Ten participants (six male, four female) took part in the study. All participants met the inclusion/exclusion criteria as detailed in chapter 2.2.1 with the additional exclusion criteria for those presenting with the condition Phenylketonuria. This disorder restricts intake of the amino acid phenylalanine which is a by-product of aspartame. However no screened participants had this extremely rare condition.

4.2.3 Experimental protocol

Participants were studied on four separate occasions and instructed to follow the pre-study standardisation protocol (chapter 2.3.1). On the day of each study, participants arrived at the Gastrointestinal Physiology research lab at approximately 9.00 hours following an overnight 11 hour fast. Upon arrival at the laboratory, an intravenous cannula was inserted into a vein in the forearm to allow repeated blood sampling (chapter 2.3.4). A fasting baseline blood sample was collected and a baseline assessment of appetite was collected using VAS (chapter 2.3.4 and 2.3.2 respectively). All participants were then asked to consume through a drinking straw, over two minutes (t= -2 min), either a) 45g glucose (180 kcal), b) 45g glucose and 150mg aspartame, c) 45g glucose and 20mg saccharin, or d) 45g glucose and 85mg ace-k dissolved in tap water to a total volume of 250ml, in a randomised fashion. All test drinks were prepared on the morning of the study at room temperature and were presented in transparent beakers as colourless liquids. On completion of the test drink, blood was sampled immediately following consumption (t= 0) and at t= 5, 10, 15, 30, 45 and 60 min. VAS questionnaires were completed immediately (t= 0) and at t= 15, 30, and 60 min. Taste ratings were also collected immediately following each test drink consumption (t= 0) (chapter 2.3.2). Blood samples were collected and

processed as described in chapter 2.3.4. Blood glucose was determined immediately using HemoCue Glucose 201^{+} Analyser (Hemocue, Angelholm, Sweden) (chapter 2.3.4.1). After 60 minutes the cannula was removed and participants were given refreshments and free to leave the laboratory with no further limitations. The study protocol is presented in figure 4.1.

4.2.4 Materials and test foods

D- (+)- Glucose (Sigma-Aldrich, Munich, Germany). For consistency, the concentration of glucose was kept the same as chapter three.

Aspartame and ace-k were a generous gift from Fuerst Day Lawson Ltd, London.

Saccharin (Hermesetas, Berkshire, UK).

The doses of NNS chosen were approximate to those used in commercial products marketed by the food industry (e.g. sweetener tablets, diet drinks).

While the combination of pure glucose with NNS is rare in common soft drinks this study was weighted more towards proof-of-concept. Glucose and NNS in combination are not representative of a commercial product but the doses of NNS used were deliberately chosen to reflect those in commercial products and the study is otherwise modeled on previous studies using NNS preloads followed by ingestion or intraduodenal glucose infusion (Brown et al., 2009, Ma et al., 2010). In whatever form sugar is ingested (generally sucrose or fructose), glucose is liberated by hydrolysis and then absorbed. A reductionist approach of administering pure glucose overcame any inter-individual differences in macronutrient handling and digestion, which would be an additional large confounding factor.

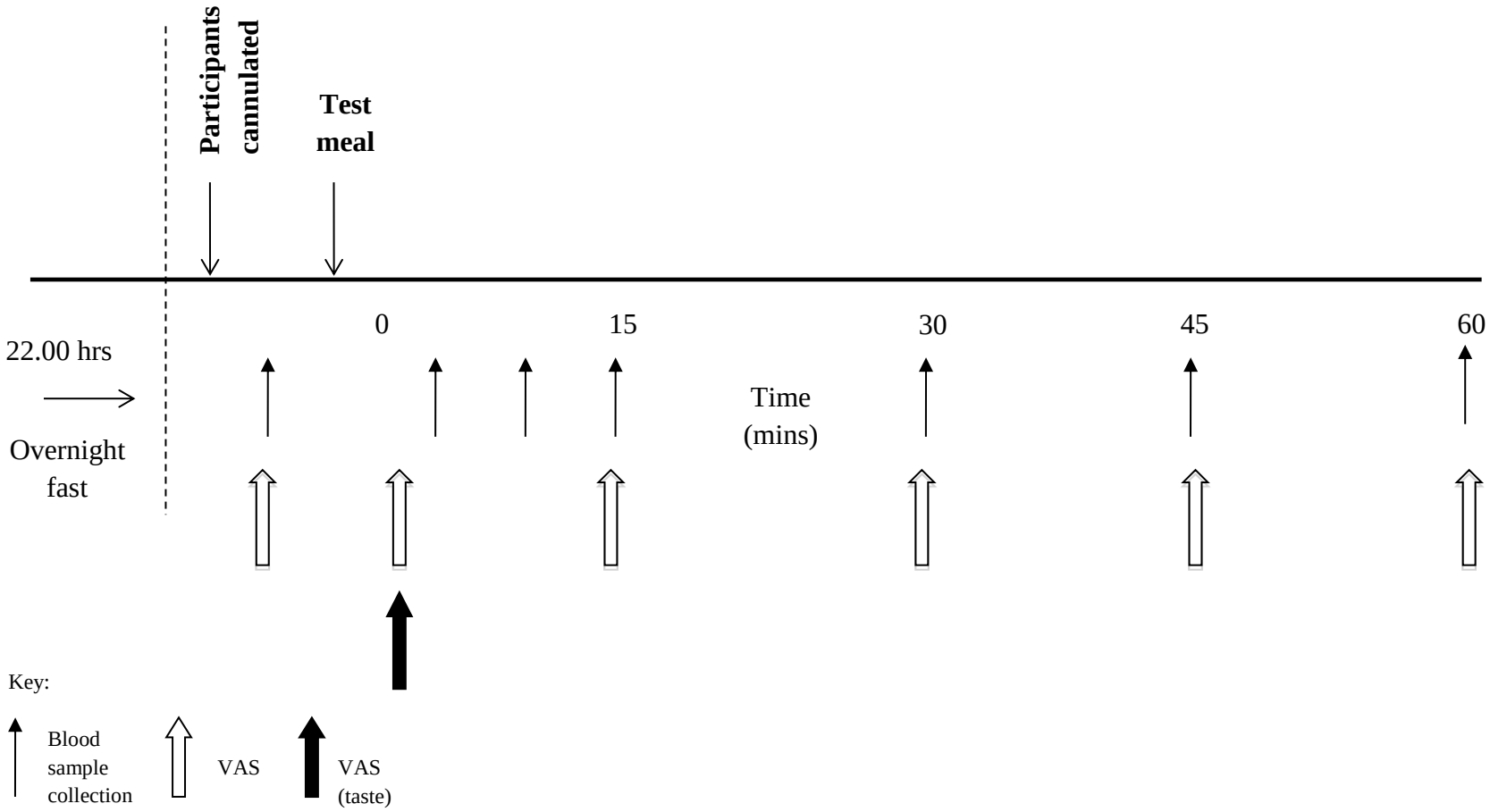


Figure 4.1 Schematic representation of study protocol

4.2.5 Statistical analysis

Data are presented and analysed as described in chapter 2.4.

4.3 Results

4.3.1 Participants

Participant characteristics are displayed in table 4.1.

		mean ± SD (*n* =10)
Age	*(years)*	21 ± 2.4
Height	*(cm)*	172.3 ± 8.4
Weight	*(kg)*	65.1 ± 8.7
BMI	*(kg/m^2)*	21.9 ± 1.8
Restraint Score[1]		2.9 $\pm$ 1.3

Values represent mean ± SD (n =10)

[1] Score of restraint using the three factor eating questionnaire (Stunkard and Messick, 1985)

Table 4.1 Participant characteristics

4.3.2 Visual analogue scales – taste

Six participants scored the ace-k sweetened glucose drink as being marginally sweeter yielding a higher mean score (86.5 ± 1.9, n=10) than the other test drinks, but the panel could not discriminate between the aspartame/saccharin sweetened or glucose drinks (79.8 ± 2.9, 78.9 ± 2.6 and 77.5 ± 4.3 respectively). The difference did not achieve statistical significance.

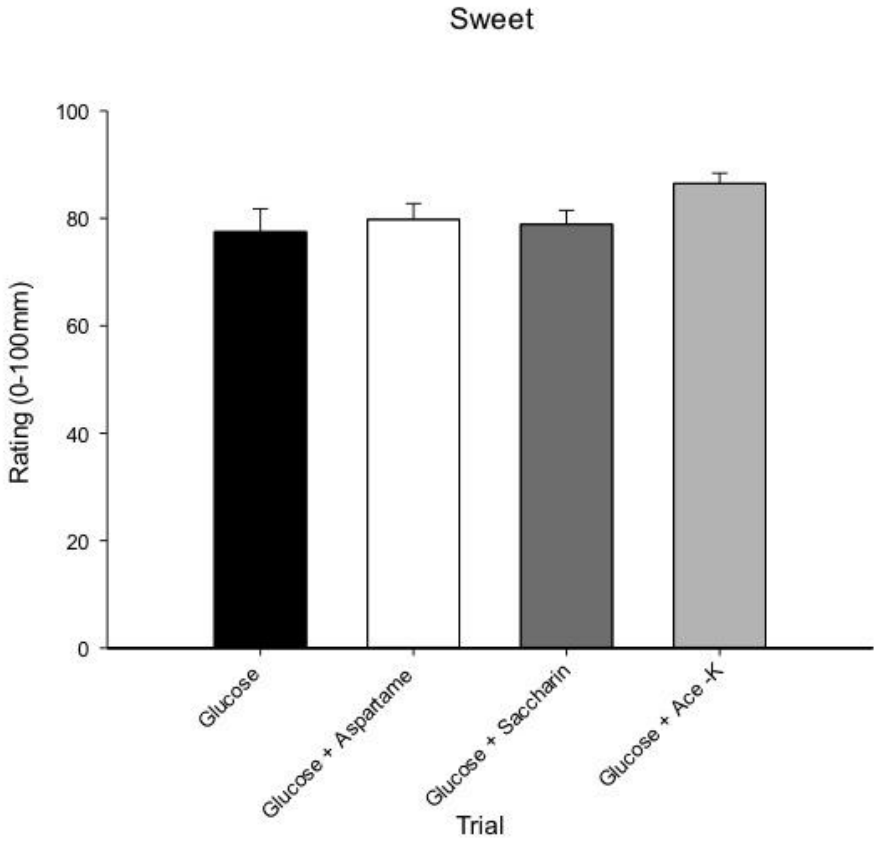


Figure 4.2 Sweetness ratings

Mean sweetness ratings for glucose (■), glucose +aspartame (□), glucose + saccharin (■), and glucose + ace-k (□) trials. Values represent mean ± SEM, (n=10).

4.3.3 Blood glucose

There was no significant difference in fasting blood glucose concentrations between the four conditions ($P > 0.05$). Following the ingestion of glucose alone, blood glucose peaked 45 minutes after consumption reaching 7.6 $\pm$ 0.5 mmol/L. The mean blood glucose also peaked identically 45 minutes after the consumption of the glucose + saccharin test drink at 7.7 $\pm$ 0.5 mmol/L; the blood glucose values were trivially greater after glucose + ace-k 8.2 $\pm$ 0.7mmol/L. The mean blood glucose concentrations peaked 30 minutes after the consumption of the glucose +aspartame test drink, reaching a similar but earlier peak value of 7.4mmol/L $\pm$ 0.3 mmol/L. There was a main effect of time ($P < 0.01$) and time x treatment interaction ($P < 0.05$) indicating blood glucose differed over time between the four conditions (figure 4.3). However post-hoc analysis revealed no differences between the glucose and NNS conditions at any time point. Analysis of AUC demonstrated a larger integrated glycaemic response following ace-k compared to glucose alone (706.8 $\pm$37.7 and 556.2$\pm$ 28.0, $P= 0.002$) (figure 4.3B) but there was no significant difference between glucose and aspartame and saccharin trials.

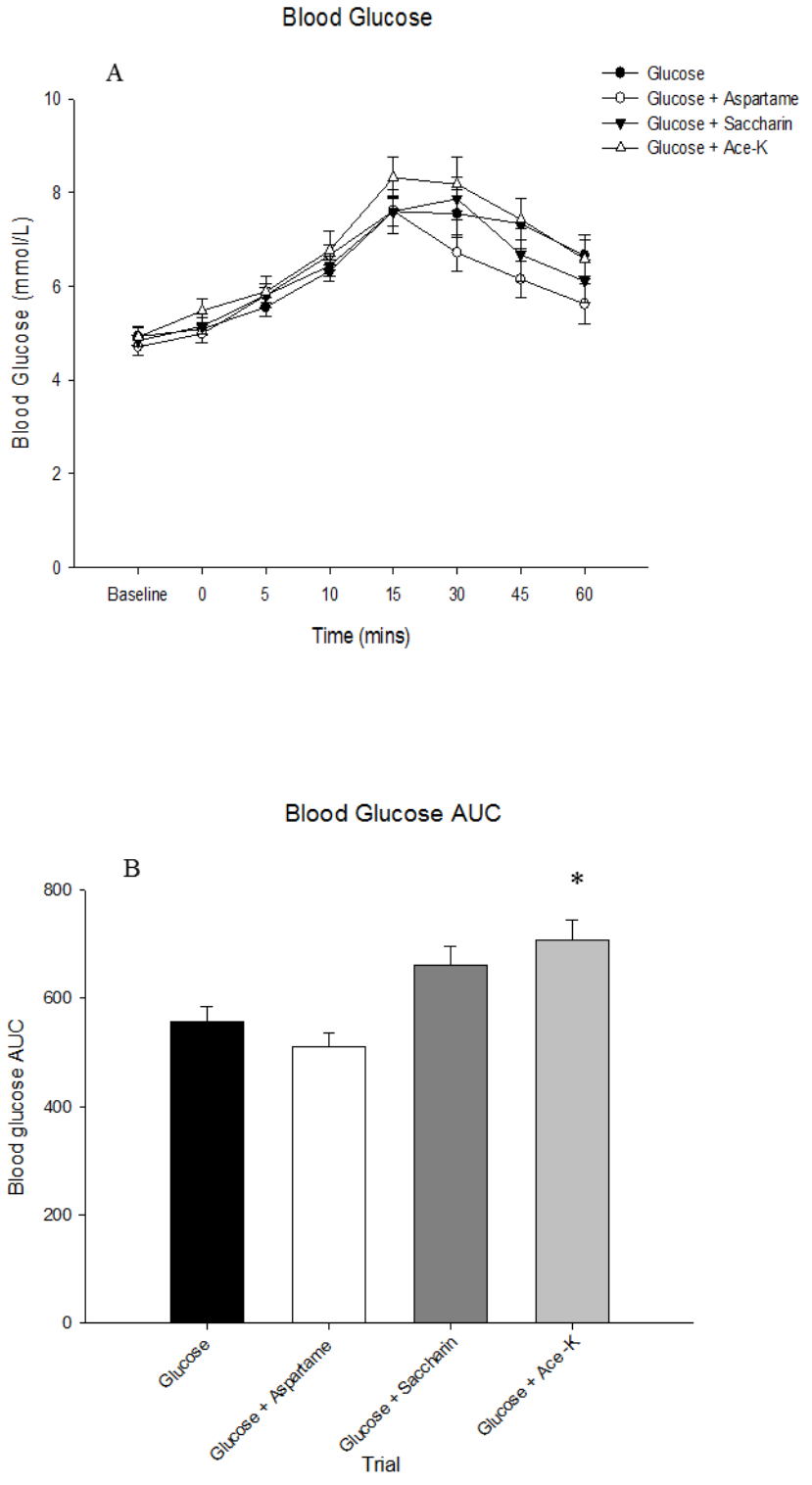


Figure 4.3 Blood glucose

Blood glucose concentrations during the glucose (✦ / ■), glucose + aspartame (-○- / □), glucose + saccharin (-▲- / ■) and glucose + ace-k (-△- / □) trials displayed over time (A) and as area under the curve (B). Values represent mean ± SEM, (n=10). * Glucose + ace-k significantly larger than glucose trial (P = 0.002).

4.3.4 Appetite perceptions

Baseline ratings of desire to eat, hunger and fullness were not significantly different between the trials ($P > 0.05$). Ratings of desire to eat and hunger decreased following consumption of the test drinks while ratings of fullness increased in all trials (figure 4.4). There was a main effect of time ($P < 0.001$) for each of the appetite perceptions assessed (desire to eat, hunger and fullness) but there were no trial or trial x time interaction effects. Following the decline in ratings of hunger and desire to eat and the increase in fullness there was no discernible patterns between the trials.

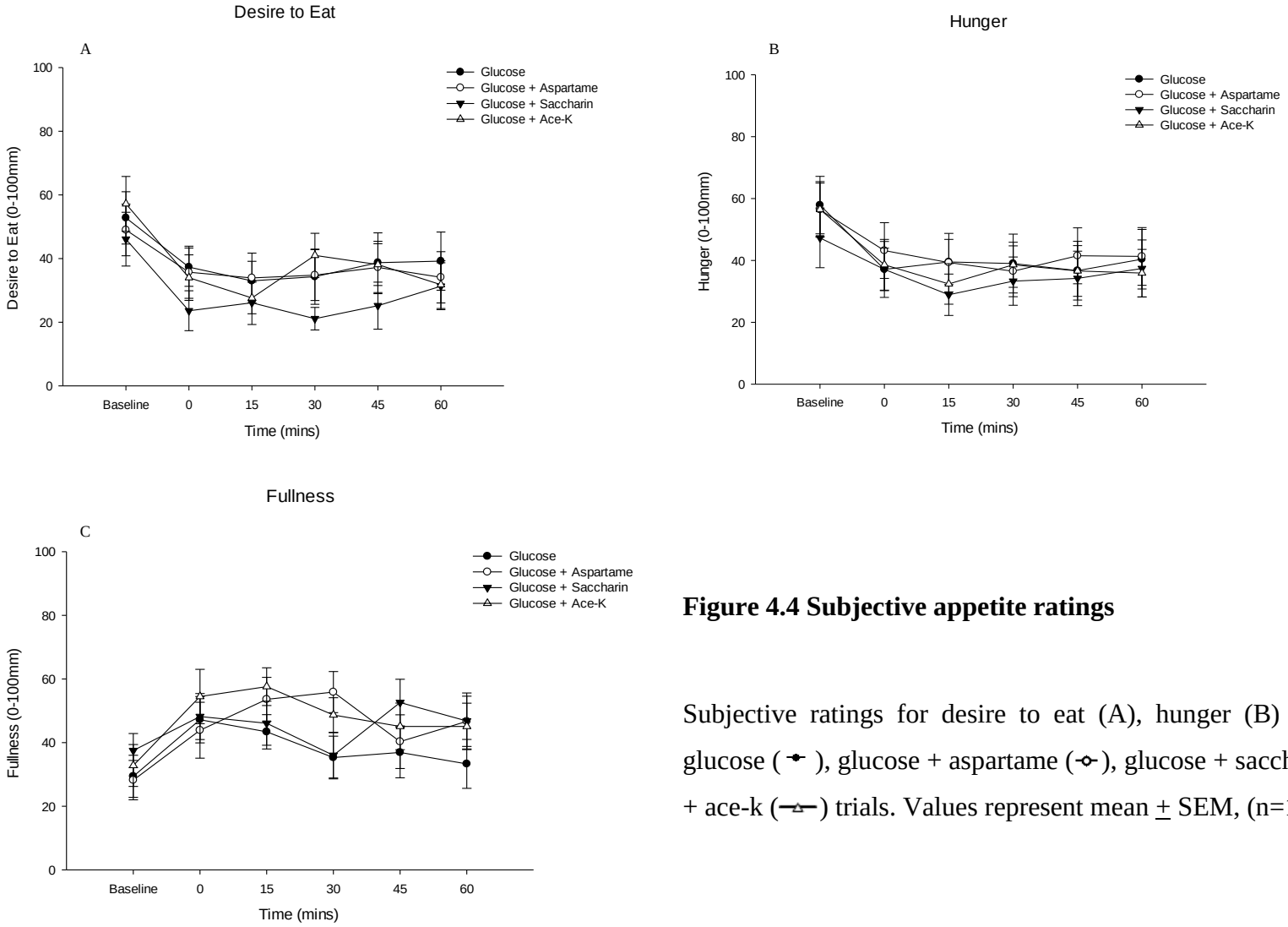


Figure 4.4 Subjective appetite ratings

Subjective ratings for desire to eat (A), hunger (B) and fullness (C) for glucose ( ), glucose + aspartame ( ), glucose + saccharin ( ) and glucose + ace-k ( ) trials. Values represent mean ± SEM, (n=10).

4.4 Discussion

This study is the first to directly evaluate the glycaemic and appetitive responses to a panel of NNS (aspartame, saccharin and ace-k) ingested in combination with a caloric sugar (glucose) to determine whether this combination could exert a readily detectable synergistic effect in healthy humans. Such an effect was considered possible based on the published data available to date. The body of research surrounding NNS and the impact on glucose metabolism and appetite is predicated on the identification of gut sweet taste receptors and their involvement in glucose absorption (Margolskee et al., 2007). Despite human *in vivo* studies failing to support any metabolic activity following NNS consumption (Ma et al., 2009, Ford et al., 2011, Steinert et al., 2011), concerns about the synergistic effect of NNS and sugars were raised following reports that such a combination could activate sweet taste receptors in the gut and increase small intestinal glucose absorption via upregulation and insertion of transporters (Margolskee et al., 2007, Mace et al., 2007). Hence, a typical human diet involving the consumption of NNS with carbohydrates raises not only a theoretical concern about raising postprandial glycaemia in healthy individuals, but even more so in diabetics who already have an over expression of SGLT1 and GLUT2 (Dyer et al., 2002).

Comparable to consumption of glucose alone, ingesting aspartame, saccharin and ace-k in combination with glucose had similar effects on blood glucose and perceptions of hunger, fullness and desire to eat. No significant differences were present at any time point and therefore reject the hypothesis that NNS in combination with glucose have a synergistic effect. There was however a small enhancement when the AUC was measured for blood glucose following the glucose and ace-k trial. The metabolic significance of this small effect is unclear and unlikely to have any biological impact. Moreover, the response to ace-k was more variable, but the data argue against a class effect of NNS enhancing responses by acting as agonists at the sweet taste receptor since the other two NNS did not have a similar profile. These broadly negative data are consistent with reports that also failed to show any effect of pre-supplementation with NNS on blood glucose in humans (Ma et al., 2010). Similarly Brown *et al* (Brown et al., 2009) also found no change in blood glucose following the consumption of a diet soda containing both sucralose

and ace-k prior to a glucose load, although they demonstrated enhanced GLP-1 release. The clinical relevance of this finding remains unclear, particularly as insulin and blood glucose were unaffected, and GLP-1 dependent factors (gastric emptying and appetite) were not measured. Absorption of glucose is dependent on the rate of delivery and the length of small intestine exposed to glucose (Little et al., 2006b) but the reason for different findings in these studies remains unclear.

In the current study, similar to that of Brown *et al* (Brown et al., 2009), the test drinks were consumed orally whereas Ma et al (Ma et al., 2010) infused directly into the duodenum raising the possibility that oral sweet taste receptors or gastric effects may have influenced the GLP-1 increase in the former study. The commercial doses of each NNS used, meant the test drinks did not impart an equal degree of sweetness. Participants failed to discriminate between the sweetness of drinks only scoring ace-k marginally sweeter despite it being the least sweet NNS out of the three chosen. Whether matching oral taste properties to downstream gastrointestinal effects would matter is not something factored into the design. However, despite activation of oral sweet taste receptors in the current study protocol the route of administration appears to exert no effect, and comparable effects were found on blood glucose to Ma *et al* (Ma et al., 2010). Furthermore, it should be noted that Brown *et al* (Brown et al., 2009) did not look at the isolated effects of individual sweeteners or the ingestion as one load, as has been performed in the present study, but used a commercially marketed beverage containing a combination of NNS given in advance of the glucose load. It is not known how this may have affected the study results but it is difficult to assume that exposure of intestinal sweet taste receptors to NNS, which in isolation do not cause a rise in GLP-1, would increase GLP-1 release following a subsequent dose of glucose unless an unknown priming effect occurs.

Unlike cell line and animal models, the concentrations of NNS used herein approximate to those used in commercial products marketed by the food industry and, although it is not certain whether these concentrations are too high/low in terms of sweet receptor biology, the study was designed to be nutritionally relevant.

The previously proposed increase in glucose absorption as a result of the synergy between a NNS and sugar could potentially influence the rate of gastric emptying and therefore appetite. However, in the current study, the addition of aspartame,

saccharin and ace-k to glucose had no additional effect on appetite compared with glucose alone. This is consistent with the existing literature that NNS do not have any unwanted effects on appetite (Bellisle and Drewnowski, 2007, Anton et al., 2010). The observed decrease in hunger and increase in fullness following consumption is presumably due to an increased intragastric volume (Schwartz, 2000). Again the negative VAS data cannot support substantive conclusions. As discussed in chapter three, VAS ratings are subjective and should therefore be interpreted with caution. The large variability, both within and between participants can result in larger sample sizes, beyond the remit of this proof of concept study, being required to detect differences.

A number of limitations in the study design need to be highlighted. Firstly, although the sample size of ten participants was small, I sought to establish whether a readily demonstrable/important effect existed – i.e. an effect that would impact on the response of an individual person consuming a commercial dose of NNS- and to that end consider the sample adequate, given the broadly negative data. A larger sample might 'squeeze out' small effects that become statistically significant, but does not lend itself to be dietetically relevant. Secondly, although blood was collected and processed for the determination of gut hormones it was decided, based on the current observations, that further analysis would not be worthwhile or economically justified. A definitive study would standardly include insulin and gut hormone profiles, but I did not consider these necessary in this small proof of concept study. Indeed the lack of differential glycaemic responses between conditions would render these superfluous and are not necessary in order to interpret the current data. If a clear effect existed, then studying its mechanism would indeed require gut hormone assays. Arguably the study is short at only 60 minutes and not comparable to other studies that have measured variables over 120 minutes. However, measurements of blood glucose after a relatively small glucose dose peak early, and fall almost to baseline by the 60 minute end point. Any dietetically meaningful effect would be expected to occur within this early phase and expecting a significant difference to subsequently arise in an additional hour does not seem realistic.

To date there is no consistent evidence that NNS intake perturbs glucose absorption and appetite in healthy human participants. However, NNS are widely consumed

including among obese and diabetic groups highlighting the need to investigate the effects not only in the short term but also the effect of repeated or long term exposure both in healthy and diabetic individuals. Participants' long term intake was not considered in the current study and future studies may need to stratify individuals into low and high NNS consumers. Different methods of administration between studies (i.e. oral vs. intragastric) also have to be considered. Appetite control is not just a physiologically controlled mechanism and the effects of oral stimulation have to be taken into account. Sensory and stimulatory influences, among which sweetness is a factor, can affect appetite, not least due to input from higher brain centres eliciting reward related signalling. Thus, it is important to explore the effects of oral vs. intragastric administration since the effects seen may be dependent on the experimental context as well as the test substance in question. The present study used oral administration to replicate 'real life' beverage intake.

In conclusion, NNS as a class did not affect the glycaemic responses to ingested glucose in healthy humans but ace-k may however merit separate attention as it may have different properties. The effects on appetite warrant further investigation using more robust methods.

CHAPTER 5

Gastric emptying and CNS responses to orally and intragastrically administered sweet tastants

Much interest lies in attempting to elucidate the gut-brain signalling mechanisms that regulate appetite and food intake. As mentioned in chapter one, human studies have focused on physiological processes such as gastric emptying, metabolic secretions, absorption and glucose homeostasis in response to sugars and NNS (Parker et al., 2010, Young, 2011, Steinert et al., 2011, Gerspach et al., 2011). The previous chapters of this thesis have focused on sweet taste receptors in the gut and their potential role in mediating the appetitive response to sugars and NNS. However, the modulation of appetite and food intake is likely to represent a combination of oro-sensory and gastric signals. Sweet taste sensing begins in the mouth and continues after ingestion with signals generated in the gut forwarded to and integrated within the brain. fMRI has enabled the study of CNS appetite pathways and much is known about parts of the brain that process sweet taste and evaluate the hedonic and reward value of sweet taste (Small, 2012, Smeets et al., 2011, Li et al., 2012). Much less is known about the relative contributions of oro-sensory and gastric stimulation to appetite regulation and the integration of both within the brain.

This chapter is split into two experimental parts. Study I investigated whether there are differential effects of oral and gastric stimulation by sweet tastants on psychological (appetite) and physiological (gastric emptying) components. The interaction of oral with gastrointestinal sweet stimuli on brain activation was then studied using fMRI in study II. Each study is described in turn.

Study I: Effect of oral sweet taste on the gastric emptying of sweet solutions

5.1 Introduction

Initially, the mouth and gut were considered to be sites of feeding stimulation and inhibition respectively (Sclafani and Ackroff, 2012). In reality oral taste sequentially precedes gastrointestinal chemo-sensation and it is likely that mechanisms that operate to control food intake in the short term include a combination of cognitive, cephalic, gastrointestinal and post-absorptive responses that all contribute to satiety (Cecil et al., 1998b, Oesch et al., 2006). Furthermore, it is not only a combination but an interaction between these factors that is necessary to elicit satiety and control food intake. The infusion of nutrients into the small intestine at a rate comparable with gastric emptying had a weak effect on appetite and food intake suppression compared to when the same nutrients were infused into the stomach or orally ingested (Cecil et al., 1998a).

Research investigating mechanisms controlling eating behaviour in humans have employed different methodologies to dissect the effect of oral, gastric and intestinal stimulation on appetite and energy intake (French and Cecil, 2001). The direct administration of nutrients into areas of the gastrointestinal tract eliminates the oro-sensory and cognitive or learned responses such as experience and expectation as well as hedonic aspects. However, caution must be exerted when results are examined in comparison to normal feeding conditions. Previous research suggests an important role for oro-sensory stimulation on the suppression of hunger and generation of fullness produced by gastric distension (Cecil et al., 1998b, Wijlens et al., 2012) which is thought to be affected at least in part by a slower gastric emptying rate following oral ingestion as opposed to intragastric infusion. Therefore it is likely that signals from the mouth to brain affect the regulation of gastric emptying, although, further studies are essential. How these pathways interact in the human brain has never been studied.

As a prerequisite to study II, the aim of study I was to establish whether sweet tastants (glucose and saccharin) have equivalent effects on gastric emptying and appetite responses if consumed orally then swallowed, or if they are directly administered intragastrically, i.e. whether the rate of gastric emptying and appetite

are dependent on the route of administration. The experimental variable in study I is whether or not oral taste has been activated. Determining changes in gastric emptying rate, a vagovagal reflex requiring brainstem activation, is once again used as an integrated proxy measure of gut-brain signalling.

5.2 Method

5.2.1 Design

This study was conducted as a single blind randomised cross-over study. Participants attended the Wellcome Trust Clinical Research Facility on six occasions (plus a screening visit) with at least three days between each trial visit. Ethical approval for the study was granted by the National Health Service North West Research Ethics Committee (ref. 10/H1016/134)

5.2.2 Participants

Ten participants (five female, five male) were involved. All participants met the inclusion/exclusion criteria as detailed in chapter 2.2.1.

5.2.3 Experimental protocol (study I)

Participants were studied on six separate occasions and instructed to follow the pre-study standardisation protocol (chapter 2.3.1). On the day of each trial visit participants arrived at the facility at approximately 9.00 hours following an overnight 11 hour fast. On arrival, participants were asked to provide a baseline, end-expiratory breath sample (chapter 2.3.3) and a baseline assessment of appetite was collected using VAS (chapter 2.3.2). On three out of six trial visits participants were then intubated with a NG feeding tube (Ryles tube 10FG) via which test solutions were administered. The NG tube was removed immediately following the infusion. Participants were asked to consume the test solutions through a drinking straw on the

remaining three trial visits. Participants received an intragastric infusion or consumed orally, over 2 minutes (t= -2 - 0min), either a) 45g of glucose (180 kcal) dissolved in tap water b) 20mg saccharin dissolved in tap water or c) tap water in a total volume of 250ml in a randomised order. All test solutions were prepared on the morning of the study at room temperature and were presented in transparent beakers as colourless liquids with no visible differences. All test solutions were labelled with 100mg of [^{13}C] sodium acetate for determination of gastric emptying rate. End–expiratory breath samples were collected immediately before and after infusion/consumption (t= -5, t= 0) and at 5 minute intervals until 60 minutes. Subjective appetite perceptions of hunger, fullness, desire to eat and nausea were collected immediately before and after infusion/consumption (t= -5, t= 0) and at 15 minute intervals until 60 minutes. Following light refreshments, participants were free to leave the facility. The study protocol is presented in figure 5.1.

5.2.4 Materials and test foods

D- (+)- Glucose (Sigma-Aldrich, Munich, Germany). The concentration of glucose was based upon previous work showing 1M glucose to have a potent effect on gastric emptying (Little et al., 2010a) and is consistent with previous studies in this thesis.

Saccharin (Hermesetas, Berkshire, UK). The amount used was based on commercially sourced dose units (i.e. sweetener tablets).

[^{13}C] sodium acetate (CK Gas Products, Hampshire, UK).

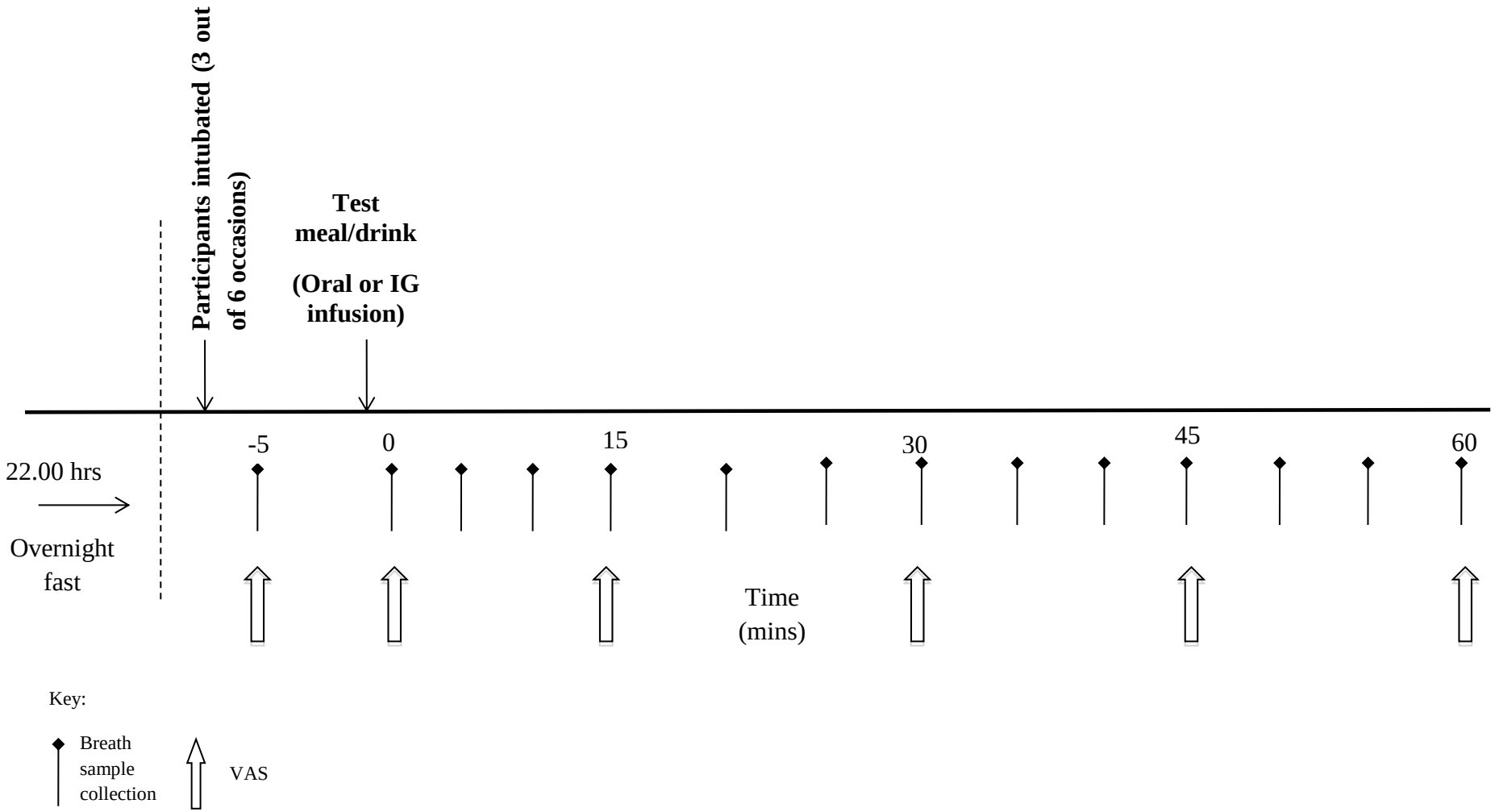


Figure 5.1 Schematic representation of study protocol

IG- intragastric

5.2.5 Statistical analysis

Data are presented and analysed as described in chapter 2.4.

5.3 Results

5.3.1 Participants

Participant characteristics are displayed in table 5.1.

	mean ± SD (*n* =10)
Age (years)	21.5 ± 1.6
Height *(cm)*	172.8 ± 5.7
Weight *(kg)*	66.2 ± 9.2
BMI (kg/m^2)	22.1 ± 1.8
Restraint Score[1]	3.2 ± 1.8

Values represent mean ± SD (n =10)

[1] Score of restraint using the three factor eating questionnaire (Stunkard and Messick, 1985)

Table 5.1 Participant characteristics

5.3.2 Gastric emptying

Following the oral consumption and intragastric infusions of test solutions, there was a rise in $^{13}CO_2$: $^{12}CO_2$ with values peaking at 25 minutes for orally and intragastrically administered saccharin and water trials, and at 35 minutes for oral and intragastric glucose, after which $^{13}CO_2$: $^{12}CO_2$ values began to decrease (figure 5.2A). For both gastric emptying rate and AUC (figure 5.2B), there was a main effect of time and condition ($P < 0.001$) but no effect of method of administration (oral vs. intragastric). Oral and intragastric glucose slowed gastric emptying when compared with the oral and intragastric saccharin and water trials. Therefore, in keeping with previous data, saccharin had no effect on gastric emptying, and no additional effect was exerted by including oral sweet tasting.

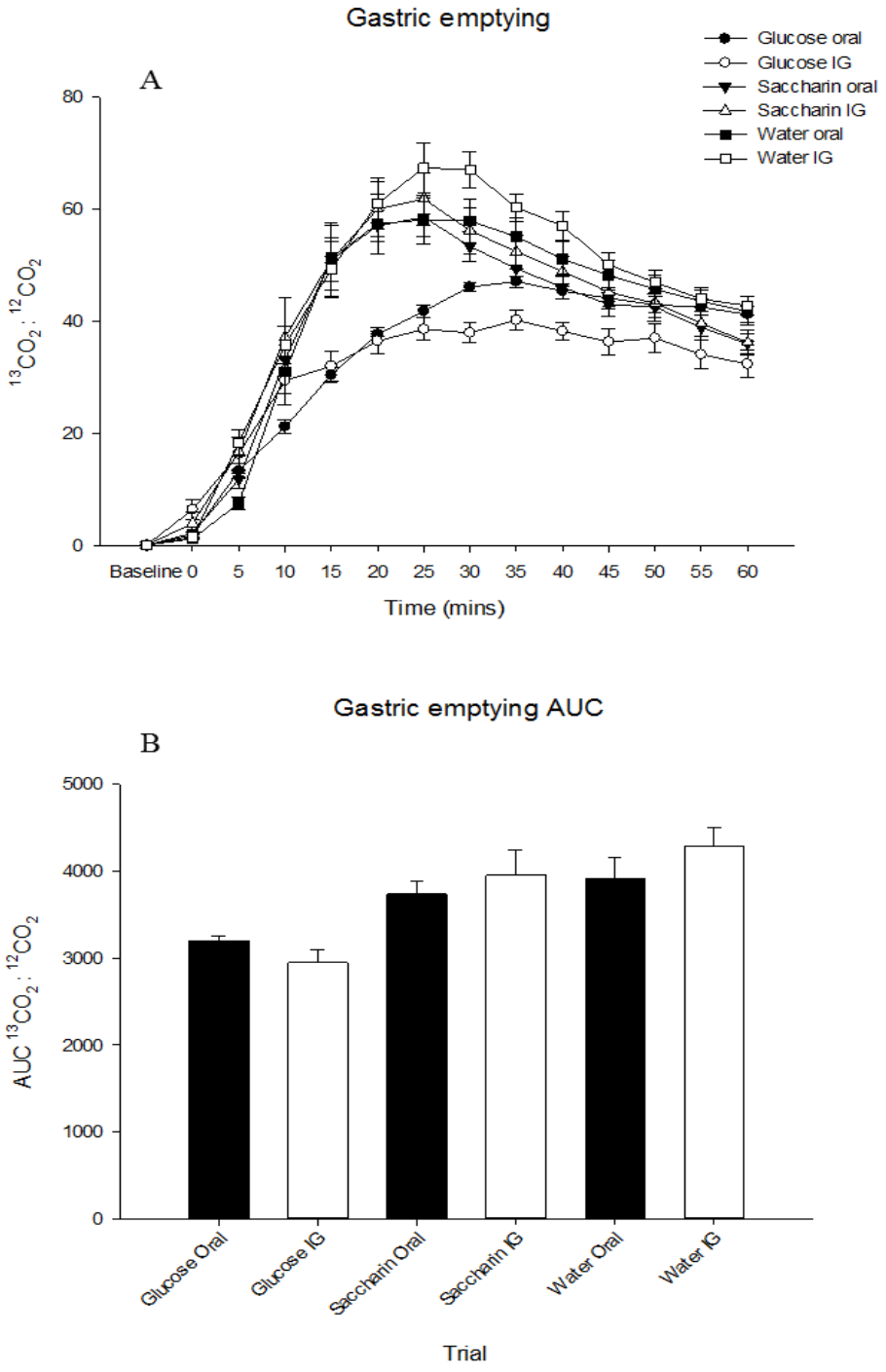


Figure 5.2 Gastric emptying rate

Gastric emptying rate of glucose oral (✦ / ■), glucose intragastric (IG) (⊸/ □), saccharin oral (▲ / ■), saccharin IG (⊸△⊸/ □), water oral (■ / ■) and water IG (□ / □) trials displayed over time (A) and as area under the curve (B). Values represent mean ± SEM, (n=10).

Although no overall effect was seen on gastric emptying rate between oral and intragastric methods, closer inspection of the data suggested emptying rates differed between the two routes of administration from baseline to 15 minutes and 15 minutes to 60 minutes, particularly in the glucose trials. When analysed in these time bins, intragastrically administered glucose emptied significantly faster than orally administered glucose for the first 15 minutes (AUC; 469.2 $\pm$ 62 vs. 346.7 $\pm$ 20.8, figure 5.3B) (P= 0.05) but the residual volume emptied significantly more slowly for the remaining 45 minutes of the study (figure 5.3C) (P < 0.05). Peak $^{13}CO_2$: $^{12}CO_2$ values reaching 47.0 $\pm$ 1.0 and 40.0 $\pm$ 1.7 (AUC; 2858.2 $\pm$ 60.2 vs. 2478.0 $\pm$ 118.5) for oral and intragastric trials respectively. Similarly, intragastric water also emptied faster for the first 15 minutes (AUC; 537.8 $\pm$ 51.4 vs. 424.0 $\pm$ 48.6, figure 5.5B) (P < 0.03). However, in contrast to glucose, intragastrically administered water emptying remained faster than its orally swallowed counterpart, albeit not significantly, for the remaining 45 minutes of the study (figure 5.5C) (P = 0.3) with peak $^{13}CO_2$: $^{12}CO_2$ values reaching 58.0 $\pm$ 4.3 and 67.3 $\pm$ 4.4 (AUC; 3485.0 $\pm$ 208.1 vs. 3571.9 $\pm$ 174.0) for oral and intragastric trials respectively. Orally and intragastrically delivered saccharin displayed similar gastric emptying rates. Similar to glucose and water, there was a trend for intragastric saccharin to empty faster compared to oral saccharin in the first 15 minutes (AUC; 559.3 $\pm$ 106 vs. 479.2 $\pm$ 34.7, figure 5.4B) but this was not significant (P= 0.5). Intragastric saccharin remained marginally faster for the remaining 45 minutes with peak $^{13}CO_2$: $^{12}CO_2$ values reaching 58.5 $\pm$ 3.4 and 61.8 $\pm$ 4.7 (AUC; 3256.8 $\pm$ 135.9 vs. 3396.5 $\pm$ 210.8) for oral and intragastric trials respectively (figure 5.4C).

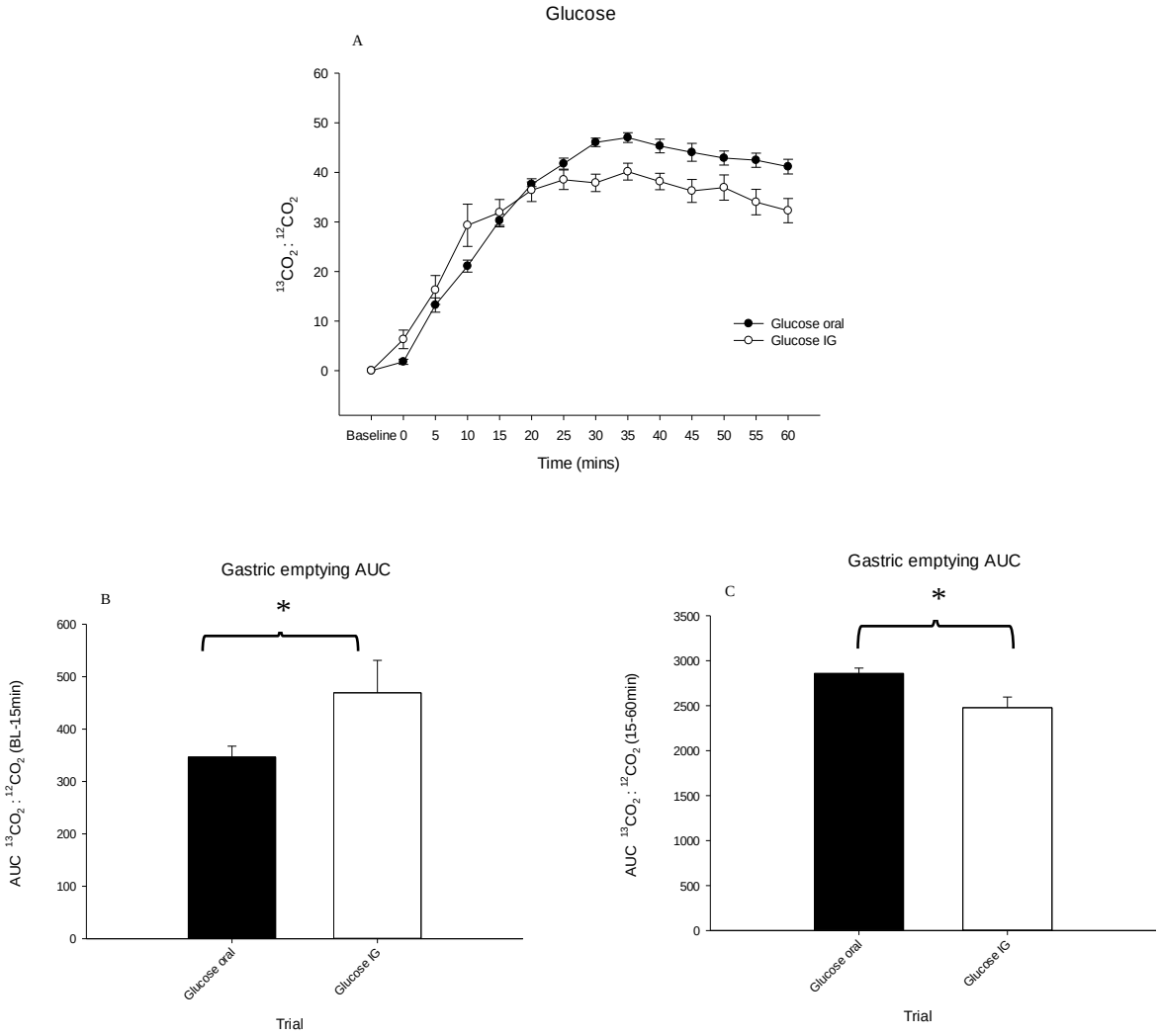


Figure 5.3 Gastric emptying rate of glucose

Gastric emptying rate of glucose oral (⊷) and intragastric (IG) (⊸) trials displayed over time (A). Area under the curve from baseline (BL) to 15 minutes (B) and 15 minutes to 60 minutes (C) for glucose oral (■) and IG (□) trials. Values represent mean ± SEM, (n=10). * Significant difference between trials ($P < 0.05$).

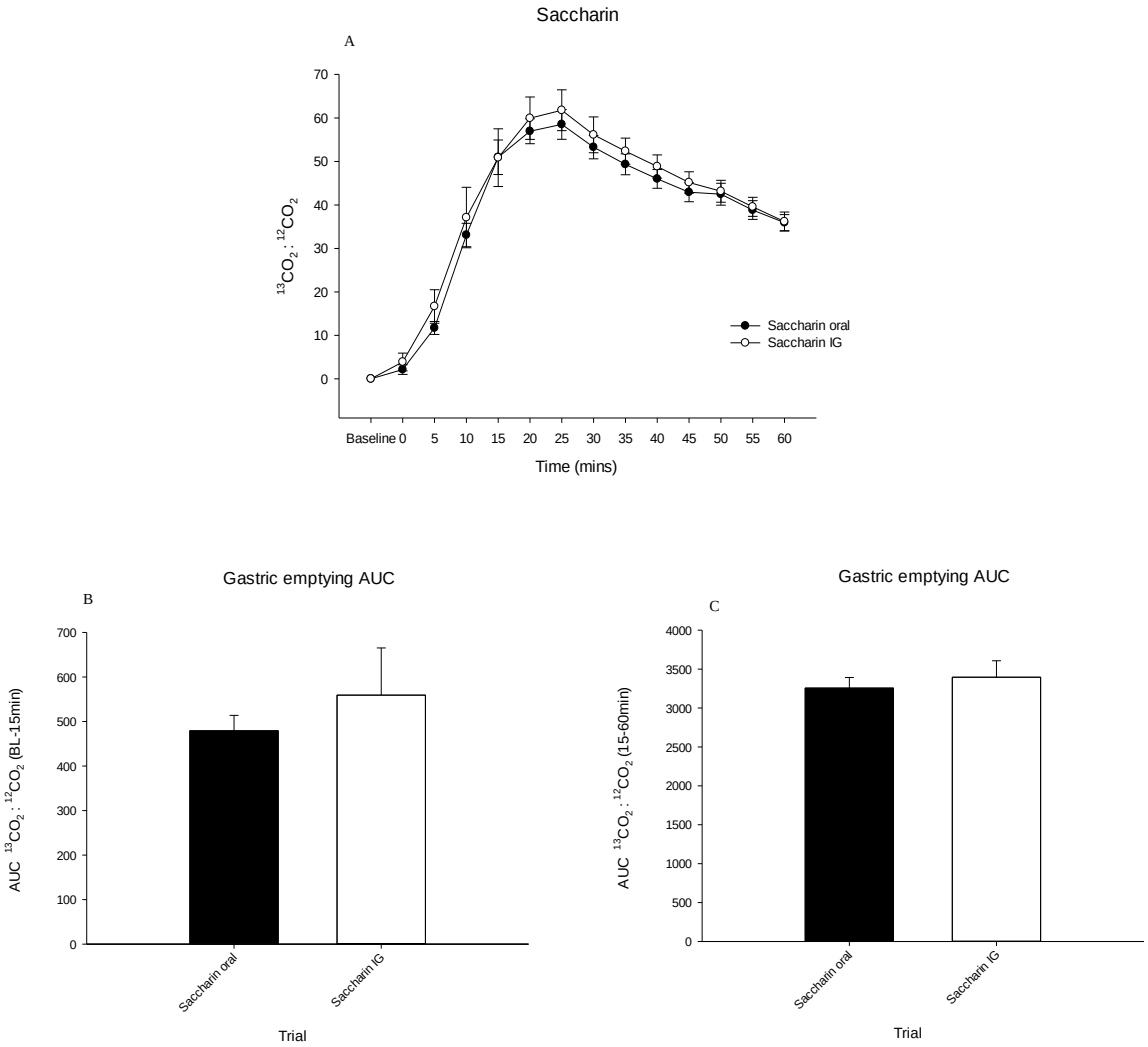


Figure 5.4 Gastric emptying rate of saccharin

Gastric emptying rate of saccharin oral (⊷) and intragastric (IG) (⊶) trials displayed over time (A). Area under the curve from baseline (BL) to 15 minutes (B) and 15 minutes to 60 minutes (C) for saccharin oral (■) and IG (□) trials. Values represent mean ± SEM, (n=10).

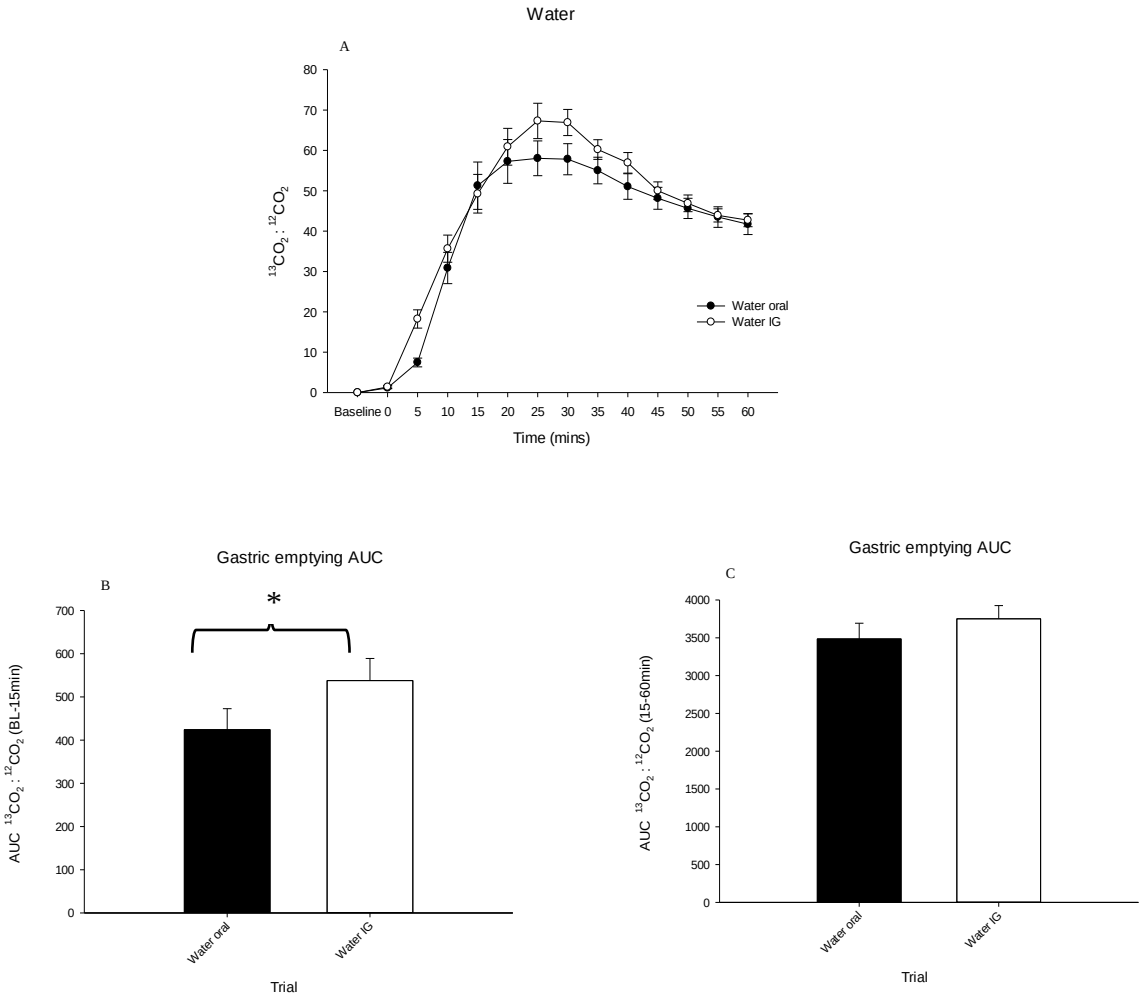


Figure 5.5 Gastric emptying rate of water

Gastric emptying rate of water oral (⊸) and intragastric (IG) (⊸) trials displayed over time (A). Area under the curve from baseline (BL) to 15 minutes (B) and 15 minutes to 60 minutes (C) for water oral (■) and IG (□) trials. Values represent mean ± SEM, (n=10). * Significant difference between trials ($P < 0.05$).

5.3.3 Appetite perceptions

Baseline ratings for desire to eat, hunger, fullness and nausea were not significantly different between the oral and intragastric trials for glucose, saccharin or water ($P > 0.05$). Ratings of desire to eat and hunger decreased following oral consumption and intragastric infusions while ratings of fullness increased. There was a main effect of time ($P < 0.001$) for each of the appetite perceptions assessed (desire to eat, hunger and fullness) for glucose, saccharin and water trials but no effect of route of administration or route of administration x time interaction. In the glucose and saccharin trials, there was a trend for ratings of desire to eat to be lower and perceptions of fullness to be higher in the intragastric trials compared to the oral route of administration, immediately following test solution consumption or infusion (Figure 5.6 and 5.7). Following their decline, ratings of hunger and desire to eat in the glucose and saccharin trials, increased. Similarly, following the increase, ratings of fullness gradually decreased. There was no discernible patterns following oral or intragastric water on any of the appetite perceptions assessed (Figure 5.8). There was a main effect of time ($P < 0.001$) and route of administration x time interaction ($P < 0.01$) for nausea. Although not significant, there was a trend for intragastric glucose, saccharin and water to have higher nausea ratings following the infusion compared to oral consumption.

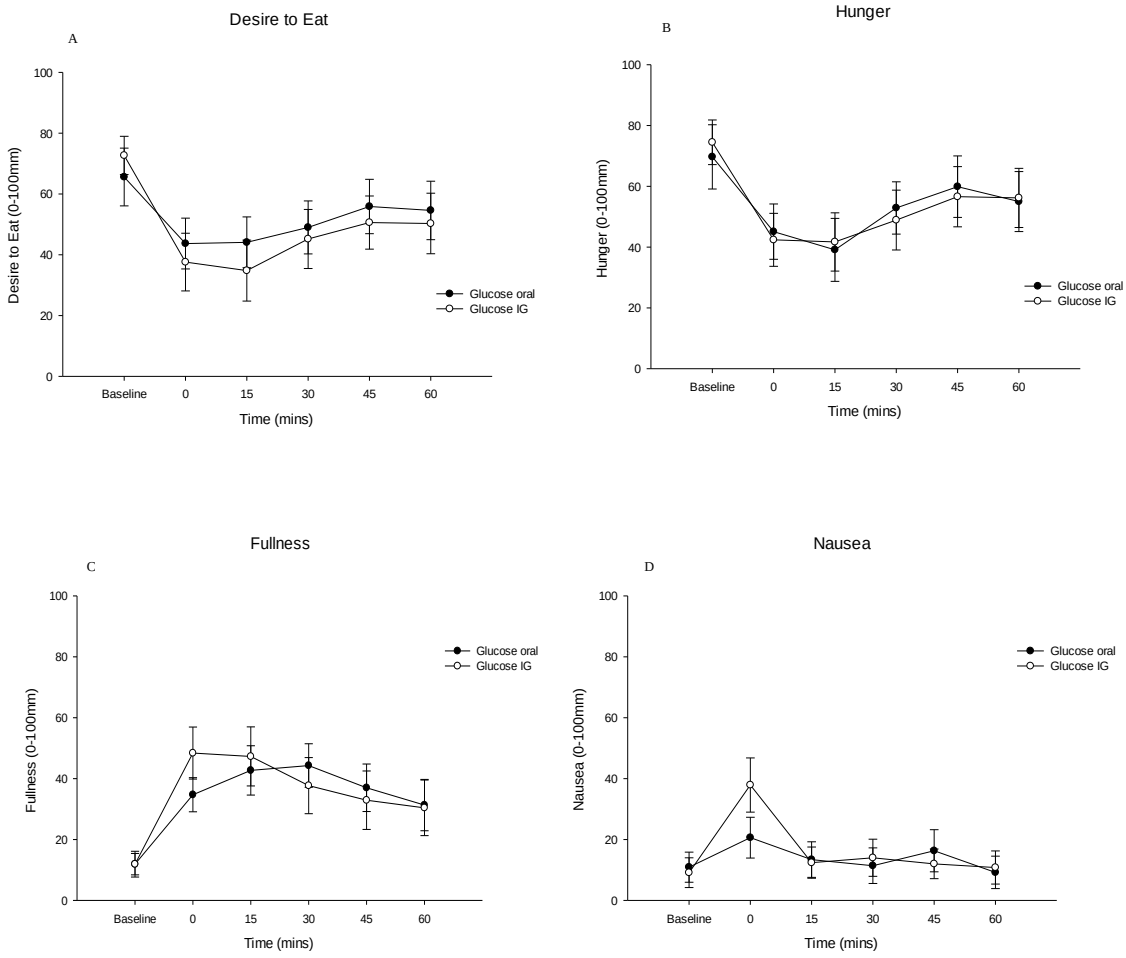


Figure 5.6 Subjective appetite ratings following glucose test meals

Subjective ratings for desire to eat (A), hunger (B), fullness (C) and nausea (D) during the glucose oral (●) and glucose intragastric (IG) (○) trials displayed over time. Values represent mean ± SEM, (n=10).

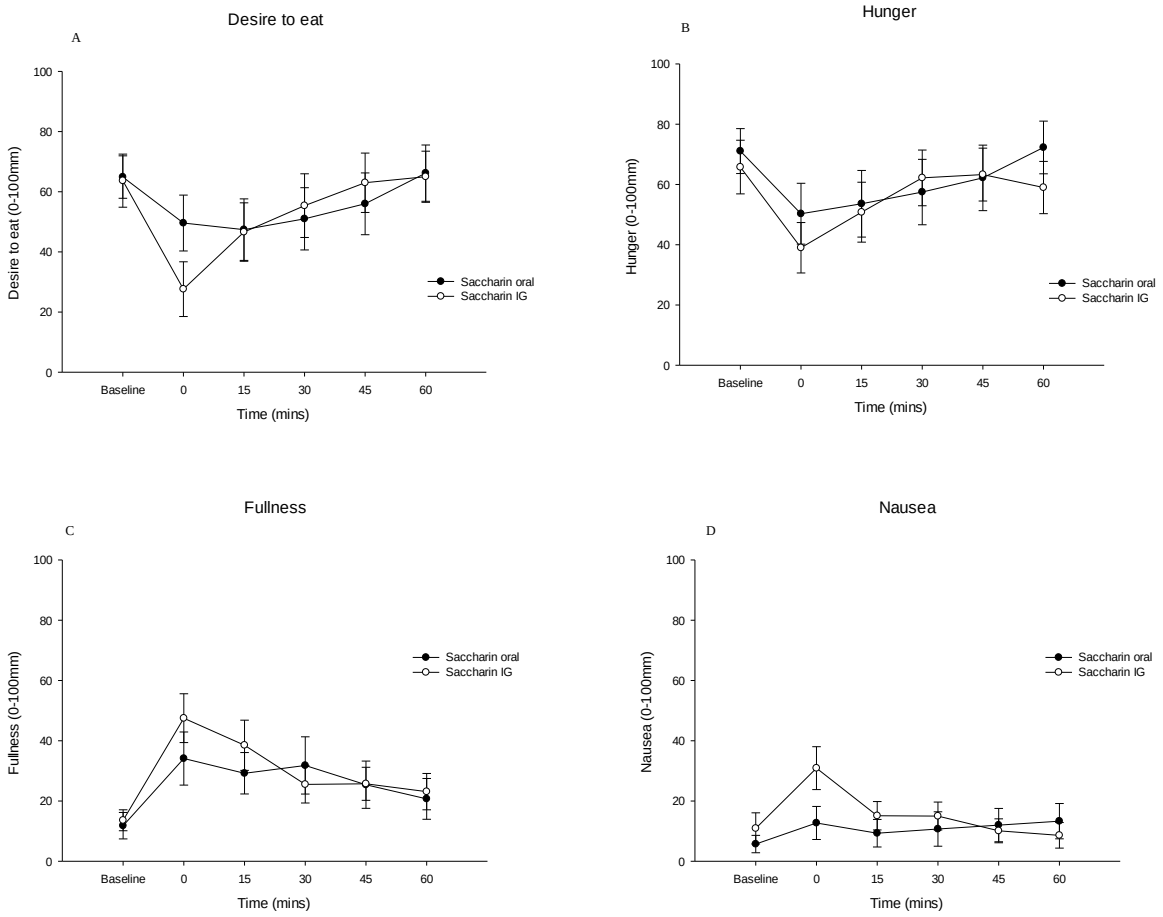


Figure 5.7 Subjective appetite ratings following saccharin test meals

Subjective ratings for desire to eat (A), hunger (B), fullness (C) and nausea (D) during the saccharin oral (●) and saccharin intragastric (IG) (○) trials displayed over time. Values represent mean ± SEM, (n=10).

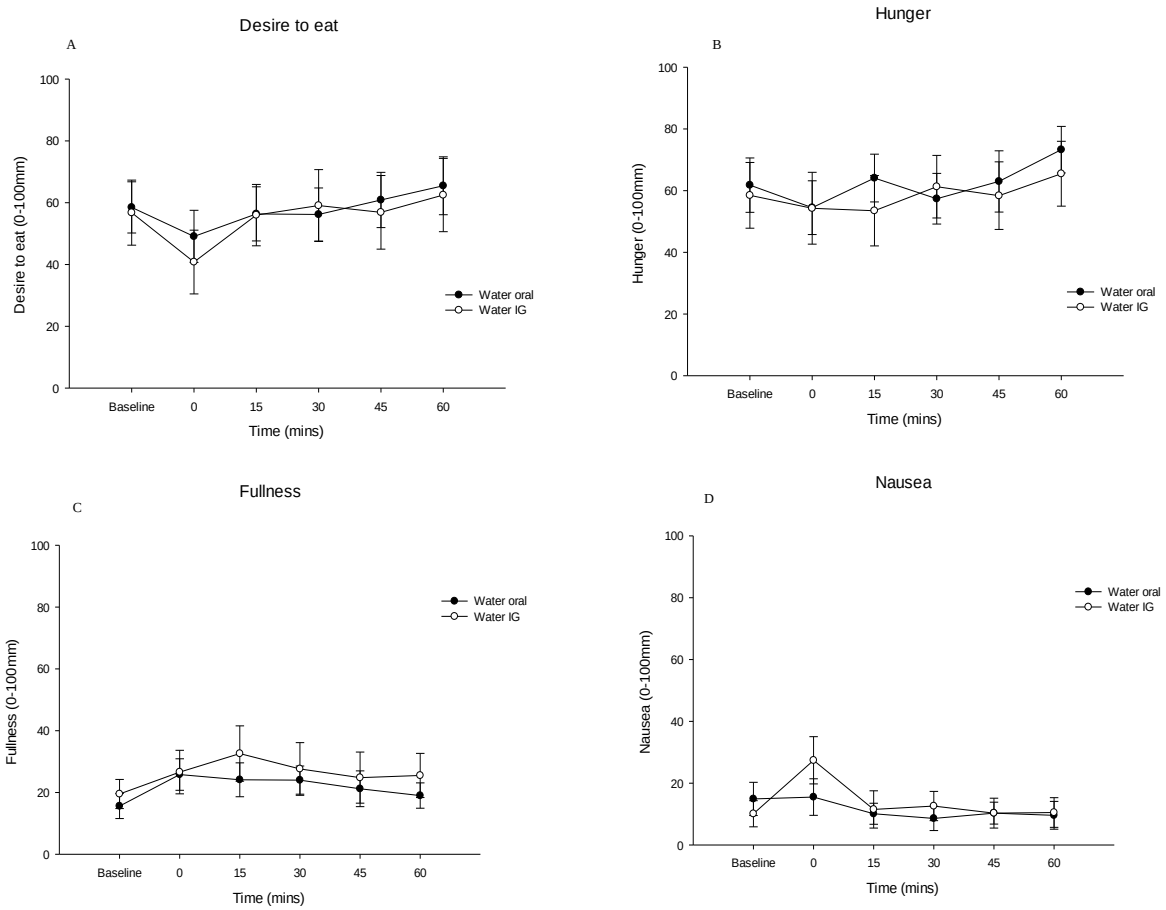


Figure 5.8 Subjective appetite ratings following water test meals

Subjective ratings for desire to eat (A), hunger (B), fullness (C) and nausea (D) during the water oral ( ) and water intragastric (IG) ( ) trials displayed over time. Values represent mean ± SEM, (n=10).

5.4 Discussion

This study has demonstrated that, the rate of gastric emptying for glucose differs depending on whether it is consumed orally or administered directly into the stomach to bypass oral taste receptors. Glucose slowed gastric emptying compared to saccharin and water, independent of route of administration, which is consistent with previous studies (Little et al., 2009, Steinert et al., 2011). The differences in gastric emptying rate for glucose occurred in two phases with intragastric glucose emptying faster then significantly slower in the first 15 and remaining 45 minutes respectively. This pattern of emptying was not apparent for saccharin or water conditions with intragastric administration of both emptying marginally faster for the duration of the study. This cannot be a confounding effect of NG tube placement inducing slower emptying for the last 45 minutes (no tube was placed for the orally consumed meal) since the effect was confined to glucose, and not seen in the control condition.

As demonstrated in chapter three, an intragastric infusion of glucose results in hormonal signals that contribute to the inhibition of gastric emptying and appetite, but the stimulation of oral taste receptors in normal consumption of food and drink may further modulate appetite by inducing cephalic phase responses (Lavin et al., 2002, Smeets et al., 2010) and by activating appetite regulating centres within the CNS (Rolls, 2007). However, there was no effect on desire to eat, hunger and fullness ratings depending on whether solutions were given by mouth or intragastrically administered, despite the significant slowing of gastric emptying by glucose. A decrease in hunger, and increase in fullness was observed immediately following all oral and intragastric infusions, although less obvious for water conditions, presumably because of the increased gastric distension. However, there was a trend for desire to eat to be lower and fullness ratings higher when glucose and saccharin were intragastrically infused compared to consumed orally. This does not support the suggestion that oro-sensory factors, including sweetness, can influence feelings of hunger and prospective consumption (Cecil et al., 1998b, Poothullil, 1995). However, despite not being significant, the trend for intragastric administration to magnify feelings of fullness may be influenced at least in part by the presence of the NG tube and the infusion itself which induces higher nausea scores than oral ingestion although the effect was short lived.

Overall, the changes in appetite responses were relatively small and any differences between the route of administration were only apparent immediately following consumption/infusion with no discernible patterns thereafter. This suggests a lack of mechanistic relationship between the gastric emptying and appetite responses. As intragastric glucose emptied more slowly than oral glucose in the last 45 minutes it might have been expected to enhance feelings of fullness and suppression of hunger due to an increased period of gastric distension. However, similar to Lavin *et al*, the present VAS data did not support this, suggesting a low sensitivity of VAS ratings to intragastrically administered solutions (Lavin et al., 2002). Although gastric distension is an important factor for inducing feelings of fullness (Rolls and Roe, 2002), oro-sensory stimulation is still crucial. For instance, despite suppressed subjective appetite responses as a result of gastric feedback this does not always translate into a reduction in food intake without prior oral stimulation (Oesch et al., 2006).

In conclusion, the pattern and rate of gastric emptying of glucose differed depending on whether it was given orally or administered intragastrically. A further study is required to ascertain the impact this might have on food intake. Given that a different pattern of emptying occurred when glucose tasting had occurred, a new hypothesis was generated, that oral glucose sensing would alter the CNS response to intragastrically administered glucose.

Study II: Effect of sequential oral and gastrointestinal stimulation on brain BOLD responses to glucose

5.5 Introduction

In humans, fMRI has become a popular and rapidly advancing research tool to study appetite. A number of homeostatic (e.g. hypothalamus) and non-homeostatic (e.g. insula, amygdala, OFC) regions associated with the regulation of appetite have been identified (De Silva et al., 2012). With regard to homeostatic control, the hypothalamus is recognised as a critical region for the integration of CNS appetite signals to regulate food intake (Heijboer et al., 2006). One of the first studies using fMRI showed a decrease in hypothalamic BOLD signal occurring 7-12 minutes after oral ingestion of glucose (Liu, Gao et al. 2000). Subsequent studies have reinforced these findings showing a dose-dependent and prolonged decrease in BOLD signal in the hypothalamus following glucose ingestion (Smeets et al., 2005b, Smeets et al., 2005a). Similarly, studies have demonstrated effects on non-homeostatic regions following the ingestion of sugars and NNS (Smeets et al., 2005a, Smeets et al., 2011, Chambers et al., 2009, Frank et al., 2008) and/or evaluating responses to food cues using visual stimuli (Page et al., 2011) with little focus on the physiological responses to nutrient ingestion. However, the aforementioned studies are technically limited by drinking associated movements during the oral consumption of test meals which induce imaging artefacts potentially obscuring the detection of possible BOLD signal changes. This is an insurmountable problem particularly when imaging the brainstem, since the areas of interest are extremely small and the changes observed may be subtle. Moreover, detailed imaging of the brainstem during nutrient ingestion had not been reported until recently and given it is the first point of contact, with signals transmitted from the gastrointestinal tract via vagal afferents, it is important to include this in CNS imaging studies. Furthermore, studying homeostatic and non-homeostatic mechanisms in isolation is not representative of appetite regulation and feeding behaviour as they do not function independently (De Silva et al., 2012). To date no studies have been undertaken to integrate brain responses to oral and gastrointestinal nutrient sensing, which more closely resembles consumption.

As mentioned in chapter one, the development of physMRI in this department has enabled the evaluation of BOLD signal responses to nutrients over time in the whole brain with improved spatial and temporal resolution (Jones et al., 2012, Lassman et al., 2010). In order to eliminate the confounding factors of oral ingestion, prior departmental work has always investigated the effect of glucose on brain activation (as with gastric emptying) by administering intragastrically, using an NG tube, an approach which enables the study of gut to brain signalling independently. However, this approach does not mimic the sequence of events that occurs when sweet substances are ingested in real life. In reality, oral taste sequentially precedes gastrointestinal chemo-sensation. The overall response to ingested nutrients will not exclusively depend on gut-to-brain signals and it is likely an interaction between the two sensory phases occurs within the CNS.

The differences in gastric emptying rate between orally and intragastrically delivered glucose observed in study I form the basis and justification for this study. The objective of the present study was to model the sequence of activation in normal ingestive patterns, whilst minimising the artefacts and difficulties inherent on imaging brain areas during swallowing. The purpose of the study was twofold: first, to establish the effect of oral and sequential gastrointestinal "sweetness" on brain activation and second, to investigate whether oral sweet taste affects the gut to brain signal.

5.6 Method

5.6.1 Design

This study was conducted as a single blind randomised cross-over study. Participants attended the Translational Imaging Unit on four occasions (plus a screening visit) with at least three days between each trial visit. Ethical approval was granted by the National Health Service North West Research Ethics Committee (ref. 11/NW/0663).

5.6.2 Participants

15 participants (six female, nine male) took part. One participant was involved in both studies I and II. All participants met the inclusion/exclusion criteria as detailed in chapter 2.2.1. In addition, participants completed a magnetic resonance imaging patient declaration form (Appendix VI) as part of the screening process to exclude anyone in whom MRI is contraindicated (e.g. pacemaker, metal foreign body).

5.6.3 Experimental protocol (study II)

Participants were studied on four separate occasions and instructed to follow the pre-study standardisation protocol (chapter 2.3.1). On the day of each trial visit, participants arrived at the Imaging Unit at approximately 9.00 hours following an overnight 11 hour fast. Upon arrival, participants were intubated with a NG tube (Ryles tube 10FG) which was then connected to adapted oxygen bubble tubing and a syringe for intragastric test meal delivery in the scanner (Bunzl Healthcare, Leicester, UK) (figure 5.9A). Participants were then positioned in the scanner (3.0T Philips Achieva MR System) (figure 5.10) and the oral tubing (figure 5.9B) placed in the participants mouth. Participants were scanned continuously for 35 minutes. After a baseline period of five minutes a 20ml solution of either a) 1M glucose or b) artificial saliva (AS) was delivered to the mouth (t= 5). Participants were instructed to hold in the mouth for 20 seconds and were cued to swallow by a tap on the leg. Participants then received a 250ml intragastric infusion over two minutes (t= 10-12) of either c) 1M glucose or d) saline. Following the 35 minute scan, the NG tube was removed and participants were free to leave the unit. The four trial visits are outlined in table 5.2 and the study protocol is presented in figure 5.11. On each visit, an additional ten minute structural scan was conducted to define each participants brain anatomy.

		Oral	
		Glucose	Artificial saliva
IG	Glucose	A	B
	Saline	C	D

Table 5.2 Trial conditions

Participants received one of four combinations of oral and intragastric (IG) test solutions (A-D) on each visit in a randomised order.

A **B**

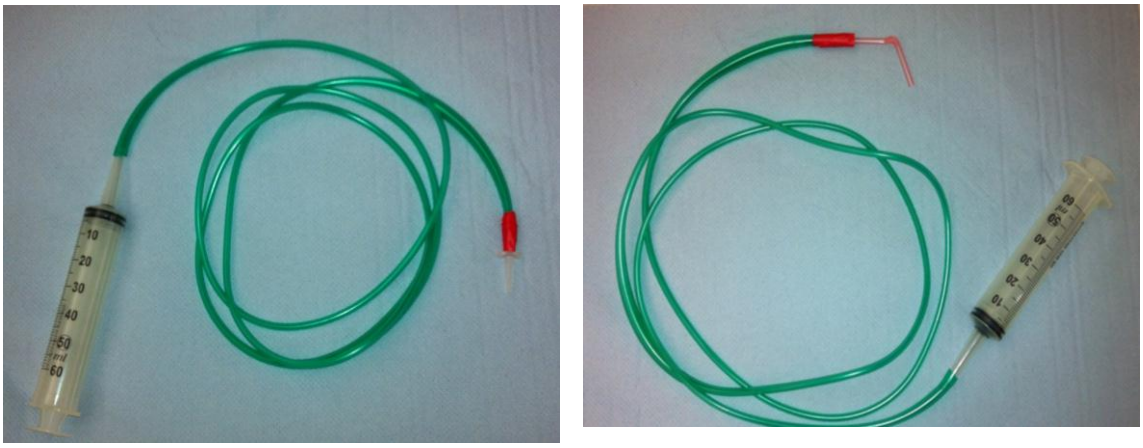


Figure 5.9 Tubing for delivery of test solutions

Connecting tubing for intragastric infusion (A) and with a drinking straw for oral test solution delivery (B).

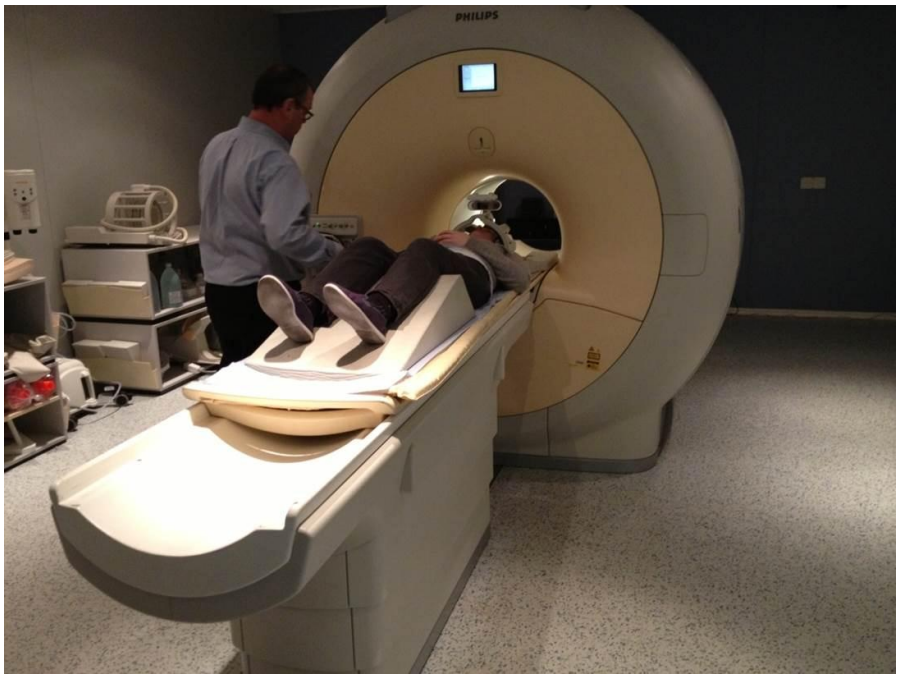


Figure 5.10 A participant in the scanner

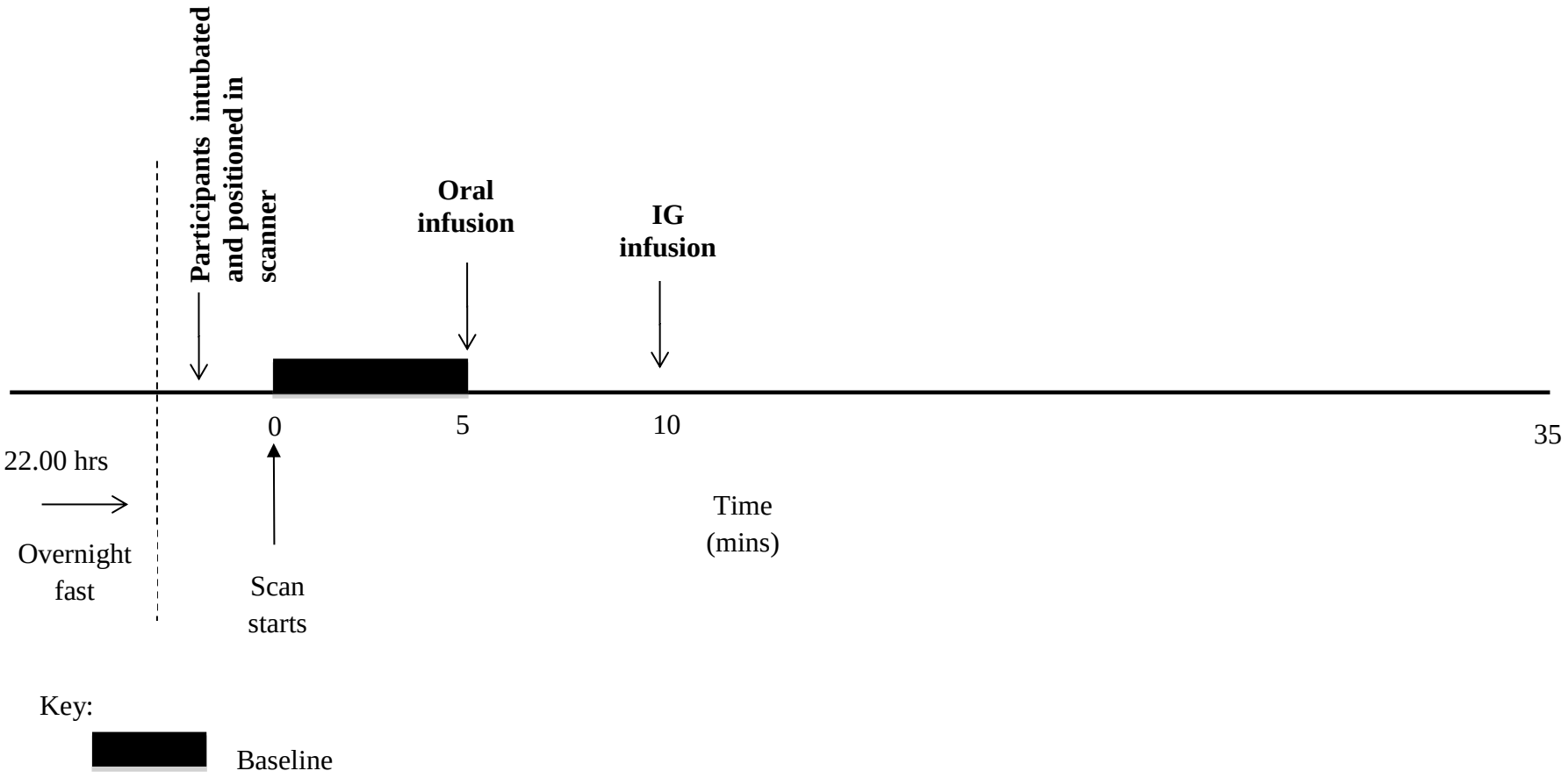


Figure 5.11 Schematic representation of study protocol

IG – intragastric

5.6.4 Materials and test foods

The glucose solution was prepared on the morning of each visit. A batch of AS solution was made up prior to the study commencing so that all participants received the same solution. All infusions were prepared and served at room temperature.

D- (+)- Glucose (Sigma-Aldrich, Munich, Germany). The concentration of glucose was based upon previous work showing 1M glucose to have a potent effect on gastric emptying (Little et al., 2010a) and is consistent with previous studies in this thesis.

AS was made up of 25 mM potassium chloride (Sigma-Aldrich, Munich, Germany) and 2.5 mM sodium carbonate (Dr Oetker Ltd, Leeds, UK) in distilled water (O'Doherty et al., 2001). AS was used as the oral control for glucose since saline would induce its own tastant effects on account of its saltiness and water is believed to activate regions of the OFC (Kringelbach et al., 2003). However, AS is not a suitable control for the intragastric phase as it empties from the stomach extremely quickly because it lacks any osmolar content and would therefore fail in its remit to act as a volumatic control in the gastric part of the study. The intragastric control used was saline (0.9%w/v sodium chloride, Baxter Healthcare Ltd, Thetford, UK). Evidence from prior departmental studies show clear differences in brainstem activation and gastric emptying between glucose and saline (Little et al., 2013, In review).

5.6.5 MRI analysis

5.6.5.1 Data acquisition

Brain volumes were acquired (50 slices, voxel size 3 x 3 x 3mm) at five second intervals (repetition time, TR= 5 secs), with 12 functional scans acquired every minute to give a total of 420 scans. Real time adjustment of motion was done using prospective motion correction.

5.6.5.2 Data analysis

Data analysis was carried out using Statistical Parametric Mapping (SPM 8, http://www.fil.ion.ucl.ac.uk/spm/) using the physMRI technique (Jones et al., 2012, Lassman et al., 2010). The data were pre-processed then split into two parts, part 1 (oral) and 2 (gastric), and analysed in two further stages as described below.

5.6.5.3 Pre-processing

Using SPM, the data were processed in the following steps:

Realignment: Participant movement in the scanner is inevitable, especially over 40 minutes, and can add to noise in the image series reducing the sensitivity of analysis. Participant movement was therefore corrected using a six parameter rigid body transformation compared to the first scan.

Co-registration: To accurately map areas of activation, the mean functional image from the realignment stages was co-registered with the high resolution anatomical image.

Segmentation and Spatial Normalisation: Individuals differ in brain size and shape so in order to extrapolate the findings to the group as a whole, the images had to be 'standardised'. The image is segmented in to grey matter, white matter and cerebrospinal fluid. The grey matter is then spatially normalised to fit a grey matter template. This involved warping the images to fit a brain template so that regions from different participants were as close together as possible and allowed direct comparison of participant scans.

Smoothing: The BOLD signal response measured may be in different but similar locations in individuals' brains. Even though each individual's brain has been spatially standardised using normalisation, the data were smoothed in order to bring these disparate locations closer together.

Due to the movement caused by swallowing in part 1, independent component analysis was run using The MATLAB Group Independent Component Analysis of fMRI toolbox (MATLAB toolbox GIFT v1.3i). The component time courses and spatial distribution were visually inspected and components associated with movement and drift were removed.

5.6.5.4 First level analysis

Analysis was performed on each participant. A diagram showing how the data were divided and analysed is shown in figure 5.12.

Part 1 (oral pre-taste)

To investigate oral glucose induced BOLD signal the glucose and AS scans were split into 11 consecutive 30 second time bins (one baseline and 10 post-infusion bins) from t= 4.5 to t= 10 min. Using regression analysis, the average BOLD signal for each time bin was compared to the baseline time bin; that is, the 30 second bin immediately prior to the oral infusion of glucose or AS. This resulted in 10 %BOLD signal intensity maps from baseline across time per condition. The AS scans were contrasted to the glucose scans to show the effect of glucose taste over time. This resulted in 10 contrast images which were then taken to second level analysis. Thus, participants acted as their own control.

Part 2 (intragastric infusion)

To investigate the effect of oral glucose taste on BOLD signal changes induced by intragastric glucose a similar first level analysis was performed with the glucose and saline scans split into 13 consecutive two minute time bins (one baseline and 12 post- intragastric infusion bins) from t= 8 to t= 34 min. The last four time bins from part 1 of the analysis formed the baseline for part 2 analysis. The last minute of the scan (t= 34-35 min) was discarded as it did not fit into a two minute time bin. Using regression analysis, the average BOLD signal for each bin was compared to the baseline time bin, that is, the two minutes immediately prior to the intragastric infusion of glucose, or saline, for all four trials as shown in table 5.2. This resulted in 12 %BOLD signal intensity maps from baseline across time per trial. The method of

analysis is always comparative and subtractive, rather than absolute, comparing the experimental condition to the relevant control condition(s). The key effect in question, of oral glucose taste on the glucose induced BOLD signal, was investigated using the following interaction contrast:

(A-C) – (B-D)

(glucose/glucose – glucose/saline) - (AS/glucose – AS/saline)

glucose(glucose–saline) - AS(glucose–saline)

This resulted in 12 contrast images which were taken to second level analysis. Again participants were their own control.

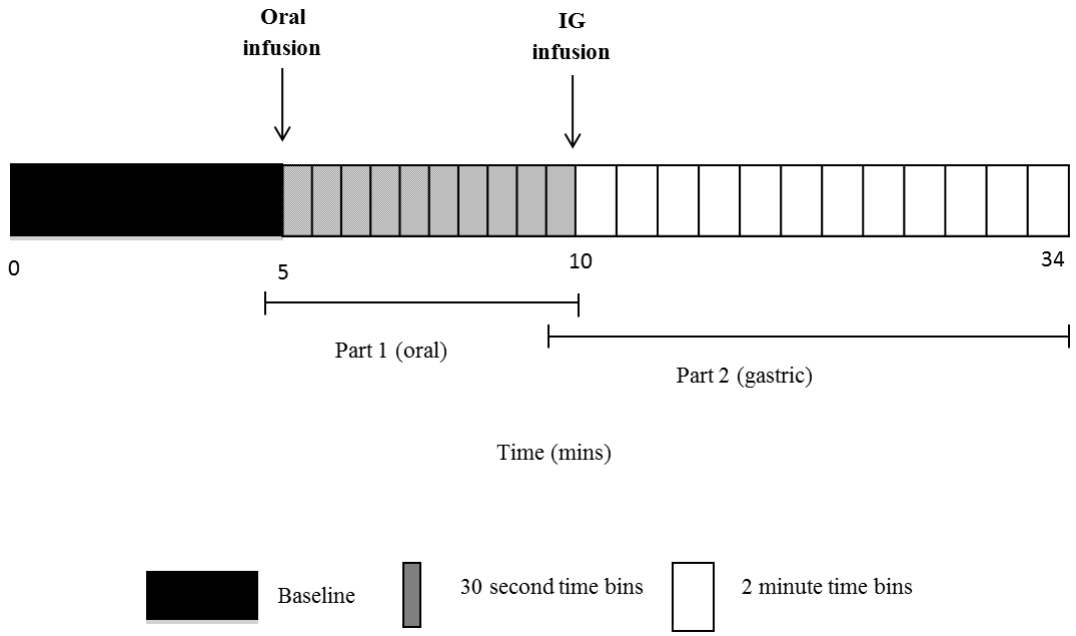


Figure 5.12 Schematic representation of data analysis

Following a five minute baseline, the oral part 1 (t= 4.5-10 minutes) was divided into 30 second time bins and the gastric part (t= 8-34 minutes) into two minute time bins for analysis. IG- intragastric.

5.6.5.5 Second level analysis

Part 1 (oral pre-taste)

To determine if statistically significant changes between glucose and AS in the BOLD signal across time occurred, a one way repeated measures ANOVA with time as the factor was conducted.

Part 2 (intragastric infusion)

To determine if statistically significant changes in the BOLD signal occurred, a one way repeated measures ANOVA exploring the interaction of taste with intragastric infusion across time was conducted.

A cluster level statistical inference of p(Family Wise Error; FWEc) < 0.05 at a peak level of p = 0.001 was used. A small volume correction (SVC) was performed for brainstem and hypothalamic regions at p(FWEc) < 0.05.

Results for each part (oral and gastric) are presented as an average cluster per time bin. For the gastric part, glucose-saline is show for both glucose and AS taste conditions. Cluster locations are expressed as centre of mass (CoM) Montreal Neurological Institute (MNI) coordinates (x,y,z).

5.6.5.6 Regions of interest

Regions of interest chosen were based on previous human studies pertaining to taste processing, reward evaluation of food and homeostatic regulation of food intake shown to be stimulated by ingested nutrients.

They are:

- Hypothalamus (Little et al., 2013, In review, Liu et al., 2000, Smeets et al., 2005b)
- Brainstem (Pons, medulla, midbrain)(Small, 2012, Little et al., 2013, In review)
- Hippocampus and parahippocampal gyrus (Haase et al., 2009)

- Thalamus (Haase et al., 2009, Small, 2012)
- Striatum (Caudate and putamen) (Chambers et al., 2009)
- Cingulate (Chambers et al., 2009, Small, 2012)
- Insula and frontal operculum (de Araujo and Simon, 2009, Frank et al., 2008, Chambers et al., 2009)
- Amygdala (Small, 2012, O'Doherty et al., 2002)
- OFC (Frank et al., 2003, Chambers et al., 2009, Small et al., 2007)

5.7 Results

5.7.1 Participants

Participant characteristics are displayed in table 5.3.

	mean ± SD (*n* =15)
Age (years)	23.2 ± 3.4
Height *(cm)*	174.6 ± 9.9
Weight *(kg)*	69.3 ± 12.3
BMI (kg/m^{-2})	22.6 ± 2.2

Values represent mean ± SD (n =15)

Table 5.3 Participant characteristics

5.7.2 Part 1 Oral pre-taste

The responses to oral glucose, revealed by the contrast with the control condition (Glucose-AS), are presented in table 5.4 and subsequent figures showing the average across all voxels in the cluster. At the point of taste (t= 5 mins) there was an instantaneous increase in BOLD signal in the brainstem (figure 5.13) and cingulate

cortex (figure 5.14a and b) following which the signal decreased. In regions of the frontal cortex and left insula (figure 5.15a, b and c) there was no or a smaller increase in the BOLD signal at the point of taste with the signal decreasing thereafter. There was signal change in the right insula but the cluster size did not reach significance. No signal change was apparent in any other regions of interest hypothesised in section 5.6.5.6.

	cluster statistics		**CoM**		
TD Region	**k**	**pFWEc**	**x,y,z (mm)**		
Brainstem - Pons/Medulla	34	0.012	-2	-38	-41
AAL Region					
Superior frontal _R/ Medial frontal_R	76	<0.001	13	59	22
Superior frontal_L/Middle frontral_L	244	<0.001	-24	54	13
Inferior frontal_L/Insula_L	49	0.003	-40	28	-10
Middle frontal_R	72	<0.001	29	50	10
Superior frontal_L	40	0.007	-18	18	39
Anterior cingulate	75	<0.001	1	27	-7
Mid Cingulate	42	0.005	-6	-32	39

Table 5.4 Significant clusters exhibiting an effect of glucose taste

TD- Talairach Daemon; k – number of voxels in cluster; pFWEc- cluster size Family Wise Error corrected p-value; CoM- centre of mass (MNI coordinates); AAL- anatomical automatic labelling; L-left; R-right.

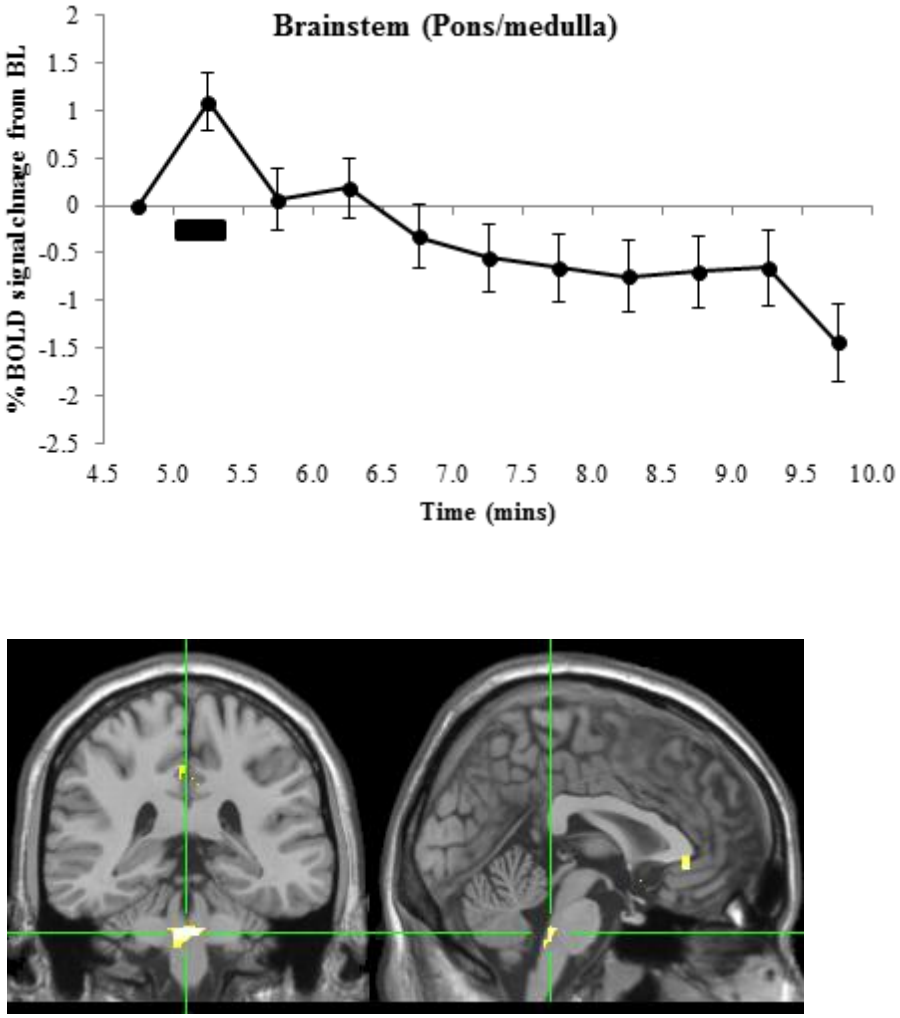
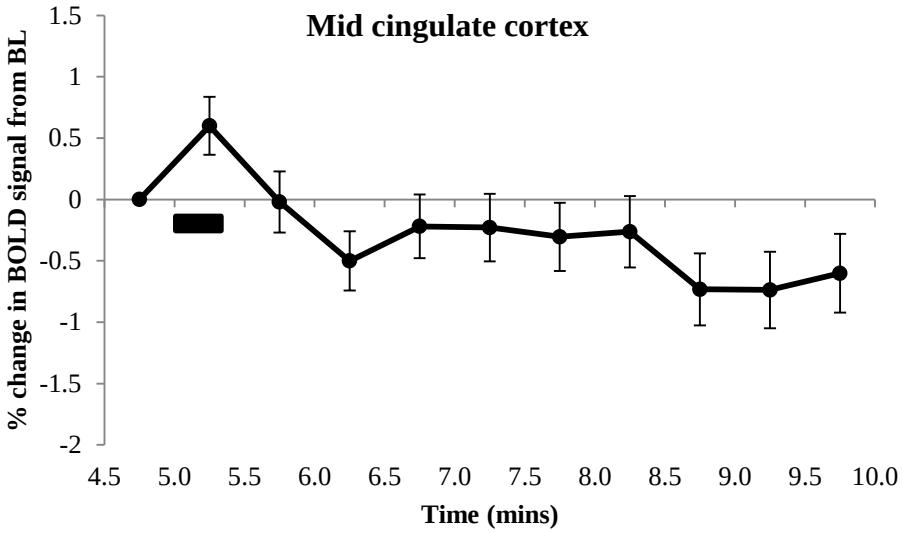


Figure 5.13 Change in blood oxygen level dependent signal over time in the brainstem in response to glucose taste

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the pons and medulla cluster following glucose taste relative to AS. Values represent mean ± SEM, (n=15). ▬ Oral taste. Brain image with cross hairs pinpointing the precise area of interest.



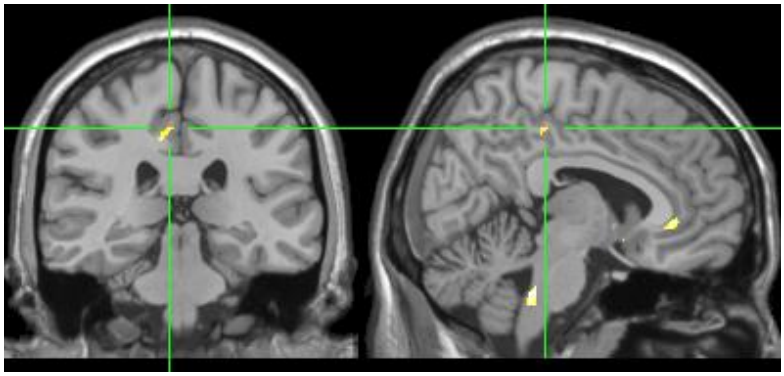
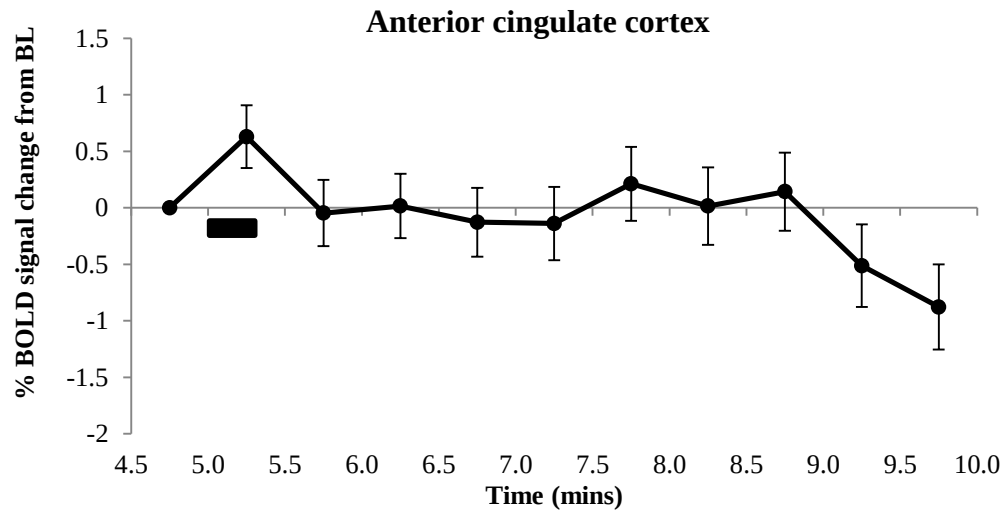


Figure 5.14a Change in blood oxygen level dependent signal over time in the cingulate cortex in response to glucose taste

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the cingulate cortex following glucose taste relative to AS. Values represent mean ± SEM, (n=15). ▬ Oral taste. Brain image with cross hairs pinpointing the precise area of interest.



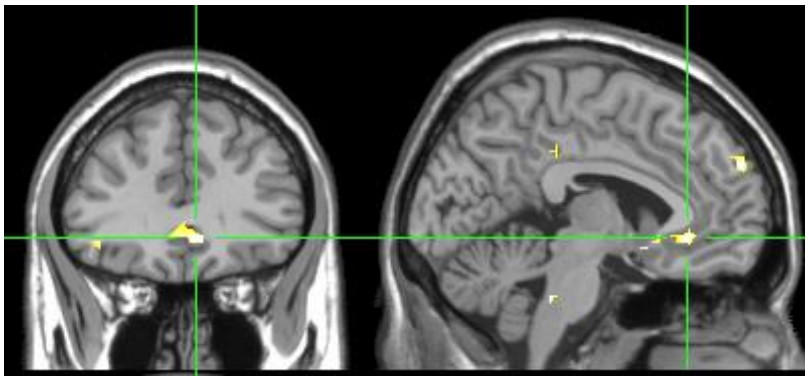
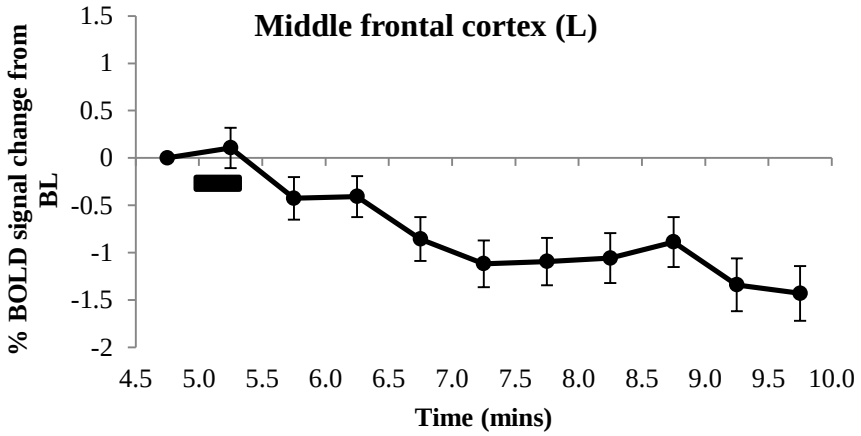
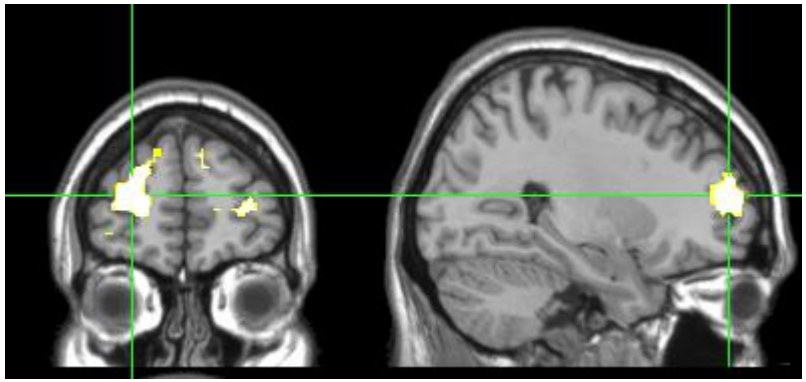


Figure 5.14b Change in blood oxygen level dependent signal over time in the anterior cingulate cortex in response to glucose taste

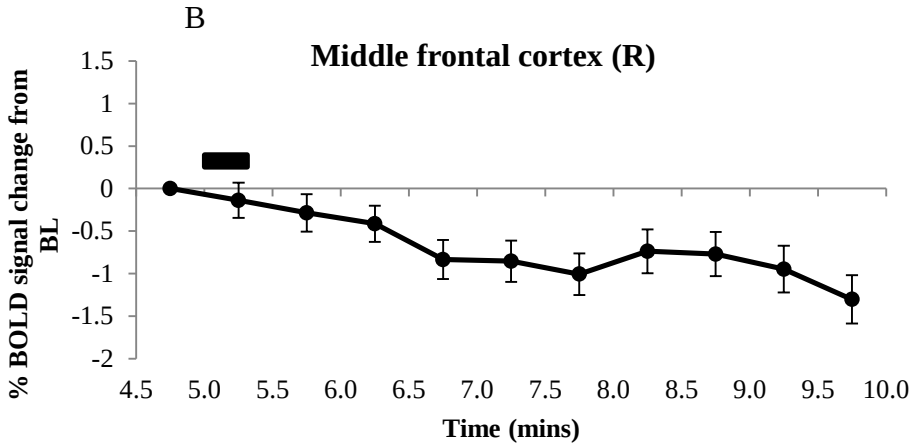
Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the anterior area of the cingulate cortex following glucose taste relative to AS. Values represent mean ± SEM, (n=15). ▬ Oral taste. Brain image with cross hairs pinpointing the precise area of interest.

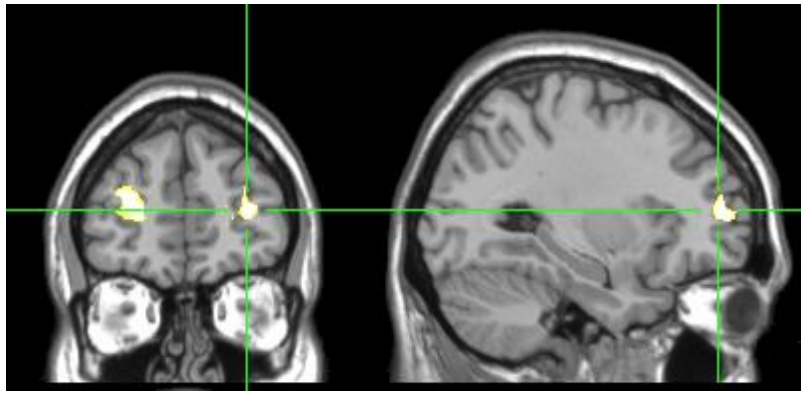
A

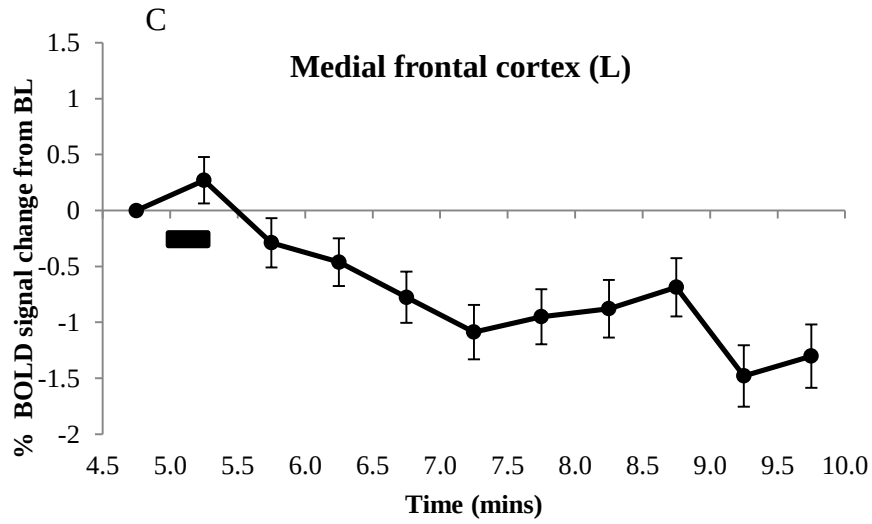

Middle frontal cortex (L)
% BOLD signal change from BL
1.5
1
0.5
0
-0.5
-1
-1.5
-2
4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0
Time (mins)



B


Middle frontal cortex (R)
% BOLD signal change from BL
1.5
1
0.5
0
-0.5
-1
-1.5
-2
4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0
Time (mins)





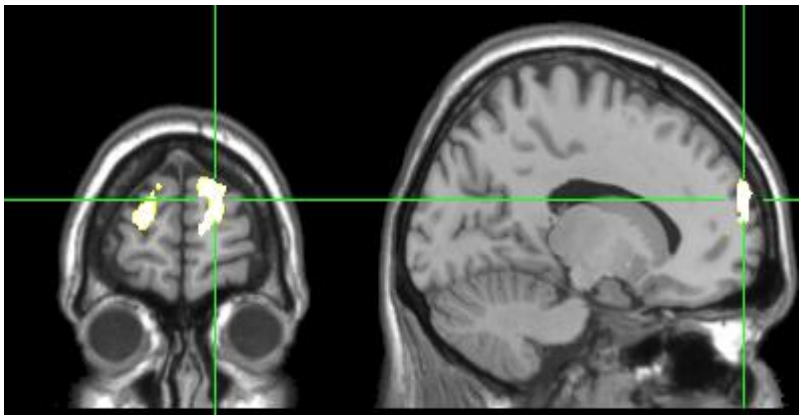
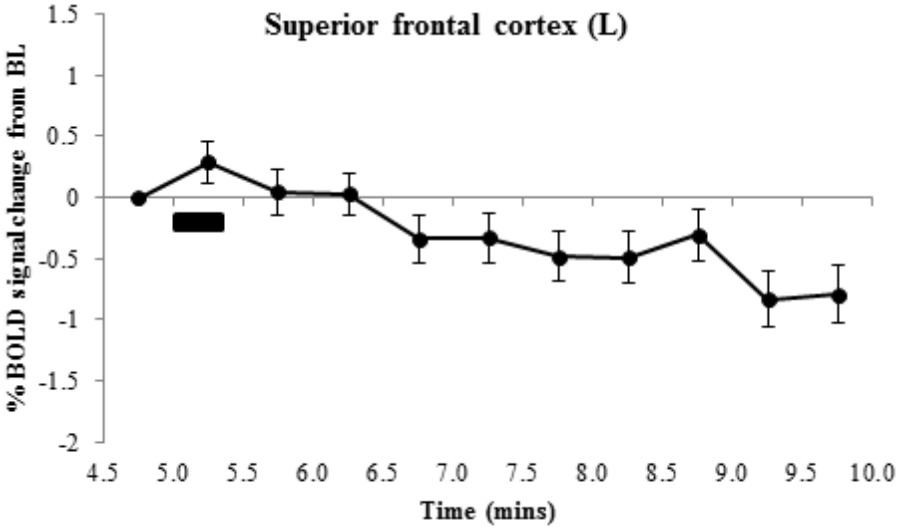


Figure 5.15a Change in blood oxygen level dependent signal over time in the frontal cortex in response to glucose taste

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the left (L) (A) and right (R) (B) middle frontal cortex and left medial frontal cortex (C) following glucose taste relative to AS. Values represent mean $\pm$ SEM, (n=15). ▬ Oral taste. Brain images with cross hairs pinpoint the precise area of interest.



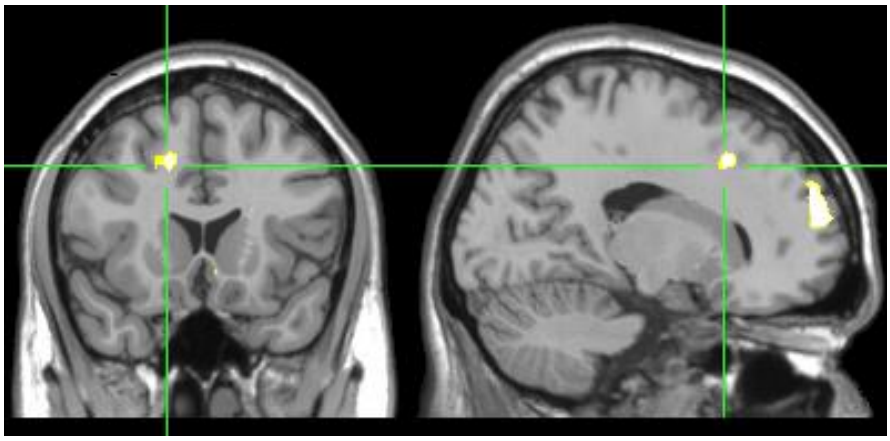
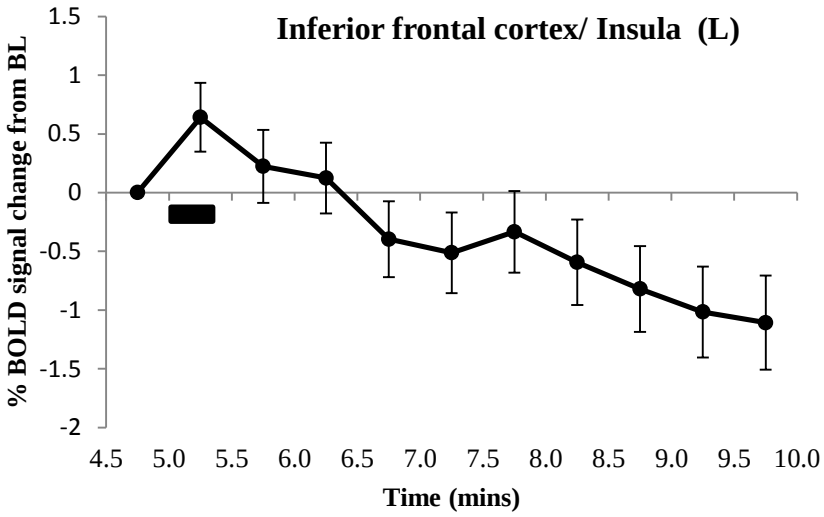


Figure 5.15b Change in blood oxygen level dependent signal over time in the superior frontal cortex in response to glucose taste

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the left (L) superior frontal cortex and left medial frontal cortex following glucose taste relative to AS. Values represent mean ± SEM, (n=15). ▬ Oral taste. Brain image with cross hairs pinpoints the precise area of interest.



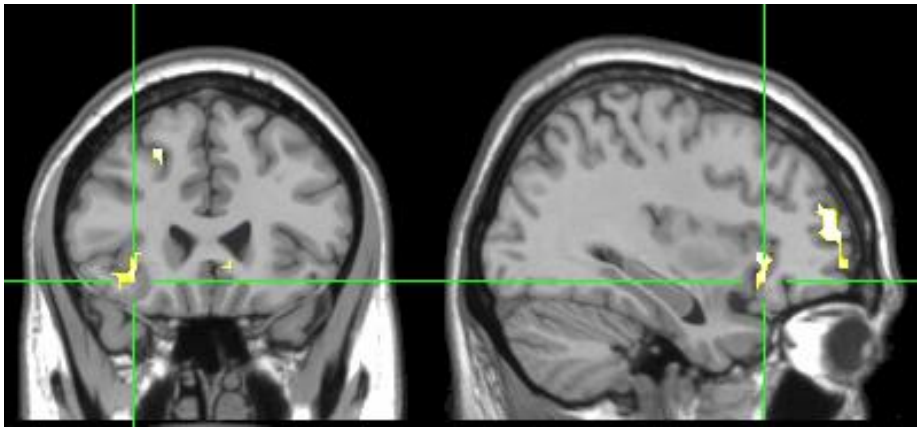


Figure 5.15c Change in blood oxygen level dependent signal over time in the inferior frontal cortex in response to glucose taste

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the left (L) superior frontal cortex and left medial frontal cortex following glucose taste relative to AS. Values represent mean ± SEM, (n=15). ▬ Oral taste. Brain image with cross hairs pinpoints the precise area of interest.

5.7.3 Part 2 Responses following intragastric infusions

There was a significant interaction between glucose taste and intragastric glucose infusion as indicated by changes in the BOLD signal bilaterally in the midbrain, pons and hypothalamus following an oral glucose but not AS taste prior to gastric infusion. In general terms, the well established reduction in BOLD signal following intragastrically administered glucose was unaffected by the AS oral taste condition, whilst the oral taste of glucose prior to intragastrically administered glucose radically altered the response observed. There were also significant clusters bilaterally in higher brain centres such as the putamen, olfactory bulb, areas of the cingulate cortex, caudate, hippocampus, thalamus and laterally in the insula (figure 5.16 and table 5.5). Areas of the frontal cortex that were activated by glucose taste in part 1 also displayed significant clusters along with occipital regions (cuneus, calcarine, lingual). Time course plots for the significant areas are presented and discussed in detail below.

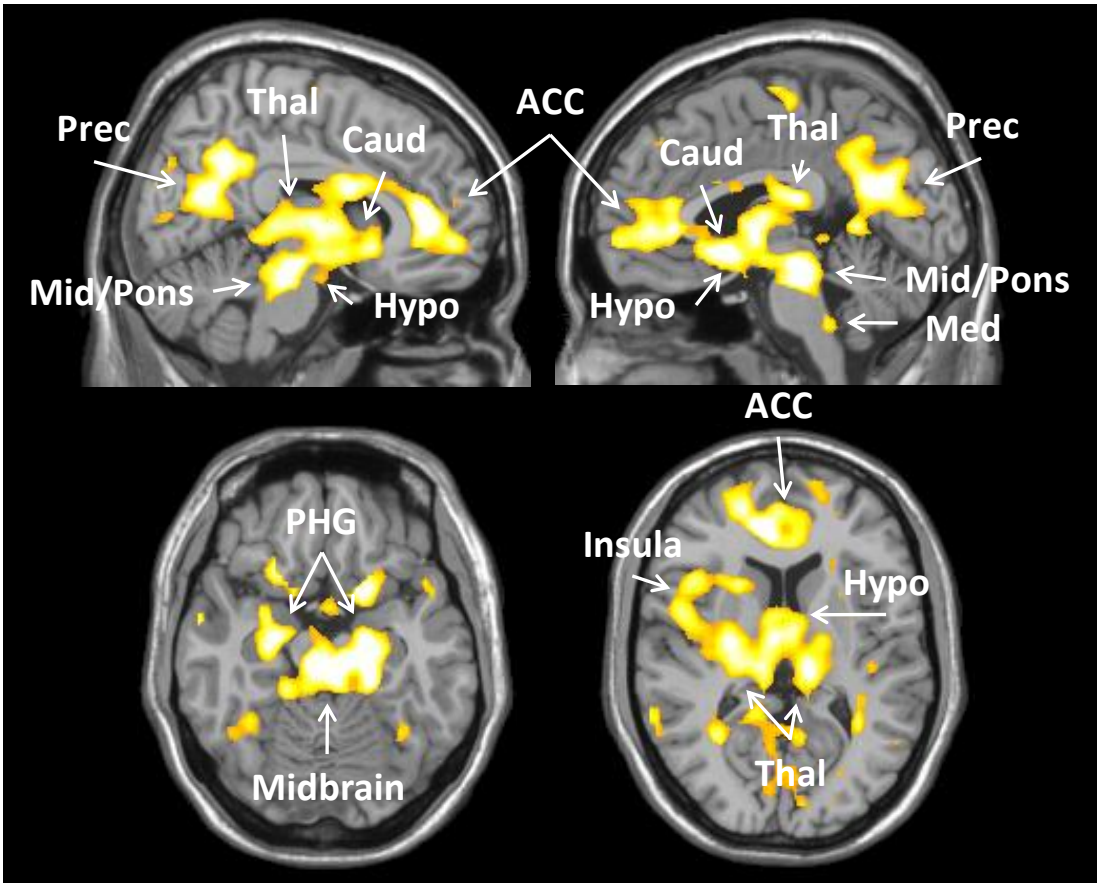


Figure 5.16 Brain images showing areas exhibiting a significant interaction between intragastric glucose infusion and glucose taste

ACC- anterior cingulate cortex; Caud- caudate; Hypo- hypothalamus; Med- medulla; Mid- midbrain; PHG- parahippocampal gyrus; Prec- precuneus; Thal- thalamus.

	Cluster statistics			CoM		
TD Region	**k**	**pFWEc**		**x,y,z (mm)**		
Brainstem - Medulla	15	0.034	*	4	-41	-45
Brainstem - Midbrain L	206	<0.001		-8	-22	-12
Brainstem - Midbrain R	230	<0.001		10	-22	-12
Brainstem - Pons L	30	0.017	*	-5	-29	-27
Brainstem - Pons R	52	0.034		8	-29	-27
Hypothalamus L	37	0.006	*	-5	-3	-9
Hypothalamus R	38	0.005	*	5	-3	-10
AAL Region						
Superior frontal_L▲	55	0.027		-21	55	5
Superior frontal _R▲	33	0.137	**	19	58	6
Orbital superior frontal _L▲	56	0.025		-20	54	-7
Orbital inferior frontal_L▲	55	0.027		-44	11	10
Rolandic operculum_L▲	58	0.021		-48	-1	8
Insula_L▲	247	<0.001		-36	5	0
Putamen_L▲	220	<0.001		-23	3	-1
Putamen_R▲	61	0.017		29	5	-2
Olfactory▲	95	0.001		8	14	-12
Medial superior frontal ▲	95	0.001		3	54	7
Orbital medial frontal_L▲	60	0.019		-8	51	-7
Orbital medial frontal_R▲	85	0.003		8	51	-5
Anterior cingulate_L▲	195	<0.001		-4	40	6
Anterior cingulate_R▲	123	<0.001		7	40	6
Caudate_L▲	213	<0.001		-11	9	7
Caudate_R▲	113	<0.001		12	16	-3
Posterior cingulate_L	49	0.042		-4	-49	29
Hippocampus_L▲	84	0.003		-25	-13	-19
Hippocampus_R▲	68	0.010		23	-21	-12
ParaHippocampal gyrus_R	71	0.008		21	-18	-19
Calcarine_L▲	161	<0.001		-7	-67	12
Calcarine_R▲	75	0.006		7	-72	12
Cuneus_L	81	0.004		-4	-71	26
Cuneus_R	54	0.029		9	-71	23
Lingual_R	55	0.027		-10	-38	-1
Precuneus_L▲	243	<0.001		-6	-57	29
Precuneus_R▲	109	<0.001		6	-58	29
Thalamus_L	218	<0.001		-11	-19	5
Thalamus_R	206	<0.001		12	-20	5

Table 5.5 Significant clusters exhibiting an interaction of intragastric glucose infusion by glucose taste

TD- Talairach Daemon; k – number of voxels in cluster; pFWEc- cluster size Family Wise Error corrected p-value; CoM- centre of mass (MNI coordinates); AAL- anatomical automatic labelling; L-left; R-right. * small volume correction (SVC) pFWEc(SVC) x 11 regions of interest. ** Not significant at pFWEc < 0.05 but bilateral. ▲denotes regions not activated by intragastric glucose alone (Little et al., 2013, In review).

5.7.3.1 Brainstem and hypothalamus

Over the duration of the scan a progressive and persistent decrease in the BOLD signal was observed in the medulla (figure 5.17) and pons (figure 5.18A) in the AS(glucose-saline) contrast. Similarly, a decrease in the BOLD signal was also observed for the same contrast in the midbrain (figure 5.18B) but this was not apparent until approximately 10 minutes post-infusion (t= 20 mins). In each case the decrease in BOLD signal was attenuated or abolished by glucose taste i.e. the glucose(glucose-saline) contrast. In the hypothalamus (figure 5.19), there was a slight increase in glucose-saline BOLD signal for both taste conditions however this was proceeded by a decrease only in the AS(glucose-saline) contrast.

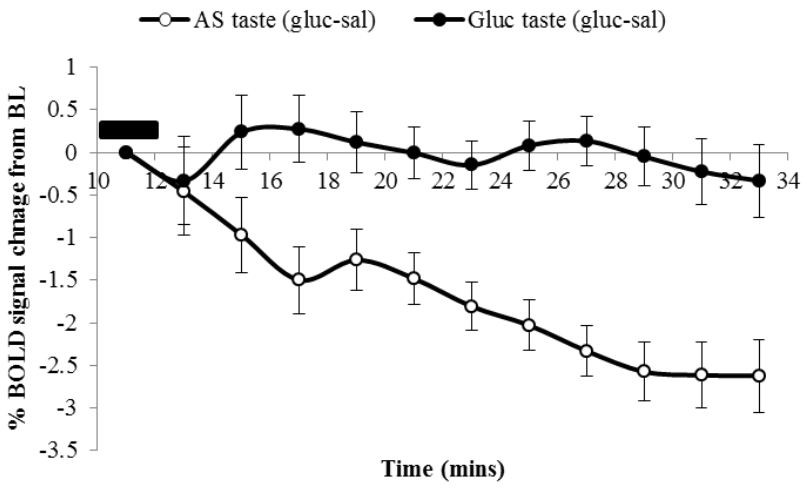


Figure 5.17 Change in blood oxygen level dependent signal over time in the medulla

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the medulla following intragastric glucose infusion preceded by artificial saliva (AS) ( ) and glucose taste ( ). Intragastric infusion. Values represent mean ± SEM, (n=15).

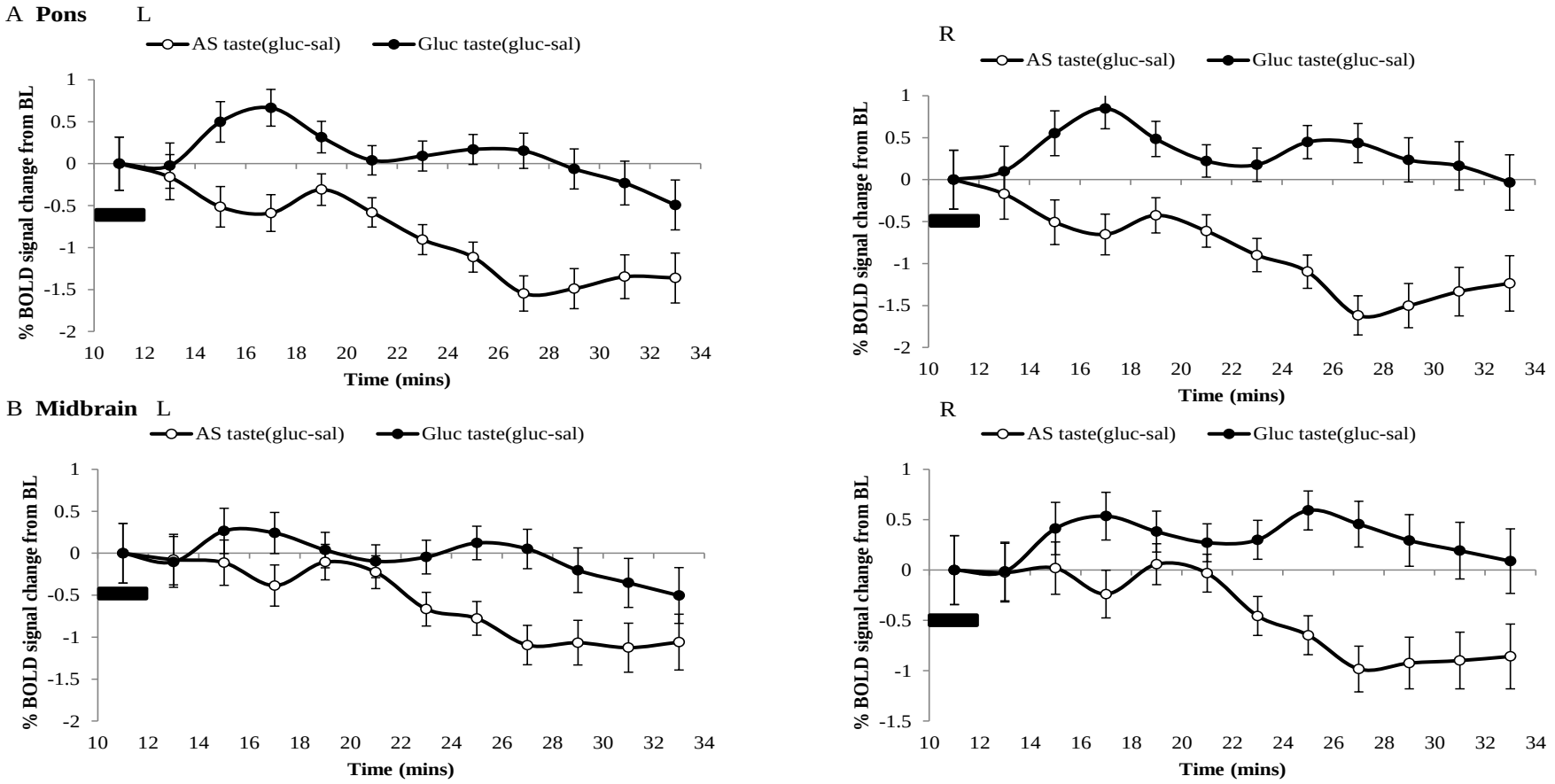


Figure 5.18 Change in blood oxygen level dependent signal over time in the pons and midbrain

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the pons (A) and midbrain (B) following intragastric glucose infusion preceded by artificial saliva (AS) ( ) and glucose taste ( ).Values represent mean ± SEM, (n=15). Intragastric infusion.

Hypothalamus

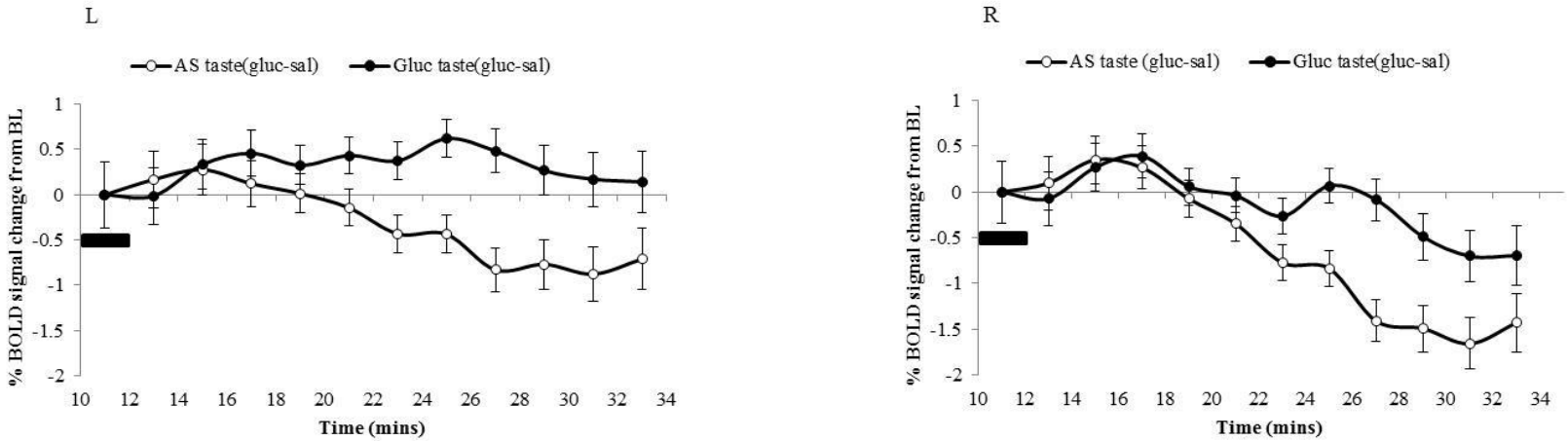


Figure 5.19 Change in blood oxygen level dependent signal over time in the hypothalamus

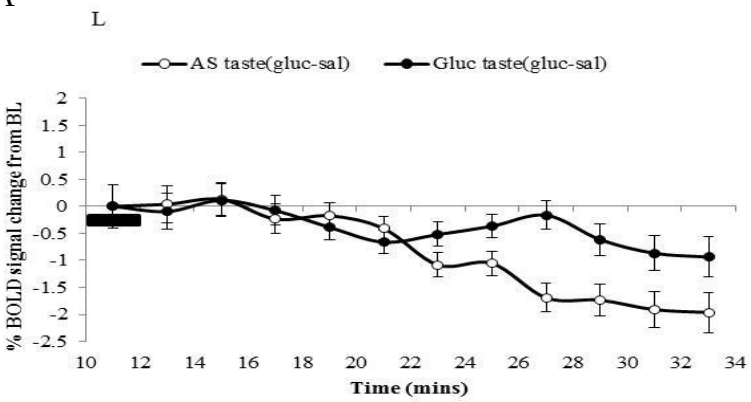
Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the hypothalamus following intragastric glucose infusion preceded by artificial saliva (AS) ( ) and glucose taste ( ).Values represent mean ± SEM, (n=15). Intragastric infusion.

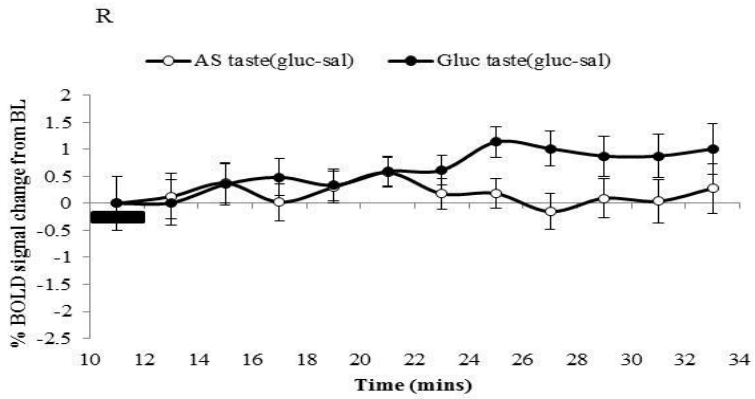
5.7.3.2 Non-homeostatic regions

There was a significant interaction between glucose taste and intragastric glucose infusion as indicated by changes in the BOLD signal in the left and right hippocampus, thalamus, caudate, anterior cingulate cortex, an area of the medial orbitofrontal cortex and insula. No interaction was found in the amygdala. Overall, with the exception of the insula, the BOLD signal for the AS(glucose-saline) contrast decreases and is always lower than the BOLD signal for glucose(glucose-saline) contrast which either increases or remains unchanged from baseline. Plotting the BOLD signal changes in the hippocampus (figure 5.20A), thalamus (figure 5.21) and putamen (figure 5.22A) the BOLD signal for each condition is similar until ~18-21 minutes (8-11 minutes post-infusion) at which point the time courses diverge. A similar pattern was seen in the caudate (figure 5.22B) however, the time courses diverged after 15 minutes (5 minutes post-infusion). In the parahippocampal gyrus (figure 5.19B), anterior cingulate (figure 5.23A) and medial frontal cortex (figure 5.24) the time courses diverged almost immediately following intragastric infusion with the AS(glucose-saline) contrast decreasing and glucose(glucose-saline) increasing. In comparison, both contrasts resulted in a decrease in the BOLD signal in the insula (figure 5.23B). However, the divergence from baseline occurred earlier for the glucose(glucose-saline) than for the AS(glucose-saline) contrast.

Hippocampus

A





Parahippocampal gyrus

B

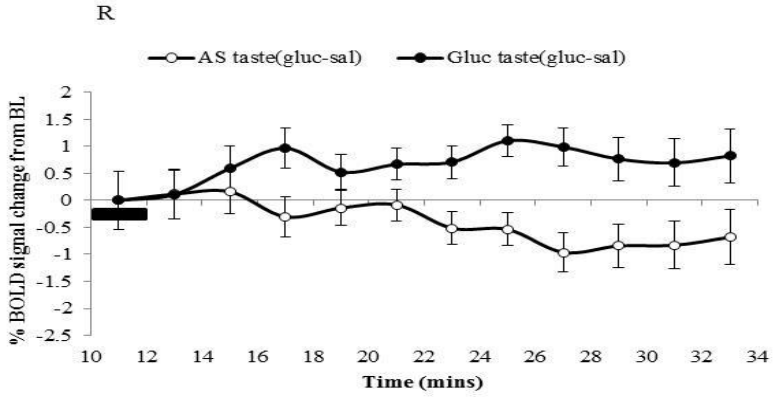


Figure 5.20 Change in blood oxygen level dependent signal over time in the hippocampus and parahippocampal gyrus

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the left (L) and right (R) hippocampus (A) and (R) parahippocampal gyrus (B) following intragastric glucose infusion preceded by artificial saliva (AS) (-○-) and glucose taste (-●-). Values represent mean ± SEM, (n=15). ▬ Intragastric infusion.

Thalamus

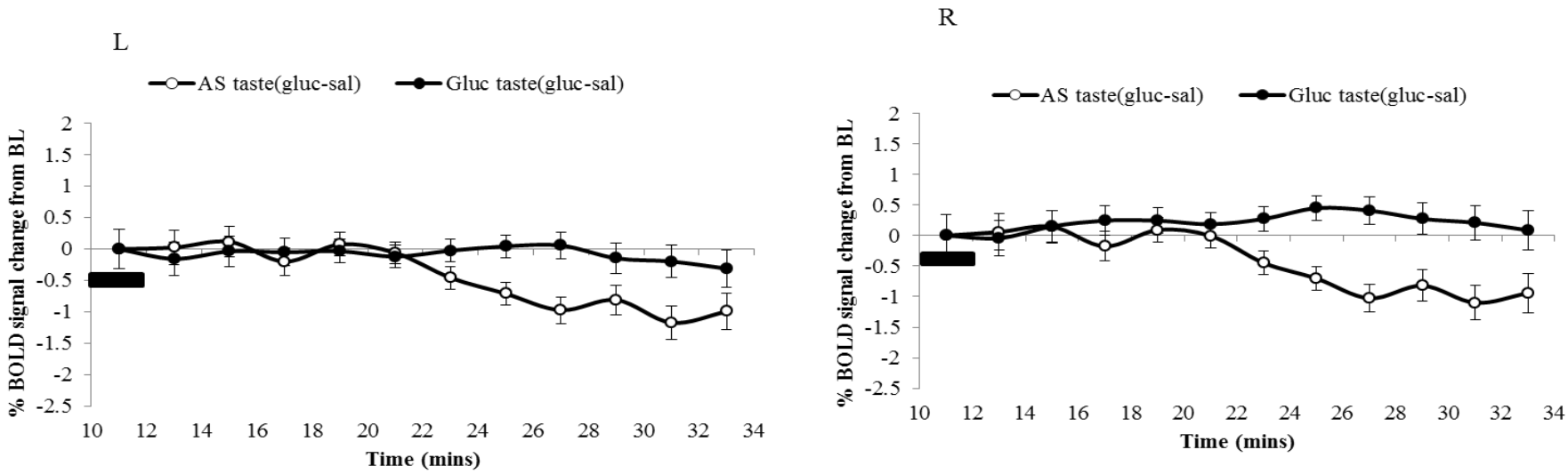


Figure 5.21 Change in blood oxygen level dependent signal over time in the thalamus

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the left (L) and right (R) thalamus following intragastric glucose infusion preceded by artificial saliva (AS) (-○-) and glucose taste (-●-).Values represent mean ± SEM, (n=15). ▬ Intragastric infusion.

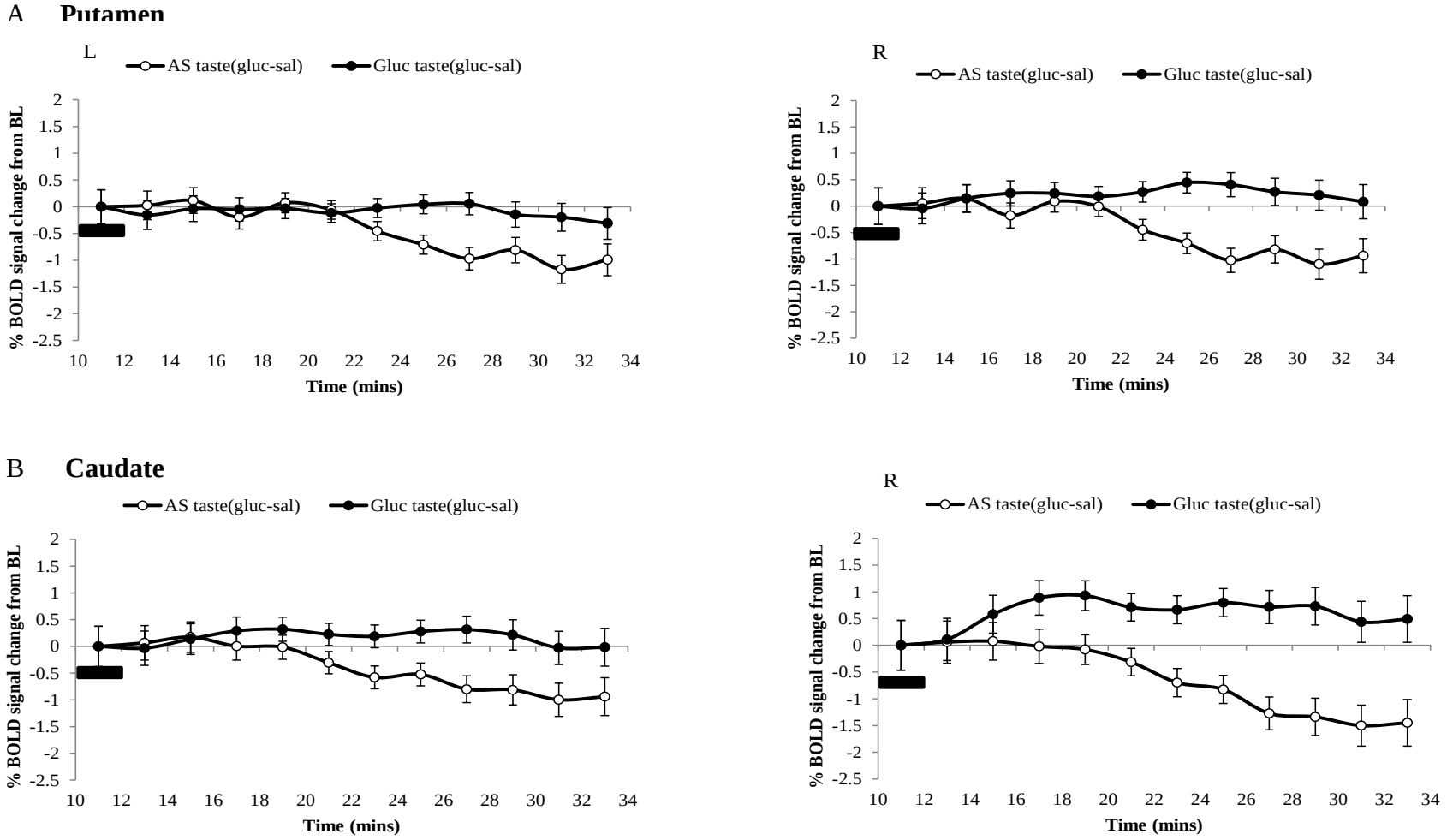
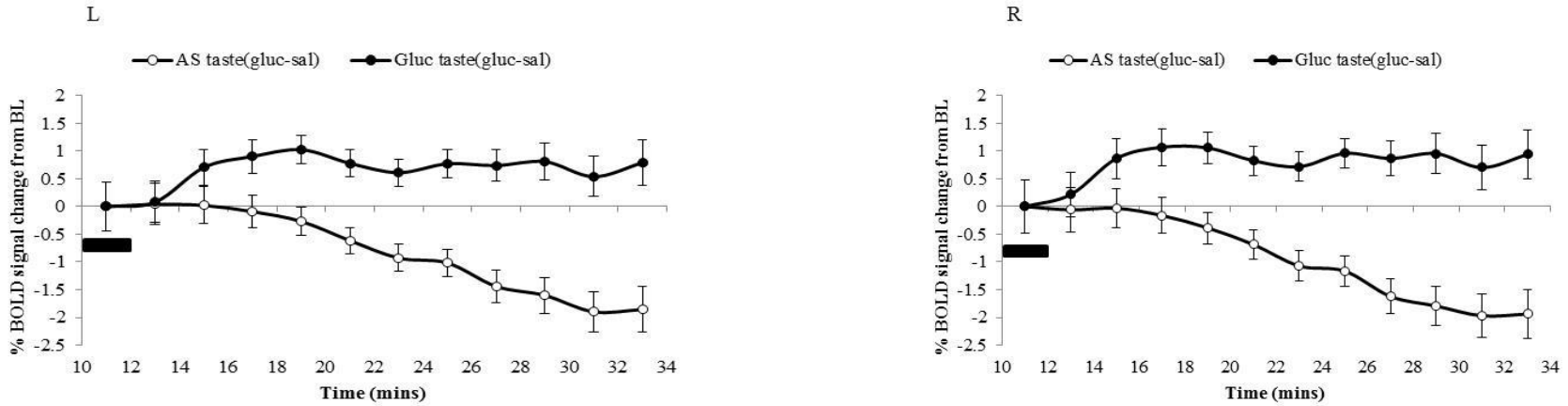


Figure 5.22 Change in blood oxygen level dependent signal over time in the putamen and caudate

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the left (L) and right (R) putamen (A) and caudate (B) following intragastric glucose infusion preceded by artificial saliva (AS) (⊸) and glucose taste (⊸).Values represent mean + SEM, (n=15). ▬ Intragastric infusion.

A **Anterior Cingulate Cortex**



B **Insula** L

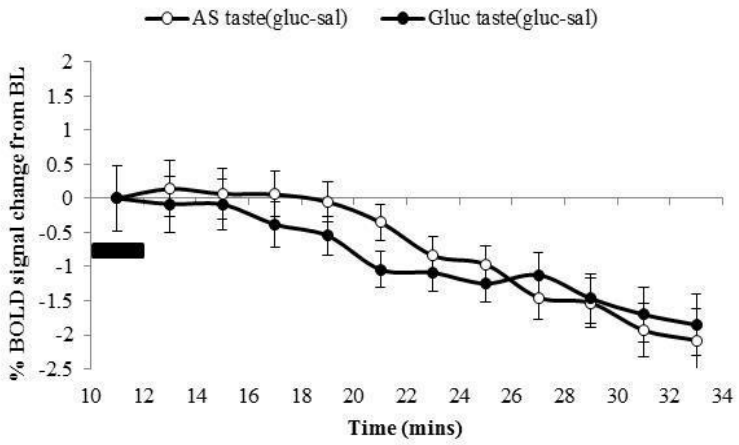


Figure 5.23 Change in blood oxygen level dependent signal over time in the anterior cingulate cortex and insula

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the anterior cingulate cortex (A) and left insula (B) following intragastric glucose infusion preceded by artificial saliva (AS) **(**) and glucose taste ( ). Values represent mean ± SEM, (n=15). Intragastric infusion.

Medial Frontal Cortex

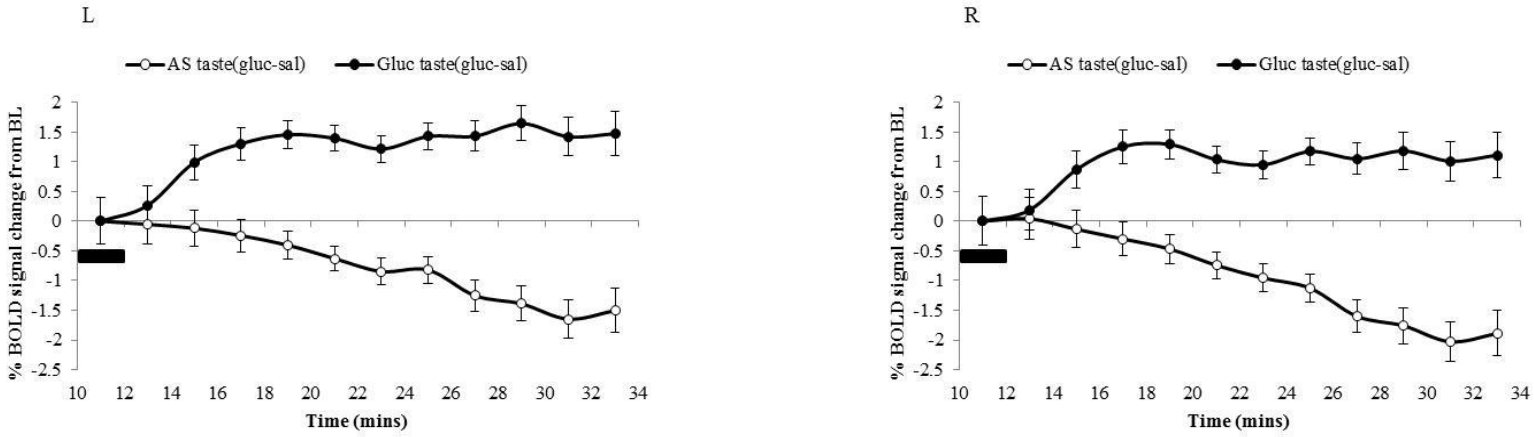


Figure 5.24 Change in blood oxygen level dependent signal over time in the medial frontal cortex

Change in blood oxygen level dependent signal (BOLD) over time from baseline (BL) in the thalamus (A) and caudate (B) following intragastric glucose infusion preceded by artificial saliva (AS) (-○-) and glucose taste (-●-). Values represent mean ± SEM, (n=15). ▬ Intragastric infusion.

5.7.3.3 Comparison to intragastric glucose infusion without oral taste stimulation

The glucose-saline responses in the brainstem and hypothalamus for the AS condition were consistent with previous findings investigating the effect of intragastric glucose infusion on brain BOLD response without oral taste stimulation (Little et al., 2013, In review). In addition, a number of non-homeostatic brain areas were identified as outlined by ▲ in table 5.5. This included regions of the frontal cortex, insula, putamen, anterior cingulate, caudate, hippocampus and parahippocampal gyrus.

5.8 Discussion

This is the first study to investigate the effects of oral and sequential gastrointestinal "sweetness" on functional human brain response in order to determine whether oral sweet taste affects gut-brain signalling. The prequel (study I) formed the basis for this, in that including an oral taste of glucose affected its subsequent rate of gastric emptying, a process known to occur under vagal and neuroendocrine regulation. Previous studies largely omitted an oral taste phase to prevent 'confounding' effects during the study of gastric emptying, when in reality it is a key part of the physiological and psychological response to food.

The objective was therefore to model the sequence as close to normal ingestive patterns as possible whilst minimising the artefacts and difficulties inherent on imaging brain responses during swallowing. There are however limitations to what can be achieved in the MR scanning scenario, so the current protocol was developed to model ingestion as closely as possible, whilst embedding appropriate control conditions. In part 1 (oral taste, t= 5-10 mins), there was a decrease in BOLD signal following the initial increase due to glucose taste in the brainstem (pons/medulla), regions of the frontal cortex, left insula and the cingulate cortex. However, a number of studies report opposite results. Increases in BOLD signal have been reported after glucose, or other sugars, in brain areas not found in the current part of this study (Chambers et al., 2009, Frank et al., 2008, Smeets et al., 2011). These inconsistencies may be attributed to differences in experimental protocol and analysis. In the current study the phase of ingestion was included in the analysis whereas most studies, due to the confounding effects of movement during swallowing, either ignore the ingestion phase completely or begin scanning post-ingestion with subjects consuming outside the scanner. Therefore this is the first study to capture the immediate effects of ingestion.

Previous fMRI studies, have observed BOLD responses in regions such as the thalamus and caudate (Haase et al., 2009, Chambers et al., 2009). These regions formed the basis of the pre-hypothesised areas in the current study. However, in the first phase of the current study a trivial amount of glucose (20ml) was used in comparison to other studies (Smeets et al., 2011, Li et al., 2012) that have involved much higher loads (e.g. 300- 450ml) therefore, meaningful signals arising from the gut via mechano- and chemo-sensitive pathways are inevitable. The current imaging protocol minimises these issues and therefore may be a more accurate representation of the initial response in human ingestion. Despite using an imaging correction protocol to minimise movement (prospective motion correction) and running independent component analysis, to remove components associated with movement, the data were still unavoidably subject to movement artefact during the oral phase. Also data from 30 second time bins (6 images) were averaged for the oral part of the study, compared to 2 minutes time bins (24 images) for the gastric part, meaning the analysis was more subject to temporal effects of movement.

This study has shown a transient increase in BOLD signal at the point of taste, as well as a subsequent decrease, in regions of the brainstem in response to oral glucose. The medulla region of the brainstem is part of the central taste pathway that receives sensory information via facial and glossopharyngeal nerves. In addition, the similar BOLD signal response observed in the anterior cingulate cortex and regions of the frontal cortex and insula reflect the function of these areas in taste processing and perception (Kobayashi et al., 2004). However, the response in these higher brain centres cannot be attributed to taste only but also to the processing of olfactory and somatosensory information.

To determine whether initial glucose taste affected the BOLD signal induced by intragastrically infused glucose, the interaction between glucose taste and intragastric glucose infusion [glucose(glucose-saline) – AS(glucose-saline)] was explored i.e. brain regions where intragastric glucose signalling (glucose-saline) was altered by a prior taste of glucose. It is important to note that the baseline for the gastric part of the study differs from the baseline used in the oral part. The 30 seconds prior to oral infusion were used for part 1 and the 2 minutes preceding intragastric infusion were used for part 2.

In the medulla, pons and midbrain brainstem regions and the hypothalamus, the BOLD signal decreased in the AS(glucose-saline) contrast compared to glucose(glucose-saline) contrast which remained unchanged from baseline. The response of AS(glucose-saline) maps to the

homeostatic regions previously identified using intragastric glucose and lipid infusion (Little et al., 2013, In review, Lassman et al., 2010, Smeets et al., 2005b). The present study also revealed a significant interaction in non-homeostatic areas such as the insula, the primary taste cortex, and medial region of the frontal cortex, assumed to be the secondary taste cortex (Rolls, 2007). The primary taste cortex is believed to project to the striatum (Fudge et al., 2005), which includes the caudate and putamen, regions which also showed a significant interaction. This region, along with the anterior cingulate, which also shows a significant interaction, encodes food reward (Schloegl et al., 2011, Rolls, 2007).

A significant interaction was also observed in the thalamus, hippocampus and parahippocampal gyrus. The thalamus is thought to have a role in processing taste and is modulated by the reward value of the taste stimuli (Kobayashi et al., 2004). The hippocampus and parahippocampal gyrus are involved in memory including learning of food stimuli (Haase et al., 2009).

In contrast to other studies a significant interaction was not observed in the amygdala (Smeets et al., 2011, Li et al., 2012). However, it is believed to be more sensitive to protein than carbohydrate (Li et al., 2012) therefore the current data further support that possibility.

It appears from these results that stimulating oral taste receptors with glucose prior to an intragastric glucose load [glucose(glucose-saline)] attenuates the BOLD signal in the usually observed homeostatic regions (Little et al., 2013, In review). In addition the significant interactions observed in non-homeostatic regions are not usually observed after intragastric infusion of glucose. This implies that the whole brain response to glucose is composite, with homeostatic and hedonic signals triggered by oral tasting, but exerting a persistent and long lasting post-taste effect on subsequent brain responses for at least the 30 minutes during which images were acquired. In seeking to explore possible mechanisms for this effect it is interesting to consider first the gastric emptying results from study I in this chapter and, second the potential effects of non-homeostatic regions. The differences in the gastric emptying rates of oral and intragastric glucose may relate to the onset of BOLD signal changes observed in the current study and the relative contribution of oral and gastric stimulation to CNS responses. The onset of decrease in BOLD signal for the AS(glucose-saline) contrast or the point at which the two contrasts diverged occurred approximately 10 minutes post-infusion in some brain regions (midbrain, hypothalamus, hippocampus, thalamus and caudate) which may contribute to the differences seen in gastric emptying rate

between orally and intragastrically administered glucose in the first 15 minutes and remaining 45 minutes of study I. Furthermore, the gastric emptying of glucose administered intragastrically was significantly slower than that of orally delivered glucose which is consistent with the decrease in BOLD signal observed in the AS(glucose-saline) contrast and the loss of decreased BOLD signal observed for the glucose(glucose-saline) contrast.

The significance of a negative BOLD signal in the current study and that of others (Smeets et al., 2007, Li et al., 2012) is only a proxy measure of the overall activity of the target brain regions, and as yet cannot be used to directly correspond to neuronal effects occurring at a neurophysiological level. However, it is assumed that a reduction in BOLD signal indicates that neuronal activations are relatively less in that region, and it appears likely that the different kinetics of gastric emptying, but not satiety, described in study I are the physiological counterpart of the changes observed.

A greater reduction in BOLD signal is observed with glucose in the gastrointestinal tract than intravenous glucose despite lower circulating glucose concentrations (Smeets et al., 2007) suggesting that signals emanating from the gastrointestinal tract play an important role. Chapter one highlighted the important role of gut hormones in regulating appetite and food intake and although not measured in the current study, previous fMRI studies have demonstrated a relationship between BOLD signal and blood glucose and gut hormone concentrations (Liu et al., 2000, Li et al., 2012). The presence of glucose sensing neurons in hypothalamic and brainstem regions are believed to be sensitive to elevated glucose concentrations.

Oral and cephalic responses are clearly important components *in vivo.* Signals from receptors in the mouth, which are bypassed by an intragastric infusion, may influence neural pathways responsible for modulating responses to food. An obvious question would be to determine the effects of other taste stimuli such as NNS which may have a similar perceived sweetness but lack caloric content. Conversely, non-sweet carbohydrates such as maltodextrin or even aversive tastes such as bitter substances may produce different brain responses and modulate the gut-brain signal differently. Future work using this paradigm should investigate the effects of other nutrient classes.

In comparison to other studies using single sex populations (usually male), the current study involved both male and female participants. It has been shown that males and females exhibit

differential brain responses in taste and reward areas in response to taste stimuli (Haase et al., 2011). However, establishing gender differences in the current study was beyond the remit of this study and would require a much higher number of participants to be statistically significant.

The study was limited by several factors. As previously discussed the confounding effects of movement during swallowing cannot be totally eliminated but the protocol was designed to minimise this as much as possible. Second, the BOLD signal is only a proxy measure of activity. Nevertheless in absence of non-invasive markers of neuronal activity, this is currently the measure utilised in fMRI studies which remains the gold standard. Third, gut hormone and glycaemic responses were not measured in the current study due to the impractical nature of taking blood while participants are positioned in the scanner and previous experience that this increases movement artefact diminishing data quality. Fourth, as participants were in a supine position in the scanner this may have had an unavoidable effect on the rate of gastric emptying rate (Ikeda et al., 2008).

In conclusion, it appears that stimulating oral taste receptors with glucose prior to an intragastric glucose load attenuates the BOLD signal in the usually observed homeostatic regions and causes a significant interaction in non-homeostatic regions not usually observed at all. The response of higher brain centres may provide a mechanism to explain choices and food preference, and now requires further study in responses to nutrient classes, and clinical scenarios such as obesity and anorexia of disease.

CHAPTER 6

General Discussion

Chapter one highlighted the multifaceted nature of the complex systems working in synergy to control appetite and food intake. The gut plays an unquestionable role in this control and its interaction with the brain has become increasingly apparent in recent years. That said, the body of existing literature is somewhat limited by species differences and experimental designs which often look at potential mechanisms in isolation or in unrealistic protocols. Conflicting data and inconsistent findings further complicate matters.

Understanding the impact and the mechanisms mediating the effects of sugars and NNS on appetite and food intake is of major nutritional and clinical importance, and may be a useful strategy for the development of novel foods in the treatment of obesity and appetite related disorders. The research presented within this thesis complements current knowledge surrounding the effects of sweet tastants (glucose and NNS) on appetite, hormones, gastric emptying and CNS responses. This chapter will collate the findings from the studies presented herein, highlight methodological issues and conclude with future directions. An overview of each study is summarised in table 6.1 and the main findings summarised thereafter.

	Chapter 3			Chapter 4	Chapter 5	
	Part I	*Part II*	*Part III*		Study1	Study 2
n	7	9	7	10	10	15
Trials	1x 45 min trial *Oral* Glucose Glucose + 250 ppm lactisole Glucose + 500pppm lactisole Lactisole	2 x 150 min trials *IG* Glucose Glucose + 250 ppm lactisole	4 x 60 min trials *IG* Glucose Glucose + 500pppm lactisole Lactisole Water	4 x 60 min trials *Oral* Glucose Glucose + Ace-k Glucose + saccharin Glucose + aspartame	6 x 60 min trials *Oral and IG* Oral x 3 Intragastric x 3 Glucose Saccharin Water	4 x 45 min trial *Oral and IG* Glucose oral + glucose IG Glucose oral +Saline IG AS oral + glucose IG AS oral + saline IG
Measurements	Taste	Gastric empting Blood Glucose Appetite Food intake	Gastric empting Blood Glucose Gut hormones Appetite Food intake	Taste Blood glucose Appetite	Gastric emptying Appetite	BOLD signal

Table 6.1 Summary of study protocols presented in this thesis

All visits were conducted on single volunteers in the morning after an overnight 11 hour fast. In total 213 completed study visits are presented, excluding subjects screened and enrolled but who did not complete the full protocols (n=13).

IG- intragastric, AS- artificial saliva, BOLD- blood oxygen level dependent

6.1 Glucose, non-nutritive sweeteners and the gut sweet taste receptor

The main findings from chapters three and four are outlined below and depicted in figure 6.1.

- The oral sweet taste of 1M glucose was effectively abolished by 250 and 500 ppm lactisole in the oral cavity (A). Following an intragastric infusion of glucose and lactisole (500 ppm), the previously described responses to glucose were preserved (B) with no effect of lactisole on gastric emptying, blood glucose, gut hormones, appetite ratings and food intake suggesting additional sweet sensing mechanism/s not blocked by lactisole or a non-taste pathway glucose specific mechanism may exist to modulate the responses to intragastric glucose infusion (C). I cannot however exclude the possibility that higher doses of lactisole would be required to block sweet taste receptors 'pharmacologically' in the gut. However the amounts used here certainly exceed those used by the food industry.

- Comparable to glucose ingestion alone, ingesting aspartame, saccharin and ace-k in combination with glucose had similar effects on blood glucose and appetite ratings (D). However, ace-k did result in a small enhancement in AUC for blood glucose although the metabolic significance of such a small effect is unlikely to have any dietetically relevant impact.

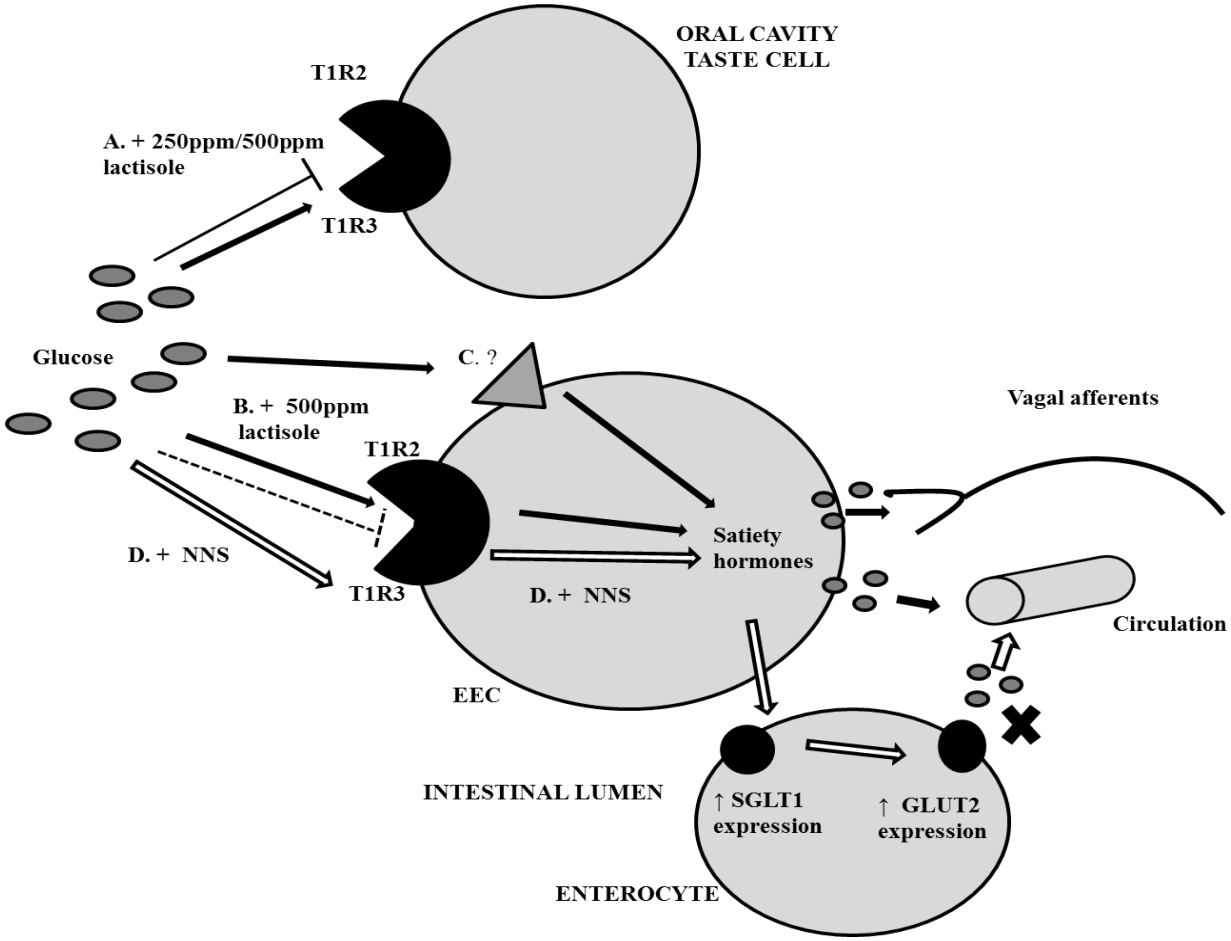


Figure 6.1 Summary schematic depicting findings from chapters three and four

Stimulates | Inhibits | Incompletely inhibits (Gerspach et al., 2011) | Proposed pathway (Mace et a., 2007) | No effect on blood glucose

The results from chapter three (*part III*) in conjunction with those found by Gerspach *et al* propose a limited role for the gut sweet taste receptor but highlight the need for further studies to fully elucidate the mechanism behind glucose sensing. Access to the human gastrointestinal tract and EEC cells directly remains a challenge in human research thus the majority of evidence stems from *in vitro* and animal models. Whether these findings are representative of human *in vivo* function is obviously questionable: my data would suggest not. An example of this issue was highlighted by chapter four which demonstrated no increase in glucose absorption, as measured by blood glucose concentrations, following ingestion of glucose in combination with a panel of NNS compared to ingestion of glucose alone despite i*n vitro* and *in vivo* animal models suggesting otherwise (Margolskee et al., 2007, Mace et al., 2007).

That said, discrepancies between human studies are just as inherent in research published to date. Some differences can be attributed to experimental approach. However variation both between individuals and within individuals can also be a confounding factor. The large variability in gut hormone responses observed in chapter three *part III* is one example of this. Furthermore, gut hormone response is only an indirect measure of the epithelial secretory response to the portal circulation and may not translate to elevated peripheral plasma levels.

The two published studies conducted in humans so far (Brown et al., 2009, Ma et al., 2010) and the results of chapter four indicate that glucose in combination with a NNS is insufficient to alter blood glucose, albeit given acutely in healthy humans. Both studies in chapter three and four focused on results derived from short-term measures and it is possible that chronic ingestion or repeated exposure to the test meals used, particularly NNS diet supplementation, may infer different results. With that in mind it may be useful for future studies to control for this and stratify participants into high and low sugar and NNS consumers as previous dietary intake of participants was not taken into account in the current studies.

6.2 Sweet tastants in the gut-brain axis: brain integration of oral and gastric sensing

As alluded to in chapter one, the control of appetite and food intake involves a combination of homeostatic and non-homeostatic mechanisms which involve

processes including sensory, cognitive, post-ingestive and post absorptive as depicted in the satiety cascade (figure 1.1).

The approach used in chapter five is the first of its kind, and I demonstrated that oral pre-tasting of sweetness has a marked impact on the subsequent CNS response to intragastric glucose. This means that in order to study whole-organism responses to nutrients it may not be appropriate to bypass the oral and cephalic phase. Indeed, the subtle but real differences in gastric emptying depending on route of administration make this point more strongly. This links altered CNS activity to changes in gut function with a temperospatial matrix that makes biological sense, within the limitations of fMRI. This has important implications for the design of healthy yet appetising food, a key interest of the food industry.

fMRI is the current gold standard, and recent imaging and analytic advances were required to obtain good enough spatial resolution to study the brainstem structures key to gut-brain interactions. The lack of ionising radiation permits repeat studies in individuals, but it provides only an indirect measure of brain activity. The physiological meaning of increased or reduced BOLD is a topic of debate, but it is clear that different nutrients induce different changes. In stark contrast to reduced BOLD in response to intragastric glucose, intragastric lipid infusion increases BOLD signal in the hypothalamus and other regions (Lassman et al., 2010) and these responses can be modulated by infusing the gut peptide ghrelin (Jones et al., 2012). Current work in my department shows that fructose exerts similar effects to lipid rather than glucose, despite also being a hexose sugar like glucose. From these studies a common anatomical nutrient sensitive matrix appears to exist, but with differential response. What if any impact oral pre-tasting of fat, or other tastants including NNS, would have on lipid- or fructose –induced BOLD changes remains to be established.

Compared with lean individuals, there is a marked attenuation of this inhibitory response in obese individuals (Matsuda et al., 1999). Furthermore, in moderately obese type 2 diabetics, ingestion of an oral glucose loads fails to inhibit hypothalamic neuronal activity (Vidarsdottir et al., 2007). Further work is clearly required to move these findings into key clinical areas.

6.3 Limitations and future directions

All participants were studied in the fully fasted state to standardise each experimental condition. There is a possibility that this outweighs any effect of the test meal in question. Future studies need to consider the effects of sugars and NNS in participants when fasted for varying times, at different times of the days and when sated, particularly as CNS responses have shown a sensitivity to the physiological state participants are in (Haase et al., 2009).

The study population was a homogenous group (young, healthy and lean) and this limits the applicability of the findings to other population groups. Of interest would be to compare the effects in overweight/obese with lean individuals. This is becoming increasingly popular in neuroimaging studies although is somewhat restricted by current scanner limitations of size and weight of participants. Furthermore, targeting gut sweet taste receptor mechanisms in type 2 diabetics may be useful particularly as elevated blood glucose concentrations have been linked to disordered control of sweet taste receptor expression (Young et al., 2009, Young et al., 2013). Potential effects of sugars and NNS on appetite and food intake also have relevance to the management of other eating disorders such as anorexia of disease and age related disturbances of appetite.

In considering the effects of sugars and NNS it is important to remember that the human diet is diverse and consumption of single nutrient classes in isolation is rare, and mainly confined to sports nutrition. Thus, the relative effects should be considered not only comparatively to other macronutrients but collectively to mimic real life intake. Furthermore, all the test meals used in the studies were liquids. The effects of solid test meals would likely infer different results not least due to differing gastric emptying rates. On the other hand, liquid emptying is rapid and less regulated than the early phase solid emptying (figure 1.3), so demonstrating the differences presented herein shows the responses are robust.

VAS scales were used in three out of the four studies in this thesis. Their use in appetite research is common and provides a basic and standard scale for self-assessment of subjective appetite in healthy adults (Blundell et al., 2010). However, there is large between subject and within subject variability requiring large numbers of participants to detect differences between experimental conditions using repeated measures design. This may explain why no significant differences were detected for

subjective appetite ratings between trials in the current studies. Despite this, the method did show a sensitivity to changes in subjective appetite ratings over time and the effect of the test meal ingestion/infusion. Indeed, attempting to capture perceived state of hunger, specific sensations (fullness) and motivation (desire) to eat using VAS may require additional measurements to fully investigate the complex nature of eating behaviour.

The use of fMRI to study CNS appetite pathways in humans is a rapidly evolving research field and brings the prospect that one day brain centres may be sufficiently well understood and manipulated by new therapies and novel food products to influence food intake and thereby control obesity. The development of BOLD has enabled the identification of a number of brain regions but its physiological basis has to be questioned as it is only an indirect measure of brain activity. At present, fMRI BOLD signals are a research mapping tool used to study appetite. The current challenge is to move this research into a clinical domain and study the effects of novel exogenous agents on CNS responses to further our understanding of the intricate nature of human ingestive behaviour

6.4 Summary

In summary, the research presented in this thesis provides valuable and novel insights into the effects of glucose and NNS in the gastrointestinal tract, and into CNS responses. It also highlights important discrepancies between humans and animal studies both *in vitro* and *in vivo.* This lays the foundations for further work in human participants within this area, and cautions against pursuing the field of enquiry in models such as rodents. Teasing out effects of putative mechanisms in isolation (i.e. oral vs. gastric) is not representative of reality and physiologically flawed, as it overlooks the interaction between these mechanisms and their effects that contribute to satiety. Only studies in whole humans can ultimately be regarded as appropriate in the study of human ingestive behaviour.

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Appendix I

Participant Name : ……Code :.....

PERSONAL DETAILS

Contact Address: ……

……

……

Contact details: (home tel): ……

(work tel): ……

(mobile): ……

(email): ……

DOB: …..……/……..…/…….

Sex: ☐ Male ☐ Female

Appendix II

Participant code: …….. Date completed: ……../ ……./……..

DOB: ……./……./ ……. Age:.....

Weight…….. Height: …….. BMI: .....

Please answer the following questions to the best of your knowledge.

If you are unsure about any question please ask the researcher.

Thank you

Has your body weight been stable over the last 3 months?

☐ Yes ☐ No

If you answered no please indicate whether your weight has increased or decreased over the last 3 months and please state the approximate amount:

…… Kg / stone/ lbs * ☐ Gain ☐ Loss

*Please delete as appropriate

Have you dieted in the past in order to lose weight?

☐ Yes ☐ No

If yes please specify the last time:

……
……

How much weight did you lose?

……
……

Are you currently taking any medication other than the contraceptive pill?

☐ Yes ☐ No

If yes please give details:

……
……
……

Females: Are you pregnant or currently breastfeeding?

☐ Yes ☐ No

Are you currently taking any dietary supplements i.e. multivitamins, cod liver oil?

☐ Yes ☐ No

If yes please give details:

……
…….....
.....

Are you currently or have you ever suffered from any of the following;

Breathing problems i.e. Asthma, exercise induced bronchospasm

☐ Yes ☐ No

If yes please give details:

……
……
……

Gastrointestinal disorders

☐ Yes ☐ No

If yes please give details:

.....
.....

Cardiovascular Disease i.e. Angina, heart attack, stroke or hypertension

☐ Yes ☐ No

If yes please give details:

……
……

Metabolic Disease i.e. diabetes, hypothyroidism

☐ Yes ☐ No

If yes please give details:

……
……

Epilepsy or nervous disorder

☐ Yes ☐ No

If yes please give details:

……
……

Have you taken part in any other research in within the last 3 months?

☐ Yes ☐ No

If yes please give details:

……
……

Do you smoke tobacco/ cigarettes?

☐ Yes ☐ No

If yes please give details:

……
……

If no have you ever smoked?

☐ Never ☐ Gave up ……. years ago

Do you suffer from any food allergies or intolerance? i.e. celiac disease, lactose intolerance.

☐ Yes ☐ No

If yes please give details:

……
……

Appendix III

THREE FACTOR EATING QUESTIONAIRE

Please answer both Part I and Part II by following the directions given at the beginning of each section

Thank you

Part I

Please answer the following questions by ticking either true or false, whichever most appropriately describes you

	True	False
1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	☐	☐
2. I usually eat too much at social occasions, like parties and picnics	☐	☐
3. I am usually so hungry that I eat more than 3 times a day	☐	☐
4. When I have eaten my quota of calories, I am usually good about not eating any more	☐	☐
5. Dieting is so hard for me because I get so hungry	☐	☐
6. I deliberately take small helpings as a means of controlling my weight	☐	☐
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry	☐	☐
8. Since I am often hungry, I sometimes wish that while I'm eating an expert would tell me that I have had enough or that I can have something more to eat	☐	☐
9. When I feel anxious I just have to eat something	☐	☐
10. Life is too short to worry about dieting	☐	☐
11. Since my weight goes up and down, I have gone on reducing diets one in a while	☐	☐
12. I often feel so hungry I just have to eat something	☐	☐

13. When I am with someone who is overeating, I usually overeat too ☐ ☐

14. I have a pretty good idea of a number of the ingredients, I usually overeat ☐ ☐

15. Sometimes when I start eating, I just can't stop ☐ ☐

16. It's not difficult for me to leave something on my plate ☐ ☐

17. At certain times of the day, I get hungry because I have gotten used to eating then ☐ ☐

18. While on a diet, if I eat a food that's not allowed, I consciously eat less for a period of time just to make up for it ☐ ☐

19. Being with someone who is eating makes me hungry and I want to eat too ☐ ☐

20. When I feel blue, I often eat ☐ ☐

21. I enjoy eating too much to spoil it by counting calories or watching my weight ☐ ☐

22. When I see a real delicacy, I often get so hungry that I have to eat right away ☐ ☐

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

24. I get so hungry that my stomach seems like a bottomless pit ☐ ☐

25. My weight has hardly changed at all in the last 10 years ☐ ☐

26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate ☐ ☐

27. When I feel lonely, I console myself by eating ☐ ☐

28. I consciously hold back at meals in order not to gain weight ☐ ☐

29. I sometimes get very hungry late in the evening or late at night ☐ ☐

30. I eat anything I want, at any time I want ☐ ☐

31. Without even thinking about it, I take a long time to eat ☐ ☐

32. I count calories as a conscious means of controlling my weight ☐ ☐

33. I do not eat some foods because they make me fat ☐ ☐

34. I am always hungry enough to eat at any time ☐ ☐

35. I pay a great deal of attention to changes in my figure ☐ ☐

36. While on a diet if I eat a food that is not allowed, I often splurge and eat other high calorie foods ☐ ☐

Part II

Please answer the following questions by circling the number above the response that is most appropriate to you

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

1	2	3	4
rarely	sometimes	usually	always

38. Would a weight fluctuation of 5lbs affect the way you live your life?

1	2	3	4
rarely	sometimes	usually	always

39. How often do you feel hungry?

1	2	3	4
rarely	sometimes	usually	always

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

1	2	3	4
rarely	sometimes	usually	always

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

1	2	3	4
rarely	sometimes	usually	always

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

1	2	3	4
rarely	sometimes	usually	always

43. How frequently do you avoid ‘stocking up’ on tempting foods?

1	2	3	4
rarely	sometimes	usually	always

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

1	2	3	4
rarely	sometimes	usually	always

45. Do you eat sensibly in front of others and splurge when alone?

1	2	3	4
rarely	sometimes	usually	always

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

1	2	3	4
rarely	sometimes	usually	always

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

1	2	3	4
rarely	sometimes	usually	always

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

1	2	3	4
rarely	sometimes	usually	always

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

1	2	3	4
rarely	sometimes	usually	always

50. On a scale of 0 to 5, where 0 means no restraint (eating whatever you want whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in') what number would you give yourself

0
eat whatever you want, whenever you want

1
usually eat whatever you want, whenever you want it

2
often eat whatever you want, whenever you want it

3
often limit food intake, but often 'give in'

4
often limit food intake, but rarely 'give in'

5
constantly limiting food intake, never 'giving in'

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

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

Appendix IV

Code ……
Date…….
Trial…….

Please make a vertical mark through the horizontal line to show how you feel at the moment.
Left and right extremes represent minimum and maximum values.

1. **How clear headed do you feel?**

Not at all _____ Extremely

2. **How strong is your desire to eat?**

Not at all _____ Extremely

3. **How energetic do you feel?**

Not at all _____ Extremely

4. **How full do you feel?**

Not at all _____ Extremely

5. **How friendly do you feel?**

Not at all _____ Extremely

6. **How happy do you feel?**

Not at all _____ Extremely

7. **How hungry do you feel?**

Not at all _____ Extremely

8. **How nauseous do you feel**

Not at all _____ Extremely

9. **How jittery do you feel?**

Not at all _____ Extremely

10. **How thirsty do you feel?**

Not at all _____ Extremely

11. **How relaxed do you feel?**

Not at all _____ Extremely

Appendix V

Code ……
Date…….
Trial…….

Please make a vertical mark through the horizontal line to show how you feel at the moment.
Left and right extremes represent minimum and maximum values.

1. **How creamy is the drink?**

Not at all ——————————————— Extremely

2. **How pleasant is the drink?**

Not at all ——————————————— Extremely

3. **How fruity is the drink?**

Not at all ——————————————— Extremely

4. **How salty is the drink?**

Not at all ——————————————— Extremely

5. **How strong is the drink?**

Not at all ——————————————— Extremely

6. **How sweet is the drink?**

Not at all ——————————————— Extremely

7. **How bitter is the drink?**

Not at all ——————————————— Extremely

8. **How sour is the drink?**

Not at all ——————————————— Extremely

Appendix VI

MAGNETIC RESONANCE IMAGING PATIENT DECLARATION

THIS MUST BE COMPLETED BEFORE EXAMINATION COMMENCES

INVESTIGATOR USE: Participant ID

Please answer the following questions by deleting yes/no as relevant:

1) Do you have a pacemaker or artificial heart valve? **YES/NO**
2) Have you ever had heart surgery? **YES/NO**
3) Do you have a hydrocephalus shunt? **YES/NO**
   If yes, is it a programmable shunt? **YES/NO**
4) Have you had any operations on your head? **YES/NO**
5) Have you had any surgery to your head or body within the last 2 months? **YES/NO**
6) Do you have any joint replacements or metal implants? **YES/NO**
7) Have you EVER had metal in your eyes or worked with metal at high speed? **YES/NO**
8) Do you have any shrapnel from a war injury? **YES/NO**
9) Do you wear a false limb, calliper or brace? **YES/NO**
10) Do you have dentures, a dental plate of a hearing aid? **YES/NO**
11) Do you have a hearing aid of any kind (including a cochlear implant)? **YES/NO**
12) Have you suffered from epilepsy or blackouts? **YES/NO**
13) Are you currently wearing a home detention curfew tag? **YES/NO**

PLEASE NOTE: If you answered YES to any questions above, please contact one of the researchers before arranging a trial visit.

For FEMALE PARTICIPANTS OF CHILD BEARING AGE ONLY:

1) Is it possible that you may be pregnant? **YES/NO**
2) Are your breastfeeding? **YES/NO**
3) Do you have any intrauterine contraceptive device or coil? **YES/NO**

I confirm that I have read the above questions and that the answers are correct to the best of my knowledge and belief:

Name: Date:

If you are suitable for inclusion in the study, you will be asked by the radiographer present at your trial appointments to complete this form again and they will make the ultimate decision about whether to proceed with MRI scanning.